

Geographic patterns and pollination ecotypes in *Claytonia virginica*

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Geographical variation in pollinators visiting a plant can produce plant populations adapted to local pollinator environments. We documented two markedly different pollinator climates for the spring ephemeral wildflower *Claytonia virginica*: in more northern populations, the pollen-specialist bee *Andrena erigeniae* dominated, but in more southern populations, *A. erigeniae* visited rarely and the bee-fly *Bombylius major* dominated. Plants in the northern populations experienced faster pollen depletion than plants in southern populations. We also measured divergent pollen-related plant traits; plants in northern populations produced relatively more pollen per flower and anther dehiscence was more staggered than plants in southern populations. These plant traits might function to increase pollen dispersal via the different pollen vectors.

KEY WORDS: Fly pollination, native bees, pollen depletion, pollinator-mediated selection, pollen presentation.

It is widely agreed that floral traits are, to a certain extent, the result of selection by pollinators (e.g., Miller 1981; Galen 1989; Campbell et al. 1996, 1997; Caruso 2000; Castellanos et al. 2004; Muchhala and Thomson 2009). Biotically pollinated flowers are commonly visited by variable assemblages of animals with varying abundances (Waser et al. 1996); this recognition has led to research on how variation in pollinators, including variation in pollinator abundance, influence selection on floral traits. The diversity and abundance of pollinators of a plant species at a given time and location make up the “pollinator climate” (Grant and Grant 1965). “Pollination ecotypes” identify plant populations that are morphologically distinct from other conspecific populations due to local adaptation to the pollinator climate (Robertson and Wyatt 1990; Johnson 1997; Johnson and Steiner 1997; Johnson 2006; Anderson et al. 2010), much the same way that ecotypes in general result from persistent differences in the abiotic or biotic environment that could select for phenotypic change.

Morphological characters and foraging motivation affect how pollinators interact with pollen grains, and therefore their role as pollinators (Harder and Thomson 1989; Thorp 2000; Thomson

and Goodell 2001). In contrast to animals that primarily collect nectar and interact incidentally with pollen grains, pollen foraging female bees often actively collect and redistribute pollen, especially by grooming it from their bodies as they fly and sequestering it in pollen transport structures (“corbiculae” or “scopae”) to be transported and fed to larvae. This pollen may be “lost from the system” and may not contribute to plant pollination (Thomson 1986; de Jong and Klinkhamer 1994; but see Parker et al. 2015).

Plant investment in pollen production can have diminishing returns for plant male fitness (Harder and Thomson 1989; Feinsinger and Tiebout 1991; Galen 1992; Richards et al. 2009). The limitation occurs in part because of ineffective pollen dispersal, which is augmented with greater pollen removal. Although all pollinators can produce diminishing returns (Lloyd 1984), female bees that actively collect pollen likely diminish fitness returns on plant male investment more than pollinators that collect nectar only because large deposits of pollen trigger bee grooming (Harder and Thomson 1989; Castellanos et al. 2006; Richards et al. 2009). Harder and Thomson (1989) demonstrated diminishing returns in pollen production for *Erythronium grandiflorum* visited by *Bombus occidentalis*; they showed that higher pollen availability in plant anthers increased pollen removal, increased grooming, and ultimately decreased the proportion of pollen that bumble bees delivered to stigmas.

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Plants can mediate the rate of diminishing returns by manipulating of the schedule of pollen presentation by which grains are made available to pollinators (Thomson and Barrett 1981; Lloyd and Yates 1982; Harder and Thomson 1989). Pollen presentation can be manipulated by dividing pollen into doses that are gradually made available through dispensing mechanisms; one example is the packaging of pollen into anther locules that sequentially dehisce (Percival 1955; Lloyd and Yates 1982; Lloyd 1984; Harder and Thomson 1989; Thomson et al. 2000; Castellanos et al. 2006; Li et al. 2014). Changes in the pollen presentation schedule allow plants some control over the number of visitors that remove and disperse pollen from a flower without affecting resource allocation to other plant functions; a flower that dehisces anthers sequentially can increase the number of floral visitors that export pollen and therefore moderate the diminishing returns of the male gain curve.

Selection on the schedule of pollen presentation might vary adaptively with the pollinator climate (Thomson and Thomson 1992; Li et al. 2014). More staggered pollen dispensing (e.g., anther locules opening slowly) should increase pollen dispersal when plants are visited by female bees that actively collect pollen and those visits are relatively frequent. More simultaneous pollen presentation (e.g., anther locules opening more quickly or simultaneously) should increase pollen dispersal when plants are visited only rarely by pollinators that actively collect pollen or visited by nectar-collecting pollinators. Models show that when pollinators cause diminishing returns to male fitness, selection favors staggered dispensing of pollen (LeBuhn and Holsinger 1998). In a comparative study of pollen presentation schedules in *Penstemon* species, Castellanos et al. (2006) found that bee-pollinated plant species staggered pollen dispensing more than hummingbird-pollinated plants; the authors linked this pattern to differences in the grooming behavior of the pollinator that dominated visitor communities. Similarly, Li et al. (2014) described variable anther dehiscence in three closely related *Epimedium* species that may be the result of selection by the pollinator climate.

