Increased Floral Rewards due to Local Adaptation Drives Plant Ecological Speciation via Learned Preferences of Pollinators

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Abstract: In animal-pollinated plants, the growth environment and pollination environment are two important agents of natural selection. However, their simultaneous effects on plant speciation remain underexplored. Here, we report a theoretical finding that if plants’ local adaptation to the growth environment increases their floral rewards for pollinators, it can strongly facilitate ecological speciation in plants. We consider two evolving plant traits, vegetative and floral signal traits, in a population genetic model for two plant populations under divergent selection from different growth environments. The vegetative trait determines plants’ local adaptation. Locally adapted plants reward pollinators better than mal-adapted plants. By associative learning, pollinators acquire learned preferences for floral signal traits expressed by better-rewarding plants. If pollinators’ learned preferences become divergent between populations, floral signal divergence occurs and plants develop genetic associations between vegetative and floral signal traits, leading to ecological speciation via a two-allele mechanism. Interestingly, speciation is contingent on whether novel floral signal variants arise before or after plant populations become locally adapted to the growth environment. Our results suggest that simultaneous selection from growth and pollination environments might be important for the ecological speciation of animal-pollinated plants.

Keywords: divergent selection, ecological speciation, labile preference, local adaptation, pollinator-mediated selection, population differentiation.

Introduction

In animal-pollinated plants, natural selection from the growth environment and pollinator-mediated selection are both important drivers of ecological speciation (Waser and Campbell 2004; Van der Niet et al. 2014). Divergent selection from different growth environments can cause vegetative traits to diverge between locally adapted plant populations, potentially leading to habitat isolation (Coyne and Orr 2004). Agents of selection from the growth environment may be abiotic (e.g., edaphic and climatic conditions; Rajakaruna 2004; Parisod 2021) or biotic (e.g., herbivory and disease; Chae et al. 2014; Maron et al. 2019). Selection from the pollination environment has been another major focus of theoretical and empirical research on plant speciation (Grant 1949, 1994; Van der Niet et al. 2014). Adaptation to different pollinators may lead to floral isolation and plant speciation (Bradshaw and Schemske 2003; Gegear and Burns 2007; Whittall and Hodges 2007). Even between populations sharing the same pollinators, floral isolation can evolve. Divergent floral morphology can cause plants to attach and receive pollen from different parts of a pollinator’s body, leading to mechanical isolation (Grant 1994). If pollinators tend to make consecutive visits to flowers of the same color (i.e., flower constancy), divergent floral color can drive ethological isolation (Wells et al. 1983; Hopkins and Rauscher 2012). From a theoretical perspective, plant-pollinator coevolution can drive plant speciation (Kiefer et al. 1984; Doebeli and Dieckmann 2000; Zhang et al. 2013; Kagawa and Takimoto 2014), and there is some empirical support for this idea (Johnson and Anderson 2010; Althoff et al. 2014).

Vegetative trait divergence is often observed together with floral trait divergence that causes reproductive isolation (Kay and Sargent 2009). Several possible causes have been proposed for this joint divergence (Waser and Campbell 2004). For example, adaptation to different habitats with divergent growth environments and pollinator assemblages
can result in the divergence of both vegetative and floral traits (Sobel et al. 2019). In addition, adaptation to a stressful growth environment can delay plant development and shift flowering time, inducing allochronic isolation (Levin 2009), and adaptation to heavy metal–contaminated soils or to local herbivores can pleiotropically induce changes in floral traits (Strauss and Whittall 2006; Rausher 2008). Finally, closely related plant species that have adapted to different growth environments may, on secondary contact, be selected to shift pollinators to avoid interbreeding that produces inferior hybrids and thus exhibit floral trait divergence (McNeilly and Antonovics 1968; van der Niet et al. 2006). Few studies, however, have considered the combined action of selection from growth and pollination environments (Waser and Campbell 2004).

Here, we propose a mechanism of ecological speciation of animal-pollinated plants. The mechanism promotes ecological speciation between plant populations in divergent growth environments when plants’ local adaptation to the growth environment increases their pollinator attraction. Because of their larger resource budget, plants that are better adapted to the local growth environment may produce more attractive rewards for pollinators because reward production is generally costly (Southwick 1984; Pyke 1991; Johnson and Schiestl 2016). Indeed, plant vigor and energy storage have been shown to increase floral reward production (Pleasants and Chaplin 1983; Harder and Cruzan 1990; Fenster et al. 2006). Moreover, genotype-by-environment interactions can affect floral reward production (Leiss and Klinkhamer 2005; Jachu et al. 2018; Parachnowitsch et al. 2019). If locally adapted plants increase floral rewards, it may further strengthen the selection for local adaptation of vegetative traits because such locally adapted plants would gain an extra fitness advantage by attracting more pollinators in addition to improved survivorship and/or seed production. For this extra advantage to be realized, two conditions need to be fulfilled. First, locally adapted plants must have specific floral signals different from those of less adapted plants. Second, pollinators must be able to distinguish the difference in floral signals and preferentially visit better-rewarding flowers. In fact, a growing body of evidence shows that better-rewarding plants present honest floral signals that inform pollinators of their rewards (Knauer and Schiestl 2015; Parachnowitsch et al. 2019; Ortiz et al. 2021). When both of these conditions are satisfied, not only is the extra fitness advantage of greater pollinator attraction realized in locally adapted plants but pollinators will also facilitate assortative mating between locally adapted plants. If different floral signals evolve between populations locally adapted to contrasting growth environments, it may lead to reproductive isolation and cause ecological speciation via a two-allele mechanism of assortative mating (Felsenstein 1981; Butlin et al. 2021).

