Floral Traits and Pollination of *Solidago altissima*: Mechanisms of Local Adaptation Among and Within Biomes

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Abstract

Solidago altissima is an herbaceous, clonal plant that has differentiated into two subspecies in the prairie and forest biomes in Minnesota. The mechanisms that have driven the divergent evolution of these subspecies are not well understood. The evolution of floral traits is influenced by the trade-off between sexual and asexual reproduction. Floral traits can evolve rapidly, and this often occurs in response to interactions with pollinators. I found differences in floral traits between plants from the two biomes, and these differences strongly affected pollinator abundance. Forest plants allocate proportionally more resources to flowering than to vegetative reproduction via rhizomes compared to plants in the prairie. I hypothesize that there is stronger competition among plants for resources in the prairie, where selection favors greater allocation of resources to vegetative reproduction.

I also tested the hypotheses that pollinator abundance is influenced by differences among plant genotypes and the genotypes of neighboring plants. I conducted an experiment and found that the number of pollinators on a plant was influenced by the genotype of a plant, but not by the genetic diversity of neighboring plants. I also found that the abundance of neighboring flowers affected pollinator abundance. Plant genotypes varied in floral size, flowering time, and nectar quantity. Floral size of the individual stem had the strongest effect on pollinator abundance. The variation in floral traits among genotypes may be a result of selection to optimize the tradeoff between vegetative growth and flowering, which can vary spatially and temporally.

Table of Contents

List of Tables

List of Figures

Introduction

Local adaptation is one of the classic hypotheses on the maintenance of polymorphism and is an important agent of natural selection (Williams 1966). Populations can become locally adapted because environmental conditions vary across space; the world is not uniform, and selection pressures vary across environments. This variation can occur on a large scale spanning biomes or on a small scale among populations separated by as little as ten meters (Craig & Itami 2011, Lenssen et al 2004, Anderson & Johnson 2007, Bischoff et al 2006). Studying the forces of local adaptation may give us an idea of the drivers of speciation, and may offer a snapshot of the divergence of one species and the evolution of new species. However, not all populations that become locally adapted are on a trajectory that leads to speciation, as gene flow or temporal variation in selection may maintain a dynamic equilibrium among locally adapted populations. Nevertheless, the effects of biological diversity at the species level or below can be seen throughout trophic levels, including in the relationships of herbivores and natural enemies that interact with the plant (Craig & Itami 2011).

Populations are locally adapted if they 1) have higher fitness in their environment than in other environments, or 2) have higher fitness in their environments than populations from other environments (Kawecki & Ebert 2004). Local adaptation has been demonstrated in many plant species, as well as the insects they interact with including herbivores and pollinators. For instance, the gallmaker *Eurosta solidaginis* is locally adapted to *Solidago altissima* subspecies from prairie and forest biomes (Craig & Itami 2011), and a geographic mosaic of coevolution is seen between a species of long-tongued fly and the flower it pollinates (Anderson & Johnson 2008).

There are many selective pressures that can lead to local adaptation in plants, including both abiotic and biotic factors. For instance, plant populations have adapted to differences in water availability and temperature (Hall & Willis 2006), competition (Prati & Schmid 2000), pollination (Johnson & Steiner 1997), and herbivory (Sork et al 1993). To learn which pressures are driving the divergent selection, researchers must isolate one variable at a time. If the traits associated with the isolated variable cause an increase in fitness in the specified 'home' habitat, but not in other habitats, then the variable may be driving local adaptation (Kawecki & Ebert 2004). Plants may also evolve in response to many selective pressures at once, and this often occurs when one abiotic factor influences many aspects of the environment (Kawecki & Ebert 2004). For instance, dry habitats may also have different pollinator communities than moist habitats, and evolution can occur in response to both pressures simultaneously. Therefore, identifying mechanisms of local adaptation can be difficult, requiring isolating and testing many potential selective pressures.

Not all traits are optimized for a single selection factor. Plants face a fundamental tradeoff in resource allocation among growth, reproduction, and survival (Bazzaz et al 1987). Selective pressures that favor increased resource allocation to one function, such as reproduction, reduce the amount of resources available for other functions and cause the evolution of those traits in response (Prati & Schmid 2000). For example, floral traits of the clonal species *Ranunculus reptans* evolved in response to below-ground competitive interactions (Prati & Schmid 2000). When populations were located in a competitive environment they produced fewer flowers than populations in competition-free environment. Flower number and vegetative growth were negatively correlated,

indicating that floral traits evolved as a tradeoff with vegetative growth in the competitive habitat, and that increased vegetative growth resulted in a fitness advantage despite smaller flowers.

Pollinators often drive the evolution of floral traits because they are involved in a process that is directly related to fitness: pollination. The ability of a flower to attract pollinators is under strong selection in plants that are pollen limited, when the number of viable seeds depends on the amount of successful pollination. Both physical and chemical characteristics, such as flower shape and nectar rewards, are important for attracting pollinators. Orchids and irises have evolved deep spurs where the nectar is stored in response to the proboscis length of their mutualist pollinators (Johnson & Steiner 1997, Goldblatt et al 1995). Similarly, generalist pollinators like bumble bees tend to favor larger corolla size (Galen 1996). Pollinator visitation is often associated with nectar production, both in the quantity of nectar produced and in sugar or amino acid content (Mitchell 1993, Sinva & Dean 2000, Hodges 1995, Rabinowitch et al 1993).

The genotypic diversity of neighboring plants may influence the selection of plant traits and local adaptation. Several recent studies have demonstrated that genotypic diversity can influence plant growth and the structure of higher trophic communities (Crutsinger et al 2006, Utsumi et al 2011). For instance, genotypic diversity increased above-ground net primary productivity of goldenrod (Crutsinger et al 2006), as well as the populations of a specialist herbivore, aphid *Uroleucon nigrotuberculatum*. Goldenrod genotypic diversity also increases pollinator abundance (Genung et al 2010). However, the mechanism that causes increased pollinator abundance in diverse plots is unclear, as there are multiple hypotheses of phenomenon that may affect pollinator behavior. The

first hypothesis suggests that pollinator abundance is influenced indirectly through plant growth via below-ground interactions. Below-ground interactions may increase plant growth through niche complementarity, a phenomenon in which different genotypes utilize slightly different niches and are able to extract more available resources from an area than plants of the same genotype that utilize identical niches (Kahmen et al 2006). The second hypothesis proposes that pollinators may be affected directly by the diversity of nectar rewards. If different genotypes have slightly different tissue or nectar composition, genotypically diverse areas may provide insects with a more nutritionally complete food source than single-genotype areas and lead to increased pollinator visitation (Ghazoul 2006). Therefore, such increases in pollinators in diverse plots could be beneficial to plants with diverse neighbors.

Solidago altissima as a model system

Solidago altissima (Asteraceae), tall goldenrod, is a clonal herbaceous species that is widely distributed throughout North America. In Minnesota, it has differentiated into subspecies across the prairie and forest biomes along a gradual cline in temperature and water availability (Semple & Cook 2006, Tester 1995). The prairie is warmer and drier than the forest, and the prairie soil consists of nutrient-rich organic matter, in contrast to the rocky clay soil in the forest (Tester 1995). *Solidago altissima altissima* inhabits the forest biome in northeastern Minnesota, and *Solidago altissima gilvocanescens* is found in western and southwestern Minnesota. The subspecies are morphologically distinct

(Semple & Cook 2006, Craig & Itami 2011), with the prairie subspecies being generally smaller in size than the forest subspecies.

Solidago altissima reproduces both sexually with showy floral displays and vegetatively by rhizomes. An individual stem can produce up to 20 rhizomes (Werner et al 1980). Rhizomes are produced in the fall and remain dormant throughout the winter, after which each rhizome emerges the following spring as a new stem, or ramet. In the forest subspecies, genets consist of dense clusters of many genetically identical ramets, and a single genet can consist of over one hundred ramets and be several meters in diameter (Semple & Cook 2006, Werner et al 1980).

The forest subspecies is typically found in mechanically disturbed sites like old fields and roadsides. They are often the dominant species, and can remain so for up to several decades (Werner et al 1980). These sites are often in mid-succession, where woody species that are more competitive eventually out-compete the goldenrod. The prairie subspecies is found in tallgrass prairie habitat, where disturbance by fire plays a large role in shaping the plant communities. Historically, fires occurred as a result of lightning strikes or American Indians, and happened once to several times per decade (Higgins 1986, as cited in Tester 1995, Weaver 1968). Fires destroy aboveground biomass, but belowground biomass like roots and rhizomes are maintained. Fires also stimulate microbial activity by reducing the litter layer and increasing the temperature of the soil, making nutrients more available for plant use. This stimulates the growth of rhizomatous grasses, but perpetually prevents succession of larger woody species. Goldenrod in prairie habitats is highly intermixed with these grasses, as genets do

monopolize resources to form dense stands as the forest subspecies does in recently disturbed sites (pers obs).

In Minnesota, *S. altissima* begins flowering in mid-late August and continues through early October. Each floral display consists of a few hundred to more than 2,000 florets, and each floret can contain up to 12 flowers (Werner et al 1980). Within a floret, 1-3 bisexual disc florets are surrounded by several female ray florets. Maturation of male and female structures is both spatially and temporally separated, which prevents self-pollination. *S. altissima* is an obligate outcrosser; self-pollination prevents pollen from other genotypes from reaching the ovules and reduces fitness. It is also pollen-limited and therefore the number of viable seeds depends on the amount of successful pollination.

Insects are the primary pollinators of goldenrod, as its pollen is too heavy to be carried by the wind. Members of the families Apidae, Syrphidae, and Vespidae are the most common pollinators, but members of many other families pollinate goldenrod in fewer numbers, including Meliodia, Lycidae, and Cantharidae. Seeds are very small and are attached to a white pappus that aids in wind dispersal. Recruitment by seed is very low, and many seeds remain dormant in the seed bank for several years (Meyer & Schmid 1999, Walck et al 1998).

Solidago altissima includes three cytotypes: diploid ($2n$=18), tetraploid ($2n$=36), and hexaploid ($2n$=54) (Halverson et al 2008). In the forest subspecies, both hexaploids and diploids are common throughout the state, with the exception of northeastern Minnesota where the populations are primarily hexaploid (Halverson et al 2008). The

prairie subspecies consists primarily of tetraploids, which are rarely seen in the forest subspecies (Halverson et al 2008). Research has suggested that plant size, flower size, and herbivore preference are related to cytotype, but that cytotype distribution among different habitat types may be the primary cause of variation in these factors (Halverson et al 2008, Richardson & Hanks 2011).

There are over 100 herbivores that feed on *Solidago* species (Messina 1978, Messina and Root 1980), including many specialist gall-makers and phloem tappers. Adaptation of the ball gall fly, *Eurosta solidaginis*, to the subspecies of *S. altissima* has been extensively researched, as well as in the parasitoids of *Eurosta* in the next trophic level (Craig and Itami 2011, Abrahamson and Weis 1997).

Several studies have looked at the effects of genotypic diversity on the goldenrod community. Genotypic diversity has a non-additive effect on above-ground net primary productivity (Crutsinger 2006) and can structure insect communities. For example, aphid density and their natural enemies increased in response to genotypic diversity (Utsumi et al 2011), as well as flower number and pollinator abundance (Genung et al 2010).

I studied populations of *Solidago altissima* to determine if they were locally adapted and to identify mechanisms driving local adaptation. I asked whether pollinators were important selection factors in this system, and what maintained variation for flower morphology among and within populations.

Chapter 1. Divergent selection on floral traits of clonal plant *Solidago altissima* in prairie and forest biomes

Many plant species have widespread distributions that span more than one biome, and populations can undergo local adaptation in response to geographic variation. In Minnesota, subspecies of *Solidago altissima* have become locally adapted to the prairie and forest biomes and have evolved several morphological and genetic differences. This study identified possible mechanisms of local adaptation in floral traits involved in pollinator interactions. I also tested the hypothesis that evolution of floral traits could be influenced as a by-product of an adaptation to another function. I found that floral morphology varied among biomes and these differences strongly affected pollinator visitation. Mean timing of flowering did not differ among biomes, but the duration of flowering was significantly lower in prairie plants. Forest plants invested proportionally more resources in flowering than prairie plants which strongly affected the ability to attract pollinators. This suggests that pollination is not the dominant driving mechanism of local adaptation in these subspecies but that variation in floral traits may be a by-product of adaptation to vegetative growth.

