

Transgressive segregation in mating traits drives hybrid speciation

Kotaro Kagawa^{1,2} , Gaku Takimoto³, Ole Seehausen^{2,4}

¹Graduate School of Life Sciences, Tohoku University, Sendai, Japan

²Department of Fish Ecology and Evolution, Center for Ecology, Evolution and Biogeochemistry, Swiss Federal Institute of Aquatic Science and Technology, Kastanienbaum, Switzerland

³Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan

⁴Aquatic Ecology and Evolution, Institute of Ecology and Evolution, University of Bern, Bern, Switzerland

Corresponding author: Graduate School of Life Sciences, Tohoku University, 6-3, Aramaki Aza Aoba, Aoba-ku, Sendai 980-8578, Japan. Email: kagawakoutarou@gmail.com

Abstract

Hybridization can rapidly generate novel genetic variation, which can promote ecological speciation by creating novel adaptive phenotypes. However, it remains unclear how hybridization, creating novel mating phenotypes (e.g., mating season, genitalia shapes, sexual displays, mate preferences), affects speciation especially when the phenotypes do not confer adaptive advantages. Here, based on individual-based evolutionary simulations, we propose that transgressive segregation of mating traits can drive incipient hybrid speciation. Simulations demonstrated that incipient hybrid speciation occurred most frequently when the hybrid population received moderate continued immigration from parental lineages causing recurrent episodes of hybridization. Recurrent hybridization constantly generated genetic variation, which promoted the rapid stochastic evolution of mating phenotypes in a hybrid population. The stochastic evolution continued until a novel mating phenotype came to dominate the hybrid population, which reproductively isolates the hybrid population from parental lineages. However, too frequent hybridization rather hindered the evolution of reproductive isolation by inflating the variation of mating phenotypes to produce phenotypes allowing mating with parental lineages. Simulations also revealed conditions for the long-term persistence of hybrid species after their incipient emergence. Our results suggest that recurrent transgressive segregation of mating phenotypes can offer a plausible explanation for hybrid speciation and radiations that involved little adaptive ecological divergence.

Keywords: hybrid speciation, sexual selection, reproductive isolation, individual-based model, transgressive segregation

Introduction

Genetic variation is the raw material for evolution including speciation. Hybridization is an important source of genetic variation because recombination between genomes of genetically distinct parental lineages can generate diverse novel genotypes and phenotypes (Abbott et al., 2013; Kagawa & Takimoto, 2018; Mallet, 2007; Marques et al., 2019). Hybrid phenotypes often involve not only intermediates of the parental forms but also novel and extreme forms that exceed the phenotypic ranges of both parental lineages combined (i.e., transgressive segregation, Rieseberg et al., 1999). Transgressive segregation in ecological traits can promote ecological hybrid speciation by allowing hybrids to utilize novel ecological niches (Abbott et al., 2013; Kagawa & Takimoto, 2018; Lamichhaney et al., 2018; Mallet, 2007; Marques et al., 2019). On top of this, transgressive segregation can create novel phenotypes in mating traits such as mating season, flower characters, genitalia shapes, sexual displays, and mate preferences (Anton et al., 2013; Barrera-Guzmán et al., 2018; Comeault & Matute, 2018; Eliason et al., 2023; Lamichhaney et al., 2018; Melo et al., 2009; Myers et al., 2022; Selz et al., 2014). Transgressive segregation in mating traits may be crucial for homoploid hybrid speciation

because if hybrids can have only intermediate mating phenotypes of the parental forms, it is unlikely that premating reproductive isolation of a hybrid population with its parental lineages becomes stronger than that between the crossable parental lineages. Meanwhile, however, the establishment of novel transgressive mating phenotypes may be difficult unless there is a strong selection for them, since novel mating phenotypes are most likely mismatched with dominant mating trait phenotypes and thus will be subject to negative sexual selection. (Kirkpatrick & Nuismer, 2004; Kopp et al., 2018; Rosenthal et al., 2018; Servedio, 2011). Therefore, despite its intuitive appeal, whether and how transgressive segregation of mating traits can promote speciation remains unclear.

Here we hypothesize that transgressive segregation of mating traits can drive the incipient formation of hybrid species (Figure 1). Hybridization and transgressive segregation can generate large standing genetic and phenotypic variations, which amplify phenotypic evolution in random directions through genetic drift. In a hybrid population receiving continuous immigration of parental lineages, recurrent hybridization will cause repeated phenotypic drifts in a hybrid population. However, the hybridization-derived replenishment of genetic variation should be halted if the hybrid population evolves a

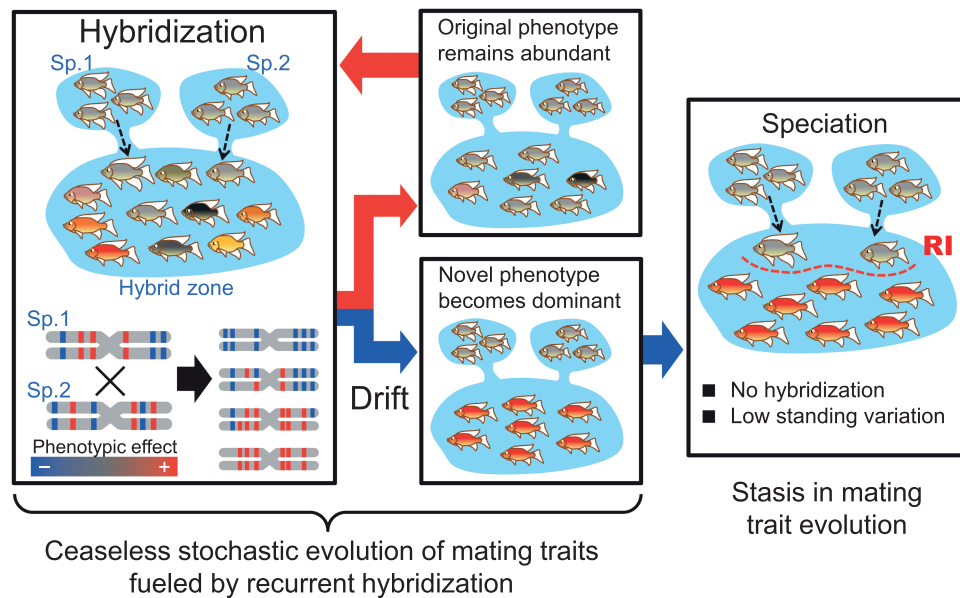


Figure 1. A hypothetical mechanism for incipient hybrid speciation. Hybridization between two genetically distinct lineages with similar mating phenotypes (“Sp.1” and “Sp.2”) can generate high standing heritable phenotypic variation, which may involve transgressive mating phenotypes, such as novel nuptial colors subjected to assortative mating preferences. Thus, hybridization can amplify the effect of genetic drift to cause stochastic phenotypic evolution of the mating trait. This process will be repeated endlessly (red arrows) until a novel transgressive mating phenotype incidentally dominates the hybrid population (blue arrows) and reproductively isolates it from both parental lineages.

novel mating phenotype that reproductively isolates it from the parental lineages. Without the replenishment of genetic variation, standing variation of mating traits will decline in the hybrid population. The loss of hybridization-derived variation will stabilize the novel mating phenotype, securing the reproductive isolation of the hybrid population. These considerations imply that hybrid speciation can become a predictable outcome of evolution when a hybrid population is receiving constant immigration of parental lineages; natural selection for novel transgressive mating phenotypes is not necessary for this speciation process. The reason for this is that recurrent hybridization caused by constant parental immigration continuously drives the stochastic evolution of mating phenotypes in the hybrid population until evolution of a novel mating phenotype leads to reproductive isolation between the hybrid population and parental lineages.