A key to our proposed mechanism is that pollinators show labile preference, a learned preference for better-rewarding flowers (Waser 1986). Labile preference occurs in a wide range of pollinators, including a honey bee (Grüter et al. 2011), bumble bee (Heinrich 1976), butterfly (Goulson et al. 1997), and hummingbird (Pyke 1978). Pollinators’ labile preference can cause frequency-dependent selection on floral signal traits. The frequency dependence can be positive among rewarding plants (Levin 1972; Cresswell and Galen 1991; Smithson 2001) and negative among non-rewarding plants (Smithson and Macnair 1997; Gigord et al. 2001; Biernaskie and Geiger 2007). When floral rewards depend on local adaptation to the growth environment, local adaptation might change the strength or even the sign of the frequency-dependent selection.

We use a population genetic model to examine our proposed mechanism of ecological speciation of animal-pollinated plants. We address two main questions. First, does the increase in floral rewards by locally adapted plants promote the evolution of reproductive isolation between populations subjected to divergent natural selection from the growth environment? Second, how does pollinators’ labile preference, which causes frequency-dependent selection on floral signal traits, affect the evolution of reproductive isolation? Our simulations indicate that ecological speciation can occur when the increase in floral rewards by local adaptation is sufficiently large and the order of mutations in vegetative and floral signal traits strongly affects the likelihood of speciation as a result of frequency-dependent selection on floral signal traits. We discuss the plausibility of our speciation mechanism, compare it with previous speciation theories, and critically examine our model assumptions.

**Model Development**

The model is a two-patch, two-locus, two-allele diploid model (for further details, see supplement A). We adopt a population genetic approach because vegetative and floral signal traits underlying species difference are usually under oligogenic control (Orr 1998, 2001). The model assumes that plant populations in habitats A and B are subjected to divergent natural selection because these habitats have different growth environments. Equal habitat sizes are assumed for simplicity. Plants have a vegetative trait (e.g., life form, functional chemistry, and morphology of leaves and roots) and a floral signal trait (e.g., color, scent, and morphology).

The vegetative trait is subjected to divergent selection and is determined by a locus with two alleles, $P$ and $p$. Plants of genotypes $P/P$, $P/p$, and $p/p$ show high, intermediate, and low vegetative performance in habitat A, respectively, and low, intermediate, and high vegetative performance in habitat B. The vegetative performance
of each plant determines its survival, seed production, and floral rewards. The relative survival rates of plants with high, intermediate, and low vegetative performance are \(1, 1 - v/2, \) and \(1 - v,\) respectively, where \(v\) is the strength of divergent selection on survival. Similarly, their relative seed production is \(1, 1 - s/2,\) and \(1 - s,\) where \(s\) is the strength of divergent selection on seed production. Relative floral rewards of plants of high, intermediate, and low vegetative performance are \(\exp(z), 1,\) and \(\exp(-z),\) respectively, where \(z\) measures the degree of floral reward increase due to local adaptation.

Another locus with two alleles, \(F\) and \(f,\) determines the floral signal trait, with different genotypes expressing distinct floral signals that pollinators can distinguish. Pollinators cannot distinguish among plants of genotypes \(F/F, F/f,\) and \(f/f,\) but they can sense the difference in floral rewards among these plants and associate it with different floral signals.

Plants are annual, and their generations are nonoverlapping. The life cycle starts with seeds produced by parental plants. Seeds are then dispersed within and between habitats, and the between-habitat seed dispersal rate is \(\tau_b (0 \leq \tau_b \leq 1; \tau_b = 1\) indicates complete mixing). Seeds germinate and seedlings experience viability selection, with survivorship determined by their vegetative performance. Mature plants produce animal-pollinated hermaphrodite flowers. Pollinators maintain a stable population size (plants other than the focal species may provide food resources to pollinators and help to maintain the pollinator population). Pollinators occur evenly across habitats. Pollinator behavior (see below) determines the frequency of mating between plants of different genotypes, which in turn determine seed genotypes. We assume free recombination by default. Lower recombination rates increase the likelihood of speciation (fig. S1B, S1D). The vegetative performance of seed parents determines seed production.