Introduction

Many plant species have widespread distributions that span more than one biome, and as a result populations of one species may experience selection to adapt to very different environmental conditions. Minnesota has forest and prairie biomes, and there are major differences in temperature, water availability, and soil quality between them (Tester 1995). Biotic interactions including pollination, herbivory, and competition, may also vary among biomes, and this may be in conjunction with abiotic variation (Craig et al. 2007; Smith 1970). Populations can undergo local adaptation as the result of divergent selection in response to this geographic variation, and as a result they may ultimately become genetically and morphologically distinct populations (Craig et al. 2007; Kawecki & Ebert 2004). In Minnesota, *Solidago altissima* has differentiated into subspecies across the prairie/forest biome boundary and they have morphological differences that may indicate local adaptation. The specific pressures driving this divergent selection remain unclear.

When populations become locally adapted, genetic changes can occur for many traits simultaneously (Franks & Weis 2008). However, not all genetically-based phenotypic changes are the result of local adaptation. For a trait to be adaptive, it must provide a fitness benefit under local environmental conditions. Some traits may be indicating an adaptation to another function; they evolve because of the trade-off among functions but are not adaptive traits (Kawecki & Ebert 2004). For instance, plants in theoretical models evolved to allocate more resources to growth than to reproduction in competitive environments (Bornhofen & Lattaud 2006). The flowers evolved a smaller

size as a by-product of the adaptation to competition because plants were allocating more to obtaining resources for growth.

Local adaptation often occurs in floral traits because there is a direct link between flowering and fitness (Galen 1996; Johnson & Steiner 1997; Hall & Willis 2006). Floral traits such as the size and timing of flowering often evolve in response to abiotic factors like water and nutrient availability, competition, and the length of the growing season. Flowering is a large resource investment, and the ability of a plant to accumulate sufficient resources for flowering may influence the evolution of floral traits. For example, differences in the length of the growing season led to divergent selection for the size and timing of flowering in coastal and mountainous populations of *Mimulus guttatus* (Hall & Willis 2006).

Pollinators can also drive the evolution of floral traits, including the size and appearance of the flower itself, timing of flowering, and nectar composition (Harder & Barrett 1996, Blionis et al. 2008; Johnson & Steiner 1997; Galen 1996**)** and this can occur on a relatively small geographic scale (Anderson & Johnson, 2008). For example, fly proboscis length and corolla tube length coevolved as a geographic mosaic with only 30 km between populations (Anderson & Johnson, 2008). Pollinator communities vary across environments, and their availability as pollinators for specific plants is influenced by many factors, including the presence of other flowers, temperature, and wind (Augspurger 1981; Arroyo et al. 1995; Corbet 1993; Vicens & Bosch 2000).

Pollinator-driven evolution may not occur as readily in clonal species, even under strong selection pressure, because the evolution of floral traits may be constrained by

their life history. Clonal plants can use vegetative reproduction to monopolize resources in their immediate area, a trait that provides a substantial fitness benefit and has become fixed in many species (Fischer & Van Kleunen 2002; Klimes 1997). Although sexual reproduction is critical for long-distance dispersal, the relative fitness benefit may be low because seed placement cannot be controlled and germination rate is often very low (Eriksson 1989). Both types of reproduction are 'competing' for the same limited supply of resources and any increase in resource allocation to sexual reproduction (i.e. floral traits) may reduce those available for vegetative reproduction (Van Kleunen et al. 2002; Prati & Schmid 2002). Instead, floral traits may evolve as a byproduct of a trait with a greater fitness benefit. For example, competition caused divergent selection of clonal traits in *Ranunculus reptans*, which indirectly caused the differentiation of floral traits, so that plants adapted to competition had fewer flowers than those adapted to competition-free environments (Prati & Schmid 2000).

Here I present results from an experiment using the clonal species *Solidago altissima* from sites in the forest, prairie, and biome boundary in Minnesota. Prairie and forest subspecies have several morphological differences that have been previously reported (Craig & Itami 2011). The primary goal of this study was to determine whether plant-pollinator interactions are different between the plants from prairie and forest biomes. I asked: 1) Are there genetic differences in the size and timing of flowering between the plants in prairie and forest? 2) If so, do differences in size and timing of flowering affect pollinator abundance? I hypothesized that smaller floral displays produced by prairie plants would result in fewer pollinator visits. 3) Is there evidence that differences in floral morphology between biomes are the byproduct of an adaptation

to another function? I hypothesized that there would be a difference in the proportion of resources invested in flowering to those invested in rhizome production between prairie and forest plants.

Methods

Study system

Solidago altissima is an herbaceous, rhizomatous plant that is widely distributed throughout North America and Southern Canada (Semple & Cook 2006). In Minnesota, it has differentiated into subspecies across the boundary of the prairie and forest biomes. *Solidago altissima altissima* is found in the forest in the eastern half of the Minnesota and is replaced by *Solidago altissima gilvocanescens* in the prairie in the west (Semple & Cook 2006). This distribution pattern follows a gradual cline in water availability, growing season length, and soil type, with the prairie being typically drier, warmer, and having richer soil than the forest (Tester 1995). The subspecies can be difficult to distinguish, although recent studies have shown that there are several morphological features that significantly differ between plants from the two biomes, including height, stem diameter, and leaf length (Craig & Itami, 2011). A genetic analysis using amplified fragment length polymorphism (AFLP) markers also showed significant differences between the plant populations (Y. Ando, unpublished data). The forest subspecies generally grows in highly disturbed, mid-succession sites, such as roadsides and old fields where it can be the dominant species for more than a quarter century (Werner et al. 1980). However, the prairie subspecies is found only in tallgrass prairie sites in which fire is the primary disturbance. It is thought that fires occurred historically at least once,

and up to several times, per decade (Tester 1995). Fires prevent the succession of woody species, but do not disturb belowground biomass. In fact, fires stimulate microbial activity and growth of rhizomatous grasses by reducing litter and increasing the temperature of the soil (Tester 1995). Therefore, the prairie subspecies is highly intermixed with rhizomatous grasses that compete for limited resources, but the early-mid succession community of the *S. a. gilvocanescens* may be maintained indefinitely (Weaver 1968).

Solidago altissima flowers in late summer, producing large floral displays composed of several hundred florets. It is an obligate out-crosser and pollinated by generalist insects (Werner et al. 1980; Gross & Werner 1983). *Solidago altissima* is pollen-limited, producing as low as 20-40% of its potential seed set in a given year (Gross & Werner 1983).

Experimental Design

To evaluate the genetic component of the morphological difference between subspecies, I used a reciprocal transplant design. Gardens were located in the prairie in Moorhead, MN, in the Forest in Duluth, MN, and at the biome boundary at Cedar Creek, near East Bethel, MN. Each garden contained 84 2x2m plots, with 14 plots designated for plants from each location. Rhizomes of nine genotypes were planted in each plot, which transplanted from field sites near each of the gardens, and each plot contained a unique combination of genotypes. Each plot was lined with 30 cm deep metal flashing to prevent rhizomes from invading neighboring plots. Gardens were planted in spring of 2010, and plants were allowed to expand through clonal growth to colonize the plot.

Plants were watered during the first year to help them become established, but they were not fertilized or watered thereafter.

In 2011, three stems were randomly selected in each plot and marked with a small piece of flagging tape tied discreetly at the base of the plant. Plant measurements, flowering phenology, and pollinator samples were taken from the flagged stems throughout the season. Plants were considered 'in flower' if 50% or more of the florets were visually estimated to be open. Flowering phenology was recorded every 10-14 days throughout the flowering season in 2011. In 2012, the flowering status of plants was evaluated twice during the season, once in late August, and once in mid September. Plants were designated as 'not flowering, no buds present,' 'not flowering, buds present,' 'in flower,' or 'done flowering.' This allowed me to distinguish between early, mid, and late flowering plants. Floral display width and stem length were measured for each plant, and vegetative branches were counted. Floral dimensions were inserted into equations to estimate the number of florets in each floral display. Two separate equations were derived from floret counts and floral display measurements of 40 unbranched plants (y=-578+169*floral display width, $d.f.$=39, p<.001, R^2=69.1%) and 36 plants with vegetative branches (y=-230+16.2*floral display height+25.2*floral display width, $d.f.$=35, p<0.001, R^2=57.9%).

Pollinator abundance was sampled using a digital camera on one day every 10-14 days on 4-5 sunny days throughout the flowering season in 2011. Photos were taken of an individual flower twice per sampling day, so that each plant was sampled 8-10 times total. Samples were collected between 1000 and 1600 hours, and to randomize the timing

of photos, plants were photographed in a designated order and each consecutive photo was 30 seconds apart.

Flowering phenology and rhizome data was collected using replicates of each of 20 genotypes collected in the field at each site that were planted in 11 liter pots in spring 2010. Plants were fertilized and watered as needed, and allowed to propagate in the same pot for 3 seasons so that each pot contained many stems of the same genotype. Once plants began flowering, bloom phenology was recorded once each week. A pot was considered 'in flower' if any stem in the pot had 50% or more open florets. After the 2012 growing season, one stem in each of 30 pots from Moorhead and Duluth was randomly selected for rhizome measurements. The size of the floral display of each stem was measured using floral branches and senesced sepals because flowers were no longer present. Floret estimates were calculated using an equation derived from floret counts and floral display measurements of 40 stems (y=-578+169*floral display width, $d.f.$=39, p<0.001, R^2=69.1%). The length and diameter at the base of each rhizome larger than 1cm in length was measured using digital calipers, and volume was calculated ($V=\pi r^2 h$). Rhizomes less than one cm in length were excluded because they were unlikely to develop into new ramets, as the plants had already gone dormant at the time of measurement. The total rhizome volume was calculated by summing the volume of all rhizomes produced by each plant.

Statistical Analysis

The effect of site, plant origin, and their interaction on stem height, floral size, and flowering time were tested using an ANOVA. An ANCOVA was used to test the

effect of site, plant origin, and the interaction on pollinator abundance, with floral size and stem height as covariates. Differences between ratios of florets to rhizomes of prairie and forest plants were tested with an ANOVA using stem length as a covariate.

Results

The effect of site and plant origin on phenotype

In both 2011 and 2012, plants grew tallest (Fig 1.1) and had the highest percent of flowering stems (86%) at the Duluth forest site. Site ($F_{2,705}$=78.63, p<0.001), plant origin ($F_{2,705}$=63.93, p<0.001), and the interaction of site and origin ($F_{4,705}$=5.16, p<0.001) explained 30.67% of the variation in plant height in 2011. In 2012, site ($F_{2,633}$=99.55, p<0.001) and plant origin($F_{2,633}$=7.37, p<0.001) explained 27% of the variance in stem height, but the interaction term was not significant at the α=0.05 level ($F_{4,633}$=2.13, p=0.076). In both years, plants from Moorhead (prairie origin) had significantly shorter stems than plants from Duluth and Cedar Creek at all sites (Fig 1.1).

At all common garden sites, plants from Moorhead had significantly smaller floral displays than plants from Duluth and Cedar Creek in both 2011 (Fig 1.2, $F_{2,474}$=15.17, p<0.001) and 2012 ($F_{2,432}$=10.2, p<0.001). Site was also significant, with all floral displays at the Moorhead site being the largest in the wet year of 2011($F_{2,474}$=21.92, p<0.001) and smallest in 2012, a drought year ($F_{2,432}$=6.03, p=0.003). There was a significant positive correlation between stem length and floral display size for both Moorhead plants grown in Moorhead and Duluth plants grown in Duluth (Pearson's correlation, *d.f.*=76, r=0.363, p=0.049, and *d.f.*=75 r=0.383, p=0.004, respectively).

Site was the only significant factor in determining the timing of flowering in the common gardens in both 2011 ($F_{2,547}$=65.25, p<0.001) and 2012 ($F_{2,432}$=13.94, p<0.001), with all plants at Cedar Creek flowering earliest. The plant origin did not affect the mean timing of flowering in 2011 or 2012. Similarly, I found no effect of plant origin on the mean timing of flowering for genotypes from all sites grown in pots in Duluth. However, I found that for genotypes planted in pots in Duluth, the variance in the mean date of first flower was significantly lower in genotypes from Moorhead than in genotypes from other sites (Fig 1.3, Levene's test, t=3.6, p=0.036). These potted plants did not vary in the duration of flowering among genotypes from different sites.

