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Contributed Paper

Phenological mismatch and the effectiveness of assisted gene flow

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Abstract: *The persistence of narrowly adapted species under climate change will depend on their ability to migrate apace with their historical climatic envelope or to adapt in place to maintain fitness. This second path to persistence can only occur if there is sufficient genetic variance for response to new selection regimes. Inadequate levels of genetic variation can be remedied through assisted gene flow (AGF), that is the intentional introduction of individuals genetically adapted to localities with historic climates similar to the current or future climate experienced by the resident population. However, the timing of reproduction is frequently adapted to local conditions. Phenological mismatch between residents and migrants can reduce resident × migrant mating frequencies, slowing the introgression of migrant alleles into the resident genetic background and impeding evolutionary rescue efforts. Focusing on plants, we devised a method to estimate the frequency of resident × migrant matings based on flowering schedules and applied it in an experiment that mimicked the first generation of an AGF program with Chamaecrista fasciculata, a prairie annual, under current and expected future temperature regimes. Phenological mismatch reduced the potential for resident × migrant matings by 40–90%, regardless of thermal treatment. The most successful migrant sires were the most resident like in their flowering time, further biasing the genetic admixture between resident and migrant populations. Other loci contributing to local adaptation—heat-tolerance genes, for instance—may be in linkage disequilibrium with phenology when residents and migrants are combined into a single mating pool. Thus, introgression of potentially adaptive migrant alleles into the resident genetic background is slowed when selection acts against migrant phenology. Successful AGF programs may require sustained high immigration rates or preliminary breeding programs when phenologically matched migrant source populations are unavailable.*

Keywords: assisted migration, assortative mating, *Chamaecrista fasciculata*, climate change, flowering time, genetic rescue, migration rate, nonrandom gene flow

La Disparidad Fenológica y la Efectividad del Flujo Génico Asistido

Resumen: *La persistencia de especies adaptadas estrechamente al cambio climático dependerá de su habilidad para migrar rápidamente junto con su entorno climático histórico o de su habilidad para adaptarse en el lugar y mantener su aptitud. Este segundo camino a la persistencia sólo puede ocurrir si hay suficiente variación genética para responder a nuevos regímenes de selección. Los niveles inadecuados de variación genética pueden ser remediados por medio del flujo génico asistido (FGA), que es la introducción intencional de individuos adaptados genéticamente a localidades con climas históricos similares al clima actual o próximo experimentado por la población residente. Sin embargo, el momento oportuno para la reproducción es adaptado frecuentemente a las condiciones locales. La disparidad fenológica entre los residentes y los migrantes puede reducir las frecuencias de apareamiento residente × migrante, lo que reduce la velocidad de introgresión de los alelos migrantes al historial genético residente e impide los esfuerzos de rescate evolutivo. Con un enfoque en las plantas, diseñamos un método para estimar la frecuencia de los apareamientos residente × migrante con base en los tiempos de florecimiento y lo aplicamos en un experimento que imitaba a la primera generación de un programa de FGA Chamaecrista fasciculata, una planta anual de praderas, que se encuentra bajo regímenes de temperaturas actuales y esperadas. La disparidad fenológica redujo el*

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potencial de apareamientos residente × migrante en un 40 – 90 %, sin importar el tratamiento térmico. Los progenitores migrantes más exitosos fueron aquellos más parecidos a los residentes en los tiempos de florecimiento, lo que sesgó aún más la mezcla genética entre las poblaciones residentes y migrantes. Otros loci contribuyentes a la adaptación local – los genes de la tolerancia al calor, por ejemplo – pueden estar en desequilibrio conectado con la fenología cuando los residentes y los migrantes son combinados en un solo pool reproductivo. Por esto, la introgresión de alelos migrantes con potencial adaptativo al historial genético residente reduce su velocidad cuando la selección actúa en contra de la fenología migrante. Los programas exitosos de FGA pueden requerir de tasas de inmigración altas y prolongadas o de programas preliminares de reproducción cuando las poblaciones fuente de migrantes correspondientes no están disponibles.

Palabras Clave: cambio climático, *Chamaecrista fasciculata*, emparejamiento selectivo, flujo génico no-azaroso, migración asistida, rescate génico, tasa migratoria, tiempo de florecimiento

Introduction

Prospects for the long-term persistence of a declining population can be improved by increasing genetic variance through managed migration. Assisted gene flow (AGF) is a conservation strategy whereby individuals are translocated from genetically distinct populations into a targeted resident population to facilitate adaptive evolutionary responses to changing environments (Aitken & Whitlock 2013; Kelly & Phillips 2016). Although managed translocations are simple in concept, biological details may complicate their execution. In particular, the successful interbreeding of migrants and residents depends on similarity in reproductive timing. Many species show geographic variation in reproductive phenology (e.g., Eckhart et al. 2004; Dambroski & Feder 2007; Conklin et al. 2010), so the reproductive phases of migrants may not match those of residents. Mismatched phenology reduces resident × migrant mating frequencies, which impedes the genetic admixture of populations.

Standard theoretical treatments of gene flow on adaptation have strongly focused on scenarios where environments are stable over time, fitness functions are stabilizing, migration is ongoing across generations, and migrants are a random sample of their natal population (Edelaar & Bolnick 2012; Bolnick & Otto 2013; Weis 2015). Under these conditions, gene flow works against local adaptation (Hendry et al. 2001; Lenormand 2002). This scenario differs fundamentally from conditions that would prompt an AGF program in response to climate change, where populations would be distressed by a severe shift in environment and expediency could dictate a single, large-scale, managed, gene-flow event. Furthermore, AGF can be nonrandom in that successful migrants (i.e., those that actually mate with residents) can be a biased subsample of their natal population at the loci governing reproductive phenology.

Consider a hypothetical northern plant population adapted to local soil conditions and local pollinators and natural enemies that is in decline due to a warming climate. Assisted gene flow to introduce loci for improved heat tolerance is attempted by a single translocation of individuals from the south. How would nonrandom mating between residents and migrants affect the pace of introgression? Suppose migrants are 20% of the mating pool, as Weeks et al. (2011) suggest. Under random mating, the frequency of resident × migrant matings would be $2(0.8 \times 0.2) = 0.32$, whereas the frequencies of within-type matings by residents and migrants would be $0.8^2 = 0.64$ and $0.2^2 = 0.04$, respectively. If, however, half the resident individuals finish mating before the southern migrants begin, hybrid mating frequency is cut in half (to 0.16), whereas the frequency of migrant × migrant matings triples (i.e., $\frac{1}{2}[0.2^2] + \frac{1}{2}[0.2] = 0.12$). Such nonrandom mating between residents and migrants by itself slows the introgression of adaptive southern alleles into the northern population. Introgression is further diminished if purebred migrant descendants are maladapted to some aspect of the resident environment, reducing their contribution to future mating pools.