Pollinator behavior involves flower choice and occasional movement between habitats. Every time a pollinator visits a plant, it adjusts its labile preference. After visiting a highly rewarding plant of high vegetative performance, a pollinator becomes more likely to choose a plant of the same floral signal in the next visit (the probabilities of pollinators visiting plants of the same signal are multiplied by the weighting factor \(\exp(z)\) (eq. [S4])). After visiting a poorly rewarding plant of low vegetative performance, a pollinator becomes less likely to visit another plant of the same floral signal (visiting probabilities are weighted by \(\exp(-z)\) (eq. [S5])). After visiting a moderately rewarding plant of intermediate vegetative performance, a pollinator randomly chooses the next plant to visit independent of floral signal (visiting probabilities have no weighting factor). We assume that pollinators have a fixed floral reward standard by which to judge whether a plant is highly or poorly rewarding, and this fixed standard corresponds with the floral rewards of a plant of intermediate vegetative performance. The fixed standard corresponds to the average energetic cost for a pollinator to travel from one plant to another and in handling flowers to collect rewards. That is, a pollinator judges a plant as highly rewarding if its floral rewards more than compensate for the average energetic cost of collecting the rewards. However, a pollinator might have a more flexible floral reward standard, changing its standard depending on the floral rewards of other plants that it visited previously. Although we assume a fixed standard throughout for simplicity, later we discuss how a flexible standard could affect our results.

During each interval of consecutive plant visitations, a pollinator may move between habitats at the between-habitat movement rate of \(\tau_m (0 \leq \tau_m \leq 1; \tau_m = 1\) indicates random habitat choice between consecutive flower visits). Pollinator movement between habitats occurs independently of their labile preference. Although it would be more logical to assume that a pollinator is more likely to change a habitat after it encounters a poorly rewarding plant, this pollinator behavior, by directly affecting between-population gene flow via pollen movement, would obscure the role of labile preference in our proposed mechanism of speciation. Therefore, our model does not incorporate this behavior. Decoupling between-habitat movement from labile preference should underestimate the effects of labile preference in promoting speciation and thus lead to a conservative estimate of the likelihood of speciation.

In addition to labile preference, fixed preference and flower constancy may affect pollinator flower choice behavior (Waser 1986). Fixed preference is a species-specific innate preference that is consistent among individuals of the same pollinator taxon, and flower constancy is short-term specialization on one floral type to avoid the cost of searching and/or handling different floral types irrespective of floral rewards (Straw 1972; Waser 1986). Fixed preference is incorporated in our model as a parameter \((w; \text{eq. [S3]})\). Positive \(w\) indicates an innate preference toward the floral signal of genotype \(F/F,\) and negative \(w\) indicates an innate preference toward the floral signal of \(f/f.\) Flower constancy is incorporated as a fraction \((c)\) of visitations that would have gone to plants of different floral signals but is directed to plants of the same signal (Straw 1972; eq. [S6]). Larger \(c\) means stronger flower constancy. When there is no floral signal variation, flower constancy does not have any effect.

To formalize pollinator behavior into the probabilities that an average pollinator visits plants of different genotypes, we use a Markov process model (Straw 1972; Janovský et al. 2017). The model yields pollinator visiting
probabilities within each plant generation as a stationary probability distribution of the Markov process (supplement A). We assume no pollen carryover by default, which yields conservative estimates for the likelihood of speciation (supplement B).

Model Simulation

Here, we briefly illustrate the protocol of model simulations (see supplement A for details). A single simulation run proceeds as follows. At the initial state, all plants in habitats A and B have the vegetative trait genotype $P/P$, meaning that population A is locally adapted and population B is maladapted. Also, all plants have the floral signal trait genotype $F/F$ at the initial state. Each simulation is run up to 10,000 generations, during which the vegetative trait mutant allele $p$ and the floral signal trait mutant allele $f$ are separately introduced at a frequency of $10^{-3}$ in population B at certain generations. We consider two scenarios of mutation order: in the VF scenario, $p$ is introduced at generation 2,500 and $f$ at generation 5,000, whereas in the FV scenario, $f$ is introduced at generation 2,500 and $p$ at generation 5,000. Genotype frequencies reach steady states before mutations are introduced and at the end of the simulation.

At the end of each simulation, we evaluate the degree of reproductive isolation as the proportion of assortative mating between locally adapted plants within populations (eq. [S7]) and consider that speciation occurs when the proportion of assortative mating exceeds 0.95 in both populations. For each vegetative and floral signal trait, we calculate a fixation index to evaluate trait divergence between populations (eq. [S8]) and consider that divergence has occurred if the index exceeds 0.75.

