Early Studies in Understanding the Genetics of Prairie Rose Gentian

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Submitted by:

Sheila Lyons
University of Illinois
Department of Plant Biology
265 Morrill Hall
505 South Goodwin Avenue
Urbana, IL 61801-3707

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Introduction:

Many organisms which are listed as endangered for a particular state or region often are not threatened with extinction along the geographic range of the species. These populations are termed peripheral populations because they are populations located outside of the central range of the species. These populations are of concern to conservation biologists for the preservation of local species diversity, maintenance of genetic diversity, and conserving the evolutionary potential for speciation (Lesica and Allendorf 1995).

From a genetic perspective, peripheral populations often have lowered genetic diversity relative to central populations due to consequences of isolation and small population size (Lesica and Allendorf 1995). Gene flow is absent in isolated populations, thereby preventing the introduction of new alleles into a population. Thus, genetic diversity is expected to decrease over time. Additionally, population sizes tend to be smaller. With smaller population sizes, the effects of genetic drift (random fluctuations in gene frequency) are much greater than in larger populations, leading to lowered genetic diversity. Small population size may also lead to an increased likelihood of inbreeding. Thus, individuals mate with close relatives resulting in lowered genetic diversity within the population. Inbreeding depression may also occur where a decrease in fitness results due to the expression of recessive lethal alleles which are unmasked as a result of mating with close relatives (Barrett and Kohn 1991).

Species which live in highly changing environments tend to have large amounts of genetic diversity (Lesica and Allendorf 1995). As previously stated, peripheral populations may have lowered genetic variation. As genetic diversity decreases, alleles within a population may be lost which could be beneficial in adaptation to environmental change. Thus, the loss of genetic diversity may be detrimental to a species because the potential for adaptation to environmental variation may also decrease (Huenneke 1991).

The importance of peripheral populations has been recognized by many. For example, prairie chickens are abundant in the Great Plains states but Illinois populations are on the verge of extinction. Additionally, the American sycamore (*Platanus occidentalis*), a state-threatened plant in Wisconsin, is commonly found in Illinois and in more southern and eastern portions of the United States (Lesica and Allendorf 1995). Another example is prairie rose gentian, *Sabatia campestris* (Gentianaceae). Prairie rose gentian is currently known from central Illinois, south to Mississippi, west into Iowa, Kansas, Oklahoma and Texas (Bolick 1991). Prairie rose gentian commonly occurs along roadsides and natural areas within the southern part of its range while it is less abundant in the northern portion of its range. Within Illinois, prairie rose gentian is currently a state-endangered plant, known to occur only within prairie remnants located along railroad right-of-ways within 3 counties in the central portion of the state.

The overall aim of this study is to investigate the genetic diversity of the prairie rose gentian within Illinois populations as well as the population structure of this species along its range. Within Illinois, I am particularly interested in quantifying migration and determining if gene flow is effective in preserving genetic variation between two populations which are separated by approximately 170m of tallgrass prairie vegetation. I am investigating gene flow from the perspective of potential gene flow which involves taking field measurements of pollen and seed movement. Additionally, I am interested in determining actual gene flow in which I will investigate the establishment of genes within populations by identifying parentage using molecular markers.

The large scope of this project necessitates repeated data collection over several years. In particular, this report covers the preliminary results of a larger dissertation research project. The first year of the study has been supported in large part by the Wildlife Checkoff Fund of the Illinois Department of Natural Resources. Most of the first year's research involves collection of data encompassing potential gene flow including studying floral visitation, seed dispersal distances, potential seed production for Twelve-Mile Prairie populations, and spatial distribution of individuals for populations near Farina. Additionally, I have included some preliminary results for isolating high quality DNA for prairie rose gentian.

Methods:

Study Organism:

Prairie rose gentian is an annual plant, typically found in sandy prairies, roadsides, and waste places along its range. In Illinois, the northernmost edge of the species range, prairie rose gentian is found only in remnant prairies along railroad right-of-ways. Prairie rose gentian is listed as an endangered plant within Illinois (Herkert 1991). Only five populations having been documented in Illinois within the last 11 years.