Effect of site and plant origin on pollinator abundance

Using stem height and floral display width as covariates, I tested the effect of site, plant origin, and the interaction between site and plant origin on pollinator abundance. Both covariates significantly influenced pollinator abundance, as did site, plant origin, and the interaction of site and origin (Table 1.1, Fig 1.4).

The dominant pollinator taxonomic groups varied among sites. Members of Apidae were the most common in Moorhead (61%), while members of Syrphidae (67%) and dipterans other than syrphids (15%) were the most common in Duluth. Pollinators in Cedar Creek consisted of a relatively even mixture of Syrphidae (29%), Vespidae (21%) and Lampiradae (14%).

Effect of biome origin on flower:rhizome ratio

Moorhead plants allocated a higher proportion of their growth to rhizome production than Duluth plants. Biome origin was the most significant influence on the

floret:rhizome volume ratio (Fig 5, $F_{1,55}$=5.51, p=0.023), and the interaction between biome origin and stem height was also nearly significant ($F_{1,55}$=3.95, p=0.052,). Stem height did not significantly affect the ratio of florets to rhizome volume.

Discussion

Plants differed significantly in floral morphology between the prairie and forest biomes, and these differences affected pollinator abundance. These differences may be the result of local adaptation, but not necessarily as adaptations to pollinators. Pollinator abundance of individual stems was highly affected by floral display size, and forest plants invested proportionally more resources in flowering than prairie plants. I hypothesize that the differences in floral morphology between the prairie and the forest plants result from divergent selection on the trade-off between flowering and vegetative growth.

I supported the hypothesis that the plants are locally adapted, because each plant population had the highest growth rate in their biome of origin. These results are consistent with those of Craig and Itami (2011), who found genetic differences in shoot and leaf morphology between the subspecies. Differences in floral morphology were maintained in all sites, which indicate that floral size is also under genetic control as a result of local adaptation. I found that smaller flowers and shorter duration of flowering resulted in Moorhead plants receiving fewer pollinators at all sites, including Moorhead. This suggests that differences between floral traits of Moorhead plants and plants from the other sites do not directly result from pollinator interactions, but that there are other factors influencing selection.

The ratio of florets to rhizomes differs between prairie and forest plants when plant height is accounted for. Moorhead plants allocate proportionately more resources to rhizome production than to flower production when compared to plants from Duluth. This supports my hypothesis that the differences in floral traits among biomes may be a byproduct of an adaptation to investment in vegetative growth. Other studies of clonal plants have shown that investment in floral traits is reduced in response to the increased vegetative growth in competitive environments (Prati & Schmid 2000; Bornhofen & Lattaud 2006). Vegetative growth is advantageous in competitive environments because connections may be maintained between clones for a time allowing for resources to be moved between ramets until they are established (Fischer & Van Kleunen 2002). It may also allow for the placement of new ramets in patches of reduced competition (Fischer & Van Kleunen 2002).

Selection for the timing of flowering varies among biomes, as is evidenced by differences in duration of flowering among plants from different biomes. Moorhead genotypes flowered synchronously over the course of a few weeks, with very little variation in the timing of flowering (Fig 1.3A). Duluth and Cedar Creek genotypes displayed a wide range of flowering strategies, which Hafdahl and Craig (2013) hypothesized was due to temporal variation in the end of the frost-free season. Some genotypes flowered early and in synchrony, some genotypes had a range of flowering dates that spanned the flowering season, and others were intermediate for mean date and duration of flowering (Fig 1.3C). In Duluth, the growing season is short and there is a tradeoff between accumulating sufficient resources for flowering and the risk associated with the end-of-the-season temperatures. Early-flowering genotypes are assured

pollination prior to the first frost, and genotypes with a range of flowering dates may be 'bet-hedging.' The average date of first frost is one week later in Moorhead than in Duluth (Minnesota Climatology Working Group). Therefore, the synchronous flowering of genotypes in Moorhead may be the result an extended growing season that diminishes the trade-off between resource accumulation for flowering and risk associated with flowering late in the season. As a result, pollination may not affect the timing of flowering in Moorhead plants.

The differences in both floral traits and floret:rhizome ratio between biomes correspond with the predictions about adaptations to the habitat differences experienced by prairie and forest populations. The cost/benefit ratio favors a relatively larger investment of rhizomes in prairie plants than in forest plants. Prairie plants typically grow in natural prairie sites where there are many rhizomatous grasses competing for resources, particularly water (Weaver 1968), and so there is strong selection for investment in rhizomes (Prati & Schmid 2000). In contrast the payoff for seed recruitment in this environment is very low because seed germination is highly affected by cover litter and the consistent grassy canopy characteristic of the prairie (Goldberg & Werner 1983). Seedlings that germinate may be outcompeted by grasses before they become established (Meyer & Schmid 1999). The prairie sites do not progress beyond mid-succession, and opportunities for seeds to germinate and establish new genets arise following each fire, and so long-distance dispersal is not essential. On the other hand, forest plants grow in recently disturbed sites where few perennial plants have become established. They may be able to monopolize resources in large areas, accumulating sufficient resources for both flowering and vegetative growth. In addition, selection for

dispersal by seeds may be stronger as the disturbed sites undergo succession, and goldenrod is eventually outcompeted. If long-distance dispersal is urgent and resources are available, pollination success may be a greater selection pressure in the forest subspecies. We can see evidence of this in floral traits of genotypes within the forest. Forest genotypes vary in nectar quality and quantity, floral display size, and the timing and duration of flowering, and these traits affect pollinator abundance (Hafdahl & Craig 2013).

Although the results of this study strongly suggest that adaptation to vegetative growth is driving local adaptation among biomes, I have not definitively pinpointed the mechanism of selection. I have presented evidence that pollinators are not driving the morphological differences between prairie and forest floral displays, and that allocation to rhizomes in response to competitive interactions may be a mechanism of adaptation. Because I measured rhizome investment on plants grown in pots where competitive interactions were limited, it is unclear whether allocation strategies themselves are under selection or the level of plasticity in allocation among functions. Prairie plants may not have the morphological plasticity to take advantage of competition-free space that forest plants may have.

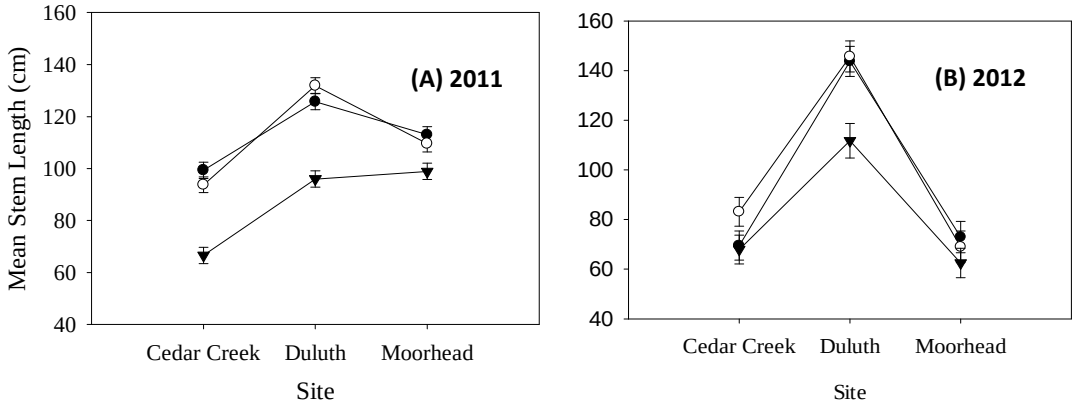


Figure 1.1. Effect of site and plant origin on stem length in (A) 2011 and (B) 2012. Points are means of 80-84 stems measured at the end of the growing season in both years. Solid circles are plants with Cedar Creek origin, open circles are plants with Duluth origin, and solid triangles are plants with Moorhead origin. Error bars are standard error.

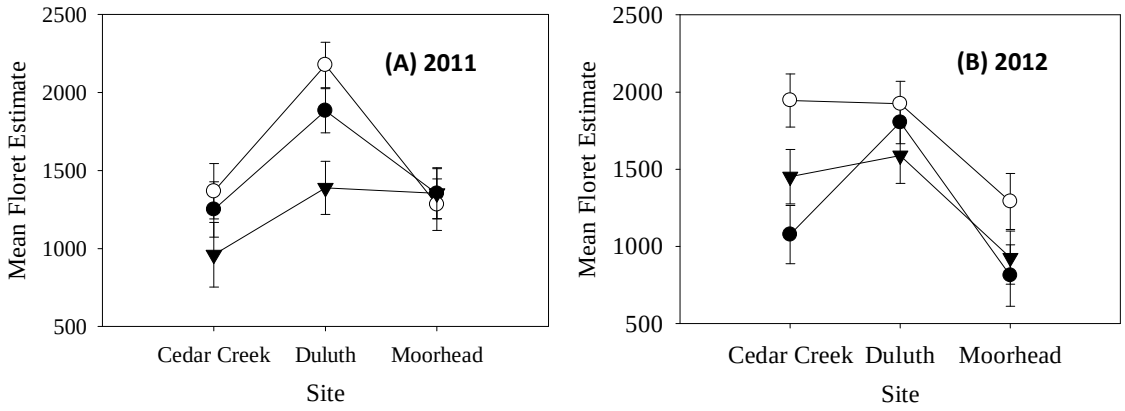


Figure 1.2. Effect of site and plant origin on floret estimate in (A) 2011 and (B) 2012. Points are means of 40-80 floret estimates. Estimates were calculated by inserting floral display dimensions into one of two equations derived from 40 plants without vegetative branches and 36 with vegetative branches. Solid circles are plants with Cedar Creek origin, open circles are plants with Duluth origin, and solid triangles are plants with Moorhead origin. Error bars are standard error.

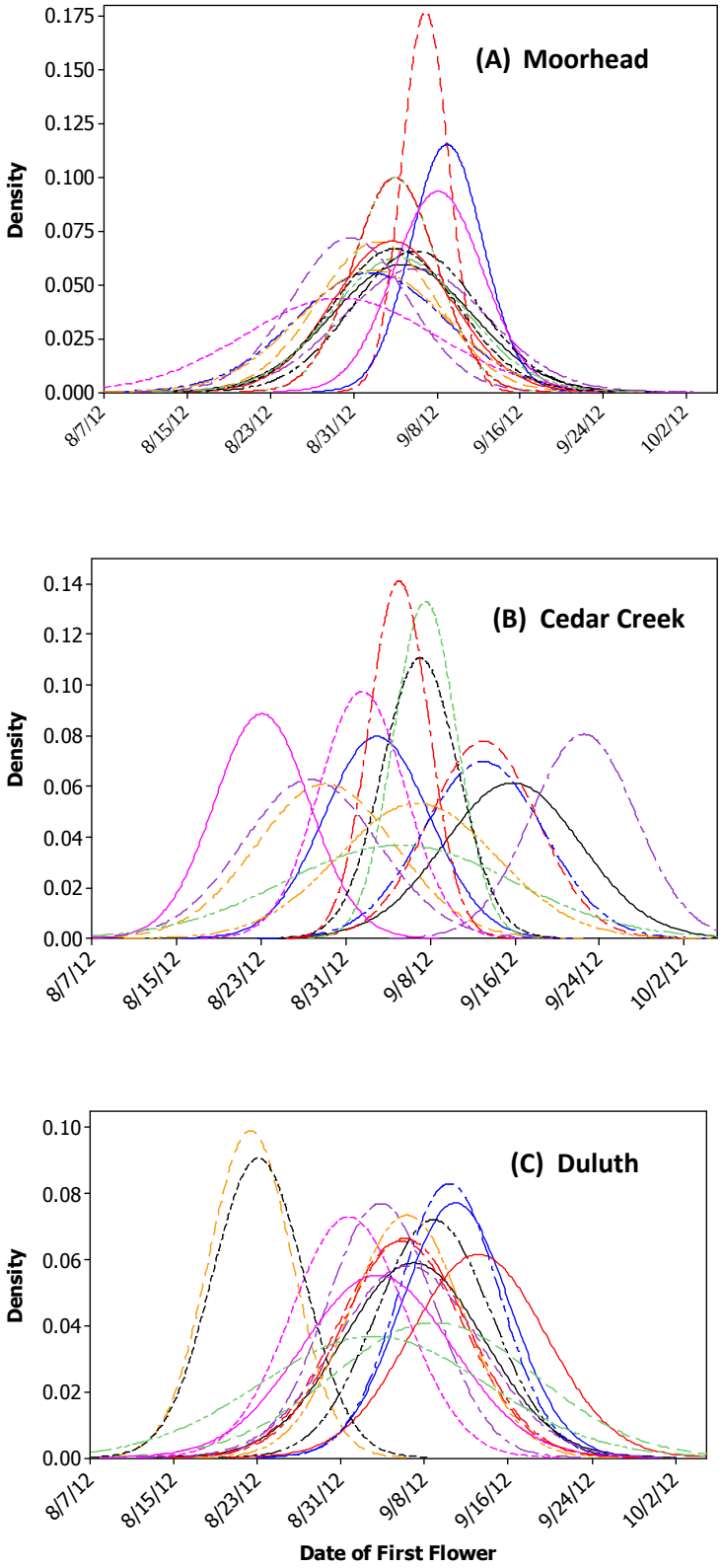


Figure 1.3. Variance in date of first flower for genotypes from (A) Moorhead, (B) Cedar Creek, and (C) Duluth in 2012. Genotypes were in pots located at a common garden in Duluth, MN. Distributions are smoothed curves, and each curve represents flowering dates from of 18-20 replicates of each of 14 genotypes from Moorhead, 16 genotypes from Duluth, and 16 genotypes from Cedar Creek.