The schedule of pollen presentation affects how a given number of pollen grains are distributed to pollinators (Thomson and Barrett 1981; Lloyd and Yates 1982; Harder and Thomson 1989), but plant investment in male function—specifically, the resources allocated to pollen production—will affect the total number of pollen grains to be distributed (Charlesworth and Charlesworth 1991; Campbell 1998). Therefore, pollen production may also vary with the pollinator climate. Theory and empirical research on the effect of pollen production on pollen dispersal is lacking, so it less clear how plant populations might respond to variation in the pollinator climate through ecotypic variation relating to pollen production; this relationship is complex and will depend on a variety of characteristics of the pollinator climate, including polli-

nator behavior and the details of the interactions between pollinators and pollen (e.g., Harder and Thomson 1989; Feinsinger and Tiebout 1991; Galen 1992; Richards et al. 2009). Even though the benefits to male fitness diminish with higher production (Harder and Thomson 1989; Feinsinger and Tiebout 1991; Galen 1992; Richards et al. 2009), higher pollen production should still increase pollen dispersal marginally in all pollinator climates, and this marginal increase in pollen dispersal may be more important when flowers are visited by active pollen-collectors because they will transfer pollen less efficiently (Thomson and Barrett 1981; Lloyd and Yates 1982; Harder and Thomson 1989). Correspondingly, the marginal increase in pollen production may not be as important to plants visited by nectar collectors because more of their pollen will be more efficiently delivered. Therefore, we expect higher pollen production when active pollen-collectors dominate visitation.

Here, we examine divergent pollen-related plant traits—the schedule of pollen presentation and total pollen production—of the spring wildflower *Claytonia virginica* that correspond to differences in pollinator climates involving the abundance of an active pollen-collector (a pollen-specialist bee) and a primarily nectar-collecting fly. We predict that *C. virginica* populations will form pollination ecotypes with divergent pollen-related plant traits. The pollination ecotype of plant populations with high visitation by the pollen-specialist bee will have relatively more staggered pollen dispensing. In contrast, the pollination ecotype of populations visited rarely by the pollen-specialist bee and more commonly by the nectar-collecting fly will have more synchronous pollen dispensing. Predictions related to total pollen production are less clear, but we expect that the pollination ecotype of plant populations whose visitor communities are dominated by the pollen-specialist bee will have higher pollen production, and the pollination ecotype of plant populations visited rarely by the bee and more often by the fly will have lower pollen production. This study will begin to fill a gap in research on how pollen production affects pollen dispersal.

Materials and Methods

STUDY SPECIES

Claytonia virginica L. (Portulacaceae) is a self-compatible, perennial wildflower common in the woodland understory of the eastern United States. It flowers in the early spring and continues flowering for 2–4 weeks, with the flowers on a raceme opening one or two at a time during the flowering period. Flowers are protandrous. Anthers dehisce to offer pollen on the first day, and the stigma opens and becomes receptive on the second day. Flowers produce nectar in both the male- and female-phase. Flowers are regular, open, and bowl-shaped, contain five anthers with two locules per anther, and produce six ovules. The corolla is usually

white or light pink, with pink or red nectar guides. Flowers open early or mid-morning and close in the early or late afternoon, depending on temperature and humidity (A. Parker and N. M. Williams, unpubl. data).

A variety of insect species visit *C. virginica* flowers. Predominant among them is the pollen-specialist bee *Andrena erigeniae*, which collects pollen primarily from *C. virginica* and the closely related *C. caroliniana* (Davis and LaBerge 1975). *Andrena erigeniae* females collect both nectar and pollen from *C. virginica*, but the males of the species collect only nectar. A second common visitor is the generalist bee fly *Bombylius major*, which hovers over flowers sipping nectar with its long proboscis. A diversity of other floral visitors also visit less frequently, including generalist bees in the genera *Lasioglossum*, *Ceratina*, and *Hylaeus*, cleptoparasitic bees in the genera *Nomada* and *Sphecodes*, a few flies, and the occasional butterfly (Schemske 1977; Motten et al. 1981). Parker et al. (2016) demonstrated that *A. erigeniae* remove more pollen (61% per single visit) than *B. major* (23.7%) and other bees (20.31%) in this system (Parker et al. 2016).