To explore the conditions necessary for speciation, we test the effects of increased floral reward due to local adaptation ($z$) on speciation and trait divergence along gradients of other parameters affecting divergent selection ($v$ and $s$), between-habitat seed dispersal and pollinator movement ($\tau_d$ and $\tau_m$), and pollinator behavior ($w$ and $c$). On the basis of a preliminary analysis, we identified a default set of parameter values with which speciation occurs in the VF scenario ($z = 2$, $v = 0.2$, $s = 0.2$, $\tau_d = 0.1$, $\tau_m = 0.001$, $w = 0$, and $c = 0$). The default values for strength of divergent selection ($v$ and $s$) were chosen on the basis of a meta-analysis of reciprocal transplant experiments examining local adaptation (Hereford 2009; supplement C). The focal range of the floral reward increase ($0 \leq z \leq 4$) covers $z$ values that induce pollinator preference of strengths observed empirically (supplement D). The other parameters range from 0 (or $10^{-4}$) to 1, which spans their theoretical bounds.

In addition to numerical simulations, we derived and analyzed a simplified version of our model. The simplified model is analytically tractable and used to confirm component mechanisms (e.g., frequency-dependent selection on floral signals) underlying our simulation results (supplement E).

Results

Frequency-Dependent Selection due to Pollinator Labile Preference

Frequency-dependent selection on the floral signal trait determines the dynamics of ecological speciation in the VF and FV scenarios. Pollinator labile preference induces this frequency dependence, which can be positive or negative depending on the local adaptation of a population (supplement E). In a locally adapted population consisting of highly rewarding plants, common floral signals have more chances than rare signals to make pollinators learn that their signals indicate high rewards and thus attract more pollinators. This results in positive frequency-dependent selection on the floral signal trait. On the other hand, in a maladapted population of poorly rewarding plants, pollinators learn to avoid flowers showing the signals of these maladapted plants. In such a population, plants of rare floral signals have fewer chances of making pollinators dislike their signals and thus receive more pollinator visitations than those of common floral signals. This drives negative frequency-dependent selection on the floral signal trait.

Ecological Speciation in the VF Scenario

The model shows that divergence in the floral signal trait can cause ecological speciation in the VF scenario (fig. 1A). When this mode of speciation occurs, the vegetative trait mutant allele $p$ successfully invades and causes a gradual increase in the frequency of genotype $p/p$ in population B. This initial invasion of the vegetative trait mutant is favored by stronger divergent selection and lower seed dispersal because strong divergent selection accelerates the invasion of fitter vegetative trait mutants and limited dispersal prevents the rare mutants from being swamped (supplement E). In the presented example (fig. 1A), local adaptation in population B is incomplete after the invasion of allele $p$ because seed dispersal and pollinator movement between habitats cause gene flow.

Next, introduction of the floral signal mutant allele $f$ occurs (fig. 1A). When $f$ invades successfully, it causes a rapid increase in the new floral signal genotype $f/f$ in population B. This invasion is favored by weaker divergent selection and increased seed dispersal. These conditions increase the frequency of maladapted, poorly rewarding
plants with the ancestral floral signal, which drives frequency-dependent selection for novel floral signals (supplement E). The successful invasion of allele $f$ leads to floral signal divergence, in which plants of genotype PF/Pf dominate population A and those of genotype pf/pf dominate population B (fig. 1A). This floral signal divergence increases the frequencies of locally favored vegetative trait genotypes in the respective populations, facilitating local adaptation in both habitats. This causes a high prevalence of assortative mating of locally adapted plants within each population, leading to ecological speciation with floral divergence.

For this mode of ecological speciation to occur, the increase in floral rewards ($z$) needs to be sufficiently high (fig. 1C–1E; speciation corresponds to reproductive isolation greater than 0.95). Divergent selection on survival ($v$) and seed production ($s$) must also be low (fig. 1C, 1D), and the rates of seed dispersal ($\tau_d$) and pollinator movements ($\tau_m$) between habitats must be intermediate (fig. 1E, 1F). The effects of divergent selection and between-habitat seed dispersal reflect the conditions favoring the invasions of vegetative and floral signal trait mutants (supplement E). Although the initial invasion of vegetative trait mutants requires sufficiently strong divergent selection and sufficiently low dispersal, the subsequent invasion of floral signal mutants depends on the maladaptation of population B, requiring weak divergent selection and high dispersal.

Ecological speciation can occur even when the floral signal trait does not diverge (fig. 1B). This situation requires that viability selection is much stronger (large $v$; fig. 1C) or seed dispersal is much more infrequent (small $\tau_d$; fig. 1E) than those required by ecological speciation with floral divergence. Because strong viability selection and limited seed dispersal reduce the frequency of locally maladapted plants in each population, a high level of assortative mating occurs between locally adapted plants within populations. This causes reproductive isolation due to immigrant inviability (Nosil et al. 2005), resulting in ecological isolation without floral divergence.

**Ecological Speciation in the FV Scenario**

Our simulations indicate that floral divergence can also cause ecological speciation in the FV scenario (fig. 1G). When the initial invasion of mutant allele $f$ is successful, it causes a floral signal polymorphism in population B. The local maladaptation of resident plants in population B at the initial state keeps their attractiveness to pollinators low and therefore promotes the invasion of the novel floral signal (supplement E). This negative frequency-dependent selection also maintains the polymorphism of floral signals because population B is kept maladapted until the vegetative trait mutant invades population B.