Table 1.1. Results of ANCOVA testing the effect of site, plant origin, flower width and stem height on pollinator abundance.

Source	**Variable type**	***d.f.***	***F***	***p***
Site	fixed	2,447	9.97	<0.001
Plant Origin	fixed	2,447	3.7	0.025
Site x Plant Origin	fixed, fixed	4,447	2.76	0.027
Floral display width	covariate	1,447	23.23	<0.001
Stem height	covariate	1,447	13.67	<0.001

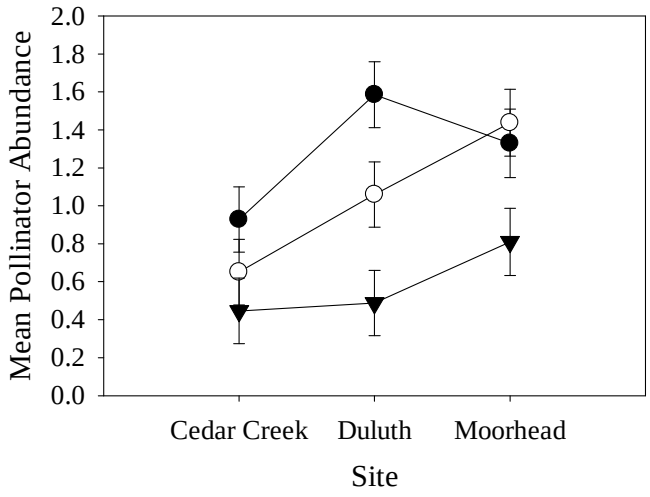


Figure 1.4. Effect of site and plant origin on pollinator abundance of plants in common gardens in Cedar Creek, Duluth, and Moorhead. Points are means of 7-10 pollinator samples on each of 50-80 flowering stems collected in 2011. Solid circles are plants with Cedar Creek origin, open circles are plants with Duluth origin, and solid triangles are plants with Moorhead origin. Error bars are one standard error.

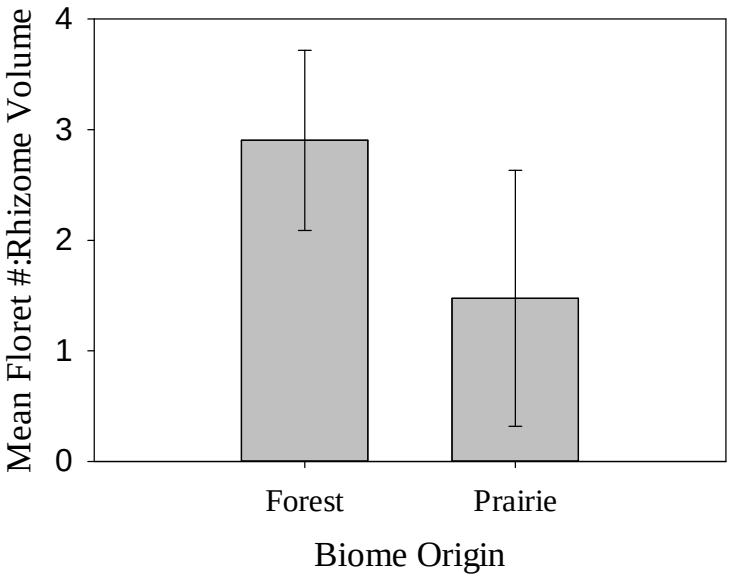


Figure 1.5. Mean ratio of florets to total rhizome volume of plants from the forest and the prairie. Each bar represents the mean of 30 ramets of randomly selected genotypes from the prairie (Moorhead) and forest (Duluth) biome. Error bars are one standard error.

Chapter 2. Genotypic diversity of *Solidago altissima* floral traits and its effects on pollinator abundance

Intraspecific genotypic diversity has been hypothesized to have a strong influence on community structure, and researchers have found genotypic diversity increases net primary productivity and arthropod diversity. The effect of intraspecific genotypic diversity on pollination is unclear. This study tested the hypothesis that genotypes vary in floral display size, nectar quantity and composition, and the timing of flowering, and that genotypic diversity of floral traits within plots of *Solidago altissima* affects pollinator abundance. I found that genotypes varied significantly in all floral traits, and that floral display size and the timing of flowering affected pollinator abundance. Genotypic diversity of plots did not influence pollinator abundance on a genotype, as pollinator visitation to genotypes did not differ between single genotype and multiple genotype plots. The number of flowers within 1 square meter affected pollination of a genotype, but the effect was even stronger at the level of the individual plant. My findings are consistent with Genung (2010), but they provide a more complete understanding of the effect of genetic diversity on pollinator interactions.

Introduction

A fundamental question in biology is the origin and maintenance of genetic variation (Williams 1966, Levins 1963, Doorn et al 2009, West-Eberhard 2005). Diversity has been hypothesized to affect many ecosystem processes and increase community resilience (Cardinale et al 2007, Lazaro et al 2009, Knops et al 1999), and genotypic diversity has been shown to influence plant interactions and ecosystem processes (Crutsinger et al 2008, Johnson and Agrawal 2005, Utsumi et al 2012). The evolution of floral traits in particular are important because of the critical role that flowers play in ecological interactions including those in agriculture (Mitchell et al 2009, Raguso 2008, Potts et al 2003, Wilcock & Nieland 2002). Plant interactions with pollinators strongly influence the evolution of floral traits (Harder and Barrett 1996, Blionis et al 2008, Johnson and Steiner 1997, Galen 1996, Totland 2001), and many studies have found a relationship between pollination and intraspecific genotypic variation in floral traits, including flower number, corolla diameter, and nectar traits (Vogler et al 1999, Silva & Dean 2000, Rabinowitch et al 1993). To demonstrate that variation in floral traits is the result of selection by pollinators requires establishing the relationship between phenotypic floral variation and plant fitness. In annual species, the impact of pollination on fitness can be quantified because floral traits, pollinator visitation, and seed set can be directly measured. Quantifying the impact in perennial species that reproduce both sexually and asexually is more difficult because fitness must be measured over multiple reproductive episodes as the fitness of flowering strategy in one year may be poorly correlated with lifetime fitness.

Perennial plants with vegetative reproduction also must produce seeds because the success of a genet ultimately depends on dispersal to new sites and establishment of new clones (Eriksson 1989). In animal-pollinated clonal species, successful seed production requires a resource investment such as flowers to attract pollinators, and floral traits will be under selection to attract pollinators. Pollinator visitation is an indicator of the return on that investment in specific floral traits.

Does genotypic diversity affect pollinators?

As is true for all characteristics of an organism, the evolution of floral characters depends on selection on phenotypes. Selection on floral characters is complex and the phenotype of a flower depends on the interaction of the genotype with its environment. The success of that phenotype may also depend on context: neighboring flowers may have competitive or facilitative impacts on pollination rates (Bell et al 2005, Potts et al 2003, Ghazoul 2006). If plants that grow in diverse environments have phenotypes that increase pollination and fitness, they may be under selection for growing in proximity to other species or genotypes.

In a pioneering study Genung et al (2010) found that increasing diversity of up to 12 genotypes per one square meter increased pollination rates of the clonal plant *Solidago altissima*. One unresolved question is the mechanism by which increasing diversity increased pollinator visitation. It could have resulted from the diversity of genotypes of flowers having a facilitative effect on pollinator visits. Facilitation occurs when the presence of one plant increases pollination of a neighboring species, and this can occur by means of many floral traits. For instance, facilitation occurs when plants offer

complementary rewards, such as nectar sugars or amino acids that attract a greater diversity of pollinators to the area, and the pollinators also visit neighboring plants (Ghazoul 2006, Hegland and Boeke 2006). Flowering in synchrony with neighboring plants may also facilitate pollination (Augspurger 1981). Alternatively, the increased pollinator visits could have resulted from indirect effects determined by belowground interactions that indirectly influenced floral characters. Diverse plots of *Solidago altissma* have increased above-ground net primary productivity and produce more flowers (Crutsinger 2006, Genung et al 2010), and the results of Genung et al (2010) support this as mechanism for genotypic interactions influencing pollination.

A second unresolved question is whether there are source-sink relationships among plant genotypes in attracting pollinators. Is the fitness of genotype context dependent: does an individual genotype have differential success in attracting pollinators based on the quality of its neighbors? In other words, in some cases is it a source exporting pollinators to other plants while in other contexts it is a sink gaining pollinators from its neighbors? To answer this question the success of a plant genotype in attracting pollinators must be evaluated in different contexts. Answers to this question are crucial in determining how selection will act on a genotype.

A third unresolved question is at what scale do the interactions with other genotypes take place? Is the pollination success influenced by flowers within 10 cm, 100 cm, or 1000 cm? At a larger scale, diversity in visual traits like flowering time may affect pollinator visitation (Augspurger 1981), but nectar traits and underground interactions are likely to operate at a smaller scale. Insect groups respond to different cues when selecting a forage patch and pollinators that use visual cues may distinguish among

patches at a larger scale than those that use olfactory cues. Bees, for example, have a greater visual response, while flies depend heavily on olfactory cues (Roy & Raguso 1997). If the response to genotypic interactions is visual, such as increased flower size, and bees are the dominant pollinators, the effect of diversity may be seen at a larger scale than if flies are the dominant pollinators and the mechanism is complementary nectar rewards.

The scale of the interaction among genotypes is a crucial question because *S. altissima* has a highly clonal growth form with one genotype being reported to form uniform stands which cover from a square meter to tens of square meters (Werner et al 1980). If this is the general case, and if the impact of neighboring plants is at a very small scale, then the importance of neighboring genotypes would be limited to the margins where genotypes met. In contrast, if plants frequently grow in plots with high-diversity on a small scale as simulated in the Genung et al (2010) study then pollination rates will be strongly influenced by neighboring plants. Goldenrod would potentially be under selection to grow in diverse patches, so that they could reduce their investment in flowers by utilizing neighboring flowers to attract pollinators.

I designed this experiment to help resolve these three questions. First, I planted plants in pots with uniform resources so that indirect, belowground impacts would be eliminated and only above ground interactions would be measured. This allowed me to assess the impact of genetic diversity of flowers on pollination success. Second, to assess potential source-sink interactions by keeping plants in pots where there genetic identities could be identified I could assess the success of genotypes in different contexts. Third, in order to measure the importance of scale I measured the impact of neighbors at various

scales. I asked whether there is genotypic variation in floral traits in *Solidago altissima*, and whether genotypic diversity within a plot affected pollinator visitation. I hypothesized that genotypes vary in floral display size, nectar quantity and composition, and the timing of flowering and that this influenced pollinator abundance. I also hypothesized that the genotypic diversity of plots would influence pollinator abundance of a genotype. If there are differences among genotypes then characteristics of neighboring genotypes could have a facilitative or deterrent effect on pollinators. A critical determinant of whether the characteristics of neighboring flowers have an impact on pollinator visitation is whether pollinators choose the patch based on individual blooms or the plot as a whole.

Methods

Solidago altissima is a rhizomatous, mid-successional species that grows in disturbed sites and is found throughout North America (Semple & Cook 2006). In late summer, it produces large, showy flowers that persist for up to ten days (Werner et al 1980), and its seed set is pollen-limited (Gross & Werner 1983). *Solidago altissima* is insect-pollinated and an obligate outcrosser (Werner et al 1980).