To provide a proof-of-concept for this hypothesis, we conducted individual-based simulations of the evolution of mating traits in a hybrid zone. Two models considering different mating rules were developed. The first model simulated the evolution of a single quantitative mating trait, which was expressed in both males and females and caused assortative mating (e.g., mating season, mating habitat selection, and flower characters). The second model, which was based on a classical sexual selection model (Lande, 1981), considered different mating traits in males and females (e.g., genitalia shapes, sex pheromones and receptors, male displays, and female mate preferences). Following the terminology of Kopp et al. (2018), we referred to the first and second models as the “matching model” and “preference/trait model,” respectively. Both models assumed polygenic mating traits, in which hybridization can cause transgressive segregation (Kagawa & Takimoto, 2018; Rieseberg et al., 1999).

While transgressive segregation may lead to the incipient evolution of reproductive isolation between hybrid and parental populations, reproductive isolation will be ephemeral if

mating trait phenotypes of either hybrid or parental population are evolutionarily unstable. Long-term persistence of species, or the continuation of speciation beyond the incipient stage, may require additional mechanisms that stabilize reproductive isolation—for example, a rugged (multi-peaked) fitness landscape for the mating trait can maintain species if hybrid and parental species occupy different local fitness peaks. As such a mechanism, we modeled a double-peaked fitness landscape, the simplest form of rugged fitness landscapes, of mating traits. However, a mechanism that stabilizes speciation at a later stage may hamper the incipient formation of new species, because the evolutionary stability of parental mating trait phenotypes can impede the invasion of any novel mating phenotypes in a population dominated by parental phenotypes. Thus, hybrid speciation can depend on whether transgressive segregation can provide ample variation in hybrid traits to successfully invade a population with an evolutionarily stable parental mating trait phenotype.

Moreover, in addition to a rugged fitness landscape, we test our mechanism with a flat fitness landscape. While a rugged landscape inevitably invokes natural selection in evolutionary dynamics, our hypothesis does not invoke natural selection for recurrent hybridization to promote incipient hybrid speciation. A rigorous test of our proposed mechanism should thus examine hybridization-driven evolutionary dynamics in isolation from confounding effects of natural selection. In this sense, our complementary simulations assuming a deliberately flat fitness landscape provide a direct test of our hypothesis.

The model

Genetics

Our individual-based models (coded in Java, available in the Dryad repository [Kagawa et al., 2023]) simulated the evolution of sexually reproducing diploid organisms with non-overlapping generations. The matching model considered

the evolution of a single quantitative mating trait, t , expressed in both sexes, for assortative mating. The preference/trait model considered two mating traits, p and s , expressed only in females and males, respectively. In both models, each quantitative mating trait was controlled by L genetic loci with additive phenotypic effects (total L loci for the mating model and $2L$ loci for the preference/trait model). The L loci were located in randomly assigned positions of the genome consisting of $2n$ chromosomes. Each chromosome was l base-pairs long, and each genetic locus was 5,000 base-pairs long. Allelic variations in these loci were generated by single-site mutations in their sequences. Mutations occurred at a constant rate of μ per base pair per generation, which replaced a nucleotide at a random position of a locus with a derived nucleotide. In our simulation implementation, we used the infinite site approximation ignoring multiple mutations on the same nucleotide site; thus, positions of derived nucleotides in the genome were implemented as continuous values instead of discrete values. Each derived nucleotide had a constant additive phenotypic effect value drawn from a normal distribution $N(0, \sigma_m^2)$. Once assigned, the phenotypic effect of a derived nucleotide was constant, regardless of the genomic background and environment. In addition to mutations, chromosomal crossover, which occurred at a constant rate of r per base pair, could generate chromosomes with novel combinations of derived nucleotides. The phenotypic effect size of each allele (i.e., a haplotype of a locus) was given as the sum of the effects of all derived nucleotides carried by the allele. Thus, the phenotypic value of a mating trait of an individual i was given as $\sum_{k=1}^L [\sum_{u \in \{M_{ik1}\}} \epsilon_u + \sum_{u \in \{M_{ik2}\}} \epsilon_u]$, where ϵ_u is the effect of the derived nucleotide u and M_{ik1} and M_{ik2} are sets of derived nucleotides in two alleles of locus k of the genome of individual i . In the ancestral genome, all loci had an allele with no derived nucleotides; thus, the ancestral trait value was 0 for all traits. This genetic modeling of phenotype determination followed our previous method (Kagawa & Seehausen, 2020).

Density regulation

A single generation consisted of three events: density regulation, natural selection, and reproduction (mating and offspring production). Density regulation inflicted density-dependent mortality on juveniles owing to limiting resources (e.g., food and space). The survival probability of juveniles was given as $K/(K + N_j(\lambda - 1)/\lambda)$, where K is the carrying capacity for surviving juveniles (i.e., prospective adults), N_j is the number of juveniles in the local population, and λ is the number of offspring per reproducing female. This form of density regulation is equivalent to the classical Beverton-Holt model of density-dependent population growth. With λ set to the default value ($\lambda = 100$), the population size immediately after density regulation was kept around K except during population establishment. This density regulation process did not exert natural selection on mating traits.

Natural selection in the matching model

While hybridization may possibly induce incipient hybrid speciation, additional mechanisms may be necessary for the long persistence of hybrid species. As such, we considered that mating phenotype evolution occurred on a double-peaked fitness landscape (optimal trait values at $t = 0$ and α (> 0), Supplementary Figure S1), on which mating phenotypes of parental and hybrid species would be kept separated after

divergence. We assumed that each individual i who had survived density regulation could participate in mating with probability $f(t_i)$, where f is a bimodal function of mating trait t . To express a double-peaked fitness landscape with f , we introduced a function $g(x) = \exp(-cx^2) - d \cdot \exp(-\beta x^2)$ and set:

$$f(t) = g(\tilde{t})/g(\gamma), \quad (1)$$

where c, d , and β are positive parameters and $\tilde{t} = 2\gamma(t - \alpha/2)/\alpha$ is a scaled mating trait with $\gamma = \sqrt{\{\ln(d\beta) - \ln(c)\}/(\beta - c)}$. We assumed $d \leq 1$ and $d\beta > c$ so that f takes a maximum of 1 at $t = 0$ and $t = \alpha$, and a local minimum of $(1 - d)/g(\gamma)$ at $t = \alpha/2$ (Supplementary Figure S1). Combinations of α, β, d , and c values determined the distance between two peaks, the steepness and depth of the fitness valley, and fitness reduction on traits deviated from the fitness peaks.

The double-peaked fitness landscape was independent of environmental variations and phenotype frequencies. Rather, the landscape reflected intrinsic fitness variation due to developmental constraints or energetic tradeoffs. Neither divergent natural selection nor negative frequency-dependent natural selection affected mating trait evolution. Hence, the double-peaked fitness landscape might assist the maintenance of once-diverged mating traits in different populations but could not facilitate the incipient divergence of mating phenotypes.

Natural selection in the preference/trait model

Different from the matching model, the preference/trait model assumed that the male trait was always subject to stabilizing natural selection with only one optimal value, $s = 0$: the intrinsic fitness of a male individual with trait value s was given as $\exp(-c\tilde{s}^2)$, where $\tilde{s} = 2\gamma s/\alpha$. For the female preference trait, the double-peaked fitness landscape was assumed as in the case of the matching model: thus, the intrinsic fitness of a female individual with trait value p was given by $f(p)$ (Equation 1). Despite the stabilizing natural selection favoring less exaggerated male mating phenotypes, evolutionary dynamics of male and female mating traits could attain multiple alternative stable equilibria since sexual selection could cancel the effect of natural selection on the male trait (Supplementary Text S1; Lande, 1981). If the fitness landscape of the female trait is flat, the evolutionary dynamics have neutrally stable line equilibria, along which female and male trait values match each other (Lande, 1981; Supplementary Figure S2). With the double-peaked fitness landscape of the female trait, the line equilibria shrank to two alternative stable equilibria associated with two fitness peaks.

