

ORIGINAL ARTICLE

Climate change alters reproductive isolation and potential gene flow in an annual plantSteven J. Franks¹ and Arthur E. Weis²¹ Department of Biological Sciences, Larkin Hall, Fordham University, Bronx, NY, USA² Department of Ecology and Evolutionary Biology; Koffler Scientific Reserve, University of Toronto, Toronto, ON, Canada**Keywords**

assortative mating, *Brassica rapa*, gene flow, global climate change, natural selection, phenology, population structure, rapid evolution.

Correspondence

Steven J. Franks, Department of Biological Sciences, Larkin Hall, Fordham University, 441 E. Fordham Road, Bronx, NY 10458, USA.

Tel.: 718 817 3609; fax: 718 817 3645;

e-mail: franks@fordham.edu

Received: 15 January 2009

Accepted: 2 April 2009

doi:10.1111/j.1752-4571.2009.00073.x

Abstract

Climate change will likely cause evolution due not only to selection but also to changes in reproductive isolation within and among populations. We examined the effects of a natural drought on the timing of flowering in two populations of *Brassica rapa* and the consequences for predicted reproductive isolation and potential gene flow. Seeds were collected before and after a 5-year drought in southern California from two populations varying in soil moisture. Lines derived from these seeds were raised in the greenhouse under wet and drought conditions. We found that the natural drought caused changes in reproductive timing and that the changes were greater for plants from the wet than from the dry site. This differential shift caused the populations to become more phenological similar, which should lead to less reproductive isolation and increased gene flow. We estimated a high level of assortative mating by flowering time, which potentially contributed to the rapid evolution of phenological traits following the drought. Estimates of assortative mating were higher for the wet site population, and assortative mating was reduced following the drought. This study shows that climate change can potentially alter gene flow and reproductive isolation within and among populations, strongly influencing evolution.

Introduction

Changes in many aspects of the climate, including temperature, patterns of precipitation and the frequency of climatic disturbances such as droughts, are occurring at a rapid rate and affecting many species (IPCC 2007). These changed conditions can cause evolution via natural selection. For example, Franks et al. (2007) showed that an extended drought in southern California selected for early flowering in the wild mustard plant *Brassica rapa*. While climate change can cause evolution via natural selection, as shown in this example, evolution can also occur by other processes such as drift and migration (Conner and Hartl 2004). As part of a series of projects investigating climate change and evolution, the purpose of this study is to investigate whether climate change can potentially cause evolution by two processes other than natural selection: assortative mating within populations and alterations in gene flow among populations. To do so, we focus on flowering phenology.

Flowering phenology, which is the timing and temporal pattern of flowering, encompasses a set of key life history traits with important effects on fitness (Burgess et al. 2007; Elzinga et al. 2007; Inouye 2008; Wilczek et al. 2009). Flowering phenology can also influence the degree of reproductive isolation within and among populations (Primack 1985; Rathcke and Lacey 1985; Fox 2003) because early flowering plants are likely to mate with other early flower plants, while late flowering plants are likely to mate with other late bloomers. Thus variation in flowering phenology is likely to lead to assortative mating, which occurs when individuals tend to mate with other individuals that are similar to them in some trait or set of traits (Hedrick 2000). Assortative mating causes evolution directly by increasing homozygosity and indirectly by expanding the additive genetic variance for the trait under assortment, which increases the potential response to selection (Wright 1921; Fisher 1958; Felsenstein 1981; Falconer and MacKay 1996). Thus when there is directional selection

on phenological traits, these traits could potentially show a rapid evolutionary response. However, it is not known exactly how much of an effect assortative mating has on the rate of evolution. Some models suggest that assortative mating may have only a small effect on response to selection (Walsh and Lynch 1998), while others predict a more substantial effect (Fox 2003). Despite the importance of assortative mating for evolution, the likelihood of assortative mating by flowering time, and the theoretical work on this topic (Fisher 1958; Felsenstein 1981; Fox 2003; Weis 2005), only one previous study (Weis and Kossler 2004) estimated assortative mating using full flowering schedule information.

While assortative mating can influence reproductive isolation within populations, variation in phenology can also affect isolation among populations by restricting gene flow (Primack 1985). For example, two populations that differ in flowering phenology would experience restricted gene flow because they are separated not only in space but also in time through phenological isolation. Thus if climate change alters phenology, this could potentially lead to changes in the amount of gene flow among populations.