STUDY SITES

We conducted this study over two seasons (2009 and 2010) on *C. virginica* populations in two general study regions: northern populations in Pennsylvania [four sites] and Maryland [one site], and southern populations in North Carolina [three sites]; *online supplement*). We collected data only on clear or lightly overcast days with a temperature of at least 15°C. We recorded temperature and wind speed every five minutes using a Kestrel 4500 weather meter (Loftopia LLC, Birmingham, Michigan).

VISITATION

To compare the visitation rates of flower visitors to *C. virginica*, we identified visitors to sets of flowers in sets of six 5-minute observation periods at each site. Observations were spread across the site, and when possible, we conducted 90 minutes of observations throughout the period of insect activity, from mid-morning until early or late afternoon. This approach allowed us to obtain enough observations overall, to collect observations spanning the main period of insect activity, to maintain observer attention, and accomplish other tasks. Prior to each 5-minute observation period, we selected a new set of flowers that we could observe simultaneously, marked them using a hula hoop or embroidery hoop (depending on floral density), and recorded the number of male- and female-phase flowers. During the observation period, we identified each visitor and counted the number of visits by that visitor by to male- and female-phase flowers. We used knowledge from multiple years of collecting (Williams and Winfree 2013) to identify *A. erigeniae* to species and sex, *Bombylius major* to species, generalist bees in the genera *Lasioglossum*, *Ceratina*, and *Hylaeus* to a group we called “small generalist bees,” cleptopar-

asitic bees in the genus *Nomada* to genus, and other visitors to species, genus, or morphological group to the best of our abilities.

POLLEN DEPLETION

To compare the rates of pollen depletion among *C. virginica* populations, we assessed the amount of pollen available in individual flowers (an estimate of the pollen standing crop) during the period of insect activity, from mid-morning until early or late afternoon. Each hour, we arbitrarily chose eight flowers scattered throughout the local plant population and collected the anthers from each flower into a microcentrifuge tube containing 1 mL of 70% ethanol. We chose flowers regardless of the number of dehisced anthers. We began anther collection each morning when the flowers' anthers began to dehisce, usually between 8:45 and 10:00, but occasionally as late as 13:15 because of cool or wet weather. We concluded anther collection when insect visitation ceased or flowers began to close, between 13:00 and 15:00.

We counted the number of pollen grains available in each flower sample using a Coulter Multisizer 3 particle counter (Beckman Coulter Inc., Brea, California). Prior to counting, we suspended pollen in 20 mL 0.9% saline. To obtain a representative count of the number of pollen grains in each flower, we took the average of four 1 mL subsamples and multiplied the mean by the total volume of the sample. These counts included the number of pollen grains in undehisced anthers because undehisced anthers opened and emptied during the counting process.

POLLEN PRESENTATION AND PRODUCTION

To estimate the pollen presentation schedule in each population, we tracked anther dehiscence in ten male-phase flowers on each day of data collection. Prior to first anther dehiscence, we arbitrarily chose ten fresh male-phase flowers and marked them with dental floss and a label. Every 30 minutes, we recorded the temperature and then returned to these flowers and recorded the number of locules dehisced (two locules per anther for a total of ten locules). We concluded tracking each flower when all ten locules had dehisced or, if the ten locules did not dehisce completely, when insect activity ceased.

We also estimated total pollen production during each day of data collection. We covered a sample of male-phase flowers with fine mesh cages prior to anther dehiscence to prevent insect visitation. After insect visitation ended for the day, we collected 5–15 samples of the unvisited flowers to estimate the total number of pollen grains. Because we collected these flowers at the end of each day, these counts exclude pollen that may have passively fallen from anthers.

DATA ANALYSIS

Analyses include data from six populations in Pennsylvania, one population in Maryland, and three in North Carolina; however, the