Next, the vegetative trait mutant allele $p$ is introduced (fig. 1G). When $p$ successfully invades, it results in a gradual increase in genotype pf/pf in population B, which eventually dominates the population. This invasion can occur because the vegetative trait mutant has a selective advantage in habitat B (supplement E). Whereas population B becomes dominated by genotype pf/pf, population A is consistently dominated by genotype PF/PF (fig. 1G). This achieves a joint divergence of vegetative and floral signal traits, which results in strong assortative mating and causes reproductive isolation.

For this mode of ecological speciation to occur, the increase in floral rewards due to local adaptation ($z$) must be sufficiently large (fig. 1I–1L; speciation corresponds to reproductive isolation greater than 0.95), as in the VF scenario. However, necessary levels of $z$ are significantly lower than those in the VF scenario (compare fig. 1C–F with 1I–L). Moreover, the ranges of divergent selection on survival ($v$) and seed production ($s$) that can cause ecological speciation with floral divergence are wider in the FV scenario than in the VF scenario (compare fig. 1C and 1D with 1I and 1J). The ranges of seed dispersal and pollinator movement rates ($\tau_d$ and $\tau_m$) that can cause ecological speciation with floral divergence are also wider in the FV scenario (compare fig. 1E and 1F with 1K and 1L). Overall, ecological speciation with floral divergence can occur under broader conditions in the FV scenario than in the VF scenario.

Invasion of vegetative trait allele $p$ does not necessarily cause floral trait divergence but can instead drive ecological speciation without floral divergence (fig. 1H). When this occurs, the frequency of floral trait genotype $F/F$ increases in population B with allele $p$ becoming associated with allele $F$, while genotype PF/FP keeps dominating population A. Ecological speciation without floral divergence

**Figure 1:** Ecological speciation with and without floral divergence and the effects of divergent selection and between-habitat seed dispersal and pollinator movement on trait divergence and reproductive isolation, A–F, VF scenario, G–L, FV scenario. A, B, G, H, Example dynamics of ecological speciation with floral divergence (A, G) and without floral divergence (B, H). To facilitate illustration, genotype frequencies are plotted every 100 generations from 1 to 2,500, from 3,000 to 5,000, and from 5,500 to 10,000 and every generation from 2,500 to 3,000 and from 5,000 to 5,500. C–F, I–L, Effects of divergent selection strength on survival (C, I), divergent selection strength on seed production (D, J), between-habitat seed dispersal (E, K), and between-habitat pollinator movement (F, L). In C–F and I–L, contour lines show reproductive isolation (defined in eq. [57]; thin lines for 0.5, 0.7, and 0.9 and thick lines for 0.95). Speciation is considered to have occurred where reproductive isolation is greater than 0.95. Divergence of vegetative and floral signal traits is considered to have occurred where their fixation indices are greater than 0.75. Specific parameter values used are $z = 4$ and $v = s = 0.1$ in A and $\tau_d = 10^{-1}$ in B and H. Default values are used for other fixed parameters ($z = 2, v = s = 0.2, \tau_d = 10^{-1}, \tau_m = 10^{-2}, w = 0, \text{ and } e = 0$).
occurs under sufficiently strong divergent viability selection (large $v$; fig. 1f) or sufficiently low seed dispersal (small $\tau_d$, fig. 1K), as in the VF scenario.

In both the VF scenario and the FV scenario, floral signal trait divergence strongly enhances the degree of reproductive isolation (fig. 1C–1F, 1I–1L). When the floral signal trait does not diverge, the degree of reproductive isolation increases gradually as divergent selection is strengthened (larger $v$ and $s$), and between-habitat seed dispersal becomes infrequent (smaller $\tau_d$).

**Effects of Pollinator Behavior**

A fixed preference of pollinators toward the mutant floral signal ($w < 0$) facilitates ecological speciation with floral divergence, while a fixed preference for the original floral signal prevents speciation (fig. 2A, 2C). This is because an innate preference for the mutant floral signal facilitates its invasion in population B. Strong flower constancy (large $c$) promotes ecological speciation with floral divergence (fig. 2B, 2D) because flower constancy causes assortative mating with respect to the floral signal trait and thus alleviates the minority disadvantage of rare floral signal mutants. Yet the speciation is precluded when constancy is perfect ($c = 1$) because perfect constancy prevents pollinators from showing labile preference. Intermediate rates of pollinator movement between habitats do not strongly promote ecological speciation with floral divergence (fig. 1F, 1L), whereas intermediate rates of seed dispersal facilitate speciation (fig. 1E, 1K).