The potential impact of phenological mismatch on AGF is readily illustrated with flowering plants, which lend themselves to the kind of bookkeeping required to predict resident × migrant mating opportunities. The number of active flowers on a plant on each day of the flowering season is called the flowering schedule (Fox 2003). Two plants can exchange pollen only if their flowering schedules overlap. Latitudinal and altitudinal clines in flowering date are very common (e.g., Ågren & Schemske 2012; Anderson & Gezon 2015) and can quickly evolve through the interplay of on-going gene flow, assortative mating, and selection (Soularue & Kremer 2014). This makes phenological mismatch likely when the migrant and resident populations come from different latitudes. Further, most plant species are monoecious (i.e., individuals reproduce as both males [via pollen] and females [via ovules]). Individual reproductive success through one sex need not be correlated to success through the other, so AGF can be asymmetric through male and female function.

We conducted a quantitative analysis of flowering schedules to illustrate how phenological mismatch between migrants and residents can impede the initial stages of an AGF effort. We reexamined a previous experiment that mimicked the initial generation of a translocation program aimed at improving the prospect of species persistence in the face of climate change. This experiment used latitudinally distinct populations of the annual legume *Chamaecrista fasciculata* (Fabaceae) as a model system (Wadgymar et al. 2015*a*, 2015*b*) and included an infrared warming treatment to mimic future thermal regimes. We considered several impacts of nonrandom mating and selection on longer-term outcomes of AGF.

Methods

System and Experimental Setup

Chamaecrista fasciculata is a frost-intolerant annual distributed across the North American Great Plains and eastward toward the Atlantic (Irwin & Barneby 1982). Its northern range limit is just below the Great Lakes and extends from Minnesota to New York. A single plant can produce several hundred flowers, which are buzz pollinated by bumblebees. First flowering date, which is apparently cued by photoperiod (Wadgymar et al. 2015*b*), varies clinally from north to south (Etterson & Shaw 2001).

In 2009, we collected seeds from 2 northern and 2 southern populations of *C. fasciculata* from two latitudinal transects throughout the United States, one in the Midwest, with collections from Minnesota (MN) and Missouri (MO), and the other along the east coast, with collections from Pennsylvania (PA) and North Carolina (NC). Further geographical and climatic information on the study site and population collection sites is in Supporting Information.

To mimic assisted migration under current and future thermal regimes, we planted 60 individuals from each of the four C. fasciculata populations in the experimental climate warming array at the Koffler Scientific Reserve, King Township, Ontario, Canada (KSR). The array is north of the plant's range limit in the eastern United States but is at a comparable latitude (44.0300°N) to the more western MN population (44.8011°N) (Wadgymar et al. 2015*a*). For our purposes, we used the MN plants to mimic a resident population in decline that lacks sufficient genetic variation for an evolutionary response to selection. The 3 southerly populations mimicked potential sources for migrants with genes favored under warmer conditions.

Seedlings were germinated under high and low temperature conditions in the greenhouse on 30 May 2011 and transplanted into the arrays 27 days later. The timing of germination among populations and treatments averaged from 3.9 to 6.0 days after planting and contributed minimally to the timing of reproduction (Wadgymar et al. 2015*a*). The warmed (experimental) plots were equipped with 6 infrared lamps mounted on a superstructure with a precise geometry that evenly warmed a 3-m-diameter area of soil surface (designed as per Kimball et al. [2008]). Ambient (control) plots had the same superstructure but no lamps. Infrared thermometers monitored canopy temperatures, and a control system adjusted power to the lamps to maintain a constant temperature difference between ambient and warmed plots (1.5 °C day/3.0 °C night) that reflected the predicted midcentury thermal regime for the area (OMNR 2007). Plants were otherwise exposed to natural conditions.

We assembled flowering schedules for all individuals by counting the number of flowers on display on each plant on each day of the flowering season (17 July to 17 October). Mature fruit were collected and counted for each plant as they ripened. The total number of seeds was highly correlated with fruit number in all populations and treatments (r = 0.68–0.99).

Predicting Hybridization Rate from Flowering Schedules

The expected frequency of resident × migrant mating can be estimated from flowering schedules. If schedules completely overlap between and within populations, all plants have equal flower production, and if all matings are equally successful, then each resident can expect a constant proportion of its flowers to be pollinated by migrant donors. This would equal the proportion of migrants in the mating pool, m^*, or the apparent hybridization rate through migrant pollen. Equivalently, we defined r^* as the proportion of flowers on migrants that are pollinated by residents, or the hybridization rate through resident pollen. The indices m^* and r^* are the expected hybridization rates under random mating; thus, they are baselines for evaluating the impact of phenological mismatch. If there were an equal number of residents and migrants in the mating pool, as in this experiment, $m^* = r^* = 0.5$.

Asynchronous flowering reduces realized hybridization rates below the apparent rate. Figure 1 illustrates a case of partial overlap in flowering schedules of individual plants both within and between resident and migrant populations. We derived an estimate for the effective hybridization rate by first evaluating the expected proportion of flowers on individual resident plants that are pollinated by migrants:

$$m_i = \sum_{d=1}^{D} f_{id}\, q_d. \tag{1}$$

The term f_{id} is the proportion of all flowers produced by resident i over the entire flowering season that are open on day d, that is $f_{id} = R_{id} / \Sigma R_{id}$, where R is the number of open flowers on resident individuals. The term q_d is the daily proportional contribution by immigrants to the pollen pool (i.e., the proportion of total resident and migrant flowers open on day d that belong to migrant