Climate change is likely to alter phenology because the timing of life-history events is strongly linked to such climatic conditions as temperature and precipitation. Several studies have shown, for many species in various parts of the world, changes in plant phenology that appear to be due to recent climate change (Fitter and Fitter 2002; Peñuelas et al. 2002; Parmesan and Yohe 2003). The degree to which these phenological shifts represent plastic adjustments or evolutionary changes remains largely unknown (Parmesan 2006).

One rigorous way to determine if evolution has occurred in a natural population is to collect propagules before and after an environmental change and raise them under common conditions (Davis et al. 2005; Franks et al. 2008). This approach was used previously to show that *Brassica rapa* evolved drought escape through earlier flowering following a series of recent dry years in Southern California, and that early flowering results in higher fitness under drought conditions (Franks et al. 2007). A related study showed that multiple phenological traits and their interactions evolved in response to the drought (Franks and Weis 2008).

In this study, we use the same pre- and post-drought collection lines from the Franks et al. (2007) experiment to quantify the full pattern of flowering phenology in both populations before and after the drought. We used flowering information to estimate both the amount of assortative mating within and the degree of phenological isolation between two *B. rapa* populations.

Materials and methods

Study system

Brassica rapa (syn. *campestris*) L. (Brassicaceae) is a self-incompatible, weedy winter annual naturalized in California (Franks et al. 2007). The plants grow first as basal rosettes, then bolt and begin to produce perfect (male and female) flowers. Once plants begin to flower, one to many new flowers may open per day, with individual flowers lasting approximately 3 days and the production of flowers continuing throughout the growing season and over most of the life of the plant. The flowers are pollinated by bees, flies, and several other species of vagile insects. There were two study populations: Back Bay (BB), located in upper Newport Harbor, and Arboretum (Arb), located near the San Joaquin Marsh and adjacent to the University of California Irvine Arboretum. Both of the sites are in Orange County, California and located approximately 3 km apart. The Back Bay site has sandy soils that drain rapidly, which tends to make this a dry area, while the Arboretum has soils higher in clay content and high water table, making this generally a more wet area (Franke et al. 2006).

In our study area, there was above average precipitation over the 5 years leading up to 1997 and below average precipitation during the 5 years preceding 2004 (Franks et al. 2007). The period prior to 2004 thus represents an extended drought, in which overall precipitation was reduced and the growing season length was shortened compared with preceding years. We collected seeds from plants in the field at the same sites and in the same locations before and after the drought. Seeds were collected from 400 to 1800 plants per site along transects at the Arboretum and Back Bay sites in May 1997 and June 2004.

Experimental design

To control environmental conditions and pollinations and to reduce maternal and storage effects, we grew the field collected seeds for a full generation in the UC Irvine greenhouse. Plants were watered daily and fertilized weekly using balanced liquid fertilizer. The plants were assigned to 1997 x 1997 (97) and 2004 x 2004 (04) crossing groups within each population, and assigned pollinations between pairs of randomly chosen plants within each group were done by hand for 100 plants per group. Seed was collected from the plants as the pods ripened. These seeds were the F_1 s that were then used in the experiment. By growing the plants for a full generation in the greenhouse, we reduced the influence of maternal effects on flowering time traits. Germination rates were >94% for both populations and collection years in the

original seed collection, and there was no difference in germination rate or seed size in the F_1 seeds between populations or generations (Franks et al. 2007). Thus there was no evidence that seed storage caused a biased sample or had any influence on flowering time. The plants have a seed bank in natural populations, but the fact that we previously found that a drought led to an evolutionary change in flowering time (Franks et al. 2007) indicates that the seed bank did not prevent the population from responding to recent episodes of selection.