**Figure 2:** Effects of fixed preference ($A$, $C$) and flower constancy ($B$, $D$) on trait divergence and reproductive isolation. $A$–$B$, VF scenario. $C$–$D$, FV scenario. Contour lines show reproductive isolation (defined in eq. [57]; thin lines for 0.5, 0.7, and 0.9 and thick lines for 0.95). Speciation is considered to have occurred where reproductive isolation is greater than 0.95. Divergence of vegetative and floral signal traits is considered to have occurred where their fixation indices are greater than 0.75. Default values are used for fixed parameters ($z = 2$, $v = s = 0.2$, $\tau_d = 10^{-1}$, $\tau_m = 10^{-3}$, $w = 0$, and $c = 0$).
Because pollinator movement causes gene flow via pollen dispersal, one might expect that pollinator movement potentially facilitates ecological speciation with floral divergence in the same manner as seed dispersal does. However, frequent pollinator movement can also disrupt the associative learning of pollinators, thus weakening the effects of labile preference in promoting speciation.

**Discussion**

Local adaptation to the growth environment often drives adapted plants have speciation mechanism is that in addition to improved viability and/or seed production, locally adapted plants gain an extra advantage by attracting more pollinators. Because this advantage is realized when locally adapted plants have specific floral signals distinct from those of maladapted plants, floral signals diverge jointly with vegetative traits and reproductive isolation develops between divergently adapted populations. When local adaptation does not affect floral rewards, ecological speciation can still occur without floral divergence but under more restrictive conditions requiring strong divergent selection and limited between-habitat dispersal of seeds and pollen. Thus, the increase in floral rewards due to local adaptation strongly promotes ecological speciation by causing floral signal divergence.

**Implications for Pollinator-Mediated Speciation in Flowering Plants**

Ecological speciation with floral divergence depends on the order of mutations in vegetative and floral signal traits (i.e., the VF or FV scenario) because the local adaptation of a population in a novel growth environment affects the sign of frequency-dependent selection on floral signal traits and thereby determines the invasion of floral signal mutants. If local adaptation occurs before floral signal mutants arise (the VF scenario), positive frequency-dependent selection prevents their invasion, making speciation difficult. If floral signal mutants arise before local adaptation (the FV scenario), negative frequency-dependent selection promotes their invasion, facilitating speciation. The key is the timing of local adaptation relative to the emergence of floral signal variants. This logic may be extended to scenarios other than the VF and FV scenarios (supplement E; fig. S2). For example, if an invading population already has a floral signal polymorphism when it invades a new habitat, this situation can facilitate ecological speciation with floral divergence. In other words, a historical contingency (i.e., mutation order and standing genetic variation in vegetative and floral signal traits) can affect ecological speciation with floral divergence. Such a historical contingency might explain empirical observations that ecotypes show floral divergence between contrasting growth environments in some plants (e.g., *Aquilegia formosa* and *A. pubescens* [Hodges and Arnold 1994]; *Mimulus aurantiacus* [Sobel et al. 2019]) but not in others (*Potentilla glandulosa* [Lewis 1966]; *Senecio lautus* [Melo et al. 2014]).

A realistic strength of pollinator preference seems sufficient to drive ecological speciation with floral divergence in the FV scenario but not in the VF scenario. Jacobs’s preference index (D) is an empirical measure of a pollinator’s preference that ranges from −1 (complete avoidance) to 1 (complete specialization; Jacobs 1974; Gegeg and Laverty 2005). In a controlled experiment in which bumble bee pollinators were presented with equal numbers of rewarding and unrewarding artificial flowers, D ranged from 0.3 to 0.6 (Austin et al. 2018). Assuming a situation similar to this experiment, we can calculate comparable D values for given z values of our model (supplement D; fig. S2). Under circumstances most favorable for ecological speciation with floral divergence in our model (e.g., the FV scenario with high flower constancy; fig. 2D), speciation occurs when $z = 0.8$, which corresponds to $D = 0.38$. Under more restrictive circumstances (the FV scenario with no flower constancy; fig. 1L–1L), speciation requires $z > 2$, which corresponds to $D > 0.76$. In highly restrictive situations (the VF scenario; fig. 1C–1F), speciation does not occur unless $z > 3$, which corresponds to $D > 0.91$. Thus, a realistic strength of pollinator preference may be sufficient to promote speciation in the FV scenario but might be too weak in the VF scenario.

**Comparison with Previous Theories of Speciation**

Reinforcement of prezygotic isolation between populations can occur when interbreeding of individuals from different populations produces unfit hybrids (Butlin 1987; Servedio and Noor 2003). In our model, interbreeding between plants with locally adapted vegetative trait genotypes ($P/P$ and $p/p$) can produce plants of the heterozygous genotype ($P/p$) that are inferior in either habitat, such that reinforcement due to extrinsic hybrid inferiority (Kirkpatrick 2001; Servedio and Noor 2003) can contribute to floral signal divergence.

Another mechanism also facilitates floral signal divergence. Consider, for example, that genotype $PF/PF$ dominates population A and genotypes $pF/pF$ and $pf/pf$ prevail...
in population B, with population A constantly supplying immigrants of genotype PF/PF to population B. In population B, these immigrants make pollinators learn the floral signal of genotype F/F as indicating poorly rewarding plants. This lowers average pollinator visitation to locally adapted plants of genotype PF/pF relative to that of genotype pf/pf. This decreases the frequency of floral signal genotype F/F and causes a floral signal divergence of population B from A. This mechanism of floral divergence is driven by a fitness advantage in pollinator attraction, unlike the reinforcement mechanism due to a fitness advantage of not producing unfit offspring.