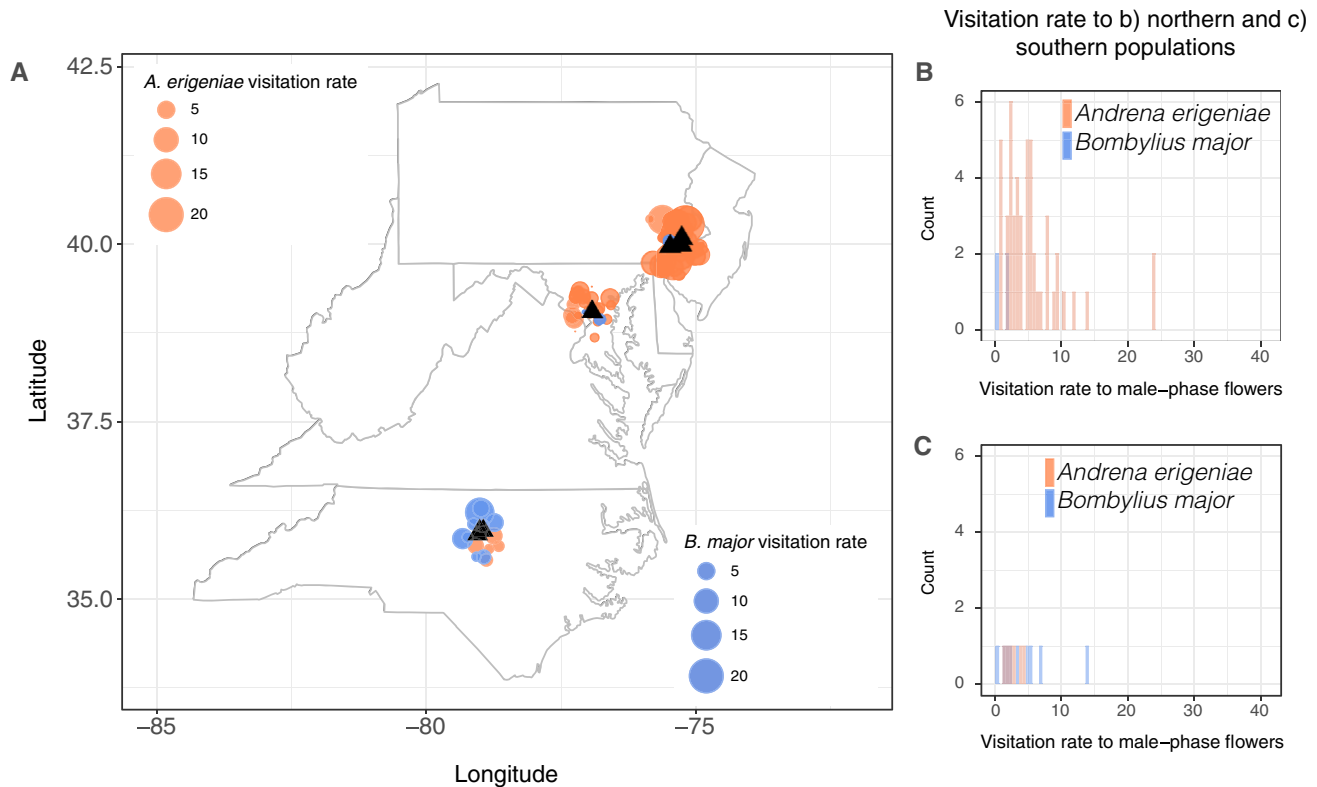


Figure 1. In northern populations, dominance by the pollen specialist bee *A. erigeniae* defines the pollination climate. In southern populations, low visitation by *A. erigeniae*, higher visitation by the bee fly *B. major*, and lower visitation overall define the pollinator climate. (A) Visitation rate by *A. erigeniae* (orange) and *B. major* (blue) to *C. virginica* in Northern populations and southern populations. Each circle represents one observation period and the size of the circles corresponds to the visitation rate; so that they may be viewed, the circles are offset (jittered) around the observation sites in both the x and y direction to 40% of the resolution of the data. Triangles represent the sites where observations were conducted, which are not offset. (B) Histogram showing the visitation rate by *A. erigeniae* (orange) and *B. major* (blue) in northern populations. Counts of zero visits were not included. (C) Histogram showing the visitation rate by *A. erigeniae* (orange) and *B. major* (blue) in southern populations. Counts of zero visits were not included.

majority of data come from Lankanau Hospital in Pennsylvania, Patuxent National Wildlife Refuge in Maryland, and Mason Farm Biological Reserve in North Carolina (Table S1). Visitation observations indicated two markedly different pollinator climates; all sites in Pennsylvania and Maryland had visitor communities dominated by *A. erigeniae*, and most sites in North Carolina had visitation dominated by *B. major* (for more details on the pollinator climate, see Results). Because of this clear difference, we pooled data with similar pollinator climates into two geographic regions that could exhibit ecotypic variation at a broad enough scale, “northern” and “southern.” The explanatory variable of interest in each of the models is this latitudinal contrast (Fig. 1). All data were analyzed in R 3.0.1 (R Core Team 2013).

To determine whether the geographic region was a significant predictor of the rate of pollen depletion, we used a negative binomial model (function *glm.nb* in the library *MASS*) with number of pollen grains in a flower as the response variable. The predictor variables were time since first anther dehiscence, ge-

ographic region, and their interaction. We used log likelihood ratios to determine if the interaction term improved model fit. Pollen presentation schedules were compared among geographic regions in a survival analysis modeling time to dehiscence (in minutes) of all ten locules by a Weibull distribution. The Weibull distribution is an extension of the exponential distribution that allows the rate at which an event occurs to increase or decrease with time (Bolker 2008); in the present analysis, the event of interest is the dehiscence of all locules. It is described by parameters shape, a , and scale, s . The mean of the Weibull distribution is calculated as $s\Gamma(1 + 1/a)$ (Bolker 2008). Exploratory plotting following Bolker (Fig. 8.5 in Bolker 2008) indicated that scale, but not shape, varied by geographic region. We therefore fit a survival model with a single shape parameter for both regions, and a scale parameter that differed by region (g , 0 = southern, 1 = northern) and with the mean temperature (t , mean-centered across all flowers for analysis) experienced during the dehiscence period:

$$T \sim \text{Weibull}(a, s(g, t))$$

$$s(g, t) = \alpha + \beta \times g + (\gamma + \delta \times g)t$$

where T = time to dehiscence, α = the intercept value for the southern population, β = a factor effect for the northern populations, γ = a linear effect of temperature in the southern populations, and δ = an interaction between temperature and geographic region for the northern populations.

We used maximum likelihood estimation in R package *bbmle* (Bolker and R Development Core Team 2016) to identify the best-fit values of a , α , β , γ , and δ . Because we did not know the precise time at which full dehiscence occurred (we knew only that it occurred between the last and second-to-last observations of a given flower), we calculated probabilities of dehiscence between these two time points when fitting the model. Complete dehiscence was not observed for 22 flowers (see Methods). Of these, we retained 14 flowers for which dehiscence of more than five locules (i.e., more than half of complete dehiscence) were observed; these were included as right-censored data in the survival analysis. Progressively simplified models were compared by Likelihood Ratio Tests; the best fit model was the most complex model (Table S2).

We compared pollen production between regions using the number of grains in an unvisited flower as the response variable and the geographic region as a predictor variable. Because pollen production might vary through the flowering season, we included the date of collection as a random effect. To include both random and fixed effects and to account for overdispersion through a negative binomial distribution, we used the R function *glmmADMB* in the library *glmmADMB* (Fournier et al. 2012).

Results

Visitation observations indicated two markedly different pollinator climates; a northern pollinator climate dominated by *A. erigeniae* and a southern pollinator climate with lower visitation overall, but higher visitation by *B. major* (Table S1, Fig. 1). Northern *C. virginica* male-phase flowers received 2.17 ± 3.91 (mean \pm SD) visits per observation period by *A. erigeniae* females but southern male-phase flowers received 0.25 ± 0.87 visits per observation period by *A. erigeniae* females (Fig. 1). Northern *C. virginica* female-phase flowers received 1.12 ± 2.51 (mean \pm SD) visits per observation period by *A. erigeniae* females but southern female-phase flowers received 0.24 ± 0.91 visits per observation period by *A. erigeniae* females, meaning *A. erigeniae* females visited northern male-phase flowers almost twice as often as northern female-phase flowers. Northern male-phase flowers received 0.04 ± 0.25 visits per observation period by *B. major* but southern male-phase flowers received 0.56 ± 2.02 visits per observation period by *B. major* (Fig. 1). Northern female-phase

flowers received 0.03 ± 0.23 visits by *B. major* per observation period but southern female-phase flowers received 0.57 ± 2.06 visits per observation period by *B. major*.

Other visitors visited rarely in both populations. Northern male-phase flowers received 0.26 ± 0.99 visits per observation period by small generalist bees and 0.03 ± 0.19 visits per observation period from bees in the genus *Nomada*. Southern male-phase flowers received 0.11 ± 0.51 visits by small generalist bees and no visits from bees in the genus *Nomada*. Visits by other visitors were very rare.

Pollen was depleted more quickly in bee-dominated northern populations than in southern populations (Fig. 2), as indicated by the significant geographic region \times time interaction since first anther dehiscence interaction (GLMM, $Z = 4.712$, $P < 0.001$) on the number of pollen grains in a flower (Table S3). We observed no autocorrelation structure in the average of the residuals over time in each time series (northern and southern), which justifies assumed temporal independence in the model.

Anther dehiscence was more staggered in populations in the bee-dominated northern populations than in southern populations (Fig. 3). The complete dehiscence of all ten locules took more time in northern populations (142 minutes predicted at the average temperature of 19.2°C) than in southern populations (101 minutes predicted at 19.2°C, Fig. 3, Table S3). Anther dehiscence was also more responsive to temperature in bee-dominated northern populations than in southern populations (Fig. 3, Table S3). In northern populations, for example, a 3°C increase in temperature to 22.2°C accelerated anther dehiscence by more than 30 minutes (mean = 105 minutes), but the same change in temperature instead tended to slow dehiscence slightly in southern populations (mean = 112 minutes; note that the effect of temperature on dehiscence rate in southern populations is not statistically significant: Table S3). The shape parameter of the fitted Weibull model was significantly greater than one (mean (95%CI) = 2.5 (2.1–2.9)), indicating that the longer a flower goes without fully dehiscing, the more the rate of dehiscence increases.