Thirty replicates were made of each of 15 genotypes using rhizomes collected from four sites in Duluth, MN. Replicates of each of six genotypes were planted in 11 liter pots in April, 2011, and replicates of each of the remaining 9 genotypes were planted in October, 2011. Seven pots were randomly arranged in 1x1.5 meter plots and each pot was spaced approximately 20 cm apart from neighboring pots. Plots were designated as either 'single-genotype' or 'diverse.' There were two replicate single-genotype plots for

each genotype, and 24 diverse plots that each contained a unique combination of seven genotypes. Pots were watered and fertilized evenly as needed throughout the growing season.

Pollinator abundance was sampled if 50% or more of the total florets were open, which was estimated visually on the day of sampling, throughout the flowering period in 2012. Samples were conducted using a digital camera, and the timing of photos was randomized by implementing a fifteen-second interval between photos. Thirty-second intervals were used early and late in the season due to longer distances between flowering plants. Genotypes in their second growing season in pots had many stems per pots, so one stem was randomly selected for sampling. The selected stem was marked with flagging tape tied discretely at the base of the stem and this stem was sampled throughout the season. Pollinators were sampled two times per day, twice each week, between 1000 and 1600 hrs on clear, sunny days. The stem length and floral display height and width were measured on the first day of flowering. Flowering phenology was recorded and the number of flowers within one square meter of each stem was estimated every 3-4 days.

Nectar was collected in the field between 1000 and 1600 hours between 5 Sept and 18 Sept in 2012. Each sample contained nectar from 10 bisexual flowers in the male phase (the stigma had not split). Nectar was collected and using 0.5µl microcapillary tubes which were placed in microcentrifuge tubes, covered with Parafilm™, and stored in a freezer. Nectar quantity was measured as the length in millimeters of the microcapillary tube that was filled and volume was calculated. Nectar composition was tested for glucose, sucrose, and fructose using high performance liquid chromatography (HPLC) analysis.

Because a single floral display may consist of thousands of florets, floret estimates were calculated using a regression equation that used measurements of floral display width and height. Two separate equations were derived and used for plants that had vegetative branches with distinct panicles (y=-230+16.2*floral display height+25.5*floral display width, $d.f.$=34, p<0.001, R^2=57.9%) and plants with a single panicle (y=-578+169*floral display width, $d.f.$=39, p<0.001, r^2=69.1%).

To test for differences among genotypes in flowering time, nectar traits, and floral display size I used an ANOVA with genotype as the independent variable. Linear regression analysis was used to test the relationship between pollinator abundance and floret estimate, flowering phenology, and nectar quantity. To determine the variable that most accurately predicted pollinator abundance, a stepwise multiple regression analysis was used.

The effect of diversity treatment on pollinator abundance was analyzed using an ANCOVA with floret estimate and the estimated number of flowers within one square meter as covariates. To test whether diversity has a non-additive effect on pollinator abundance, we calculated expected values for each plot and compared them to the observed number of pollinators in each plot. Expected values were generated by summing the mean pollinator abundance for each genotype in diverse plots. Mean pollinator abundance for each genotype was calculated using all pollinator samples (n=24) for all seven stems in the two replicate single-genotype plots. Expected values were compared to observed values using a one-way t-test.

Results

Plant traits

Genotype explained 24.1% of the variance in the floral display width (Fig 2a, $F_{14,261}$=5.92, p<0.001) and 25.1% of the variance for mean flowering date (See Fig. 2b in Hafdahl and Craig 2013, $F_{14,258}$ = 6.32, p< 0.001). Genotypes also varied significantly in the quantity of nectar production (Fig 2c, $F_{14,250}$=2.11, p=0.016) and composition. Sucrose concentration was significantly different between genotypes ($F_{10,77}$=2.24, p=0.024), but the concentrations of glucose, fructose, and the ratio of sucrose:hexoses (glucose and fructose) were not. Total sugar concentration (sucrose, glucose, and fructose) was not significant at α=0.05 level, but this may be due to the small sample sizes used for testing for nectar composition (between 5 and 10 samples per genotype), which made it difficult to detect significant differences.

Pollinator abundance

Genotype explained 25.6% of the variance in pollinator abundance (Fig 1, $F_{14,358}$=8.8, p<0.001). I used an ANCOVA with pollinator abundance as a covariate to test the relationship between genotype and taxonomic richness and found that taxonomic richness was explained by total pollinator abundance ($F_{1,343}$=429.55, p<0.001) genotype ($F_{14,343}$=1.73, p=0.048), and the interaction between pollinator abundance and genotype ($F_{14,343}$=6.61, p<0.001).

Using floret estimates calculated with the regression equations discussed above, I tested the effect of floret estimate on pollinator abundance using regression analysis and found a strong relationship between the average floret estimate for each genotype and

mean pollinator abundance for each genotype (Fig 1, y=-0.0484+0.00018x; r^2=80.6%, p<0.001, n=15). Similarly, I tested the effect of mean flowering date (independent variable) and mean nectar traits (independent variables) on the mean pollinator abundance per genotype. The regression between mean genotype pollinator abundance and mean genotype flowering date was significant (See Figure 2b in Hafdahl and Craig 2013, y = 405 - 0.00983x, r^2 = 26.5%, p > 0.049, n = 15). The regression was not significant at the α=0.05 level between mean genotype pollinator abundance and mean genotype nectar quantity (y=0.0367+0.00634x, r^2=22.0%, p=0.078, n=15), nor was the regression between mean pollinator abundance and mean nectar sucrose concentration significant.

I used regression analysis to test the relationship between floret estimates of individual ramets and mean pollinator abundance of individual ramets (mean of 24 pollinator samples for each ramet) for each genotype. There was a significant relationship between the floret estimates of individual ramets and pollinator abundance for 10 of the 15 genotypes**.** An ANCOVA with floret estimate as a covariate indicates that floret estimate explains 50.5% of the variance in pollinator abundance among genotypes ($F_{1,348}$=176.03, p<0.001), but that genotypic differences are still significant when floret estimates are accounted for ($F_{1,348}$=5.06, p<0.001).

I conducted several regression analyses by pooling all samples of individual stems to determine which traits that had the strongest effect on the pooled samples of pollinator abundance. Using floret estimate of individual stems as the independent variable and pollinator abundance of individual stems as the dependant variable, I found floret estimate to have a strong effect on pollinator abundance (y=0.03+0.000096x, r^2=40.4%;

p=<0.001, n=364). The regression analysis using the pooled sample of flowering date of all individual stems (independent variable) and pooled samples of pollinator abundance on all individual stems was also significant (y = 297 - 0.00072x, r^2 = 15.1%, p < 0.001, n = 273), as well as the regression of pooled samples of pollinator abundance on individual stems and pooled samples of nectar quantity of individual stems (y=2.92+0.071x; r^2=5.4%, p<0.009, n=124). Floret estimate was the best predictor of pollinator abundance using stepwise multiple regression analysis. Floret estimate alone was the strongest predictor of pollinator abundance (r^2=15.39, n=119, p<0.001), Flowering date only increased the strength of the regression by one percent, and nectar quantity was not a significant predictor of pollinator abundance when floret estimate and flowering time were accounted included in the regression.

Diverse versus single-genotype plots

To address the question of whether plants in diverse plots and single-genotype plots differ in pollinator abundance, I used an ANCOVA with floret estimate and the number of open flowers within one square meter as covariates. I found no difference between the diverse and single-genotype treatments in pollinator abundance (Table 1). Variance among plots was largely explained by the floret estimate of each stem, the number of open flowers within one square meter of that stem, and by the interactions of each covariate with genotype. Variation due to genotypic and block effects was not significant.

I compared observed and expected values for diverse plots using a t-test that compared the mean observed pollinator abundance for each diverse plot to the expected

values calculated by summing the means of each genotype in each plot. I found no difference between observed and expected values (Fig 4, one-way t-test, t=0.15, p= n.s., n=30).

Discussion

My results complement those of Genung et al (2010) and provide a more complete understanding of the impact of genetic diversity on pollinator visitation. First, my study indicates that there is genotypic variation in attractiveness of plants to pollinators. Genotypes varied in the size and timing of flowering and in the quantity of nectar produced, and the size and timing of flowering influenced pollinator abundance. Genotypes also varied in nectar sugar composition but differences did not influence pollinator abundance, possibly due to the taxonomic composition of the pollinator community. Honeybees respond strongly to visual cues versus olfactory cues. Because honeybees were the dominant pollinators in my study which this study does not eliminate the possibility of nectar composition influencing pollinator abundance in other taxonomic groups.

Second, this study indicates that there is not a significant impact of genetic diversity on floral traits alone. It is likely, then, that the non-additive effects of diversity on pollinator abundance reported by Genung et al (2010) are the result of below-ground interactions. I found no non-additive effects of genetic diversity when genotypes with different attractiveness to pollinators where in close proximity in pots and did not have any underground interactions. Third, I found that neighboring plants can impact pollination at the intermediate spatial scale, but stronger effects are more local, at the

level of the individual stem. Pollinators were attracted to patches with more flowers within 1 square meter, indicating that some genotypes were involved in source-sink relationships, but floral characters of individual stems, particularly floral display size had a stronger effect on pollinator abundance.

My results indicate that in the absence of underground interactions pollinators choose forage patches partially based on the total floral density of a patch, but that the floral traits of an individual genotype within the patch have a greater impact on pollinator numbers. Since *S. altissima* often forms large genotypically-uniform stands through vegetative spread it is likely that the impact of genotypic diversity in influencing the pollination success of an individual plant is limited to the edges of clonal patches where adjoining genotypes have underground interactions. Based on published literature (Werner et al 1980) and personal observations, a high diversity of *S. altissima* genotypes within a one-meter-square area, the scale on which Genung et al (2010) conducted their study, is probably rare. Genotypes may benefit from enhanced floral traits where underground interactions occur, but the effect on selection for floral characters of the genotype as a whole would be negligible. Future studies should be conducted to better understand the scale at which interactions among plant genotypes influence pollination occur including measuring the distribution of genotypes in a natural setting.

If most selection on floral characters occurs in genotypically-uniform stands produced by vegetative spread, then the evolution of floral traits will primarily result from selection to optimize the allocation of resources between vegetative and sexual reproduction. In this study the most important factor determining attractiveness to pollinators was floral display size. Thus a plant would have to optimize allocation

between rhizome production which increases vegetative spread, and flower production which increases seed production. *Solidago altissima* grows in mid-succession disturbed sites where there is gradual temporal variation in biotic interactions and sexual reproduction is critical for dispersal to new sites before *S. altissima* is outcompeted. However, large investments in floral traits may not be the only way to maximize the lifetime fitness of a genet (Winkler & Fischer 2001). Lifetime fitness may be increased by investing more resources in vegetative growth early-on, producing a large base from which to then reproduce sexually. Further, there is evidence that allocation strategies may not be static and genotypes may increase relative allocation to seed production with genet age (Werner & Platt 1976).

In this study, a second important factor determining pollinator attractiveness was timing of flowering, and selection to adapt to local environmental variation could also produce genetic diversity within and among populations (Hafdahl and Craig 2013). Duluth has high temporal variation in the end of the frost-free season (Minnesota Climatology Working Group), and so the flowering phenology with the highest pollination success varies among years. Genotypes that flower early may have less time for resource accumulation, and so produce small floral displays (Werner et al 1980), but are ensured some pollination prior to the onset of cold temperatures. Late-flowering genotypes may have accumulated resources to produce larger floral displays, but risk the onset of cold temperatures that reduce pollinator activity and signal the end of the growing season. Because genotypes can persist for many years between years when they receive sufficient pollination, genotypes with a variety of flowering strategies can be maintained in the population. The source-sink relationships among neighboring plants

may provide some pollination to plants without the optimal flowering strategy. If the optimal strategy for successful pollination varies among years, then genotypes may be a source in some years and a sink in others.

In conclusion, the results of this study indicate that genotypic variation for floral traits is maintained in the *S. altissima* population and that variation for these traits affects pollinator abundance. In the absence of belowground interactions, I found no effect of diversity on pollinator abundance but that pollinators are affected by neighboring plants on a relatively small scale. Floral traits of an individual stem had the strongest effect on pollinator abundance, and I hypothesize that these traits are the result of the optimization of the tradeoff between vegetative reproduction and sexual reproduction or an adaptation to environmental variation.