Theoretical and empirical studies have shown that an intermediate amount of migration can most efficiently promote reinforcement (Kirkpatrick 2000; Nosil et al. 2003). Similarly, in our model intermediate seed dispersal maximizes the likelihood of ecological speciation with floral divergence (fig. 1E, 1K). This result can be explained by floral divergence due to pollinator attraction as well as reinforcement due to hybrid inferiority because between-habitat migration triggers both mechanisms. Moreover, migration can decrease the local adaptation of diverging populations and facilitate the invasion of novel floral signals, which also contributes to intermediate seed dispersal facilitating speciation.

In animals, condition-dependent expression of sexually selected mating traits has been proposed to promote local adaptation (Proulx 2001; Lorch et al. 2003) and speciation (van Doorn et al. 2009). That is, if locally adapted animals exaggerate the display of secondary sexual characters and are thus more successful in mate acquisition, it increases local adaptation and drives reproductive isolation between populations under divergent natural selection. This is similar to our speciation mechanism in that the increase in floral rewards due to local adaptation promotes local adaptation and speciation. Despite this similarity, however, our plant theory differs in several points. For example, while locally adapted animals directly increase their attractiveness to their mating partners, locally adapted plants in our model increase their attractiveness to pollinators that indirectly mediate mating between plants. Moreover, while reproductive isolation can evolve without mating signal divergence in animals via a one-allele mechanism (van Doorn et al. 2009; Thibert-Plante and Gavrilets 2013), our model shows that floral signal divergence drives speciation via a two-allele mechanism.

**Examination of Model Assumptions**

The speciation mechanism that we consider here is rather complex, which allows us to analyze only a limited set of possible situations. For example, our model assumes that vegetative and floral signal traits are each controlled by a single locus. This assumption may be appropriate for traits under oligogenic control (Orr 1998), including many vegetative and floral signal traits (Orr 2001). In particular, mutations in single genes often strongly affect the production of major flower color pigments, such as anthocyanin (Rausher 2008) and carotenoids (Zhu et al. 2010). On the other hand, previous theory suggests that ecological speciation is less likely when traits affecting mating are controlled by many genes of small effect (Dieckmann and Doebeli 1999; Kondrashov and Kondrashov 1999). If floral signals are a quantitative trait and pollinators can distinguish only a sufficiently large difference in floral signals (Dyer and Chittka 2004; Kagawa and Takimoto 2016), then evolution of a novel floral signal may require a large number of mutations and may be more difficult than expected in our model. Thus, it remains to be shown whether our speciation mechanism can work when vegetative or floral signal traits are quantitative traits.

Our model assumes that pollinators have a fixed standard for floral rewards. On the basis of this standard, they judge a plant as highly or poorly rewarding and change their labile preference. A more realistic assumption, however, might be that pollinators have flexible standards. For example, a pollinator might lower its standard if it encounters only poorly rewarding plants. Pollinators with lowered standards will keep visiting poorly rewarding plants or can leave a habitat that contains only poorly rewarding plants. If this happens in our model, it will reduce the likelihood of ecological speciation in the FV scenario because such flexible standards will weaken or negate the negative frequency-dependent selection on the floral signal trait in a maladapted population and act against the invasion of novel floral signals.

Although we assumed that diverging plant populations share the same pollinator species, plant ecotypes in divergent growth environments are sometimes associated with different pollinators (Anacker and Strauss 2014; Forest et al. 2014; Sobel et al. 2019). Our model could be applied to situations where distinct pollinators utilize the diverging populations by considering no between-habitat movement of pollinators. The conditions for ecological speciation with floral divergence may then be rather relaxed because between-habitat movement does not disrupt associative learning of pollinators.

A crucial assumption of our model is that locally adapted plants with high vegetative performance are superior in survival (v), seed production (s), and floral reward production (z). Although we varied the corresponding parameters independently in our model analysis, it may be more realistic to consider that survival and seed production are correlated positively with floral reward production and the correlation strength depends on how locally adapted plants allocate their resource to these fitness components.
Our model reveals that ecological speciation with floral divergence can occur relatively easily when selection pressure on viability and seed production is low and the increase in floral reward is large (fig. 1). These conditions might be unlikely if survival, seed production, and floral reward production are strongly positively correlated or if locally adapted plants allocate most of their resources to survival and seed production but little to floral rewards. On the other hand, if the resource allocation of locally adapted plants is biased toward floral reward production, it can relax the conditions for speciation. We might further expect that allocation strategy could evolve in response to the relative strength of selection from growth and pollination environments. Future work should explore these possibilities.