Pollen production differed between populations. In the model describing *C. virginica* pollen production, there was a significant effect of the geographic region on the number of pollen grains in an unvisited flower. The number of pollen grains in an unvisited flower was significantly higher in flowers from bee-dominated northern populations (2941 ± 916) than flowers in southern populations (2330 ± 861 , Table S3, GLMM, $Z = -2.31$, $P = 0.021$).

Discussion

The pollinator climate for *C. virginica* populations differed markedly between northern and southern geographic regions, driven by striking differences in two functionally very distinct pollinators: *Andrea erigeniae*, an active pollen-collector with the

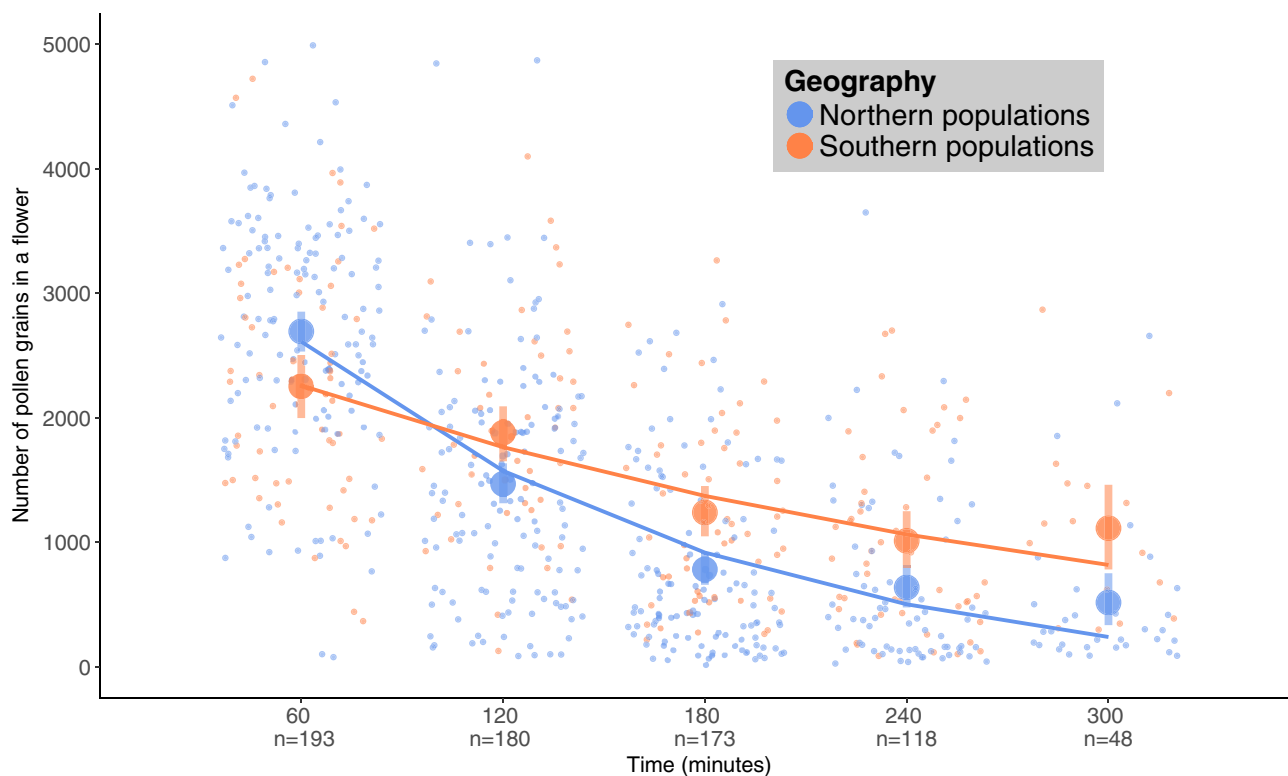


Figure 2. Pollen depletion in northern (blue) and southern (orange) populations. Pollen depletes faster in northern *C. virginica* populations than southern populations. Each point represents the number of pollen grains in one sample male-phase flower. The curves display fitted lines for each geographic region from the final negative binomial generalized linear model. There was a highly significant interaction between the time since first anther dehiscence and the geographic region (GLMM, $Z = 4.712$, $P < 0.001$).

potential to rapidly deplete pollen from flowers, and *B. major*, a nectar-collector, that only weakly interacted with pollen during flower visits. These differences appear to have selected for divergent pollen-related plant traits by *C. virginica* between geographic regions. Whereas other studies have documented strong morphological responses of flowers to pollinator-mediated selection (e.g., Miller 1981; Robertson and Wyatt 1990; Johnson and Steiner 1997; Boyd 2004; Castilla et al. 2015), examples of changes to pollen-related plant traits and reproductive phenology are rare (Castellanos et al. 2004; Castellanos et al. 2006; Li et al. 2014).