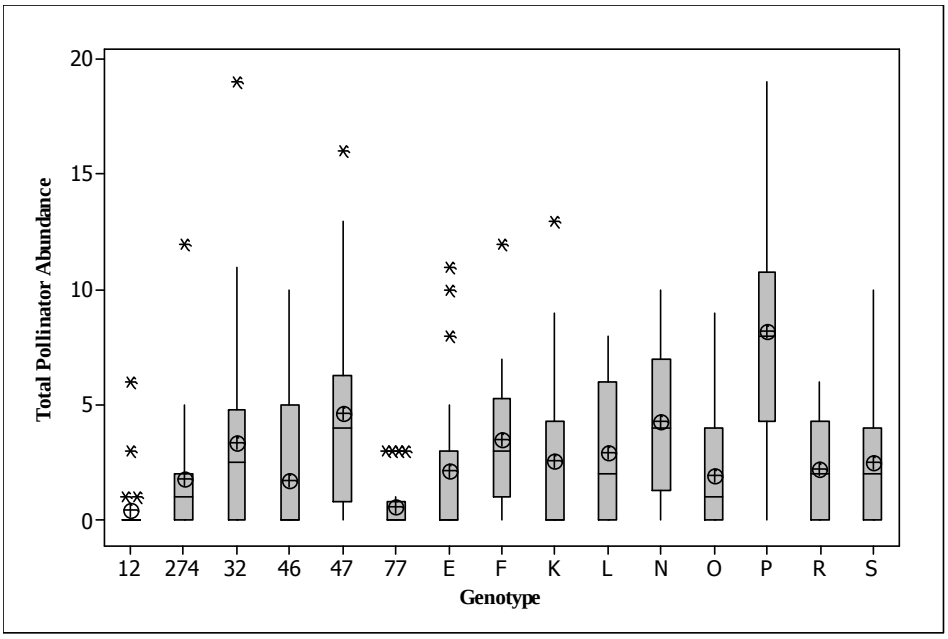
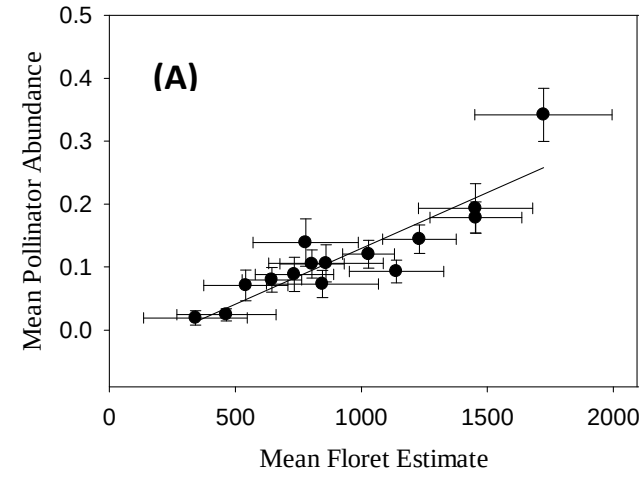
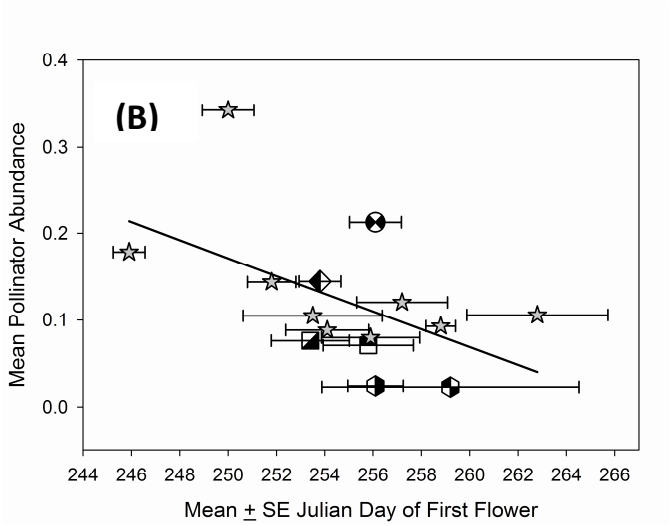


Figure 2.1. Variance in pollinator abundance of replicates of 15 genotypes. Each boxplot represents the total pollinators observed on 15-25 stems per genotype over 24 samples. Each shaded box contains the interquartile range, with crosshairs representing the mean and asterisks representing the outliers.





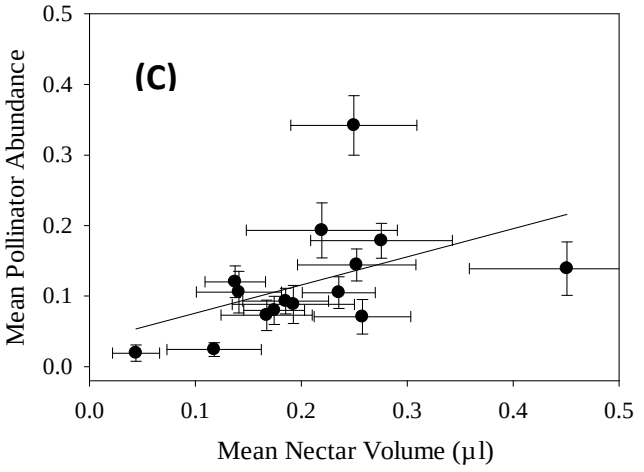


Figure 2.2. Effect of (A) floret estimate, (B) flowering date (from Hafdahl & Craig 2013), and (C) nectar volume on pollinator abundance for 15 genotypes in 2012. Each point represents the mean of 24 pollinator samples on 15-25 stems per genotype, paired with mean floret estimate and flowering date 15-25 stems per genotype (A and B) and 5-12 nectar samples from 15-25 genotypes (C). Error bars are ±1 standard error.

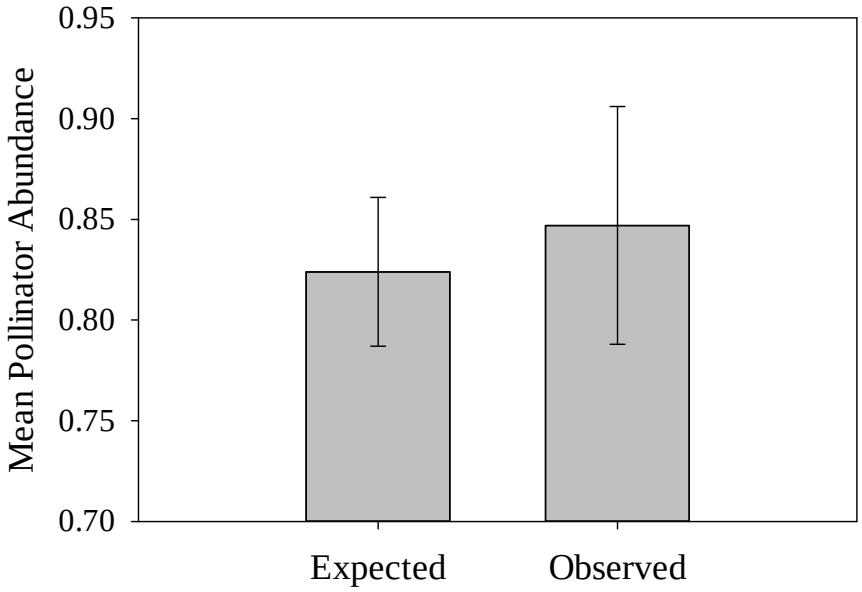


Figure 2.3. Observed versus expected values for pollinator abundance in diverse plots. Bars are means of 25 plots, which are the sum of the average of 24 pollinator samples for seven stems in each plot. Error bars are standard error.

Table 2.1. Results of ANCOVA testing the effect of diverse versus single-genotype plots on pollinator abundance.

Series	Effect Type	*d.f.*	*F*	*p*
Diversity treatment	fixed	1,266	0.00	0.972
Genotype (G)	fixed	14, 266	0.61	0.860
Block	random	52, 266	1.04	0.4
Flowers in 1x1 m (Fl 1x1)	covariate	1, 266	41.18	<0.001
G x Fl 1x1	fixed, covariate	14, 266	8.113	0.038
Floret estimate (FE)	covariate	1,266	61.27	<0.001
G x FE	fixed, covariate	14, 266	3.43	<0.001

Chapter 3. Flowering phenology in *Solidago altissima*: adaptive strategies against temporal variation in temperature

The evolution of flowering phenology is the result of a trade-off that balances many factors, including growth, reproductive capacity, and temporal overlap with pollinators. When there is a large temporal variation in temperature, particularly in the onset of frost, the optimum flowering strategy will vary from year to year. In Duluth, MN, USA, the end of the growing season can vary by more than 30 days. In this study, we observed flowering phenology and pollinator abundance on 15 genotypes of *Solidago altissima* in Duluth, MN. We predicted that temporal variation in temperature would lead to a range of flowering strategies in the *S. altissima* population; some genotypes flower early and in synchrony, some 'hedge their bets' by flowering over a range of dates, and others have an intermediate strategy. Our results indicate that genotypes vary in mean flowering date and duration of flowering and, for the two observed years, pollinator abundance was highest for early-flowering genotypes.

Introduction

Flowering phenology strongly affects plant fitness. Several ecological factors exert selection on the timing of flowering, and species are under selection to reach an optimum strategy to balance these factors. For example, animal-pollinated plants must flower when pollinators are available, but only after they have had time to obtain sufficient resources for flowering and seed production (Gross & Werner 1983; Widen 1991). Species in harsh, variable environments are also under strong selection to adapt to the timing of the onset of low temperatures which terminates the pollination season (Kudo 1993; Widen 1991). Many species have evolved to flower early to avoid the end of the season temperatures, despite reduced time for resource accumulation (Widen 1991; Lacey et al. 2003). In clonal species, high temporal variation in temperature can result in a range of strategies within a population because plants can persist and reproduce asexually for a number of years between years when they are successful in sexual reproduction (Werner et al. 1980).

Many plant species are known to 'hedge their bets' with regards to the timing of reproduction (Cohen 1966, Forrest & Thomson 2010, Tarayre et al. 2007). Bet-hedging is hypothesized to provide a buffer against risks like temporal variation in environmental conditions by employing a variable strategy. Cohen (1966) modeled a classic example of this concept using desert annuals which employ a bet-hedging strategy by which individual plants produce some seeds that germinate immediately and some that remain dormant for a time. In his model, the seeds that germinated immediately risked mortality if the environment was unfavorable, but also had a high reproductive potential because of their short generation time. Later germinating individuals had lower reproductive

potential because of their longer generation time, but allowed the plant to germinate at times when the environment might be favorable, reducing it probability of total reproductive failure. 'Bet-hedging' has also been observed for the timing and duration of flowering. For examples, the spring wildflower *Mertensia fusiformis* bet-hedges by delaying the opening of some flowers to ensure pollination and seed development in years with late spring frosts (Forrest & Thomson 2010). Similarly, the timing and duration of flowering in *Ulex europaeus* has been suggested to follow a bet-hedging strategy to spread out the risk of freezing, rotting, and seed predation (Tarayre et al. 2007).

In areas with high temporal variability at the end of the growing season, flowers face a similar situation to that of the desert annuals studied by Cohen (1966). Genotypes that flower early are more likely to avoid frosts which will end pollination and seed maturation, but they may have a limited capacity to produce flowers and seeds because of the reduced time to accumulate resources. On the other hand, those that flower late may accumulate more resources to invest in reproduction, but risk the onset of cold temperatures before their seeds are mature. Genotypes that 'hedge their bets' have a range of flowering dates to spread out the risk between many ramets if the onset of end of the growing season is highly variable. The success of early and late flowering ramets will vary, but the genet will have some reproductive success in all years. Areas with high temporal variation, then, may select for a bet-hedging strategy. However, in clonal species, the risk of flowering late may be offset if genotypes that flower late have a high pay-off in years when the first frost is late. Because they can persist through years with little to no reproductive success, late-flowering, non-'bet-hedging' genotypes may still

maintain a strong presence in the population because high reproductive success in some years may compensate for years with low or absent sexual reproduction.