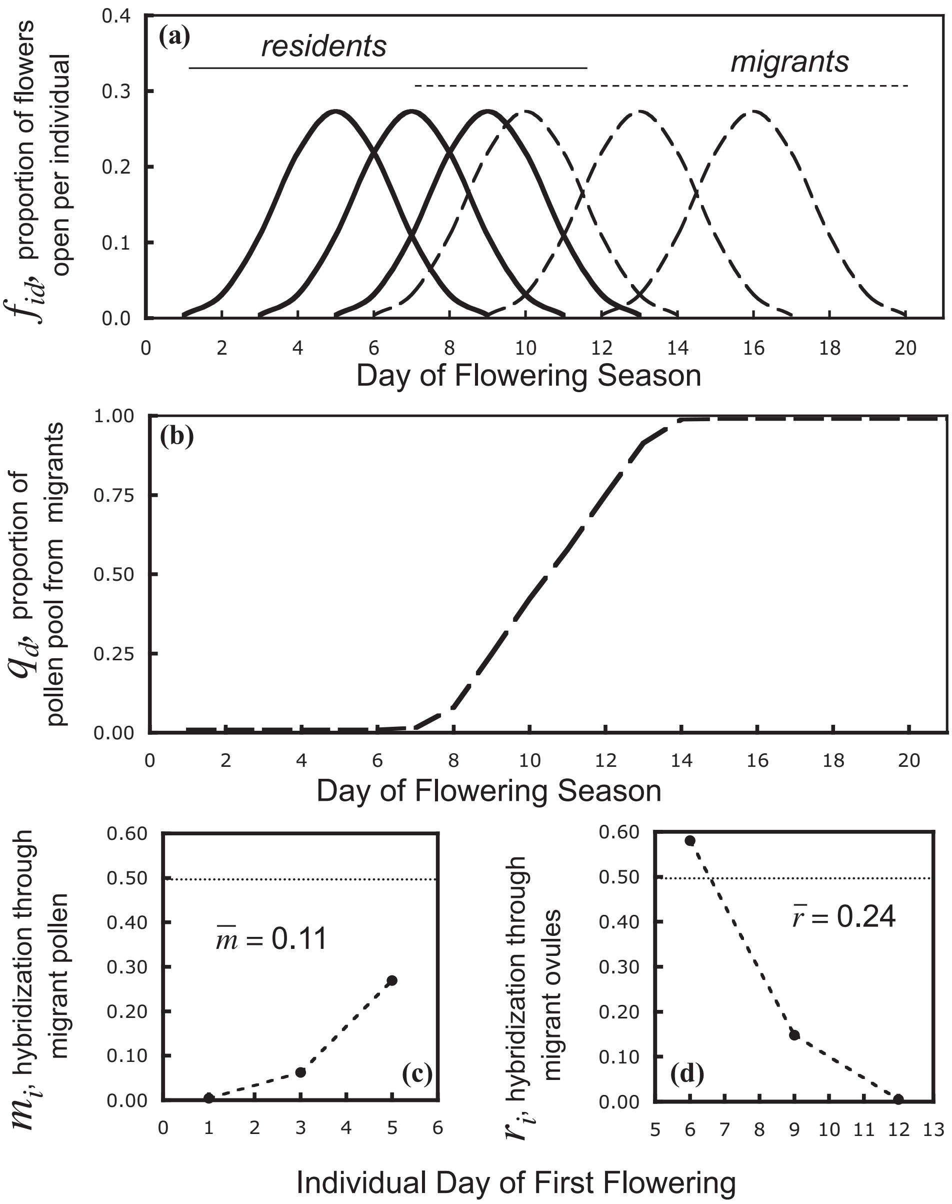

Figure 1. The effect of phenological mismatch on hybridization rates between residents (northern population) and migrants (southern population) of a hypothetical plant species: (a) flowering schedules for 3 residents and migrants (day of flowering onset for each plant is the lowest x-intercept of its flowering schedule curve), (b) shift in the proportional contribution of migrants to the pollen pool over the season (only residents contribute before day 6 and only migrants contribute after day 13), (c) expected proportion of seeds produced by each resident dam with migrant sires ($\bar{m}$, mean hybridization rate through migrant pollen), (d) expected proportion of seeds produced by each migrant dam with resident sires ($\bar{r}$, mean hybridization rate through migrant ovules).

individuals) (Fig. 1b). The last plant stops flowering on day D; thus, $f_{id}q_d$ is calculated for each day that any of the plants are in flower. The mean of this index across residents, $\bar{m}$, estimates the effective hybridization rate through migrant pollen (Weis 2015). Equivalently, r_i and $\bar{r}$ describe the proportion of migrant flowers pollinated by residents and the effective hybridization rate through resident pollen, respectively. These metrics are calculated through Eq. (1) with the migrants' flowering schedules (i.e., M_{id}) in lieu of resident flowering schedules (R_{id}) and substituting $1 - q_d$ for q_d.

Variation among individuals in flowering schedules (Fig. 1a) produces a covariance between flowering onset date and m_i. The last resident to flower produces more F_1 hybrids through seed than the first (Fig. 1c). Figure 1d shows the complementary relationship for the migrants, where the first plant to flower produces the most hybrids. Thus, when flowering schedules differ between populations, $\bar{m} \neq \bar{r}$. (Fig. 1c vs. d).

Values of m_i and r_i should be weighted by total flower and fruit numbers per plant when calculating means (Weis 2015). It is typical for the first several flowers opening on a plant to produce fruit, preempting resources for later fruit development (Austen et al. 2015). Thus, pollen produced early in the flowering season is more likely to fertilize flowers that actually go on to set fruit. To account for the age-related change in fruiting success on m_i, we modified Eq. (1) so that f_{id} is calculated as the ratio $l_{id}\,R_{id}$ / $\Sigma\ l_{id}\,R_{id}$, where l_{id} is the proportion of open flowers on individual i on day d that go on to produce fruit (assuming an equal number of seeds and fruit). Again, the calculation for r has M_{id} and $1 - q_d$.

The expected frequencies (P) of the 4 mating combinations (RR, resident × resident; RM, resident × migrant; MR, migrant × resident; and MM, migrant × migrant) proceeds from the hybridization rates $\bar{m}$ and $\bar{r}$:

$$\begin{aligned} P_{\mathrm{RR}} &= f_{\mathrm{R}}(1-\bar{m}), \\ P_{\mathrm{RM}} &= f_{\mathrm{R}}\bar{m}, \\ P_{\mathrm{MR}} &= f_{\mathrm{M}}\bar{r}, \text{ and} \\ P_{\mathrm{MM}} &= f_{\mathrm{M}}(1-\bar{r}) \end{aligned} \tag{2}$$

where f_{R} and f_{M} are the proportion of the entire seed crop produced by resident and migrant plants, respectively. Reproductive isolation between populations can be indexed as

$$I = (P_{\mathrm{RR}} + P_{\mathrm{MM}}) - (P_{\mathrm{MR}} + P_{\mathrm{RM}}), \tag{3}$$

which scales from 0 for random mating (i.e., $\bar{m}$ and $\bar{r}$ equal m^* and r^*, respectively) to 1 for complete phenological isolation.