Prairie rose gentian grows in a variety of soil types and at varying moisture levels. The soil is usually dry and sandy, yet plants grow in wet areas as well (Perry 1971). Illinois populations typically grow in clay soils (pers. obs.).

Illinois populations of prairie rose gentian flower synchronously from mid-July to mid-August with peak flowering in late July and early August. Prairie rose gentian plants typically range in size from 10 to 35 cm tall. Flowers are pink and typically have 5 petals but can to range from 4 to 12 petals (Bolick 1991). Illinois populations range from 3 to 5 petals (pers. obs.).

Prairie rose gentian is self-compatible. However, inbreeding is avoided by having a protandrous breeding system with anthers and stigmas physically separated within a flower. At anthesis, anthers are prominent and upright while the 2 stigmas are coiled together and bent almost perpendicular to the anthers. Between day 3 and 5 after anthesis, anthers recoil and stigmas uncoil and stand upright. There is partial overlap in time between the receptivity of stigmas and the presence of pollen indicating that within a flower, self pollination can occur (Perry 1971). Immediately after flowering, fruit production begins and plants begin to senesce (pers. obs.).

Each flower can produce one fruit capsule. Fruits contain many tiny seeds which disperse passively after the capsule dehisces (Perry 1971). Some evidence suggests that seeds may be stored in a seed bank as prairie rose gentian in Illinois is generally found in recently burned or disturbed prairie habitats (W. Handle, pers. obs.). Still, the presence of a seed bank needs to be confirmed scientifically.

Overall, little is known about this species. The most widely published papers on the prairie rose gentian involve studies of the systematics of the genus *Sabatia* by Wilbur (1955) and Perry (1971).

Study Area:

The study sites are located along a 35.8 km stretch of tallgrass prairie within a railroad right-of-way along Route 37 between the towns of Watson and Kinmundy, Illinois in Effingham and Marion Counties. This area is commonly referred to as "Twelve-Mile Prairie" and contains remnants of tallgrass prairie and savannah vegetation. It is currently maintained by a scenic easement agreement between the Illinois Central Railroad and the Illinois Department of Transportation in cooperation with the Illinois Department of Natural Resources. Three of at least five known Illinois populations of prairie rose gentian are located on Twelve-Mile Prairie: one population is located northwest of Edgewood (referred to as the Edgewood site) and the other two populations are approximately 1 mile southwest of Farina (referred to as the Farina A site and Farina B site). The Farina populations are separated by approximately 170 m of prairie vegetation. The Edgewood population and Farina populations are separated by a distance of approximately 12 miles.

Mapping and Collection of Plant Tissues:

Mapping and tissue collection are imperative to determine actual gene flow distances of individual plants. Individual prairie rose gentian plants at both Farina locations were marked and mapped within the study area. Plants were individually marked by placing a small piece of green tape coded with an unique number on the plant near the base. Towards the end of flowering, a numbered golf tee was placed within 1 cm of the base of the plant; the tee facilitates locating plants after flowering has ceased. As plants were marked, one small leaf was collected, placed in a small zip lock bag, and put immediately on ice to preserve genomic DNA. Leaf tissues were placed in -80°C freezer upon arrival to the University of Illinois within a few hours following collection.

Plants were mapped relative to a straight line transect since the populations generally had a linear pattern. For each population, a single transect was made by placing a fiberglass rod at either end of the population. Using measuring tape, I measured the distance between the two ends of the population. The measuring tape was kept taut and in place while plants were mapped. Using a geographer's compass, I determined a precise compass reading for the line. To check the precision of

the line, a bright orange golf tee was placed at each 1 m interval, and a wooden stake at each 5 m interval. The transects were made permanent by placing a stake at the end of each transect.

I mapped plants relative to the transect by measuring the perpendicular distance from the individual plant marker to the line transect; perpendicular distance is the distance from the plant to the transect where the intersection of the lines forms two right angles.

Floral Visitors:

Over a three week period during the summer of 1996, I conducted a series of 10 minute observations of individual prairie rose gentian plants or plant clusters (which comprises a small group of plants) to identify the main floral visitors of prairie rose gentian. All visitors, the number of flowers present on a plant, the number of visits to flowers on a plant, and the duration of visitation were recorded. Floral visitation was observed between the hours of 9:00 am and noon.