Temporal overlap with pollinators is an important factor in the evolution of flowering phenology, particularly for species whose seed production is limited by successful pollination. Selection pressure by temperature and photoperiod is often paired with top-down pressures like pollination and pre-dispersal seed predation (Elzinga et al. 2007). Many studies have shown correlations among flowering phenology, pollinator abundance, and fitness (de Jong & Klinkhamer 1991; Totland 1993, Brody 1997; Irwin 2006). For example, plants that share the same pollinators may experience competition for pollinator visitation, which may select for staggered flowering dates (Wright & Calderon 1995). In contrast, synchronous flowering can attract additional pollinators with large, showy floral displays through a process called 'facilitation' (Staggemeier et al. 2010). It has also been suggested that the evolution of flowering phenology may be constrained by pollinators, as climate change has advanced flowering in some species and not others (Rafferty & Ives 2011).

In general, pollinators tend to be most abundant during peak flowering when floral rewards are highest (Totland 1993; Staggemeier et al. 2010). For this reason, some plants may have evolved to flower in synchrony (Augspurger 1981). Further, pollinators are more active at warmer temperatures **(**Arroyo et al. 1985; Gilbert 1985). While not all pollinators have the same temperature threshold, cooler daytime temperatures near the end of the growing season may reduce activity of some pollinators. One hypothesis is that top-down selection by pollinators will favor early, synchronous flowering. However, the degree and direction that pollinators shift reproductive timing is likely influenced by

many other factors, including optimization of the trade-off between growth and the risk associated with the timing of the end of the growing season.

Solidago altissima in Duluth, MN, USA

Tall goldenrod, *Solidago altissima*, is a mid-successional rhizomatous plant that inhabits disturbed sites throughout North America (Semple & Cook 2006). It forms dense clonal clusters, but sexual reproduction is critical for dispersal as succession progresses. In Duluth, *S. altissima* is one of the last species to bloom, beginning in late August and continuing through the end of the growing season (Pors & Werner 1989). *Solidago altissima* is an obligate out-crosser, and it is pollinated by several species of generalist insects including honeybees and syrphid flies (Werner et al.1980). Its seed production is limited by pollinator abundance, and experimental hand-pollinations have shown that late-flowering *S. altissima* has a higher capacity for seed production than those that flower early due to increased resources (Gross & Werner 1983).

In Duluth, Minnesota, USA, the growing season is short and the timing of the end of the season is variable. The average first frost occurs on 13 September, and 90% of first frosts occur before 28 September (NOAA 2012). However, in the last 90 years, first frost dates have varied by more than 30 days and have occurred as early as August 25th (Fig. 1).

Due to the short growing season in Duluth and high temporal variability in the end of the season, *Solidago altissima* faces a trade-off between acquiring sufficient resources for seed production and ensuring seed maturation prior to the end of the growing season. I hypothesized that climatic variation will maintain a polymorphism in the population for both the initiation and the duration of flowering. In this study, I asked: (1) is there variation for the mean date of flowering among genotypes of *S. altissima* in Duluth, MN? and (2) do these genotypes differ in the duration of flowering? Because successful pollination limits production of viable *S. altissima* seeds (Gross & Werner 1983) and pollinator activity is highly temperature dependent (Corbet et al. 1993), I asked (3) does pollinator abundance vary among genotypes with different flowering strategies? I hypothesized that genotypes with different flowering strategies will have differences in pollinator visitation, and that this will differ among years.

Methods

Flowering phenology of *S. altissima* clones was observed during the summers of 2011 and 2012 at the University of Minnesota Research Farm, in Duluth, MN, USA. In April, 2011, thirty replicates of each of six genotypes were planted in 11 liter pots using rhizome fragments. These genotypes are distinct, as was found by AFLP analysis (Y. Ando unpublished data). Flowering phenology was recorded once per week, starting late August, 2011. A plant was considered to be 'in flower' if 50% or more of its florets were open, which was estimated visually on each sample day.

In October, 2011, 30 replicates of nine additional genotypes were planted. These were originally collected from greater than 10 meters apart to ensure that genotypes were

distinct. During the 2012 field season, flowering phenology was recorded every 3-4 days, and included plants from both years. Second-year replicates had multiple stems per pot, so one stem per pot was randomly selected prior to flowering to be used for all flowering phenology observations and pollinator samples. Selected stems were marked with colored flagging tape tied discretely at the base of the stem. Flowers were evaluated according to the protocol used in 2011. All plants were uniformly watered and fertilized as needed throughout the growing season.

Pollinators were sampled twice per week in September and early October, except for one week in late September in 2011, and four times per week from late August though September in 2012. Samples were conducted using digital photography on sunny days between 1000 and 1600 hours, when pollinators were most active. To randomize the timing of photos, each successive photo was taken at a pre-determined time interval, so that each photo was exactly 15 or 30 seconds apart. Thirty-second time intervals were used for samples with fewer plants in flower due to longer distances between plants. The photographer stood one meter away from the flower being sampled to prevent pollinator disturbance.

A one-way ANOVA was used to test genotypic differences in flowering time and pollinator abundance. Regression analysis was used to test the relationship between flowering phenology and pollinator abundance for genotype means and individual stems. Levene's test was used to test for equal variance among flowering dates of genotypes.

Results

Genotypes varied in both the timing and variance of flowering. Genotypes varied significantly in mean flowering date in both 2011(Fig 3.2a, $F_{5,116} = 12.88$, $p < 0.001$) and 2012 (Fig 3.2b, $F_{14,258} = 6.32$, $p < 0.001$). Mean flowering date of the genotypes ranged from 5 September to 14 September in 2011 and from 9 September to 18 September in 2012. Genotypes grown for two years flowered at relatively the same time in both years, although the correlation was not significant at α=0.05 level (Pearson's correlation, r=0.751, p=0.085) likely because of a small sample size. The duration of flowering significantly differed between genotypes, which was tested using data from each plant's first season (genotypes 12, 32, 46, 47, 77, and 274 from 2011; genotypes E, F, K, L, N, O, P, R, and S from 2012) (Fig 3.3, Levene's test, t=3.01, p<0.001). This was done to control for possible influence of competitive interactions on the timing of flowering within individual pots, as pots may contain several ramets after their first year.

In support of my second hypothesis, I found that total pollinator visitors were significantly different among genotypes in both 2011 and 2012 (one-way ANOVA, $F_{14,358}$=8.8, p<0.001). There was a strong trend between mean pollinator abundance and mean flowering date for each genotype in 2011, but the regression was not significant (Fig 3.2a, $y = 4627 - 0.113x$; $r^2 = 51.3\%$, $p < 0.109$, $n = 6$). However, the regression between mean pollinator abundance and mean flowering date for each genotype was significant in 2012 when the sample size was increased (Fig 3.2b, $y = 405 - 0.00983x$, $r^2 = 26.5\%$, $p > 0.049$, $n = 15$). The regression of flowering dates of individual stems and mean pollinator abundance of one sample on individual stems was significant in 2011 ($y = 5493-0.135x$, $r^2 = 13.4\%$, $p < 0.001$, n=122) and in 2012 ($y = 297 - 0.00072x$, $r^2 =$

15.1%, $p < 0.001$ $n = 273$). Pollinators from the families Apidae and Syrphidae were the most abundant with 60% of the total pollinators being honeybees and 29% syrphids. The remaining 11% consisted of vespid wasps and dipterans other than syrphids.

Discussion

I propose temporal variation in the timing of the end of the growing season has produced variation in the timing and variance of flowering among genotypes of *S. altissima*. In this study, I found that genotypes varied significantly in flowering phenology (Fig. 3.2a and 3.2b) and in their variance for flowering date (Fig. 3.3).

Environmental variability has led to genotypic variation in life-history traits in other plant species (Johnson 2006). Temporal variation can produce a variety of strategies for flowering time because each year selection will favor genotypes with different flowering strategies. In some species, temporal variation may favor only early-flowering genotypes that avoid the onset of low temperatures. In species like *S. altissima*, however, late-flowering plants can have a fitness advantage in warmer years because they have accumulated more resources to invest in reproduction, and so temporal variation generates variation for flowering time.

In both 2011 and 2012, the early-flowering strategy was apparently advantageous. In 2012, I found a significant negative relationship between later flowering date and pollinator abundance. Genotypes that flowered late received significantly fewer pollinators than those that flowered early. In 2011, the relationship between flowering date and pollinator abundance showed a strong trend similar to that in 2012, but our small sample size made it difficult to detect a significant pattern. In 2011, Duluth had two

nights beginning 15 September with lows below freezing, and this began 19 Sept in 2012. Both frost dates fall in the early 40% of the first frost dates for the previous 90 years in Duluth, MN (Fig 3.1).

However, late flowering periods may be favored in some years maintaining the polymorphism for phenology in the Duluth population. There were several years during the past 90 years when the frost-free period extended more than 20 days beyond that seen in the two years of our study (Fig 3.1). In these years an extended growing period could produce larger amounts of resources to be invested in reproduction that would put them at a competitive advantage with conservative plants that had closed down growth early and missed the opportunity to gain additional resources. In clonal plants, the progeny from one successful year can persist for many years when their strategy does not give them a fitness advantage. It has been estimated that a single genotype can persist more than 100 years by propagating asexually (Werner et al. 1980).

Genotypes with moderate to late mean flowering dates and large variance were strongly represented in our sample. Five out of six genotypes had a mean flowering date before the first frost in 2011as did all 15 genotypes in 2012. However, genotypes with later mean flowering dates also had ramets that flowered later than the date when the first frost occurred. It is possible that genotypes with extended flowering periods have evolved to use a 'bet-hedging' strategy. In both 2011 and 2012, this strategy would have been successful because although many ramets flowered late and had low pollinator abundance, some ramets flowered prior to the freeze dates, ensuring at least some pollination success. Becoming adapted to use a bet-hedging strategy may be the stable state for populations in highly unpredictable environments (Sasaki & de Jong 1999).

In this study I focused on a small subset of strategies for flowering, but it is likely that there is a continuous distribution of timing and variance in flowering phenology. In the 15 genotypes I observed, there was a trend that suggests early-flowering genotypes may have smaller variance in flowering duration than late-flowering genotypes. However, the sample size was not large enough to test whether early-flowering genotypes had significantly smaller variance than late-flowering genotypes. It is also possible that the late-flowering genotypes are using a bet-hedging strategy, although I do not have conclusive data to support this hypothesis.

There may be factors other than temporal variation in temperature and pollinator abundance that select for flowering phenology. For instance, honeybees are the most effective pollinators of goldenrod (Werner et al. 1980), and were the most abundant pollinators in this study. They can be active at temperatures as cool as 12°C (Corbet et al. 1993; Vicens &Bosch 2000), much cooler than many other pollinators including Syrphids (Gilbert 1985). Additionally, *S. altissima* is one of few plants in Northern Minnesota to flower late in the growing season, so it is possible that late-flowering genotypes achieve higher pollen efficiency if honeybees are only pollinating other *S. altissima* plants. This may select for genotypes with late-flowering clones.

In summary, I have shown that *S. altissima* genotypes employ a variety of flowering strategies. In two years with average timing of the onset of temperatures below freezing, I found pollinator abundance was greatest for early-flowering clones, which may translate to higher seed production. Others have shown that late-flowering *S. altissima* has more resources for seed production (Gross & Werner 1983), indicating that there is a trade-off between resources accumulated and ensuring pollination prior to the

end of the season. While there are obviously many alternate strategies, it is possible that some *S. altissima* clones moderate the growth/end-of-season trade-off by employing a bet-hedging strategy.