F_1 seeds were planted in the greenhouse, following stratification for 5 days at 4°C on moist filter paper, under the same conditions as the parents. Plants were grown 6 per pot in 25 cm pots, with each pot containing at least one of each combination of population (Arb and BB) and cross (1997, 2004). Pots were assigned either a short season (drought simulated) or long season treatment in order to determine the direct effects of drought on flowering phenology and to verify that changes in estimated assortative mating and phenological isolation were due to the drought. The watering treatment was applied at the level of the pot in a randomized block design, with pot considered the experimental unit for all statistical analyses involving watering treatment. The watering treatments were short season (watered daily to saturation for 33 days) and long season (watered 81 days). There were 100 plants from each population by generation by treatment combination, for a total of 800 plants. Almost all seeds germinated, and the few that failed to germinate were replaced by seedlings that we grew in separate extra pots. We checked all plants daily and recorded germination and date of first flowering. Every 3 days, we hand pollinated all plants (both to obtain fitness estimates based on seed weight for other studies and because lack of pollination alters reproductive allocation) and also counted all open flowers on all plants to obtain the complete flowering schedules.

Statistical analyses

For organisms where mating events can be observed, levels of assortative mating can be determined directly. But for outcrossing plants, the pollen donor is generally not known in natural populations without paternity analysis, which is impractical for populations containing numerous individuals (Weis 2005). In this case, levels of assortative mating can be estimated indirectly by regressing the flowering time of mothers against the flowering time of potential fathers, as determined by flowering schedules (Weis and Kossler 2004). This prospective analysis of assortative mating is appropriate for outcrossing plants and was used in our study.

To calculate assortative mating by flowering time, we determined the time of first flowering of all plants as the number of days between germination and the opening of the first flower. Assortative mating is the correlation between mates (Falconer and MacKay 1996), so the level of assortative mating by flowering time is the correlation between maternal and paternal flowering time. Because *B. rapa* flowers are perfect, all plants are both mothers and fathers. The maternal flowering time for each plant is known, but the paternal flowering time in this obligately outcrossing species must be estimated. For each maternal plant, we estimated paternal flowering time as the average flowering time of all potential fathers (all of the other plants in the experimental population) weighted by the number of shared maternal and paternal open flowers, following the analyses given in Weis and Kossler (2004) and further developed in Weis (2005). The level of assortative mating is then determined by regressing average paternal flowering time over maternal flowering time for each individual (Weis and Kossler 2004; Weis 2005). The degree of assortative mating, ρ (rho), is estimated by the regression coefficient, and varies between zero (random mating) and one (completely assortative mating). Because average paternal flowering time is calculated from all potential fathers in the population for each mother, the same data are used multiple times. We therefore used bootstrapping in SAS (version 9.1, SAS, Cary, NC) to generate standard errors. We used bootstrapping by pairs with replacement, which is appropriate for this type of data in which maternal and paternal individuals were statistically paired together, and used 1000 bootstrap iterations. Because of the nature of this data, it is not possible to compare among groups (such as generations or treatments) using parametric tests. Standard errors and confidence intervals can be used in this case as a guide to determining significance, although caution should be used with this approach (Payton et al. 2003).

Like the estimate of assortative mating, we determined potential reproductive isolation and gene flow also by using information on flowering schedules. We used the method of Elzinga and Bernasconi in Elzinga et al. (2007), which takes advantage of the full flowering schedule information and is appropriate for population comparisons (Elzinga et al. 2007). With this method, the index of phenological isolation between two populations, I , is based on the proportion of overlap in flowering over time for all of the individuals in the two populations. Essentially, I is the relative amount of non-overlap in the total flower schedules for the two populations. The index is zero for complete overlap in population flowering schedules and one for complete isolation. This analysis was conducted separately for the 1997 and 2004 collection years. 95% confidence intervals around I were determined using a bootstrapping procedure in R (version 2.3.1) with

replacement and with 1000 iterations. Using the overlap in the number of open flowers between populations is an appropriate measure of phenological reproductive isolation in this context because each open flower indicates a potential mating opportunity, and each time two flowers are open in two different populations, this represents a potential gene flow event.

Results

Reproductive isolation within populations

Our estimate of assortative mating by time of first flowering was positive within both populations for both collection years, with levels of assortative mating (ρ) significantly (based on bootstrapped standard errors) greater than zero for all groups (Fig. 1). Mating therefore appears to be highly non-random within all populations.