Flowers in all study populations are morphologically very similar including in male morphology. All flowers (with very few exceptions) had five anthers, each with two locules, for a total of ten pollen packages. However, the schedule of pollen dispensing and total pollen production differed substantially (Fig. 3). In bee-dominated northern populations, *C. virginica* populations had more staggered dispensing and higher overall pollen production, and in southern populations, *C. virginica* populations had more synchronous dispensing and produced less pollen overall. These changes in plant traits may be the result of selection mediated by the abundance of *A. erigeniae*; in bee-dominated northern pop-

ulations, staggered dehiscence may restrict *A. erigeniae* females to collect less pollen per visit, groom less, and deliver a higher proportion of removed pollen to receptive stigmas. In addition, higher overall pollen production may mitigate the loss of pollen to bee nests. In southern populations, rare visitation by *A. erigeniae* may allow for more simultaneous pollen dehiscence.

These observations on pollen presentation schedules correspond with theory. Harder and Thomson (1989) showed that pollen packaging can be adaptive for pollen dispersal when pollinators cause diminishing returns in pollen production, as they likely do in bee-dominated northern populations. Likewise, LeBuhn and Holsinger (1998) measured strong selection for pollen packaging and weak selection on the schedule of pollen dispensing when pollinators exhibited diminishing returns.

Despite striking inter-region differences, there was surprisingly low variation in pollinator climate among populations within a region. For example, the number of visits by *A. erigeniae* to male-phase flowers in two of the northern sites (Lankanau hospital in Pennsylvania and Patuxent National Wildlife Refuge in Maryland) was 2.33 ± 5.06 and 1.60 ± 1.77 visits per observation period, respectively, and the number of visits by *A. erigeniae* to male-phase flowers in two of the southern sites (Mason Farm and

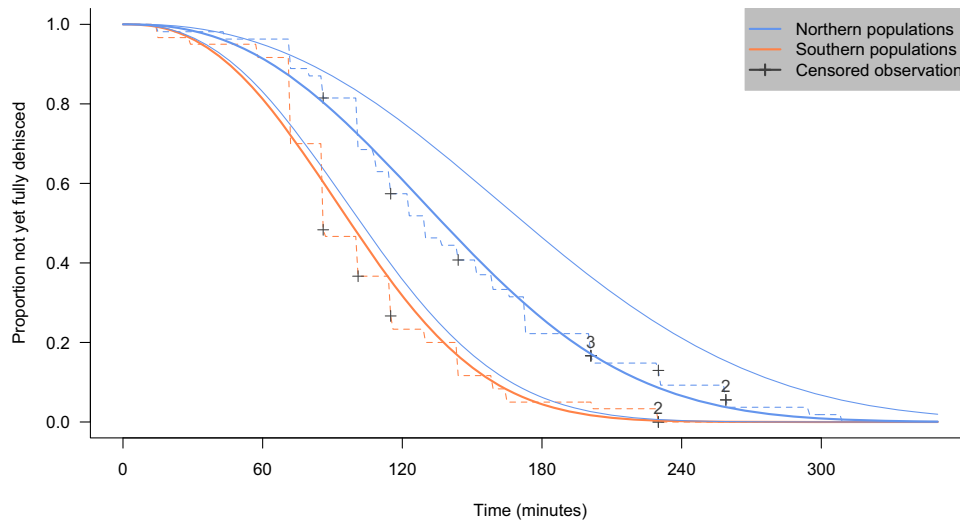


Figure 3. Complete dehiscence of anthers took longer in northern populations (blue) than in southern populations (orange). Dashed lines depict raw observations of proportion of flowers not yet fully dehiscent as a function of time since onset of flower dehiscence; for plotting, full dehiscence was assumed to occur at the midpoint between the last and second-to-last flower observations for uncensored data, and at the time of censoring for right-censored data. Where more than one observation is censored at a given time point, the number censored is provided near the censor marker. Solid lines depict Weibull distributions fit by survival analysis. Within the trio of solid lines, the middle line is the predicted distribution of flower dehiscence times for Northern populations at the average observed temperature (19.3°C), the upper and lower fine lines are the predicted distributions at -3°C and $+3^{\circ}\text{C}$ from the average, respectively; both regions regularly experienced this temperature range. For clarity, the nonstatistically significant effect of temperature in southern populations is not depicted here.

a site located off of Martin Luther King Jr. road, both in North Carolina) was 0.19 ± 0.86 and 0.42 ± 1.06 visits per observation period, respectively (Table S1). These patterns of visitation within and among regions are matched by differences in pollen-related plant traits with the main differences occurring between Northern and Southern regions, but similar pollen-related plant traits within each region.