Applying Estimation Methods to *C. fasciculata* Flowering Schedules

The expected frequency of resident × migrant mating depends on the timing of flower production, the number of flowers produced, and the probability that each flower successfully matures into a seed-bearing fruit (Eq. 1). To gauge the potential impact of age-related decline in fruiting success (Austen et al. 2015), we incorporated the individual plant's success level, l_i (number of fruit collected at the end of the season/number of flowers counted across the season), under two extreme scenarios. In the age-independent success scenario, each $l_{id} = l_i$ (i.e., each flower had the same chance of producing a fruit regardless of plant age). At the other extreme, for the age-dependent success scenario, we assumed each $l_{id} = 1.0$ at the beginning of the individual's flowering period and stayed there until proportion l_i of their total flower production was achieved; thereafter, $l_{id} = 0$. For instance, if a plant produced 100 flowers that in aggregate produced 50 fruit, each flower was given a 50% chance of making one of those fruit under the age-independent scenario. Under the age-dependent scenario, the first 50 flowers were given a 100% chance of producing a fruit, whereas the remainder had a 0% chance. Thus, these 2 scenarios bracket the possible effects of age-related variation in fruiting success. Almost all of the 30 plants survived in each of the 8 treatment combinations of population and thermal regime; thus, this experiment mimics a situation in which the apparent migration rates, m^* and r^*, are 0.5. Compensating weighting factors were applied where survival was less than perfect (in all cases, survival was >90%). When $\bar{m}$ or $\bar{r}$ was <0.5, there was some degree of phenological isolation between residents and migrants ($I > 0$, Eq. 3).

We estimated opportunities for hybridization under two additional cases. First, the migrant population contained equal proportions of the PA, MO, and NC source populations, again with the residents constituted half the breeding pool. For this calculation, we used the population-level flowering schedules (e.g., the number of open flowers on day d produced by all resident plants combined); schedules for each migrant source populations were weighted by one-third. Second, we asked what would be the potential for production of F_2 and backcross genotypes in the following generation, where mating would occur between purebred plants of both types and F_1 offspring, to occur at frequencies as predicted by Eq. (2). We used a population-level flowering schedule for the resident and migrant populations and assumed additive contributions of early and late flowering alleles to the phenology of offspring (i.e., no dominance or epistatic effects). The F_1 flowering schedule was estimated by first aligning the 2 parent types to the midpoint between them (assumes no dominance) and then averaging the number of flowers produced by the parents on each day. For both of these additional cases, we calculated the matrix of mating probabilities, as developed by Weis (2005). Details of this method are presented in Supporting Information.

We tested the null hypotheses that the logistic regressions of m_i and r_i over date of flowering onset are different from 0 for each population and treatment combination and that $\bar{m}$ and $\bar{r}$ are different from 0.5. Statistical tests of additional quantities based on these estimates (e.g., isolation index I) are redundant.

Results

Opportunities for Hybridization

Populations exhibited a latitudinal cline in flowering phenology (Fig. 2). (See Wadgymar et al. [2015*a*] for complete results.) Under ambient temperatures, plants from the northernmost MN (resident) population flowered 64.4 days after sowing, on average. The PA, MO, and NC (migrant) populations flowered 12.1, 16.8, and 34.5 days later than the residents, respectively. Artificial warming advanced flowering onset by 4.4–9.4 days in all populations. The MN plants continued to flower for approximately 27 days regardless of thermal treatment; thus, the overlap in resident and migrant flowering schedules reflected the differences in mean start dates.

Clinal differences in flowering time made late flowering resident plants more likely to hybridize with migrants than their early-flowering counterparts in the age-independent fruit set scenario (Fig. 3a & Supporting Information). Reciprocally, the early-flowering migrants were more likely to be pollinated by residents (Fig. 3b). The exception was the NC migrants, for which the