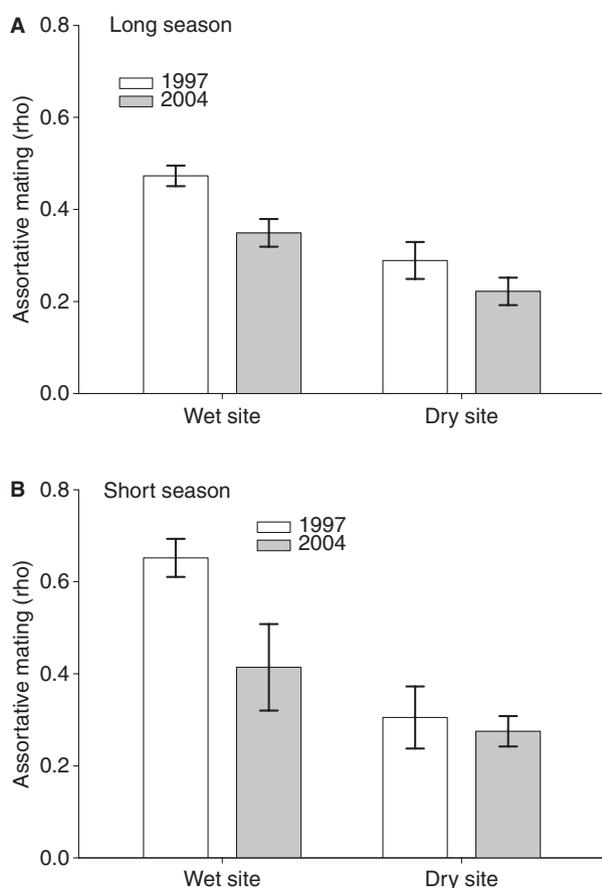


Figure 1 Prospective estimates of assortative mating (ρ) by time of first flowering ± 1 bootstrapped standard error for *Brassica rapa* plants grown in the greenhouse under the long (A) and short (B) season treatments. Plants are from the Arboretum (wet site) and Back Bay (dry site) populations and from the 1997 (white bar) and 2004 (gray bar) lines. $\rho = 0$ indicates random mating, while positive values indicate a correlation in flowering time among mates.

The average estimated level of assortative mating was 0.37. Estimated levels of assortative mating were significantly lower for the post-drought (2004) lines than the pre-drought (1997) lines (Fig. 1), indicating a decrease in assortative mating following the drought. Estimated assortative mating was also lower in the dry (BB) than in the wet site (Arb) population and lower in the long than in the short season treatment (Fig. 1).

To examine causes of variation in assortative mating, we compared variance in flowering time among groups, based on the hypothesis that increased flowering time variance could potentially be linked with increased assortative mating. Using an *F*-test of variance equality (Sokal and Rohlf 1995), we found that variance in flowering time was significantly greater for the Arboretum than for the Back Bay population ($F_{790,890} = 3.8$, $P < 0.0001$) and significantly greater for the 1997 than for the 2004 lines ($F_{541,577} = 2.0$, $P < 0.0001$). The difference in the amount of assortative mating among groups was therefore related to differences in the amount of variation in flowering time.

Reproductive isolation among populations

To examine reproductive isolation and potential gene flow among populations, we calculated the index of phenological isolation *I*, which is zero for complete overlap in population flowering schedules and one for complete isolation (Elzinga et al. 2007). We calculated *I* between the Arboretum and Back Bay populations for the 1997 and 2004 lines and the long and short season watering treatments (Fig. 2). Isolation indices were generally significantly greater than zero, indicating restrictions in potential gene flow. The isolation indices were $I = 0.33$ (0.24, 0.40) for the 1997 lines, long season treatment; 0.14 (0.08, 0.20) for the 2004 lines, long season treatment; 0.16 (0.09, 0.22) for the 1997 lines, short season treatment and 0.06 (−0.01, 0.11) for the 2004 lines, short season treatment (values in parentheses are 95% bootstrapped confidence intervals). Phenological isolation was lower in the 2004 lines than in the 1997 lines for both the long season and short season. Thus, based on flowering times, the Arboretum and Back Bay populations were less phenologically isolated following the drought. In addition, phenological isolation was lower under short season (drought simulated) than under long season treatments. Total flowering time was more compressed under the short season treatments (Fig. 2), which decreased the amount of phenological isolation between the two populations.