Reproduction

Mating took place after natural selection. At the beginning of the mating season, a mating group was formed that included all surviving individuals in a population. The mating season consisted of a series of encounter events between a female and a male who were randomly selected from the mating group. Each female individual i encountered a male individual up to E_i times. The value of E_i was drawn from a Poisson distribution, $Po(eN_m)$, at the beginning of the mating season, where e is the encounter rate between male and female individuals and N_m is the number of males in the mating group. In the matching model, the mating probability upon an encounter was $\exp(-(t_j - t_i)^2/(2\theta^2))$, where t_i and t_j were the mating trait values of female and male individuals, respectively. In the preference/trait model, the mating probability was $\exp(-(p_i - s_j)^2/(2\theta^2))$, where p_i and s_j are mating traits of female and

male individuals, respectively. The larger the parameter θ , the greater the degree of random mating. Each male could mate with up to M_m females and was removed from the mating group when they had mated with M_m females. Each female i was removed from the mating group once she mated with a male or when she experienced E_i encounters without mating (i.e., females can mate only once). The mating season terminated when all females or males were removed from the mating group. After the mating season, females produced offspring. The number of offspring per each female was randomly drawn from the Poisson distribution $Po(\lambda)$. Mutation and crossover recombination occurred during meiosis to produce gametes. Sex was randomly assigned at birth.

Simulation scenarios

Our simulations described evolutionary dynamics in a secondary contact hybrid zone. To simulate hybridization, we first simulated the evolution of two geographically isolated populations (the allopatric phase) starting from a common ancestral species with one of the optimal mating trait values ($t = 0$ or $s = 0$, $p = 0$). At the beginning of a simulation, the common ancestor species was introduced in two allopatric areas. Then the two populations independently evolved for T_0 generations. Although allopatric speciation could occur if the mating trait in one of two populations shifted to the other optimal value owing to genetic drift, stabilizing selection inhibited phenotypic evolution away from the ancestral optimal trait value. Therefore, parental lineages were very unlikely to undergo speciation in the allopatric phase across a wide parameter region including our default parameter values (Supplementary Figure S3). When allopatric speciation did not occur, the mating trait phenotype of both parental lineages mostly stayed near the ancestral trait value throughout the allopatric phase. However, during the phenotypic stasis, genomes of both parental lineages fixed many mutations, of which phenotypic effects compensate for each other (Supplementary Figure S4). Such accumulated mutations could give rise to transgressive mating phenotypes in a hybrid population (Kagawa & Seehausen, 2020; Kagawa & Takimoto, 2018; Rieseberg et al., 1999).

After the allopatric phase, we simulated evolutionary dynamics in a hybrid zone, another habitable area where two parental lineages went into secondary contact. In our default simulation scenario, the “recurrent hybridization” scenario, the hybrid zone receives a constant mean number of immigrants per generation, m , from both parental populations. Immigration occurred after reproduction and before density regulation. Every generation, the number of immigrants from each parental population to the hybrid zone was independently drawn from the Poisson distribution $Po(m)$. For ease of interpretation of simulation results, we did not incorporate migration from the hybrid zone to parental populations. Therefore, expansion of the hybrid zone to parental areas, competitive exclusion of parental lineages by a hybrid species, and mating character displacement between parental populations by reinforcement could not occur in our simulations. This assumption is valid when immigration is one-way or when both parental populations are large enough that gene flow from the hybrid zone is negligible.

Additionally, we also simulated the “one-time hybridization” scenario wherein the hybrid population did not receive further parental immigration after 50 founder individuals from each parental population established the hybrid

population. Comparison between the scenarios of one-time hybridization and constant parental immigration will shed light on the effects of recurrent hybridization on hybrid speciation.

Conditions for hybrid speciation

We explored the conditions for hybrid speciation by simulating evolutionary dynamics with systematically varied parameter values. In the case of a double-peaked fitness landscape, we varied 12 parameters, including those controlling the shape of the fitness landscape, phenotypic effect size of mutations, the number of loci determining mating traits, carrying capacity of the hybrid population, the strength of sexual selection, and the relative contributions of two parental lineages to the hybrid population (Supplementary Text S2). Table 1 summarizes the parameters of the model as well as their default and alternative values for exploration. Empirical basis of default values is described in Supplementary Text S3. For each parameter combination, simulation was replicated 30 times.

To systematically analyze simulation results, evolutionary outcomes were sorted into categories. In the matching model, categorization was based on the average and standard deviation of the mating trait t in hybrid and parental populations at the end of simulation (i.e., 10,000 generations after the beginning of the secondary contact). Whenever the standard deviation in the hybrid population exceeded 1, simulation results were categorized as “persistent drift,” in most of which hybridization continued to generate high standing phenotypic variation until the end of simulation. Remaining simulations were categorized into either “speciation” or “no change.” Categorization was based on whether the average mating trait value of populations was in a circle of radius $\alpha/2$ around either fitness peaks ($t = 0$ or $t = \alpha$). Simulation result was “speciation” if both parental populations occupied the same fitness peak and the hybrid population occupied the other fitness peak. Simulation result was “no change” if the hybrid population and both or either of parental populations occupied the same fitness peak. Technical details of categorization are in Supplementary Text S4.

Additionally, the strength of premating reproductive isolation between hybrid and parental populations were quantified in simulations of recurrent hybridization scenario. In brief, we measured the strength of premating reproductive isolation based on the per-capita probability that immigrants from parental lineages mated successfully with residents of the hybrid population. Strengths of reproductive isolation between immigrant females and resident males and between immigrant males and resident females were separately quantified and averaged to describe the overall strength of reproductive isolation (Supplementary Text S4). For this analysis, we recorded all mating events in the hybrid population every ten generations. Then, the strength of reproductive isolation in a given period was evaluated from all records during this period. For example, reproductive isolation in the period between generations 10 and 200 was calculated from records at generations 10, 20, 30, ..., and 190.

Simulations with the flat fitness landscape

We conducted complementary simulations in which the fitness landscape was relaxed to become flat in the hybrid zone. This complementary analysis examines our proposed mechanism of hybrid speciation (Figure 1) in isolation

Table 1. Model parameters that were varied.

Definition	Symbol	Default value ^a	Alternative values examined ^b
The number of loci controlling each mating trait	L	200 [50]	50, 100, 400, 800 [12, 25, 100, 200]
Phenotypic effect size of mutations	σ_m	0.4	0.025, 0.05, 0.1, 0.2, 0.8, 1.6 [0.1, 0.2, 0.8, 1.6, 3.2, 6.4]
Carrying capacity (at the adult stage) of the hybrid zone	K_H	200	50, 100, 400, 800, 1600
Expected number of immigrants per generation	m	10	10^0 , $10^{0.25}$, $10^{0.5}$, ..., 10^2
Degree of asymmetry in parental immigration (symmetrical with $\gamma = 1$)	γ	1	2^{-3} , 2^{-2} , 2^{-1} , 2^1 , 2^2 , 2^3
The duration of allopatric evolution of parental lineages (generations)	T_0	5×10^5 [3×10^5]	5×10^4 , 1×10^5 , 2×10^5 , 3×10^5 , ..., 9×10^5 , 10^6
The range of male traits that females accept (i.e., randomness of mating)	θ	1	0.5, 0.75, 1.25, 1.5, 1.75, 2
Upper limit number of mating per male individual	M_m	5	1, 2, 4, 8, 16, 32
Encounter rate between female and male individuals within a population	e	0.5	0.1, 0.2, 0.4, 0.8, 1.6, 3.2
Initial ratio of two parental lineages	r_0	NA ^c	0.1, 0.2, 0.3, 0.4, 0.5
Depth of fitness valley	d	0.2	0.05, 0.1, 0.15, ..., 0.35
Distance between two fitness peaks in the phenotype space	α	5	3, 4, 6, 7, 8, 9