Data were analyzed using an analysis of variance to determine if differences in duration of visit were present between insect visitors. Additionally, correlation analyses were performed to detect if any relationship existed between number of flowers, and number and duration of visits.

Seed Dispersal:

Seed dispersal distances for four groups of plants were measured directly in the field using seed traps. Traps consisted of petri dishes brushed with Tanglefoot® to "catch" seeds (Dudash 1991), with the outer bottom portion of the petri dishes painted bright orange to facilitate relocating dishes under prairie vegetation. Small seed traps were made using petri dishes with a 5.7 cm diameter and large traps had a 9.0 cm diameter. Large traps were used within the outermost trapping perimeter to increase manageability of setting up the seed traps by reducing the number of petri dishes used.

In total, I set up four seed dispersal arrays. Two arrays were large and sampled seed dispersal out to 1.25 m, while two arrays only sampled a 0.50 m radius around the focal plants (Figure 1). For the large arrays, I placed small seed traps in circles at distances of 0 cm, 0.1 m, 0.2 m, 0.3 m, 0.4 m, 0.5 m, 0.75 m and 1.0 m from focal plants using 1, 8, 16, 24, 32, 40, 60, and 80 traps respectively for a total of 261 small seed traps per array. Sixty large seed traps also were placed around the array at a distance of 1.25 m from the focal plants. The small arrays were set up in an identical manner except that the last perimeter of seed traps was placed at 0.5 m for a total of 121 small seed traps per array. For each perimeter around the focal plants, 53% of the area within the designated perimeter was sampled using seed traps. A large area was sampled to ensure that the distribution of the seed shadow could be determined due to the small number of plants sampled.

Large arrays included arrays 1 and 2. Array 1 contained 3 plants ranging from 16.5 to 25 cm tall with 7 fruits. Array 2 contained 1 plant, 30.5 cm in height with 4 fruits. The small arrays included arrays 3 and 4. Array 3 had two plants with 7 fruits ranging from 22.5 - 33.5 cm in height. Array 4 contained 2 plants, which ranged from 25 - 28.5 cm in height and contained 6 fruits.

When a group or cluster of plants was used, plants were located within 8 cm of one another with most plants located within 5 cm of each other. By increasing the number of fruits within the array, I could increase the likelihood that seeds might fall into seed traps. Plants or plant clusters were chosen by locating relatively isolated plants or plant clusters within the Edgewood population; this selection of plants was necessary to decrease negative impacts on this population. I removed all fruits from a few plants which were not the plants of interest, but were within 2 m of the focal plants.

Traps were placed in the field before seed dispersal began. Seed traps were collected approximately 2 weeks after the last fruit in the array opened to ensure that seeds in the capsule were given enough time to disperse. Traps were then collected, individually stored, and later examined for seeds using a dissecting scope.

Potential Seed Production:

Over the course of the growing season, reproductive parameters were regularly monitored and recorded including number of flowers per plant, number of fruits, and size of fruits for most individual plants at both the Farina and Edgewood populations. Approximately 95% of the prairie rose gentian plants at the Farina population were individually identified and followed from flowering to fruiting. The population size at Edgewood was estimated to be approximately 500 plants of which 347 plants were individually identified during the 1996 growing season.

In early October 1996, 36 intact fruits were randomly collected from the Edgewood site. Fruits which were collected had not yet dispersed seed. Thus, number of seeds could be counted per fruit to estimate total seed production for the population. Each fruit was classified by relative size as small, medium, or large. Seeds were counted and average seed weight per fruit was determined. Estimates of the number of viable seeds were recorded. Seeds were arbitrarily assigned as viable if they appeared half full when magnified through a dissecting scope. Fruits were stored dry at 5°C. Seeds from this portion of the study will be used to translocate prairie rose gentian to a state-protected natural area.