Discussion

Our greenhouse study showed that a change in climate altered flowering phenology in the annual plant *Brassica*

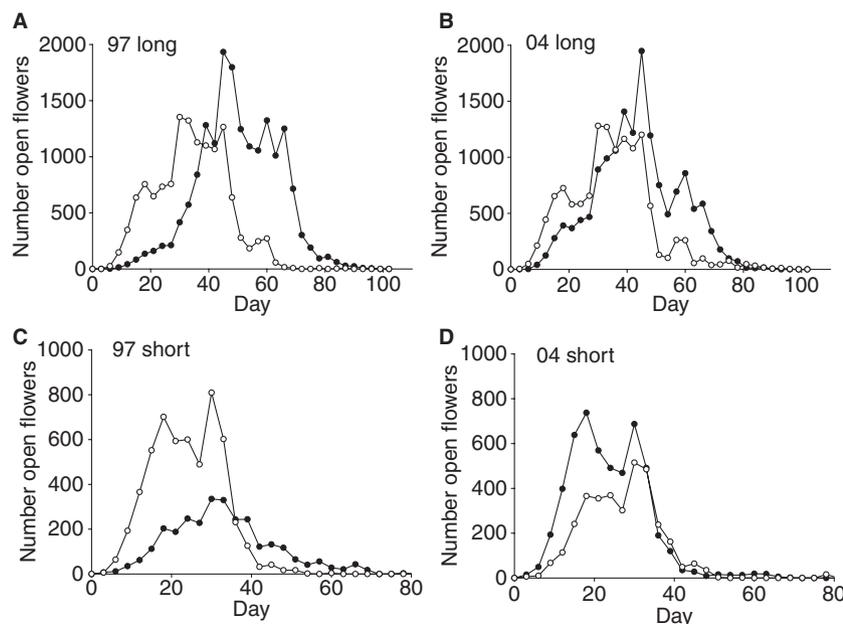


Figure 2 The degree of phenological isolation among *Brassica rapa* populations. Shown are population total flowering schedules, which are the total number of open flowers for all plants from a given population, grown under greenhouse conditions, over time. White circles show the dry site (BB) population and black circles show the wet site (Arb) population. The index of phenological isolation (I) is the relative area of non-overlap between two population flowering schedules, and varies from zero for complete overlap and one for complete isolation. Note the difference in scale between the long and short season treatments.

rapa, which in turn changed the estimated amounts of reproductive isolation both within and among populations. The high levels of estimated assortative mating within populations we found may have contributed to the rapid evolution of early flowering time following a natural drought seen in a previous study (Franks et al. 2007). Evolutionary changes in flowering phenology also altered estimated amounts of reproductive isolation and potential gene flow among populations. This study thus provides evidence that changes in climatic conditions can alter genetic structure and influence the rate of adaptive evolution in natural populations.

Within populations, we estimated substantial levels of assortative mating, with ρ ranging from 0.22 to 0.65 ($\rho = 0$ with random mating and 1 for completely assortative mating). This amount of assortative mating is comparable to that found in Weis and Kossler (2004), which is the one previous study to prospectively estimate assortative mating based on flowering phenology. Weis and Kossler (2004) found a ρ of 0.43 in an open pollinated, experimental population of the same species as this study (*B. rapa*). In this and our study, there was positive assortative mating by flowering time, clearly indicating a departure from random mating.

Non-random mating can substantially influence evolution (Lynch and Walsh 1998). Specifically, assortative mating can speed the response to selection by expanding

the phenotypic variance of the trait under assortment (Felsenstein 1981; Walsh and Lynch 1998; Fox 2003). In our study, we estimated a high level of assortative mating by flowering time in *B. rapa*. In a previous study with this species, we demonstrated a strong evolutionary response in flowering phenology to a change in climate, with a natural drought leading to an evolutionary shift to earlier flowering time after just a few generations (Franks et al. 2007). This rapid evolutionary response may have been due both to direct selection for earlier flowering as well as to the combination of selection on and assortative mating by this trait. Thus assortative mating by flowering time is likely a contributing cause of rapid evolution in flowering phenology in response to selection.

Assortative mating can not only influence the rate of evolution but can also change as a result of selection on flowering phenology. We found that a natural drought led to earlier flowering and reduced the amount of assortative mating by flowering time, with ρ lower in the post-drought than in the pre-drought lines for both populations and under both watering treatments (Fig. 1). This makes sense since a compression of flowering schedules should lower assortative mating because of a reduction in variation in flowering time. This reduction in assortative mating could slow further responses to selection.