^a Default parameter values in the preference/trait model are shown in squared brackets when they differ from the default values in the matching model.
^b Values examined in the preference/trait model are shown in squared brackets when they differ from the values examined in the matching model.
^c Parameter r_0 is not applicable to the default simulation scenario where the hybrid zone is initially vacant.

from confounding effects of natural selection in the double-peaked fitness landscape. We retained the double-peaked fitness landscape in parental populations to avoid additional confounding effects on evolutionary dynamics in the hybrid zone. If parental populations also evolve on a flat fitness landscape, their stochastic phenotypic evolution would affect evolutionary dynamics in the hybrid zone. Additionally, the stochastic evolution during the allopatric phase would generate variable degrees of interlineage divergence in the mating trait, which could affect the outcomes of secondary contacts.

To test our proposed mechanism of hybrid speciation, we compared evolutionary outcomes between recurrent and one-time hybridization scenarios with the flat fitness landscape. Our hypothesis predicts that recurrent hybridization can drive rapid incipient speciation by inhibiting the maintenance of parental mating trait phenotypes. Thus, we expect that rapid evolution of novel mating trait phenotypes will be more likely in the recurrent hybridization scenario than the one-time hybridization scenario. We note that the feasibility of speciation itself was not of interest with a flat fitness landscape, where any mating trait values constitute neutrally stable equilibria and gradual stochastic evolution through spontaneous mutations and genetic drift should eventually cause reproductive isolation between isolated populations (Uyeda et al., 2009).

Results

Below we first present the dynamics and conditions of hybrid speciation on the double-peaked fitness landscape. Next, we use simulations on the flat fitness landscape to show that our speciation mechanism does not hinge on natural selection on the double-peaked fitness landscape. Unless otherwise noted, the matching and preference/trait models supported essentially the same conclusions.

On the double-peaked fitness landscape

Speciation dynamics in the recurrent hybridization scenario

In the recurrent hybridization scenario, a hybrid zone received continuing immigration from parental populations. In both matching and preference/trait models, hybridization between two genetically diverged parental lineages generated high standing heritable variation of mating trait phenotypes in the hybrid zone (Figure 2A and B). Inflation of hybridization-derived variation continued until the mean mating trait value in the hybrid population shifted from the ancestral value ($t = 0$) to the other optimal value ($t = 5$). Once the novel optimal mating phenotype dominated the hybrid population, the hybrid population was reproductively isolated from both parental lineages. Then, the standing phenotypic variation in the hybrid population dropped due to the lack of hybridization, which stabilized the novel mating trait phenotype and further strengthened the premating reproductive isolation from the parental lineages (Figure 2A and B). The reduction of standing phenotypic variation also reduced the mortality caused by natural selection against maladaptive transgressive phenotypes, but this effect was slight (Supplementary Figure S5). Hybrid speciation occurred almost certainly in 30 simulation replications with the default parameter set (Figure 3, $T_0 = 500,000$ and $m = 10^1$ in matching model; $T_0 = 300,000$ and $m = 10^1$ in preference/trait model), despite that the intrinsic fitness of the novel phenotype ($t = 5$) was no larger than that of the original optimal phenotype of parental lineages ($t = 0$).

Speciation dynamics in the one-time hybridization scenario

The evolution of novel transgressive mating trait phenotypes was feasible also in the one-time hybridization scenario, where the hybrid population received parental immigration only once when it was established (Supplementary Figure S6a and b). However, the maintenance or re-evolution of the parental mating trait phenotype also occurred frequently with this scenario (Supplementary Figure S6c and d). Therefore, although