Reproductive potential in terms of potential seed production for prairie rose gentian was determined by multiplying the average number of seeds for a specific size fruit by the number of fruits in a size category for a specific plant for each population. Some fruits did not have a size designation and hence were assigned in the small category to allow for a conservative estimate of seed production. The number of seeds per fruit at the Edgewood population was assumed to be consistent with the number of seeds per fruit at the Farina populations.

DNA Extraction:

Determination of actual gene flow via establishment of seedlings during the consecutive growing season requires the use of molecular markers. For the large scope of this study, I plan to use microsatellite markers. These markers are the genetic technique of choice as they can detect a fairly large amount of variation within the genome of individuals which are thought to contain low levels of variation. Genetic markers will allow estimates of gene flow level at the level of the individual for prairie rose gentian at the Farina sites. Additionally, the genetic markers can be used to study the population genetics of prairie rose gentian across its range.

To investigate gene flow in Illinois populations of prairie rose gentian, a genomic library will have to be developed. The first step in this process is to extract high molecular weight genomic DNA. Leaf tissues were ground into a fine powder in liquid nitrogen. DNA was extracted using a urea extraction buffer; urea acts to denature proteins and salts. Phenol and chloroform are added to stabilize DNA by destroying enzymes which can break down DNA. Isopropanol and ammonium acetate are added to precipitate DNA. The procedure is basically followed by another phenol-chloroform extraction to purify DNA. I extracted DNA from individuals from Oklahoma, Texas, and Illinois. Additionally, DNA was extracted from marsh pink (*Sabatia angularis*) for comparison. For detailed protocols, please contact me at the University of Illinois.

Comparison of the Genetics of Central and Peripheral Populations:

Prairie rose gentian populations from Oklahoma and Texas were sampled during 1996 by collecting leaf tissues. Leaf tissue samples from approximately 100 individuals per population were collected for five populations from Oklahoma and Texas. Sample collection was performed as follows: Two parallel transects were set up which were 100-m long and separated by approximately five meters. Sampling began approximately 10-m from the edge of a population. At 2-m intervals, leaf tissues from the prairie rose gentian plant closest to the transect were collected. This sampling scheme allowed for an appropriate size comparison with the Edgewood population at Twelve-Mile Prairie. Samples were collected and frozen. I plan to collect tissues from Missouri, Arkansas, and Oklahoma to consider the population genetics along the entire range of *S. campestris* using microsatellite markers. These data will be used to determine the extent by which Illinois populations may have diverged from central populations. I am currently in the process of searching for genetic markers, hence data for this portion of the study will be provided at a later date.

Results:

Mapping:

At Twelve-Mile Prairie, prairie rose gentian populations basically have a linear, yet clumped distribution within both Farina populations (Figure 2). While not mapped, the Edgewood population also appeared to have a linear shape with plants in a clumped distribution during the summer of 1996. While the overall distribution is linear, plants often were clumped in small patches. For both populations, the length of each of the Farina populations spanned approximately 40 m while the width

was approximately 7 m for the southern populations (site A) and only 2.5 m for the more northern population (site B).

Floral Visitation:

Flower visitors were grouped as follows: halictid bees, syrphid flies, small bees, and other bees. Halictid bees and syrphid flies were grouped as they were common visitors and were relatively easy to identify. Of approximately 130 observations of individual plants, 52% of insects visiting flowers were halictid bees (n = 61) and 31% of visitors were syrphid flies (n = 37). Other insects visiting flowers were not identified to family level. These insects which were grouped into 'other bees' and 'other flies' categories which were comprised 15% and 2% of the visits respectively.

Data for comparing duration of visit for insect visitors were not normally distributed. To normalize the distribution, data were transformed using natural log transformation. Using analysis of variance, no differences were detected for duration of visit depending upon visitor. On average, halictid bees visited prairie rose gentian flowers more often and for a longer duration than other visitor groups, however this difference is not significant (Figure 3). A weak relationship between number of visits and total number of flowers on a plant was apparent ($R^2 = 0.4098$). The number of flowers on a plant was not correlated with duration of visit ($R^2 = 0.0909$) or number of visits ($R^2 = 0.2571$).