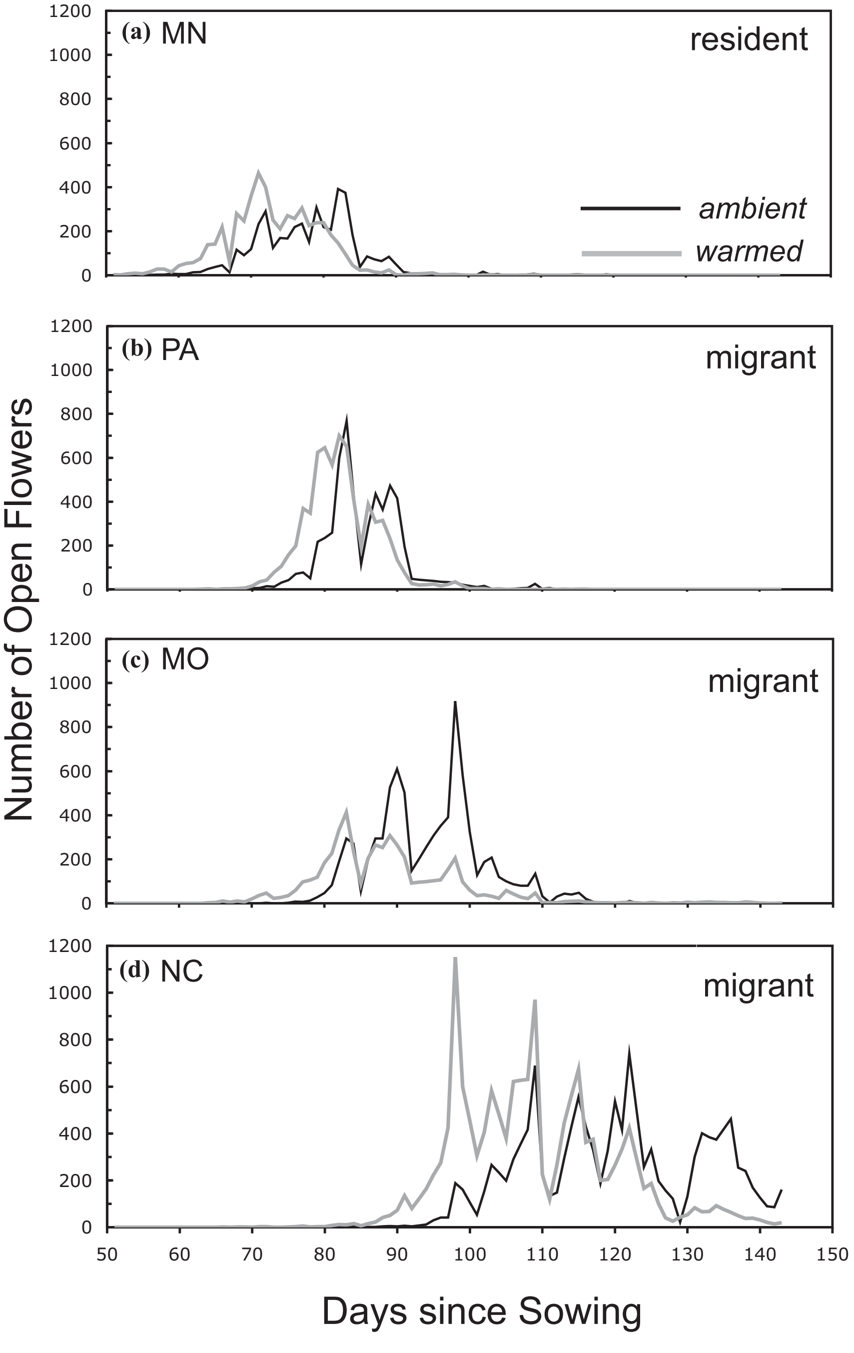

Figure 2. Population-level flowering schedules under ambient and warmed temperatures for (a) the resident Minnesota (MN) population and migrant populations of C. fasciculata *from (b) Pennsylvania (PA), (c) Missouri (MO), and (d) North Carolina (NC).*

overlap in the flowering schedule with residents was too slight to allow more than sporadic opportunities for pollen exchange. The coefficients for the logistic regressions of m_i and r_i over flowering onset day were significantly positive and negative, respectively (Supporting Information), when PA and MO acted as migrants. Results were similar under ambient and warmed regimes (Fig. 3a, e vs. b, f). The relationship between m_i and r_i and day of flowering onset for the age-dependent fruit-success scenario were qualitatively the same but stronger (Figs. 3c, d, g, h & Supporting Information), except for NC, again because of minimal schedule overlap.

The overall potential for hybridization was substantially less than expected under random mating. For the age-independent scenario, all estimates of $\bar{m}$ and $\bar{r}$ were significantly lower than the panmictic expectation of 0.5 (Table 1 & Fig. 3), regardless of temperature regime. In all instances of the age-independent scenario, $\bar{m} > \bar{r}$ (Table 1). This reflected the longer duration of flowering in the migrant populations, which gave them slightly more opportunities for within-type mating. When all mating combinations were considered, within-type matings exceeded between-type matings (Fig. 4), but when considering only the between-type matings, resident

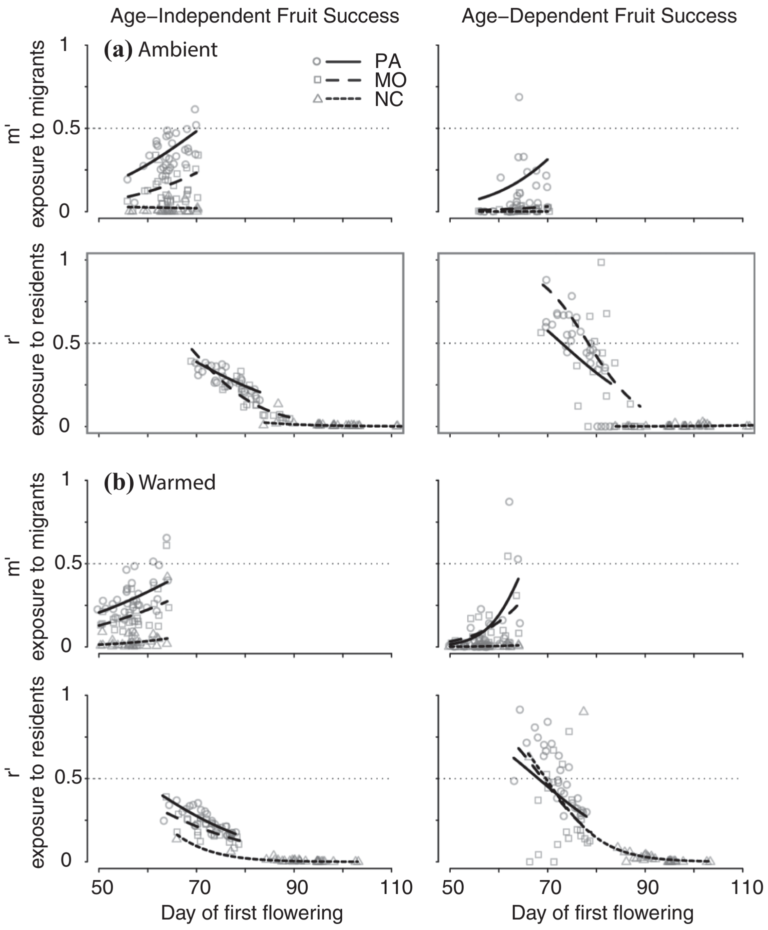

Figure 3. Expected hybridization rates for the resident (Minnesota) population of C. fasciculata *through migrant pollen (*m_i*) and migrant ovules (*r_i*) under the age-independent and age-dependent fruiting-success scenarios: (a) ambient temperatures, (b) experimentally warmed temperatures (horizontal dashed lines, hybridization rates under random mating). Logistic-regression lines are depicted for the Pennsylvania (PA), Missouri (MO), and North Carolina (NC) migrant populations. Regression coefficients and results of significance tests are in Supporting Information.*

dams had more opportunities to hybridize than migrant dams.

Under the age-dependent scenario, between-type matings were, on the whole, also below random expectations (Fig. 4), but the roles of residents and migrants were reversed. In all cases, $\bar{r} > \bar{m}$ by several standard errors (Table 1). In fact, migrant ovules were exposed to resident pollen at a rate no different from the random

Table 1. Average hybridization rates through migrant (*m*) and resident (*r*) pollen under ambient and experimentally warmed conditions between the resident Minnesota population and each of the Pennsylvania (PA), Missouri (MO), and North Carolina (NC) migrant populations of *Chamaecrista fasciculata*.