This study showed a change in the estimated amount of assortative mating with a change in climate and also

provides some insight into the factors influencing assortative mating by comparing pre-drought and post-drought lines, wet site and dry site populations, and long season and short season treatments. Our estimate of assortative mating was greater in the wet site than the dry site and greater in the pre-drought than in the post drought lines. This indicates that assortative mating should be greater under wet than under drought conditions, especially if wet conditions cause greater variation in flowering time. But surprisingly, assortative mating tended to be higher under the short than the long season treatment. This pattern was mainly driven by the high degree of assortative mating in the wet site, pre-drought lines grown under the short season treatment. This population would have been the least well adapted to the drought. In this population, some individuals flowered very late, which may have indicated drought stress. Other plants, especially those from the dry site, were able to avoid the drought by flowering early. The late flowering of the potentially drought stressed plants may have led to the especially high degree of assortative mating in this group. In general, the amount of assortative mating was more directly related to the amount of variation in flowering time than to particular environmental conditions.

There was evidence in this study not only of reproductive isolation within populations due to assortative mating but also of reproductive isolation among populations due to phenological separation. We found that the two *B. rapa* populations differed in flowering time, which resulted in isolation indices greater than zero and indicates that these two populations were separated in mating opportunities not only in space but also in time due to variation in flowering phenology. Variation in the timing of flowering thus restricts potential gene flow among populations. While isolation due to variation in phenology is expected, there has been little work documenting the degree of isolation due to the timing of flowering (Fox 2003; Antonovics 2006; Elzinga et al. 2007).

Like assortative mating within populations, phenological isolation among populations can both influence and be influenced by selection on the timing of flowering. While assortative mating can speed the response to selection, reductions in gene flow can either slow adaptive evolution by promoting drift or can increase the likelihood that populations will become locally adapted (Conner and Hartl 2004). We previously found evidence for local adaptation in this system, with plants from the dry site flowering earlier than those from the wet site when both were grown under common conditions and plants from the dry site having higher fitness under drought conditions than plants from the wet site (Franks et al. 2007). Restricted gene flow due to phenological isolation among these populations could

potentially have contributed to this degree of local adaptation.

Although the populations in our study were potentially isolated due to differences in phenology, the drought actually reduced differences in flowering time and thus decreased phenological isolation, increasing potential gene flow, between them. The index of phenological isolation, I , in the post-drought descendants was half of what it was in the pre-drought ancestors. The fact that the drought treatment reduced I provides additional evidence that drought itself, rather than other factors occurring between 1997 and 2004, was the mechanism for reducing isolation. One reason for this is that the drought caused earlier flowering in both populations, but this change in flowering time was greater in the Arboretum than in the Back Bay population. This differential effect was expected, since the Back Bay is a drier site, so the plants there should already be more closely adapted to drought-like conditions (Franke et al. 2006). Additional changes in the duration of flowering and the shapes of the flowering schedule curves (Franks and Weis 2008) also contributed to reducing the phenological separation between the populations.

The fact that the drought reduced phenological separation between the populations has important implications for evolution. The reduction in isolation could lead to increased gene flow between the populations. This increased gene flow could expedite the spread of drought-adapted, earlier flowering alleles from the dry site to the wet site, which could then accelerate the rate of adaptive evolution for drought escape in the wet site following drought. Alternatively, an increase in gene flow could potentially reduce the ability of the populations to become locally adapted.

Previous studies have used genetic markers and male-sterile plants to estimate pollen movement in *Brassica* crop plants. One study found that although most pollen movement was fairly local, around 13% of seeds were produced from pollen from plants >1 km away, and 1% from plants >3 km away (Devaux et al. 2005). It is also possible that some of the plants whose pollen sources could not be identified (12%) could represent very long distance dispersal (Devaux et al. 2007). Furthermore, only a very small amount of gene flow is needed to maintain genetic coherence among populations (Hedrick 2000). There is thus the potential for at least some gene flow between the two sites in our study, which are separated by about 3 km. The type of change in phenology following a change in climatic conditions that occurred in our sites and that decreased phenological isolation could also potentially increase gene flow between crops and their wild relatives or between genetically modified plants and natural conspecifics. There is also now growing evidence

that changes in climate can directly affect plant-pollinator interactions, further influencing ecological interactions and evolutionary change (Hegland et al. 2009).