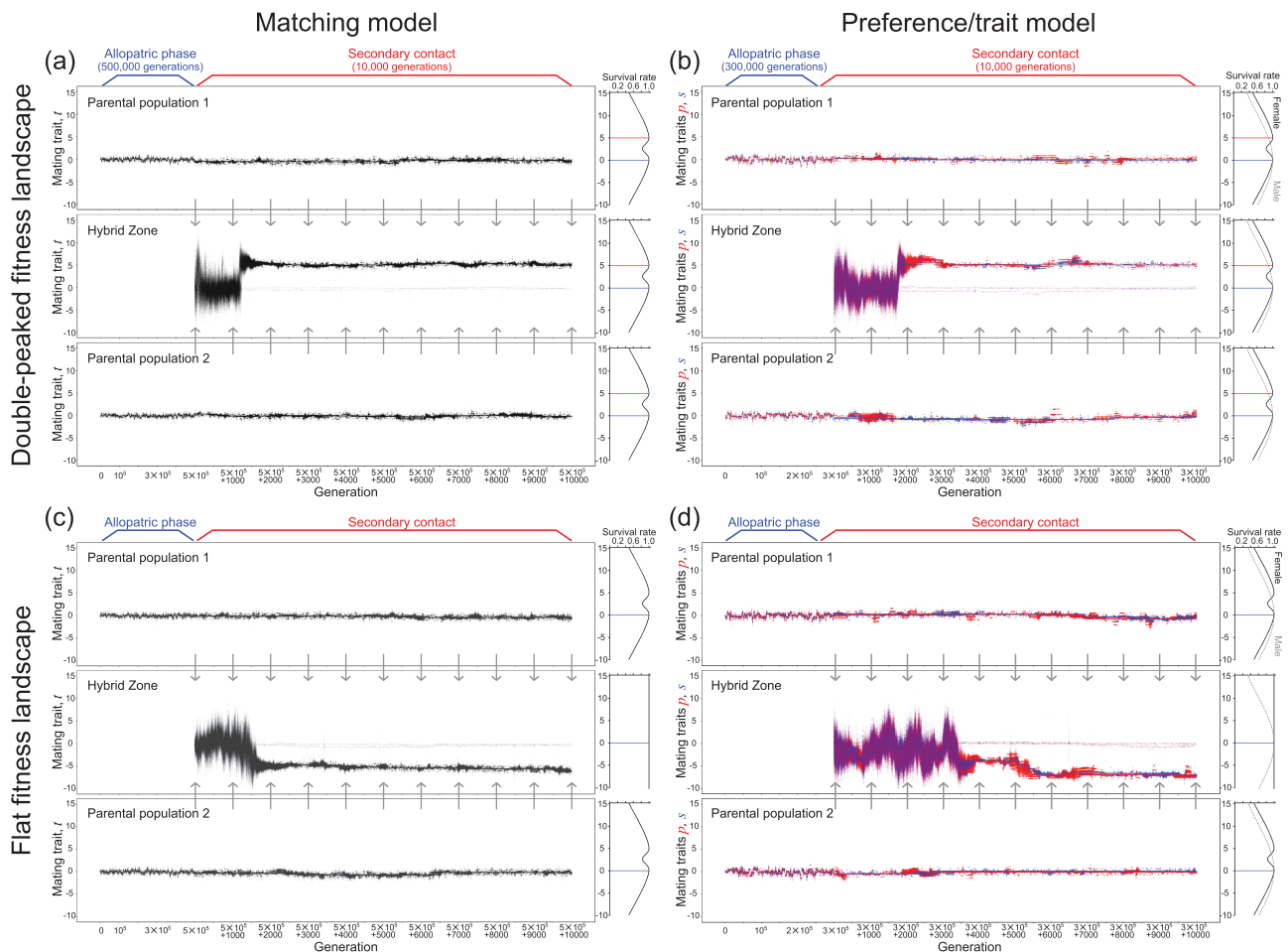


Figure 2. Speciation dynamics in the recurrent hybridization scenario. (A) Matching model with a double-peaked fitness landscape. Mating trait values of all individuals (the vertical axis) in each population are plotted for each generation shown on the horizontal axis. The first 500,000 generations are the allopatric phase and plotted at intervals of 1,000 generations. The following 10,000 generations are the period with a hybrid zone and plotted every generation. Right panels show the survival rates of individuals with each mating trait value (same as [Supplementary Figure S1](#)). Blue and red lines show positions of the ancestral and the other optimal mating trait values, respectively. (B) Preference/trait model with a double-peaked fitness landscape of the female trait. Mating trait values of female, p , and male, s , are plotted with red and blue, respectively. The first 300,000 generations are the allopatric phase (plotted at intervals of 500 generations), and the following 10,000 generations are the period with a hybrid zone (plotted every generation). Black bold line and gray dotted line in right panels show fitness landscapes of female and male traits, respectively. (C) Matching model assuming a flat fitness landscape in the hybrid zone. (D) Preference/trait model assuming a flat fitness landscape of the female mating trait in the hybrid zone. Parameters were set to the default values shown in [Supplementary Table S1](#).

hybrid speciation could occur, the likelihood of hybrid speciation was lower compared to the recurrent hybridization scenario under the default parameter values ([Figure 3](#), the bottom-most row. $T_0 = 500,000$ in matching model; $T_0 = 300,000$ in preference/trait model).

Conditions for hybrid speciation in recurrent versus one-time hybridization scenarios

The most fundamental requirement for hybrid speciation in both recurrent and one-time hybridization scenarios was that the duration of the allopatric phase before hybridization, T_0 , was not too small ([Figure 3](#), $T_0 \geq 300,000$ in matching model; $T_0 \geq 200,000$ in preference/trait model). This was because hybridization between lineages with small genetic differentiation usually did not generate large transgressive phenotypic variation required for speciation ([Supplementary Figure S4](#)). More specifically, the potential of transgressive segregation to generate novel phenotypes increased as more mutations with large phenotypic effects accumulated between parental

lineages; hence, the length of the allopatric phase necessary for hybrid speciation depends on other parameters that affect the tempo of genomic divergence in the allopatric phase, such as the number of loci affecting mating traits (L). Although hybrid speciation required a sufficiently large genomic differentiation between parental lineages, hybridization generating excessive phenotypic variation compared to the distance between fitness peaks may rather inhibit speciation. Indeed, too large values of L and T_0 reduced the likelihood of hybrid speciation in the recurrent hybridization scenario ([Supplementary Figure S7](#)).

In the recurrent hybridization scenario, the likelihood of hybrid speciation strongly depended on the mean number of immigrants per generation, m . Especially, recurrent hybridization with moderate values of m resulted in speciation almost certainly ([Figure 3](#), $10^1 \leq m \leq 10^{1.5}$) as long as T_0 was not too small. With more intense parental immigration, hybridization persistently generated high standing variation of mating phenotypes ([Figure 3](#), $m \geq 10^{1.75}$; i.e., “persistent drift”). In this case, fixation of a novel mating phenotype, which was

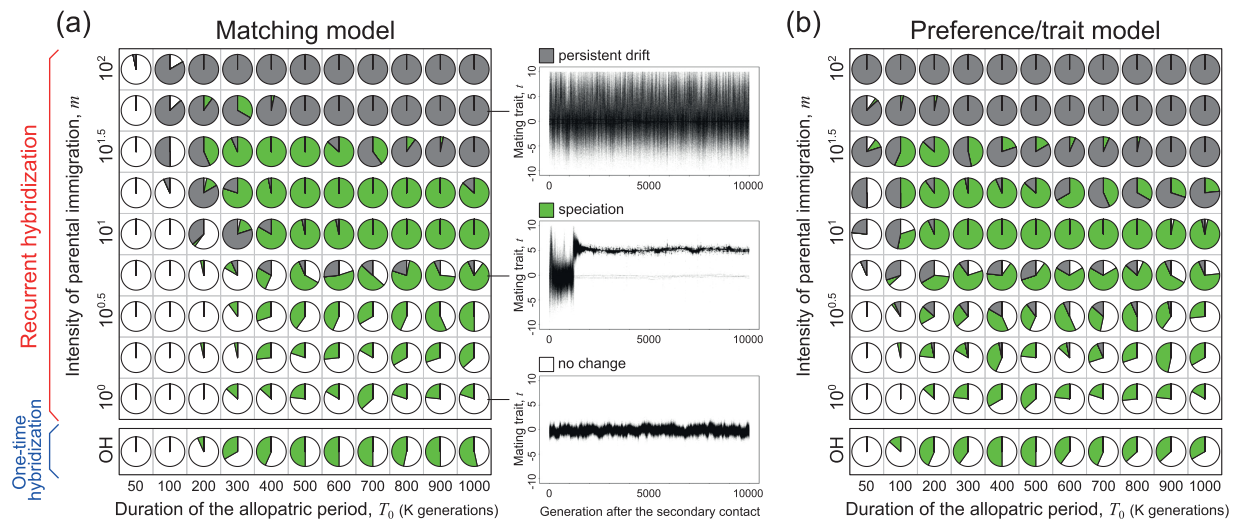


Figure 3. Conditions for hybrid speciation in recurrent versus one-time hybridization scenarios on the double-peaked fitness landscape. (A) Matching model results of 3,300 simulations under various durations of the allopatric period, T_0 (horizontal) and intensities of parental immigration to the hybrid population, m (vertical). Pie charts depict the frequencies of speciation (green), persistent drift (gray), and no change (white) among 30 simulations with given parameter combinations. Right panels show examples of three categories of evolutionary dynamics in the hybrid zone. The bottom-most row (OH) summarizes outcomes in the one-time hybridization scenario. (B) Preference/trait model results of 3,300 simulations under various values of T_0 and m . Detailed categorization methods for preference/trait model outcomes are in [Supplementary Text S4](#). The bottom-most row (OH) summarizes outcomes in the one-time hybridization scenario. No allopatric speciation occurred between parental lineages in simulations presented here. Unchanged parameters were set to the default values ([Supplementary Table S1](#)).

required for the establishment of reproductive isolation, was difficult owing to an excessive supply of hybridization-derived genetic variation that maintained polymorphisms in the mating trait. In contrast, the low likelihood of speciation with very low immigration rates ([Figure 3](#), $m < 10^{0.5}$) was owing to the lack of hybridization-derived genetic variation.

Carrying capacity in the hybrid zone, K_H , also influenced the evolutionary outcome of hybridization. In the recurrent hybridization scenario, the range of m favorable for hybrid speciation shifted to larger values with increase of K_H ([Supplementary Figure S8](#)). This is because the impact of gene flow caused by single immigrant individual is diluted in large populations. Additionally, large K_H shrunk the range of m favorable for hybrid speciation. Large population sizes limiting conditions for hybrid speciation implies that the incipient hybrid speciation in the recurrent hybridization scenario is driven mainly by genetic drift, since the relative importance of genetic drift to natural (sexual) selection decreases in larger populations.