Thermal treatment	*Migrant source*	*Age-independent fruit success (SE)*[a]		*Age-dependent fruit success (SE)*[b]	
Ambient		$\bar{m}$	$\bar{r}$	$\bar{m}$	$\bar{r}$
	PA	0.377[c] (0.059)	0.292[c] (0.043)	0.104[c] (0.024)	0.471 (0.041)
	MO	0.173[c] (0.032)	0.148[c] (0.021)	0.011[c] (0.004)	0.467 (0.111)
	NC	0.023[c] (0.012)	0.008[c] (0.001)	0[c] –	0.003[c] (0.001)
Warmed					
	PA	0.309[c] (0.054)	0.240[c] (0.013)	0.089[c] (0.033)	0.451 (0.049)
	MO	0.223[c] (0.039)	0.179[c] (0.039)	0.076[c] (0.021)	0.413 (0.087)
	NC	0.028[c] (0.006)	0.010[c] (0.005)	0.003[c] (0.001)	0.068[c] (0.035)

[a] *Assumption is that all flowers have an equal probability of maturing into a fruit.*
[b] *Assumption that the probabilities of early and late flowers producing fruit are 100% and 0%, respectively.*
[c] *Values differ by >2 SE from the random expectation of 0.5.*

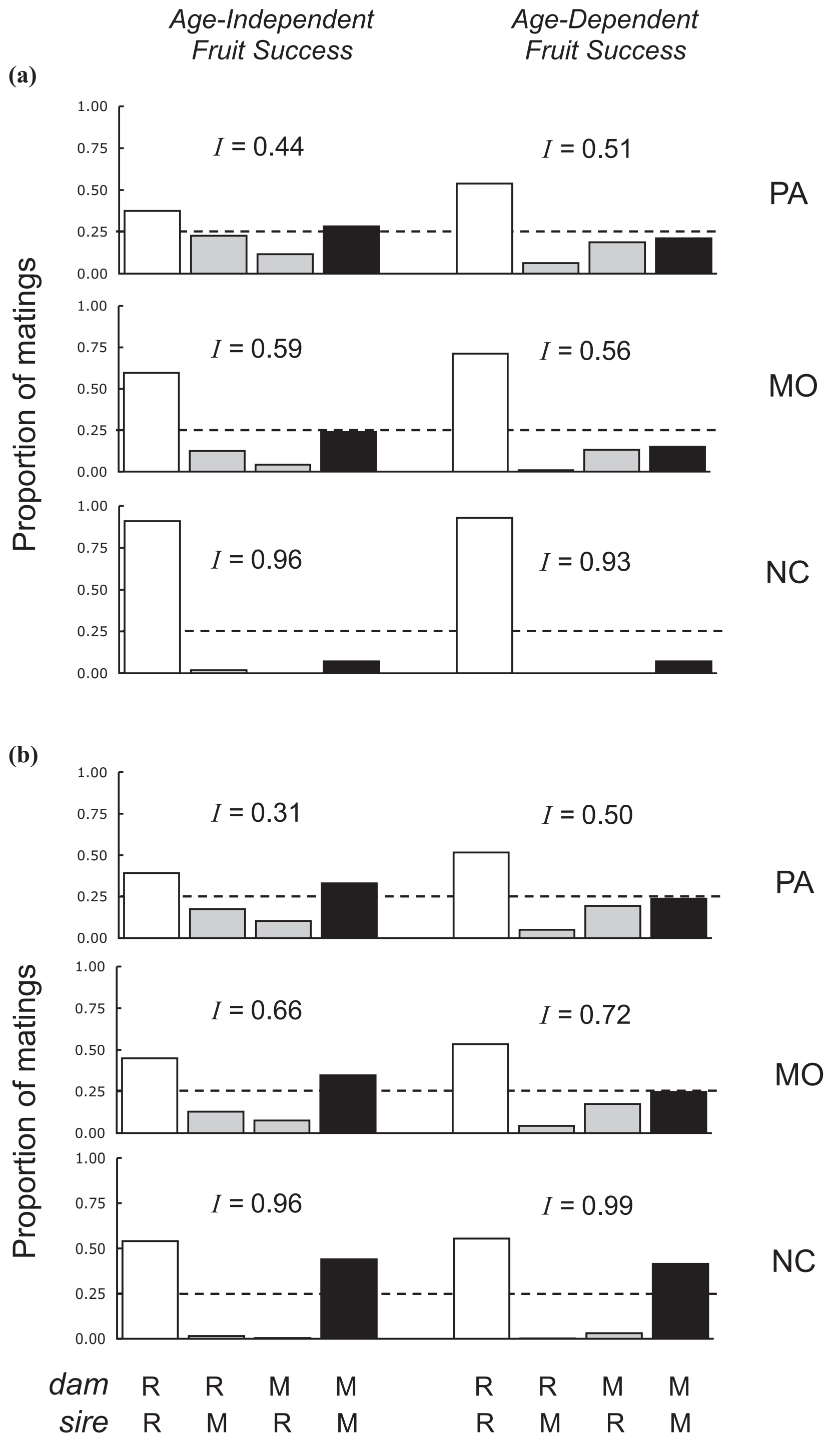

Figure 4. Predicted frequency of matings between (white and black) and within (gray) the resident (R) and migrant (M) populations for the age-independent and age-dependent fruiting-success scenarios under (a) ambient and (b) warmed temperatures (horizontal dotted lines, frequencies expected under equal fertility and random mating; I*, degree of reproductive isolation between residents and migrants;* I = *1, complete isolation;* I = *0, panmixia).*

expectation of $r^* = 0.5$. This makes biological sense because the earlier flowering residents stopped fruit production earlier than pollen production. Hence, migrant pollen had restricted access to the most successful resident flowers, yet residents were still producing pollen during the same time that migrants were producing their most successful flowers. Thus, more hybrids were formed on migrant than resident dams in all cases (Fig. 4).

Hybridization Rate When Using a Mixed Migrant Pool

Calculations indicated that using a mixture of migrant populations did not appreciably increase the opportunity for alleles from the late-flowering, heat tolerant NC population to pass into the resident genetic background (Supporting Information). In this scenario, NC plants hybridized with PA and MO plants. These migrant

× migrant hybrids, with intermediate phenology, then hybridized with the early-flowering residents. Under random mating, when the 3 source populations contributed equally to the migrant population, 11.2% of potential pairings were between NC and either PA or MO (Supporting Information). Phenological mismatch of NC to the other migrants reduced this to 7.4% under the age-dependent artificial-warming condition (Supporting Information) and to 0.6% under the age-independent artificial-warming condition (Supporting Information). The predicted frequency of purebred residents ranged from 43% to 68%, whereas 25% was expected under random mating. Thus, using a mixture of migrants in the initial generation created a small number migrant × migrant hybrids that could pass NC alleles to resident in the next generation, but this event would be countered somewhat by high resident × resident mating frequency.

Projecting to the Next Generation

Calculations for hybridization and backcrossing in the subsequent generation showed that phenological mismatch and fitness variation further impeded introgression of migrant alleles into the resident genetic background. Figure 5 illustrates the point with the MO migrant population under the age-independent scenario.