It is important to note that we based our estimates of assortative mating and phenological isolation on the flowering phenology of plants in a greenhouse population. Actual levels of assortative mating within and gene flow among natural populations could differ due to factors such as pollinator behavior and differential pollen success. However, flowering phenology should strongly influence the degree of reproductive isolation. A plant cannot be pollinated by another plant that has not yet come into flower by the time the first plant has finished flowering, so flowering phenology sets absolute limits on potential reproductive opportunities. Similarly, the more two plants share open flowers over time, the more likely it is for them to mate. Furthermore, our use of phenological information allowed us to address the main objective in this study, which was to determine if climatically driven changes in phenology could lead to evolution via changes in reproductive isolation. Additional work examining the effects of changes in climate on assortative mating within populations and gene flow among populations using genetic markers would augment this work and be greatly valuable.

In conclusion, we provide evidence that climate change can influence evolution in at least three ways. We previously demonstrated that climate change can act as an agent of natural selection, leading to evolutionary changes in traits affected by climate (Franks et al. 2007; Franks and Weis 2008). Here we show that climate change can alter plant phenology, which can change patterns of assortative mating within populations. This assortative mating can directly change genotype frequencies and can also increase the rate of evolution by interacting with selection. We further show that climatically driven changes in phenology can potentially influence gene flow among populations due to changes in overlap in flowering schedules. These changes in gene flow can also influence both the rate and pattern of evolutionary change. This study indicates that flowering phenology is a highly unique and important trait because it can change in response to direct selection, influence the rate of response to selection by causing assortative mating, and then further change the degree of assortative mating with populations and the amount of gene flow among populations when there is selection on phenology. The high degree of interdependence of flowering time, assortative mating, selection and gene flow make predicting evolutionary responses to changes in climate particularly complex and challenging. Further work focusing on flowering phenology is thus likely to provide key insights into the effects of climate change on evolution.

Acknowledgements

We thank K. Afshar, V. Chandrasekaran, A. Dick, A. Franks, L. Gonzalez, C. Herman, L. Hua, E. Ko, T. Kossler, P. Le, K. Musser, A. Ng, M. Ngugen, A. Ogura, P. Rath, S. Sim, K. Torosian, P. Tran, W. Yang, A. M. Weis, and E. Weiss for greenhouse work. D. Franke made the 1997 seed collections. This research was supported by NSF grants DEB-0345030 and DEB-0440595 to A. Weis and a Fordham University Faculty Research Grant to S. Franks.

Literature cited

- Antonovics, J. 2006. Evolution in closely adjacent plant populations X: long-term persistence of prereproductive isolation at a mine boundary. *Heredity* **97**:33–37.
- Burgess, K. S., J. R. Etterson, and L. F. Galloway. 2007. Artificial selection shifts flowering phenology and other correlated traits in an autotetraploid herb. *Heredity* **99**:641–648.
- Conner, J. K., and D. L. Hartl. 2004. *A Primer of Ecological Genetics*. Sinauer, Sunderland, MA.
- Davis, M. B., R. G. Shaw, and J. R. Etterson. 2005. Evolutionary responses to changing climate. *Ecology* **86**:1704–1714.
- Devaux, C., C. Lavigne, H. Falentin-Guyomarc'h, S. Vautrin, J. Lecomte, and E. K. Klein. 2005. High diversity of oilseed rape pollen clouds over an agro-ecosystem indicates long-distance dispersal. *Molecular Ecology* **14**:2269–2280.
- Devaux, C., C. Lavigne, F. Austerlitz, and E. K. Klein. 2007. Modeling and estimating pollen movement in oilseed rape (*Brassica napus*) at the landscape scale using genetic markers. *Molecular Ecology* **16**:487–499.
- Elzinga, J. A., A. Atlan, B. Arjen, L. Gigord, A. E. Weis, and G. Bernasconi. 2007. Time after time: flowering phenology and biotic interactions. *Trends in Ecology and Evolution* **22**:432–439.
- Falconer, D. S., and T. F. C. MacKay. 1996. *Quantitative Genetics*. Prentice Hall, Harlow.
- Felsenstein, J. 1981. Continuous-genotype models and assortative mating. *Theoretical Population Biology* **19**:341–357.
- Fisher, R. A. 1958. *The Genetical Theory of Natural Selection*. Dover Publications, New York.
- Fitter, A. H., and R. S. R. Fitter. 2002. Rapid changes in flowering time in British plants. *Science* **296**:1689–1691.
- Fox, G. A. 2003. Assortative mating and plant phenology: evolutionary and practical consequences. *Evolutionary Ecology Research* **5**:1–18.
- Franke, D. M., A. G. Ellis, M. Dharjwa, M. Freshwater, M. Fujikawa, A. Padron, and A. E. Weis. 2006. A steep cline in flowering time for *Brassica rapa* in southern California. I: Population-level variation in the field and the greenhouse. *International Journal of Plant Sciences* **167**:83–92.
- Franks, S. J., and A. E. Weis. 2008. A change in climate causes rapid evolution of multiple life-history traits and their