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Statement of Authorship

G.T. conceived and coordinated the study, developed and analyzed the model, interpreted results, and wrote the manuscript. K.K., T.Sat., and T.Sak. checked the model, interpreted model results, and reviewed the manuscript.

Data and Code Availability

Model codes and output data are available through a Zenodo repository (https://doi.org/10.5281/zenodo.6426848; Takimoto et al. 2022).

Literature Cited

Althoff, D. M., K. A. Sgraves, and M. T. J. Johnson. 2014. Testing for coevolutionary diversification: linking pattern with process. Trends in Ecology and Evolution 29:82–89.

Anacker, B. L., and S. Y. Strauss. 2014. The geography and ecology of plant speciation: range overlap and niche divergence in sister species. Proceedings of the Royal Society B 281:20132980.

Austin, M. W., P. Horack, and A. S. Dunlap. 2018. Choice in a floral marketplace: the role of complexity in bumble bee decision-making. Behavioral Ecology 30:500–508.

Bierinaskie, J. M., and R. J. Gegear. 2007. Habitat assessment ability of bumble-bees implies frequency-dependent selection on floral rewards and display size. Proceedings of the Royal Society B 274:2595–2601.

Brashaw, H. D., and D. W. Schmierk. 2003. Allele substitution at a flower colour locus produces a pollinator shift in monkeyflowers. Nature 426:176–178.

Butlin, R. 1987. Speciation by reinforcement. Trends in Ecology and Evolution 2:8–13.

Butlin, R. K., M. R. Servedio, C. M. Smadja, C. Bank, N. H. Barton, S. M. Flaxman, T. Giraud, et al. 2021. Homage to Felsenstein 1981, or why are there so few/many species? Evolution 75:978–988.

Chae, E., K. Bomblesies, S.-T. Kim, D. Karelina, M. Zaidem, S. Ossowski, C. Martin-Pizarro, et al. 2014. Species-wide genetic incompatibility analysis identifies immune genes as hot spots of deleterious epistasis. Cell 159:1341–1351.

Coyne, J. A., and H. A. Orr. 2004. Speciation. Sinauer, Sunderland, MA.

Cresswell, J. E., and C. Galen. 1991. Frequency-dependent selection and adaptive surfaces for floral character combinations: the pollination of Polemonium viscosum. American Naturalist 138:1342–1353.

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

Doebeli, M., and U. Dieckmann. 2000. Evolutionary branching and sympatric speciation caused by different types of ecological interactions. American Naturalist 156:577–510.

Dyer, A. G., and L. Chittka. 2004. Biological significance of distinguishing between similar colours in spectrally variable illumination: bumblebees (Bombus terrestris) as a case study. Journal of Comparative Physiology 190:105–114.

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

Fenster, C. B., G. Cheely, M. R. Dudash, and R. J. Reynolds. 2006. Nectar reward and advertisement in hummingbird-pollinated Silene virginica (Caryophyllaceae). American Journal of Botany 93:1800–1807.

Forest, F., P. Goldblatt, J. C. Manning, D. Baker, J. F. Colville, D. S. Devey, S. Jose, et al. 2014. Pollinator shifts as triggers of speciation in painted petal irises (Lapeirousia: Iridaceae). Annals of Botany 113:357–371.

Gegear, R. J., and J. G. Burns. 2007. The birds, the bees, and the virtual flowers: can pollinator behavior drive ecological speciation in flowering plants? American Naturalist 170:551–566.

Gegear, R. J., and T. M. Laverty. 2005. Flower constancy in bumblebees: a test of the trait variability hypothesis. Animal Behaviour 69:939–949.

Gigord, L. D., M. R. Servedio, C. M. Smadja, C. Bank, N. H. Barton, S. M. Flaxman, T. Giraud, et al. 2021. Homage to Felsenstein 1981, or why are there so few/many species? Evolution 75:978–988.

Goulding, D., J. Ollerton, and C. Sluman. 1997. Foraging strategies in the small skipper butterfly, Thymelicus flavus: when to switch? Animal Behaviour 53:1009–1016.

Grant, V. 1949. Pollination systems as isolating mechanisms in angiosperms. Evolution 3:82–97.

———. 1994. Modes and origins of mechanical and ethological isolation in angiosperms. Proceedings of the National Academy of Sciences of the USA 91:3–10.

Grüter, C., H. Moore, N. Firmin, H. Hanelt, and F. L. W. Ratnieks. 2011. Flower constancy in honey bee workers (Apis mellifera) depends on ecologically realistic rewards. Journal of Experimental Biology 214:1397–1402.

Harder, L. D., and M. B. Cruzan. 1990. An evaluation of the physiological and evolutionary influences of inflorescence size and flower depth on nectar production. Functional Ecology 4:559–572.
“One of the largest and most formidable looking, though perfectly harmless, insects we have, is the Corydalus cornutus... Insects like this were characteristic of the Coal Period, probably breeding in the marshes and fens of Carboniferous times.” From “The Horned Corydalus” from the Natural History Miscellany (The American Naturalist, 1867, 1:434–439).