There also is evidence that relatively high visitation by *B. major* that we documented in southern populations is consistent over time. Motten et al. (1981) documented high visitation of *B. major* to *C. virginica* in North Carolina 28 years before our data were collected; he estimated that *B. major* visited each *C. virginica* flower on average 0.60 visits per hour, a rate that is very similar to our rate of 0.56 visits per hour. Moreover, Parker (2014) documented a latitudinal gradient in *B. major* visitation of *C. virginica* across the plant's range, with higher visitation in Southern populations.

Because of *A. erigeniae*'s intimate and intense interactions with *C. virginica* pollen, it seems most likely that the pollination ecotypes presented here are driven by the changes in *A. erigeniae* abundance. We documented faster depletion in the bee-dominated northern populations than in southern populations. Parker et al. (2016) demonstrated that *A. erigeniae* remove more pollen (61% per single visit) than *B. major* (23.7%) in this system (Parker et al. 2016), and simulation models have demonstrated that these empir-

ical removal rates by *A. erigeniae* would deplete pollen faster than *B. major* (Parker 2014). In fact, simple bookkeeping shows that if a male-phase *C. virginica* flower containing 2764 pollen grains (the mean pollen production, as measured by Parker et al. 2016) is visited by an *A. erigeniae* female removing 61% each time, the number of pollen grains could deplete to just 420 grains after just two visits (similar to the visitation rate of *A. erigeniae* females in northern populations). Two visits by *B. major* could deplete a male-phase *C. virginica* flower less substantially, to just 1566 grains. Although not definitive, this evidence supports the conclusion that *A. erigeniae* causes faster pollen depletion, as we saw in the northern bee-dominated populations (Parker et al. 2016). Moreover, the mean-variance relations of the number of pollen grains remaining in a flower between the two geographic regions are similar, which also suggests a single pollinator species dominating pollen dispersal. However, we were not able to examine that possibility directly with these data because in many of our sites, we saw either *A. erigeniae* or *B. major*, but not both. Ultimately, differences in the anther dehiscence and pollen production could be driven by changes in the abundance of *A. erigeniae*, changes in the abundance of *B. major*, overall changes in the visitation rate by all floral visitors, or some combination of factors that are indistinguishable here. For example, the timing of pollinator visits will influence depletion rate, and Parker et al. (2016) documented *A. erigeniae* visiting more often in early morning, but *B. major*

visiting more consistently when *C. virginica* flowers were open (in Pennsylvania populations). Future work should take a more mechanistic approach to this question.

The other floral visitors in this system—small generalist bees, bees in the genus *Nomada*, and other rare visitors—may have an additional contribution to selection on pollen-related plant traits, but we did not include them in the results because we did not detect notable differences in these groups between geographic regions. Therefore, in this case they probably do not contribute significantly to distinct pollinator climates. Bees in the “small generalist bee” category—genera *Lasioglossum* and *Ceratina*—may also contribute to pollen depletion because they also collect and redistribute pollen, groom pollen from their bodies as they fly and sequester it (in scopae, or—interestingly in *Hylaeus*—internally). Bees in the genus *Nomada* are likely to act more like *B. major* and have high pollen transfer efficiency because, as cleptoparasites, they do not collect pollen for offspring.

This study is an initial investigation into a new kind of pollination ecotype, in which pollen-related plant traits—schedule of pollen dispensing and total pollen production—vary with the geography of the pollinator climate. These results provide some insight into evolutionary processes and the implications of consistent geographic differences in pollinator diversity and abundance. We should continue to examine the prevalence and importance of pollination ecotypes, including their role in local adaptation and reproductive isolation. The results presented here can be further clarified by looking at other factors, because numerous biotic and abiotic variables can also result in trait differences between geographic regions generally and the sites where we worked specifically. These variables may include climatic variables, other ecological interactions, and variation in the land use of the surrounding area. Moreover, each of the plant traits measured is likely to also be affected by plant characteristics not measured here, such as plant or flower size. Finally, future work should examine how pollinators' interactions with pollen grains impact pollination and reproductive success, how differences in pollinator climates is correlated with pollen-related plant traits, and how selection acts on pollen-related plant traits mediated by different pollinators and pollinator climates.

AUTHOR CONTRIBUTION

AJP, JDT, and NMW contributed to study concept and design. AJP conducted data collection and data analysis and interpretation. AJP drafted the article with critical revision by AJP, JDT, and NMW. AJP, JDT, and NMW approved the version to be published.

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DATA ARCHIVING

Data is archived at <https://doi.org/10.5061/dryad.8nj84>.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Table S1. Summary of observation periods, visitation rates, samples collected, and flowers observed in each geographic region.

Table S2: Comparison of survival analysis models for time to complete dehiscence by Likelihood Ratio Tests.

Table S3. Summary of model results.