The depth of the fitness valley, d , affected both likelihoods of allopatric speciation and hybrid speciation. Simulations of the allopatric phase demonstrated that even a shallow fitness valley between two fitness peaks efficiently prevented parental populations to undergo allopatric speciation through the stochastic evolution of mating traits ([Supplementary Figure S3c and g](#)). However, hybridization could cause speciation under the same fitness landscape, although deep fitness valleys shrank conditions for hybrid speciation ([Supplementary Figure S3a, b, e, and f](#)). Therefore, hybridization was essential for speciation with the double-peaked fitness landscape (see also [Supplementary Figure S9](#) for further support for this conclusion).

While conditions for hybrid speciation were generally similar between matching model and preference/trait model ([Supplementary Text S2](#)), we identified one qualitative difference between them: monogamy suppressed hybrid speciation

in the preference/trait model, but not in the matching model ([Supplementary Figure S10](#)). Hybrid speciation in the preference/trait model was feasible since, consistent with the classical theory ([Lande, 1981](#)), female mate choice could generate a disparity in reproductive success among males, inducing sexual selection on the male trait. Monogamy, however, reduced the disparity in male mating success and weakened sexual selection in the preference/trait model, making speciation unfeasible ([Supplementary Text S1](#)).

In [Supplementary Text S2](#), we provide more comprehensive analyses for the effects of parameters, including additional simulations that consider: various values of the distance between two fitness peaks (α) and the randomness of mating (θ) ([Supplementary Figure S11](#)), asymmetric immigration intensities from two parental lineages ([Supplementary Figure S12](#)), and an alternative scenario where the hybrid zone is initially occupied by a population containing two parental lineages in a given ratio ([Supplementary Figure S13](#)).

On the flat fitness landscape: recurrent hybridization could promote speciation more effectively than one-time hybridization

Irrespective of matching and preference/trait models, simulations with the flat fitness landscape in the hybrid zone supported that our proposed mechanism ([Figure 1](#)) can drive incipient hybrid speciation without the aid of natural selection. First, as in the case of the double-peaked fitness landscape, hybrid speciation could occur through evolution of a transgressive mating phenotype ([Figure 2C and D](#)), although phenotypes of new hybrid species varied among simulation runs. Second, evolutionary trends across replicated simulations demonstrated that recurrent hybridization could drive the evolution of reproductive isolation. [Figure 4A](#) shows trajectories of the average trait value in the hybrid zone in 120 simulations of the recurrent hybridization scenario with the default parameter set. In the recurrent hybridization

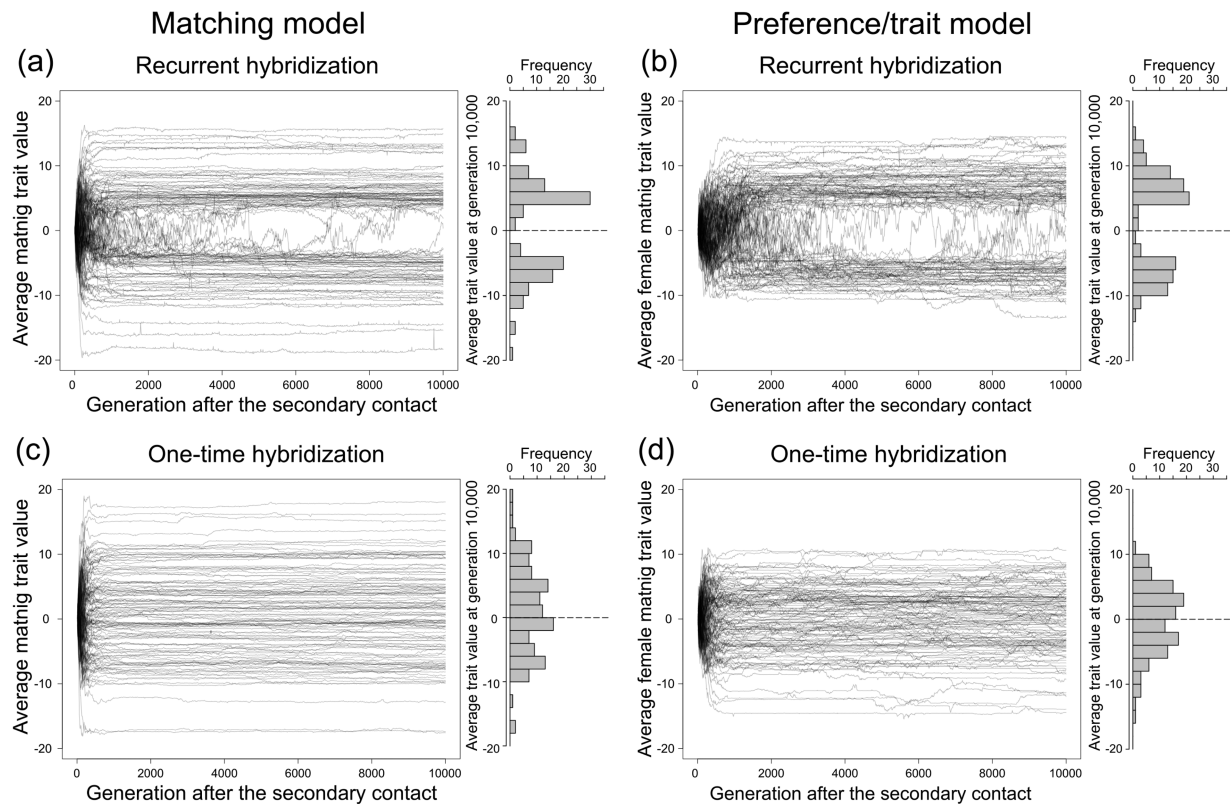


Figure 4. Evolutionary dynamics on the flat fitness landscape. Each panel shows evolutionary dynamics in 120 simulation runs with the same condition. Each line shows the trajectory of average mating trait value in a single simulation run, and the histogram presents the frequency distribution of the average mating trait value at the generation 10,000. (A) Recurrent hybridization scenario with matching model. (B) Recurrent hybridization scenario with preference/trait model. (C) One-time hybridization scenario with matching model. (D) One-time hybridization scenario with preference/trait model. Parameters were set to the default values (Supplementary Table S1). In the recurrent hybridization scenario, evolution of a novel mating trait value was seldom reversed. In 116 of 120 simulations of the matching model (A), the mean mating trait value at generation 5,000 was out of the circle of radius 3 around $t = 0$ (i.e., the neighborhood of the origin); subsequently, in only 4 of the 116 simulations, mean mating trait value reentered the neighborhood of the origin at least once until generation 10,000. Similarly, in the preference/trait model (B), re-evolution of the original female mating trait value ($|\bar{p}| \leq 3$) after the evolution of a novel female mating trait value ($|\bar{p}| > 3$) occurred in only 15 of 118 simulations.

scenario, mating trait evolution of hybrid populations tended to approach a stasis at trait values considerably different from those of parental lineages (Figure 4A and B). The stasis occurred only when reproductive isolation was established to halt hybridization generating standing genetic variation. By contrast, in 120 simulations of the one-time hybridization scenario, the hybrid population frequently maintained or re-evolved the parental mating phenotype (Figure 4C and D); thus, no trend of hybrid mating trait evolution toward phenotypes diverged from parental phenotypes was observed.

Dynamics of standing genetic variation and reproductive isolation can explain the differentiation of hybrid mating trait from parental traits in the recurrent hybridization scenario (Figure 5). Hybrid populations exhibited high variances in the mating trait when their mean mating trait values were not distant from the parental mating trait value ($t = 0$). In contrast, the mating trait variance dropped to very low levels in hybrid populations with novel mating trait values that were distant from the parental mating trait value ($t = 0$); such mating trait values reproductively isolated the hybrid population from parental populations (Figure 5B).