Under the assumptions of completely random mating and equal fitness, expected frequency of second-generation purebred residents was only 6.25% because effective gene flow mixes resident and migrant alleles. With phenological mismatch and observed fertility levels, however, the expected purebred-resident genotype frequency was 9-fold higher (56%) after 2 generations of impeded gene flow (Fig. 5a). Under the warmer temperatures, where seed production by residents and migrants is more equitable, the expected frequency of purebred residents was 35% (Fig. 5b), which was still 5-fold greater than random. Backcrossing of F_1s to residents, expected to be 25% under random mating and equal fitness, fell below 20% under both thermal regimes. Most importantly, phenological mismatch reduced the expected frequency of F_2s in the second generation to 5%, compared with 25% under random mating (Fig. 5). A low frequency of F_2s would be particularly troublesome in an AGF program because it is the genotypic class with resident and migrant alleles at equal frequency and segregating independently (i.e., the class out of which genotypes with the most favorable combinations of resident and migrant alleles emerge). The expected frequencies of backcross and hybrid genotypes in the subsequent generation were slightly greater with PA migrants (data not shown), given the larger overlap between resident and PA flowering schedules. The negligible overlap of resident and NC flowering schedules (Fig. 2) led to negligible hybridizing potential even in the subsequent generation.

Discussion

Our fine-scale analyses of *C. fasciculata* flowering schedules showed 2 impacts on AGF programs if the migrant source population is phenologically mismatched to the residents. First, more migrant individuals are needed to achieve a targeted hybridization rate in a single gene-flow event than under mating synchrony. Second, both the resident and the migrant individuals with the greatest opportunity to hybridize are biased subsamples of their respective populations; hybridizing residents are migrant like in phenology, and hybridizing migrants are resident like. In the case we considered, where the phenology of migrants was maladaptive in the resident's current environment (Wadgymar et al. 2015*a*), the prospects for passing adaptive genes into the resident population are poor. However, prospects were improved somewhat in the anticipated future environment.

We also found that when fruiting success rate declined with maternal age, most of the F_1 hybrids were formed on migrant plants fertilized by resident pollen. Because chloroplasts and mitochondria are inherited through the maternal line, hybrids carry migrant cytoplasmic genes disproportionately.

Sources of Mismatch among Populations in Mating Phenology

Many taxa show latitudinal or altitudinal clines in reproductive phenology, including insects (Dambroski & Feder 2007), amphibians (Guttman et al. 1991), birds (Conklin et al. 2010), and plants (Eckhart et al. 2004; Anderson & Gezon 2015). Although clinal differences can have a genetic basis, reproductive onset frequently has a plastic component triggered by environmental cues that vary across latitude. In some cases, the plastic shift is concordant with the shift in optimal phenology across latitudes (e.g., warm temperature both accelerates flowering and favors early-flowering genotypes), which has milder effect on realized gene flow than when plasticity and selection are discordant (Soularue & Kremer 2014). In either case, a migrant's home-site phenology may be a weak predictor of mating phenology at the resident site.

Consider spring-flowering species, which tend to bloom after they accumulate a characteristic heat sum (i.e., a number of heating degree days above a threshold developmental temperature) (Thompson & Clark 2008). If residents and migrants differ in either threshold or heat-sum requirements, they will flower asynchronously when grown at the same site. In many species, heat sums do not accumulate until an initiating cue is received. For instance, the accumulation of winter chilling degree days is the initiating cue in many tree species (Laube et al. 2014). Translocation to colder northern latitudes is unlikely to delay migrant flowering in such cases because the initiating cue would be received well in advance of spring warming. In a warmer future, however, mismatch could

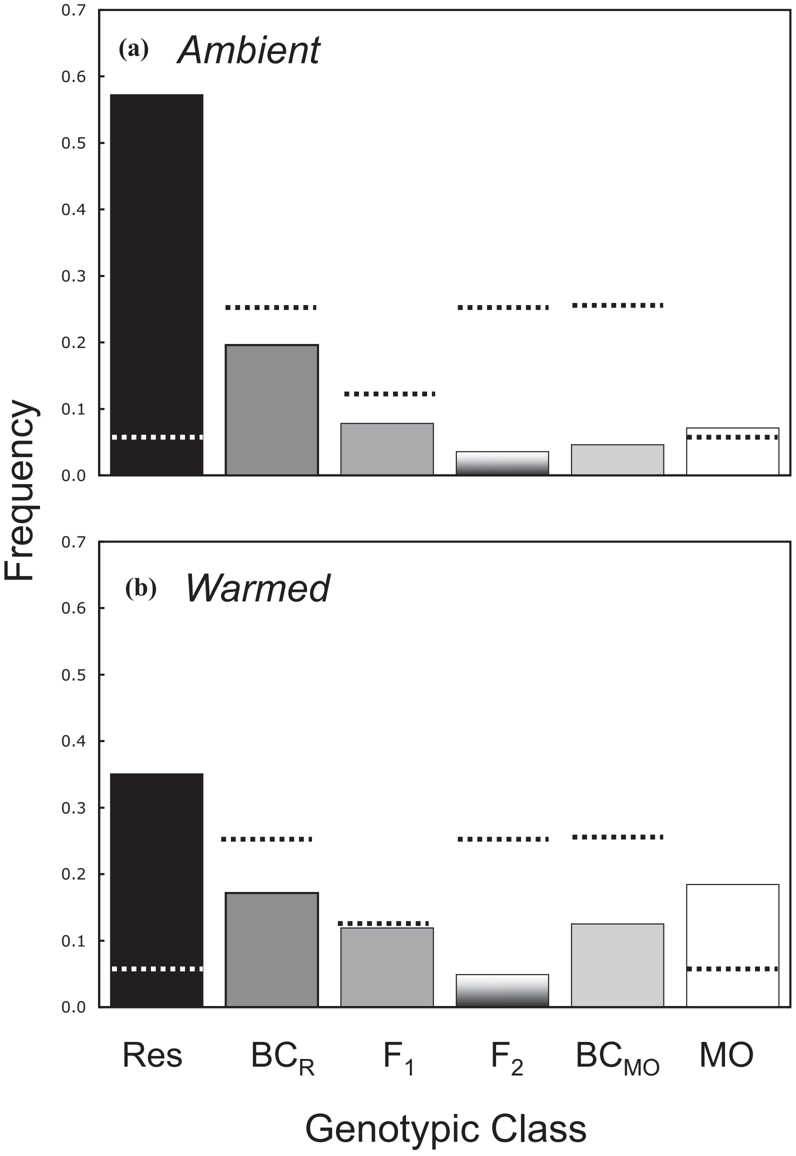

Figure 5. Expected frequencies of purebred resident (Res), purebred Missouri migrants (MO), backcross (BC_R and BC_{MO}), and hybrid (F_1 and F_2) genotypic classes of C. fasciculata *2 generations after the hypothetical assisted gene-flow event (horizontal dashed lines, expected frequencies when resident and migrant flowering phenologies are matched; bars, expectations under observed phenological mismatch).*

arise if northern residents are delayed by insufficient chilling, causing them to flower after southern migrants.