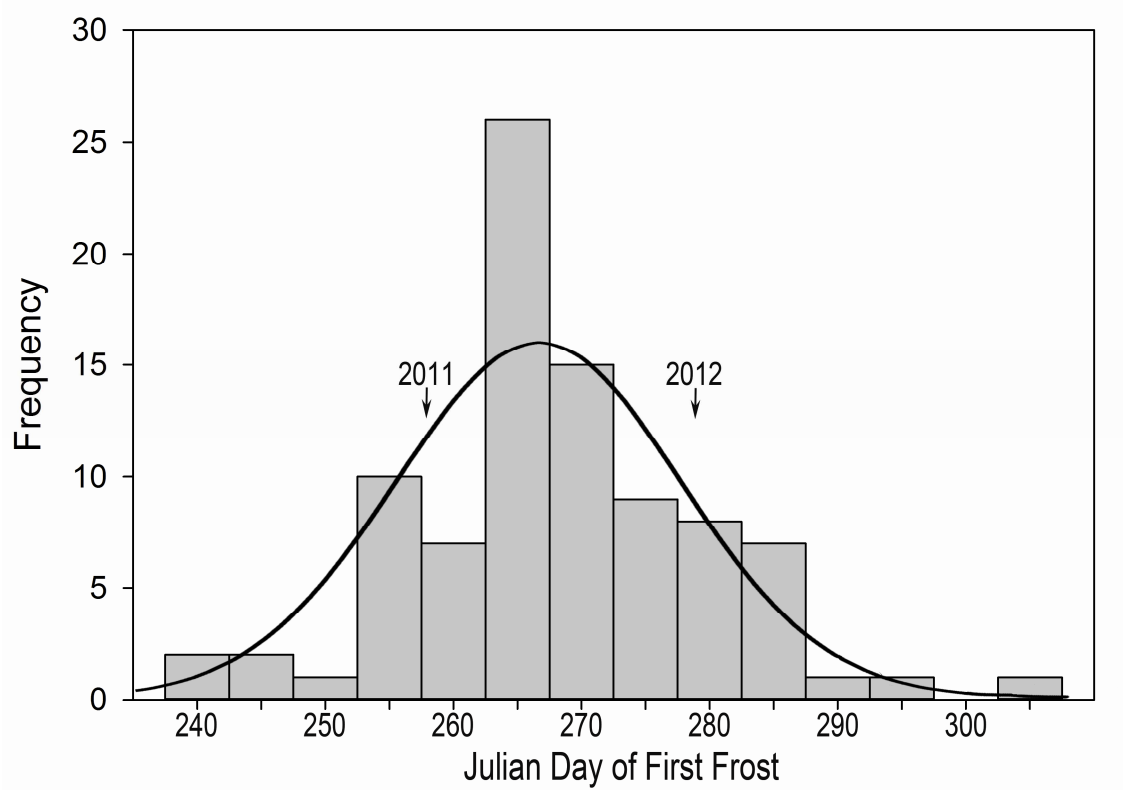
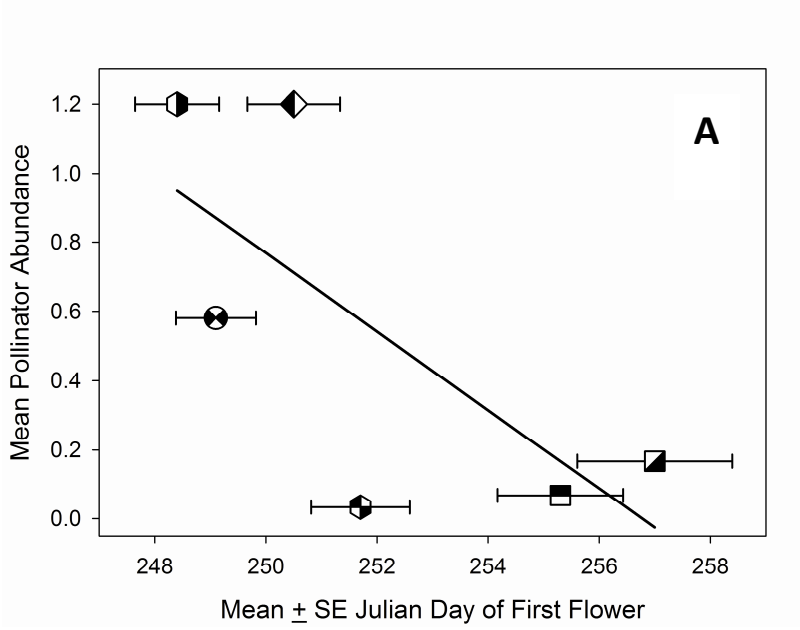


Figure 3.1. Distribution of the dates when the first frost occurred from 1922-2012 in Duluth, MN, USA. Data was provided by the Minnesota Climatology Working Group.



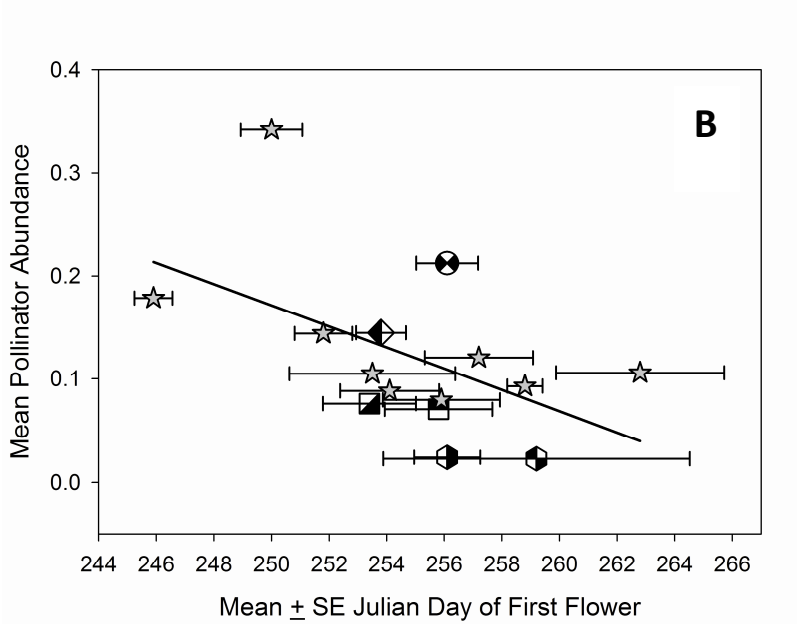


Figure 3.2. Effect of mean flowering date on pollinator abundance for (A) 6 genotypes in 2011 and (B) 15 genotypes in 2012. Black and white symbols demarcate genotypes that were sampled both years, with 2012 being their second year in pots. Stars represent new genotypes sampled only in 2012. Sample size for flowering date varies between 13 and 25 in 2011 and between 5 and 25 in 2012. Pollinator abundance is the mean of 7 samples on 30 replicates of each genotype in 2011 and 24 samples on 30 replicates in 2012.

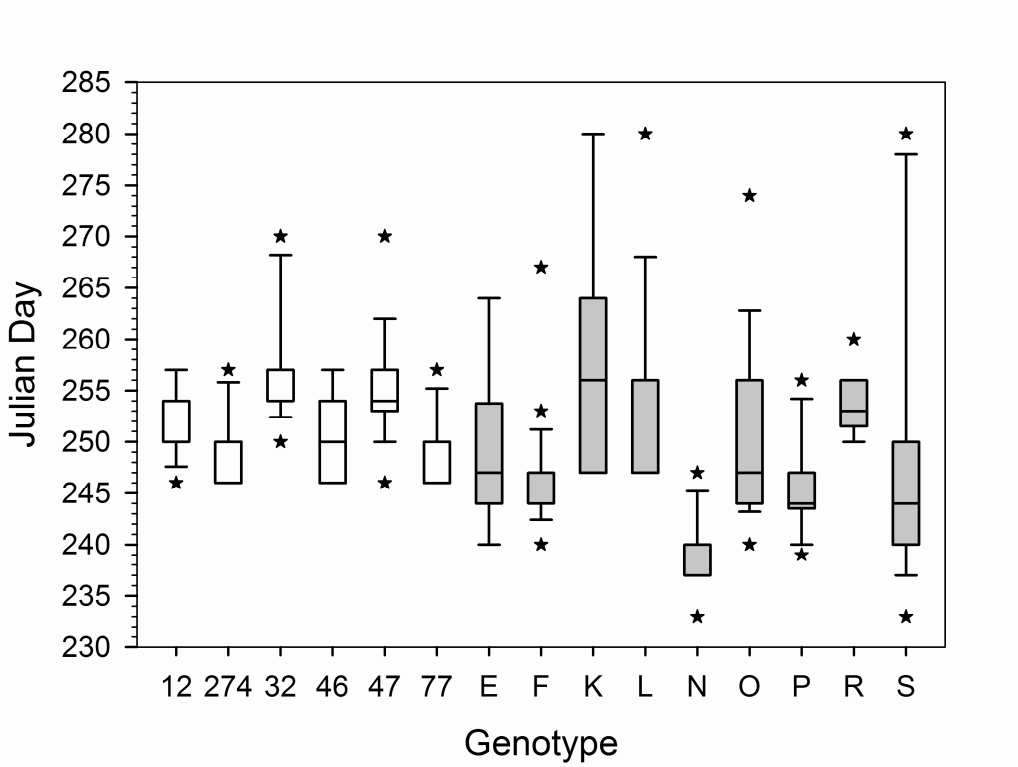


Figure 3.3. Variance of the date of first flower for individual stems of 15 genotypes in their first year in pots. Unshaded boxes represent data from 2011 and shaded boxes represent data from 2012. Each box represents the inter-quartile range, and lines within boxes are the mean date of first flower for 13-25 ramets. Stars represent outliers.

Conclusion

The general goal of these studies was to quantify variation in floral traits among and within biomes, and to try to understand mechanisms that may be driving or maintaining variation in these traits. I used pollinators as a proxy for success at sexual reproduction, as the seed set of *S. altissima* is pollen-limited, and tested how variation in floral traits affected pollinator visitation. By isolating the effects of floral traits on pollinator visitation, I was able to determine 1) if pollinators were driving the evolution of floral traits, and 2) which traits were most important.

In my first study, I found that floral traits of plants from the prairie and forest biomes follow the pattern of local adaptation among biomes. Differences in floral display size and pollinator abundance suggest that pollination is not the primary selection pressure in differentiation of these subspecies. However, differences in the relative allocation to vegetative growth suggest that floral traits may be a by-product of an adaptation to competition. Forest plants allocated proportionally more resources to flowering than prairie plants. This supports the hypothesis that prairie plants are experiencing strong selection by competition or fire to reproduce vegetatively, whereas selection in the forest favors high investment in sexual reproduction for long-distance dispersal.

In the second experiment, I studied how the selection pressure of pollination may be acting on many phenotypic traits, including both physical characteristics of the individual and context. I found differences in flowering time, floral display size, nectar quantity, and nectar sucrose composition. I also found that pollinator abundance is affected most strongly by flowering date and floral display size. However, when

genotypes were placed in different contexts, source-sink interactions appeared, in which plants that were relatively large in comparison to neighboring plants acted as a 'source' of pollinators to floral displays that were smaller relative to its neighbors. Location in a dense floral patch increased pollinator visitation to a genotype, whereas location in a sparse patch decreased it. These results suggest that selection may be acting on the phenotype of the individual and on its location relative to other flowering *S. altissima*. There may be many strategies for allocating resources among sexual reproduction and vegetative growth in *S. altissima*, and genotypes have optimized their strategy in different ways. The level of genotypic diversity of the plot did not affect pollinator abundance. This is consistent with the life-history of *S. altissima*. Ramets in dense clonal patches in the forest are likely to experience little genotypic diversity, with the possible exception of the ramets at the margins of a genet, and so pollinators select flowers to visit based on traits of the individual stem and density of the stems within the genet.

In my third study, I found significant variation in mean flowering date among genotypes, and in the variance of timing of flowering. In both 2011 and 2012, genotypes with early, synchronous flowering received the highest pollinator visitation. However, genotypes with moderate to late mean flowering dates and large variance were strongly represented in the sample. In years when the first frost is late, late-flowering ramets may have high fitness due to increased time for resource accumulation. It is possible that genotypes with extended flowering periods have evolved to use a 'bet-hedging' strategy, minimizing the risk of the onset of colder temperatures that indicate the end of the season. Late-flowering genotypes remain in the population for many years when they are

not successful at sexual reproduction, which may explain the large variation of flowering strategies maintained in the *S. altissima* population.

These studies have provided a better understanding of the interactions between *S. altissima* and its pollinators and how they vary among and within biomes, and they have highlighted traits that may play an important role in the evolution of floral traits. However, using pollinators as a proxy for sexual reproduction may not precisely reflect genet fitness. First, pollinators vary in their efficiency of pollen transfer. These studies treated all pollinators equally, but giving more weight to visits by particular pollinators like honeybees, which are known to be highly efficient pollinators of goldenrod, may more accurately estimate the amount of successful pollen transfer. Second, future studies should assess genet fitness by taking into account the number of ramets produced as well as viable seed production.

Research of this nature, in which ecological processes are studied at both large and small scales, adds to our understanding of large, macroevoutionary patterns as well as the history of individual organisms. Natural selection acts on all scales simultaneously, whether it be populations within biomes or individuals within a population. By trying to understand mechanisms at both scales, we can make comparisons and observe similarities in order to get a better understanding of the mechanisms of local adaptation.

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