Notably, the shapes of fitness landscapes (doubled-peaked vs. flat) did not qualitatively affect parameter conditions for recurrent hybridization to cause hybrid speciation (Supplementary Figure S14). Namely, recurrent hybridization almost certainly caused a rapid incipient hybrid speciation

when T_0 was not too small, K_H was not too large, and m was in a certain range.

Overall, the results with flat fitness landscapes demonstrate the core mechanism of our hypothesis. That is, recurrent hybridization continues to promote the stochastic evolution of hybrid mating trait until the hybrid population is reproductively isolated from parental lineages (Figure 1).

Discussion

Hybridization can create novel transgressive mating phenotypes, but how they affect hybrid speciation process remained unclear. We hypothesized that transgressive segregation of mating traits can drive the incipient stage of hybrid speciation even in the absence of ecological or intrinsic incompatibilities contributing to speciation. Using two models considering different mating rules (matching model and preference/trait model), we examined the theoretical validity of this hypothesis by simulating evolutionary outcomes of hybridization between allopatrically evolved lineages. Supporting the hypothesis, simulations with both models demonstrated that transgressive segregation of mating traits could trigger the evolution of a new reproductively isolated species with a novel mating phenotype (Figures 2 and 4). Under favorable conditions, rapid speciation occurred with a probability of nearly 1, demonstrating that this mode of incipient hybrid

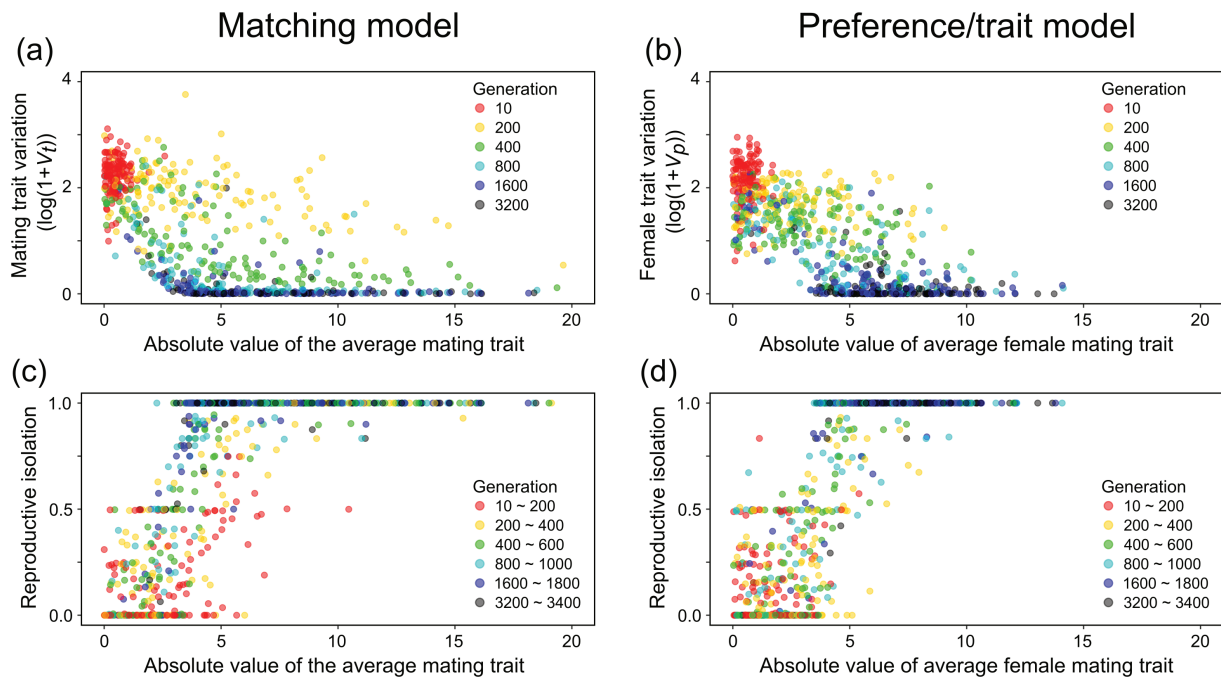


Figure 5. Evolutionary dynamics of mating trait variation and reproductive isolation in the recurrent hybridization scenario. (A) The relationship between mean and variance of mating trait values of the hybrid population in 120 simulations of the matching model (Figure 4A). Points represent the absolute mean and log variance of the mating trait in a hybrid population at generations 10 (red), 200 (yellow), 400 (green), 800 (cyan), 1,600 (blue), and 3,200 (gray). (B) The relationship between mean and variance of female mating trait values of the hybrid population in 120 simulations of the preference/trait model (Figure 4B). (C, D) The relationship between mean mating trait value in a hybrid population and the strength of reproductive isolation (RI) between the hybrid population and parental populations in the matching model (C) and preference/trait model (D). Points represent the absolute mean mating trait and the strength of reproductive isolation in the following time periods: 10–200 (red), 200–400 (yellow), 400–600 (green), 800–1,000 (cyan), 1,600–1,800 (blue), and 3,200–3,400 (gray).

speciation was not a mere product of chance, but driven by a firm biological mechanism. A key condition favorable for speciation was that hybrid populations received continued parental immigration with a moderate intensity (Figure 3). In such hybrid populations, recurrent hybridization continued to generate standing variation of mating phenotypes until the hybrid population evolved a novel mating phenotype that reproductively isolated it from parental lineages (Figure 5). Subsequently, the reproductive isolation halted the hybridization-derived replenishment of standing variation of mating phenotypes, thereby stabilizing the novel mating phenotype and forming an incipient hybrid species. These results support that transgressive segregation of mating traits can serve as a primary driver of hybrid speciation.

Notably, transgressive segregation of mating traits could trigger hybrid speciation even under conditions where the double-peaked fitness landscape suppressed allopatric speciation (Figure 2, Supplementary Figure S3). While the double-peaked fitness landscape of the mating trait promotes the persistence of species after incipient speciation has already occurred, it also hinders the evolution of new species by causing stabilizing selection around fitness peaks of the mating trait. Indeed, even a shallow fitness valley between two fitness peaks inhibited allopatric speciation caused through stochastic evolution of mating traits in isolated populations (Supplementary Figure S3c). Transgressive segregation in hybrid zones enabled rapid hybrid speciation under the assumption of such fitness landscapes. This result also suggests that hybrid speciation by transgressive segregation of mating traits is compatible with the subsequent long-term persistence of species.

Our results also demonstrate that (a) the novel mating phenotype of new hybrid species need not confer a fitness advantage over the original parental mating phenotype and (b) recurrent hybridization can drive incipient speciation without the aid of natural selection. These conclusions were rigorously validated by complementary simulations in which potential contributions of natural selection to hybrid speciation were deliberately excluded by assuming a flat fitness landscape of mating traits in the hybrid zone (Figures 4 and 5). The effect of recurrent hybridization to drive rapid speciation was supported by the comparison of recurrent versus one-time hybridization scenarios. Namely, under certain parameter conditions, the recurrent hybridization scenario almost certainly led to rapid speciation, whereas the one-time hybridization scenario did not cause rapid speciation with comparable probabilities under any parameter conditions that we explored (Supplementary Figure S14).

In addition to recurrent hybridization and natural selection, two other mechanisms may potentially drive hybrid speciation in our models. First, in certain situations, sexual selection in the hybrid population can generate a reinforcement selection favoring females that avoid mating with immigrant males of parental lineages (i.e., reinforcement between hybrid and parental populations by sexual selection). Second, assortative mating per se can develop linkage disequilibrium between alleles affecting the mating trait, which drives a slow but steady increase in frequencies of transgressive mating trait phenotypes (i.e., “allele sorting by assortative mating”). In Supplementary Text S5, we investigated how these mechanisms might affect our results. Supplementary simulations supported that, although both mechanisms could affect

evolutionary dynamics, recurrent hybridization could drive speciation independently from them (Supplementary Figures S15 and S16).