Seed Dispersal:

Based upon four seed dispersal arrays, primary seed dispersal in prairie rose gentian is highly localized (Figure 4). The seed distribution was skewed, with most seeds dispersing within 0.3 m of the plants. Figure 4 illustrates seed dispersal distributions of number of seeds located in traps. Seeds located within dishes at 1 m from the focal plants may be attributed to plants which inadvertently were not removed from the area when the seed traps were placed in the field. In cases where large numbers of seeds fell in an area, entire fruits fell into seed traps resulting in dispersal of many seeds within a small area.

Potential Seed Production:

The fruit size assignment was fairly consistent with field observations of fruits. The mass of small and medium fruits were significantly different from large fruits (ANOVA, $p < 0.05$). While no significant differences were found between the mass of small and medium sized fruits, medium fruits did tend to be slightly heavier than small fruits.

A similar pattern is present when looking at number of seeds per fruit. Both small and medium sized fruits did not have significantly different number of seeds. However, medium sized fruits did tend to have more seeds within a fruit than small fruits. In contrast, small and medium sized fruits had significantly fewer seeds than large fruits (Figure 5). Additionally, differences in fruit size were noted for number of viable seeds per fruit (ANOVA, $p < 0.0001$). No differences in number of viable seeds were apparent between small and medium sized fruits. However, large fruits have significantly more viable seeds than small and medium sized fruits (Figure 6). Additionally, estimates for seed production per population were based upon the average number of seeds within fruits of similar size from the Edgewood site (Table 1).

DNA extraction:

The DNA was extracted from leaf tissue using a urea extraction buffer, two treatments with phenol-chloroform solution and isopropanol yielded high molecular weight DNA (Figure 7). The bright bands indicate that samples migrated by electric charge from the wells. Bands were present in wells 7 and 8, however they were faint. This weaker result may have occurred as these samples thawed in shipping from Oklahoma to Illinois. Using the polymerase chain reaction (PCR), the DNA which was extracted from the Oklahoma samples can probably be amplified. Thus, these samples still may be useful, although these initial bands are not as defined as the others. The distance that the bands moved was consistent with good quality, high molecular weight DNA.

Discussion:

Overall, floral visitation data indicated that the duration of visits among pollinators was approximately the same. While the average length of each visit did not differ, the number of visits by

insects appeared to differ. Halictid bees were most frequently encountered based on the number of floral visitor observations, followed by syrphid flies. If the quantity of visits influences pollination by increasing the probability of pollen transfer to a stigma, then the main pollinators of prairie rose gentian may be halictid bees and syrphid flies. Still, observations of floral visitors do not necessarily indicate that pollination is occurring. Schmske and Horvitz (1984) discovered that floral visitors are not necessarily effective pollinators. Thus, during the summer of 1997, I will identify if floral visitors are indeed pollinators by inspecting visitor's bodies for prairie rose gentian pollen. Additionally, during the summer of 1997, I will expand my study of pollen flow in prairie rose gentian by taking field observations of pollinator flight movements to obtain measures of potential gene flow by pollen. Studies by Rasmussen and Brødsgaard (1992) indicated that most pollinators tended to visit near-neighbor plants. Based upon my observations from 1996, this trend may also exist with prairie rose gentian. If so, then pollen movement is restricted in space. Explanations for pollen dispersal would include pollen carryover on the bodies of pollinators over long distances and between different species of plants. Additionally, pollen transfer might be facilitated if a pollinator was carried by a wind current to another prairie rose gentian population.

Additionally, a weak relationship between number of visits on a plant and number of flowers was present indicating that flower number may be important for attracting greater number of floral visitors. For large plants with many open flowers, this may have both positive and negative consequences. Many flowers may enhance outcrossing by attracting pollinators over greater distances. However, it may result in more visits to flowers within the same plant as pollinators typically travel between neighboring plants and hence, potentially increasing the chance of inbreeding.