Photoperiod is another common initiating cue in flowering plants, whereby flowering occurs after day lengths exceed or deceed a critical duration. A northward translocation can either accelerate or delay flowering, depending on whether the species' critical day length is longer or shorter than that of the vernal equinox. Because of the greater amplitude of the annual photoperiodic cycle at higher latitudes, the 11-hour day in spring occurs at later calendar dates going north, while the 13-hour day occurs earlier. Correspondingly, the degree of phenological mismatch among transplanted populations with photoperiodic requirements for reproduction depends on the latitudinal difference between the source and relocation sites.

Summer-flowering, short-day plants like *C. fasciculata* initiate reproduction after day length drops below a critical value. Experimental warming accelerated flowering in all four of the *C. fasciculata* populations (Wadgymar et al. 2015*a*; Fig. 2), but the relative timing was maintained. The NC population flowered later at the experimental site than it typically does at its home site, even when experimentally warmed (Wadgymar et al. 2015*a*, 2015*b*). This confirms that increased temperature did not compensate for the delay due to the northern photoperiod.

Finally, in some species, late-summer short days initiate the development of flower buds, which remain dormant until they are cued by winter chilling and then spring warming. Translocation across latitudes will decouple thermal and photoperiodic cues. If buds then resume development too early or too late, they could be exposed to desiccation, frost (Inouye 2008), or natural enemies (Elzinga et al. 2007).

Temperature and photoperiod affect the timing of life-history transitions across a wide range of taxa, which will complicate the latitudinal translocation of locally adapted populations (Bradshaw & Holzapfel 2008). Basic knowledge of the mechanisms controlling plasticity can provide qualitative predictions on phenological shifts upon translocation to a new latitude, but precise quantitative prediction must rely on experimentally measured parameters.

Phenological Mismatch, Selection, Rescue, and Replacement

Several conservation goals can be served by translocating genetic variants into resident populations. For instance, small populations in decline due to inbreeding depression can be genetically rescued by introducing migrants from similar populations, thereby reducing the load of deleterious recessive alleles (Frankham 2015). The goal of AGF, as defined by Aitken and Whitlock (2013), is to augment natural gene flow to introduce alleles that improve specific adaptive traits. The accelerated influx of adaptive alleles thus facilitates population persistence through evolutionary rescue (Gomulkiewicz & Shaw 2013). This approach can be expanded to what Kelly and Phillips (2016) call "targeted gene flow" between populations that would not otherwise exchange migrants. This practice could introduce alleles for disease resistance or disrupt dispersal in invasive species. Each these practices will be impeded if mating phenology differs between resident and translocated individuals.

The immediate impact of phenological mismatch is reduced hybridization, whereas the longer-term impact depends on the direction of local selection on migrant phenology. Late flowering was disfavored in our experiment, even when the growing season was extended by artificial warming (Wadgymar et al. 2015*a*, 2015*b*). Incoming adaptive alleles for thermal tolerance would likely have been in linkage disequilibrium with late phenology alleles. That is, heat tolerance and flowering time would be tightly and positively genetically correlated among populations, constraining joint selection for increased tolerance and early flowering. Under random mating, recombination could break down linkage disequilibrium in just a few generations, reducing the genetic correlation and thereby relaxing the constraint. However, assortative mating between residents and migrants caused by phenological mismatch restricts recombination and thus slows the decay of linkage disequilibrium (Gianola 1982) and prolongs this evolutionary constraint. Conversely, if selection favors both heat-tolerance and the late phenology (i.e., the trait combination in the migrant), assortative mating would prevent the breakup of the favored combination.

Thus, the long-term outcome of an AGF effort depends not only on the degree of phenological mismatch but also on local selection on the novel migrant phenotypes. If migrants are generally disfavored, assortative mating impedes genetic admixture and the resident population is little changed. Conversely, when migrants are highly favored, admixture is again impeded, but in this case the resident population gets replaced by the high-fitness migrant genotypes. This latter outcome would be counterproductive if the conservation goal is to maintain a valued local variety (Moritz 1994; Fraser & Bernatchez 2001). Conversely, this same process could facilitate the conservation of the genetic variety from the migrant's location. For instance, variants from the southern, trailing edge of a shifting climate envelope could be maintained when translocated northward when assortative mating limits the introgression of local, northern alleles into the favored southern genetic background.

One way to mitigate the impact of phenological mismatch on AGF is to create later-generation hybrids through captive breeding, perhaps by staggering planting dates or manipulating temperature or photoperiod and then releasing these hybrids into the declining resident population. Another measure that can be used with plants is to allow gene flow only through migrant pollen by translocating flowering migrants, allowing pollen transfer to residents, and then removing them. The seeds matured on the resident plants would then be a combination of purebred and F_1 genotypes. Some purely migrant genotypes will reappear from matings between hybrids, but there would be fewer of these genotypes than if the translocated migrants were allowed to set seed. However, if migrants are more likely than residents to produce hybrid seed, as in our age-dependent success scenario, this approach fails. Easier approaches include introducing a larger number of migrant individuals than are found in resident populations and maintaining sustained translocation over time. Finally, translocating multiple populations that can act as phenological stepping stones for genetic introgression between the populations with the most disparate phenologies is possible, although its success depends on the fitness of the migrants in the resident's location.

As our experiment indicates, assortative mating between residents and migrants impedes the introgression of adaptive migrant alleles into the resident population. The prevalence of phenological differences among populations suggests that AGF programs must deploy strategies to overcome reduced resident × migrant mating frequency.

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Supporting Information

Details of the mating-matrix method for estimating proportion of purebred, hybrid, and backcross matings (Appendix S1); geograhic and climatic data for the study sites (Appendix S2); logistic regression coefficients for exposure to allo-pollen as a function of date of flowering onset (Appendix S3); and predicted frequencies of purebred residents, the 3 purebred migrants, and all reciprocal F_1 genotypic classes produced in the first generation (Appendix S4) are available online. The authors are solely responsible for the content and functionality of these materials. Queries (other than the absence of material) should be directed to the corresponding author.

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