Our novel results extend the conclusions of previous speciation theories in a number of ways. First, transgressive segregation can permit a novel mating phenotype to evolve under assortative mating. Assortative mating has been thought to hinder the evolution of novel mating phenotypes because assortative mating induces sexual selection against rare mating phenotypes (Kirkpatrick & Nuismer, 2004; Kopp et al., 2018; Otto et al., 2008; Rosenthal et al., 2018), leading to an idea that assortative mating poses a major obstacle to the incipient stage of speciation. Our results suggest that transgressive segregation of mating traits can overcome this obstacle by rapidly creating abundant variation of mating phenotypes. The increased standing variation of mating phenotypes then relaxes the sexual selection against novel mating phenotypes, which further promotes the evolution of novel mating phenotypes (Supplementary Text S6; Supplementary Figure S17).

Second, transgressive segregation in mating traits may generally promote species diversification when there are multiple alternative stable equilibria in mating trait evolution. In our models, the double-peaked fitness landscape generated two alternative stable equilibria in mating trait evolution and transgressive segregation could generate a new mating phenotype that represents the previously unoccupied fitness peak. Especially, recurrent hybridization efficiently promoted the evolution of a hybrid population toward the new fitness peak by destabilizing the mating phenotypes of parental lineages. These results suggest that recurrent hybridization that causes transgressive segregation may generally facilitate the evolution of new hybrid species that occupy as yet unoccupied potential stable equilibria of mating trait evolution. For instance, the handicap process of sexual selection can generate multiple alternative stable equilibria for mating trait evolution (Iwasa & Pomiankowski, 1994). In such a situation, recurrent hybridization might facilitate the evolution of diverse species that occupy different stable equilibria of mating trait evolution, thereby promoting sexual radiation.

Third, homoploid hybrid speciation may be more feasible than suggested by previous theoretical studies. Previous models have not considered transgressive segregation of mating traits but examined the roles of recombination in sorting out parental genetic incompatibilities to establish a hybrid species (Blanckaert & Bank, 2018; Buerkle et al., 2000; McCarthy et al., 1995; Schumer et al., 2015). However, without transgressive segregation, strong reproductive isolation between hybrids and their parental lineages was generally unlikely, because hybrids tended to have intermediate phenotypes that could isolate themselves from their parental lineages no more strongly than the isolation between the parental lineages. With transgressive segregation, by contrast, hybrids can have extreme phenotypes that can strongly isolate themselves from the parental lineages, increasing the potential of homoploid hybrid speciation.

Finally, continued gene flow from parental populations can promote hybrid speciation. While continued parental gene flow has generally been thought to hinder hybrid speciation (Blanckaert & Bank, 2018; Schumer et al., 2015), our model showed that moderate levels of continued parental gene flow could promote hybrid speciation because of transgressive segregation of mating traits. This result calls for further

empirical research on how the intensity of parental gene flow affects hybrid speciation processes. At the same time, more theoretical investigation is required. For instances, temporal variation in migration and back migration from hybrid zones to parental populations could affect the roles of transgressive segregation in hybrid speciation.

We note that in addition to the double-peaked fitness landscape that increased the persistence of newly formed hybrid species, another mechanism might lead to persistence in our model. After evolution of reproductive isolation, incipient hybrid species became immune to the invasion of parental lineages owing to sexual selection against locally rare mating phenotypes; therefore, hybrid and parental species could coexist for long term in parapatry without ecological divergence. Our models incorporated not only sexual selection on males by female mate choice but also sexual selection on females by weak mate search cost: In our models, females that rejected all potential mating partners failed to reproduce. Without mate search cost, mating trait differentiation of hybrid species was insufficient to prevent female immigrants from causing gene flow (Supplementary Figure S18). This is because any females, including immigrants that prefer locally uncommon male phenotypes, have the same expected mating success if there is no mate search cost. Similar effects of mate search cost have been suggested in previous theoretical studies (M'Gonigle et al., 2012; Servedio & Bürger, 2015).

An important limitation of our model is that it considered only genetic variants with additive phenotypic effects. Owing to this assumption, transgressive segregation could not occur in the F1 hybrid in our model. In the presence of nonadditive genetic effects such as epistasis; however, transgressive segregation can occur in the F1 hybrid by interactions between genes from different parental lineages (Dittrich-Reed & Fitzpatrick, 2013; de los Reyes, 2019). Some empirical studies have reported that transgressive segregation of mating traits occurring in F1 hybrids leads to assortative mating between hybrids, which may contribute to hybrid speciation (Selz et al., 2014). Future studies should theoretically assess the roles of epistasis in hybrid speciation through evolution of novel transgressive mating trait phenotypes.

Our results suggest that we may have been underestimating the roles of nonadaptive stochastic evolutionary processes in hybrid speciation. Most previous theories explaining the evolution of premating reproductive isolation invoke its adaptive advantages, such as the avoidance of maladaptive hybridization (i.e., classical reinforcement and reinforcement during ecological speciation; Calabrese & Pfennig, 2020; Kirkpatrick, 2001). This requires that the evolution of intrinsic or ecological hybrid incompatibilities precedes the evolution of premating reproductive isolation. Similarly, most previous theories of hybrid speciation consider either ecological divergence or evolution of intrinsic incompatibilities as the first step of speciation between hybrid and parental populations (Blanckaert & Bank, 2018; Buerkle et al., 2000; Duenez-Guzman et al., 2009; Kagawa & Takimoto, 2018; McCarthy et al., 1995; Schumer et al., 2015). However, our simulations demonstrated that transgressive segregation of mating traits and genetic drift can be sufficient for accomplishing the incipient stage of speciation, implying that the evolution of premating reproductive isolation can precede the accumulation of hybrid incompatibilities. The preceding evolution of premating isolation seems plausible especially when hybridization is less harmful.

Our theory may explain why spatially confined adaptive and sexual radiations often generate more species than the number of ecomorphs. For instance, radiations of cichlid fishes in African great lakes (Seehausen, 2000), Hawaiian crickets (Mendelson & Shaw, 2005), South American capuchino seedeaters (Turbek et al., 2021), or Japanese *Ohomopterus* ground beetles (Sota & Nagata, 2008) have many species-pairs with marked differentiation in mating traits albeit with minor ecological differentiation. Since members of such rapid radiations often lack both strong postzygotic hybrid incompatibility and clear geographic isolation, evolutionary differentiation of mating phenotypes may have contributed to speciation in these radiations. Genetic evidence from many of these radiations supports the prevalence of introgressive hybridization among members of radiation (Shaw, 2002; Sota & Nagata, 2008) or even the hybrid origin of entire radiations (Meier et al., 2017, 2019; Svardal et al., 2020). Taken together, we speculate that recurrent hybridization events among parental lineages and members of radiation may have led to multiple episodes of hybrid speciation with transgressive segregation in mating traits, thereby contributing to the generation of many ecologically similar species with distinct and arbitrary mating traits in these radiations.

Supplementary material

Supplementary material is available online at *Evolution* (<https://academic.oup.com/evolut/qpad072>).

Data availability

Java source codes for the individual-based evolutionary simulations, R scripts to analyze and visualize simulation results, and final output files of all simulations presented in this article are available in the Dryad repository (Kagawa et al., 2023; doi:10.5061/dryad.7sqv9s4pw).

Author contributions

K.K. designed the study with inputs from O.S. and G.T.; K.K. performed the simulations and analyses; K.K. wrote the manuscript together with G.T. and O.S.

Conflict of interest: The authors declare no conflict of interest.

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