- interactions in an annual plant. *Journal of Evolutionary Biology* **21**:1321–1334.
- Franks, S. J., S. Sim, and A. E. Weis. 2007. Rapid evolution of flowering time by an annual plant in response to a climate fluctuation. *Proceedings of the National Academy of Sciences of the United States of America* **104**:1278–1282.
- Franks, S. J., J. C. Avise, W. E. Bradshaw, J. K. Conner, J. R. Etterson, S. J. Mazer, R. G. Shaw *et al.* 2008. The resurrection initiative: storing ancestral genotypes to capture evolution in action. *Bioscience* **58**:870–873.
- Hedrick, P. 2000. *Genetics of Populations*, 2nd edn. Jones and Bartlett Publishers, Boston.
- Hegland, S. J., A. Nielsen, A. Lazaro, A. L. Bjerknes, and O. Totland. 2009. How does climate warming affect plant-pollinator interactions? *Ecology Letters* **12**:184–195.
- Inouye, D. W. 2008. Effects of climate change on phenology, frost damage, and floral abundance of montane wildflowers. *Ecology* **89**:353–362.
- Intergovernmental Panel on Climate Change [IPCC]. 2007. *Climate change 2007: The physical science basis. Summary for policy makers. Contribution of working group I to the fourth assessment report of the Intergovernmental Panel on Climate Change.* (<http://www.ipcc.ch>).
- Lynch, M., and J. B. Walsh. 1998. *Genetics and Analysis of Quantitative Traits*. Sinauer, Sunderland, MA.
- Parmesan, C. 2006. Ecological and evolutionary responses to recent climate change. *Annual Reviews in Ecology and Systematics* **37**:637–669.
- Parmesan, C., and G. Yohe. 2003. A globally coherent fingerprint of climate change impacts across natural systems. *Nature* **421**:37–42.
- Payton, M. E., M. H. Greenstone, and N. Schenker. 2003. Overlapping confidence intervals or standard error intervals: what do they mean in terms of statistical significance? *Journal of Insect Science* **3**:34.
- Peñuelas, J., I. Filella, and P. Comas. 2002. Changed plant and animal life cycles from 1952 to 2000 in the Mediterranean region. *Global Change Biology* **8**:531–544.
- Primack, R. B. 1985. Patterns of flowering phenology in communities, populations, individuals and single flowers. In J. White, ed. *The Population Structure of Vegetation*, pp. 571–593. Kluwer, Dordrecht.
- Rathcke, B., and E. P. Lacey. 1985. Phenological patterns of terrestrial plants. *Annual Reviews in Ecology and Systematics* **16**:179–214.
- Sokal, R. R., and F. J. Rohlf. 1995. *Biometry*. Freeman, New York.
- Walsh, J. B., and M. Lynch. 1998. *Genetics and Analysis of Quantitative Traits II*. Sinauer Press, Sunderland, MA.
- Weis, A. E. 2005. Direct and indirect assortative mating: a multivariate approach to plant flowering schedules. *Journal of Evolutionary Biology* **18**:536–546.
- Weis, A. E., and T. M. Kossler. 2004. Genetic variation in flowering time induces phenological assortative mating: quantitative genetic methods applied to *Brassica rapa*. *American Journal of Botany* **91**:825–836.
- Wilczek, A. M., J. L. Roe, M. C. Knapp, M. D. Cooper, C. Lopez-Gallejo, L. J. Martin, C. D. Muir *et al.* 2009. Effects of genetic perturbation on seasonal life history plasticity. *Science* **232**:930–934.
- Wright, S. 1921. Systems of mating. *Genetics* **6**:111–178.