Seed dispersal data indicate that gene flow via passive seed movement from a fruit capsule is restricted. While the sample size is small, the data indicate that most seeds do not typically travel distances greater than 0.4 m from the maternal plant, with most seeds falling within 30 cm. Limited movement is typically what might be expected from a plant with passively dispersed seeds. However, a study on a related species, *Sabatia angularis*, which has a similar seed dispersal syndrome, indicated that seeds dispersed from maternal plants between 12 and 38 cm in height traveled at least as far as 225 cm (Dudash 1991). This measure of seed movement did not include the tail of the seed shadow indicating that seeds traveled further than 225 cm. Prairie rose gentians on Twelve-Mile Prairie with a similar height stature to the previous study may have more restricted dispersal than it's congener due to environmental conditions. *Sabatia angularis* in the previously mentioned study was monitored at the Indiana Dunes. On the dunes, seed dispersal may have been influenced secondarily by strong winds, extending the tail of the seed shadow.

Still, other hypotheses may be formulated to explain how seeds might be transported. Prairie rose gentian seeds are small, and may utilize secondary dispersal methods. The small size of seeds may be suitable for secondary movement via water in the event of large rainfalls. Additionally, mud might act as an adhesive where seeds might stick to animal fur or feathers. To investigate if prairie rose gentian seeds travel relatively long distances, molecular methods will need to be used.

Thus, gene flow via seeds appears to be restricted. If so, then homogenization of the two Farina populations via gene flow (if it occurs between populations) may depend almost exclusively on pollination. Studies by Rasmussen and Brødsgaard (1992) indicated that most pollinators tended to visit near-neighbor plants. While pollinators typically may travel to the nearest neighboring plant, pollinators could travel farther distances by using plants of other species as 'stepping stones' to another prairie rose gentian population or a wind gust might simply move insects from one population to another.

I estimated seed production in the three populations based upon a small sample from the Edgewood population. Using fruits from the one population may not have been the best method to determine seed production for the Farina sites. The Edgewood population was a large population with several hundred plants, with many plants larger than those at the Farina sites. Additionally, the more northern Farina site tended to be overall dryer than either of the other sites, whereas the more southern Farina site tended to encounter a very high amount of cover by grasses. These potentially stressful environmental factors may be influencing the success of prairie rose gentian at these sites. Overall, using fruit size as an way to determine seed output in a population of an endangered plant may be an alternative to harvesting seeds annually.

Besides field work, I also performed laboratory research to extract DNA from prairie rose gentian tissues. Obtaining high molecular weight DNA is critical for building a genomic library to determine microsatellite markers. These data represent the first steps in the process of finding molecular markers to determine actual gene flow distances of pollen and/or seeds.

Another aspect of gene flow can be considered on a temporal level involving the soil seed bank (viable seeds which remain dormant in the soil after germination has ceased). Seed banks can cause delays in recruitment into a population (Templeton and Levin 1979, del Castillo 1994) and may explain the maintenance of genetic variation in plant populations after a bottleneck (Rasmussen and Brødsgaard 1992). I had proposed to incorporate the seed bank in this year's data set. However, germination requirements for prairie rose gentian have been difficult to pinpoint. For example, in a pilot germination study I planted seeds from the Edgewood site as well as seeds from a Texas source. After approximately 12 weeks, a high percentage of the Texas seed germinated in contrast to a complete lack of germination for the Illinois seeds. As a result of the preliminary data, I have decided to pursue a field study to investigate the soil seed bank.

Learning about the dynamics of gene flow and the importance of it to population genetics can be a very useful tool in conservation biology. By understanding how far genes may travel in space and time we may be better able to manage rare and endangered populations. Depending upon the goal of the manager, conservation genetics can be used to assess the genetic status of a population and to determine how a population should be managed genetically in addition to demographic concerns. By better understanding the population genetics, conservation biologists will make more educated decisions regarding various issues such as increasing the amount of genetic variation within the population or conserving the potential for speciation within the population.

Summary:

A thorough understanding of gene flow in plant populations involves an understanding of the dispersal distance of both pollen and seeds as well as the establishment of offspring into future generations. This report represents the initial phase of a detailed study of gene flow and population genetics of a regionally endangered plant. By utilizing both field and laboratory techniques, I aim to better understand the genetics of small populations and how data can be used for species management. This report provides data concerning floral visitation, seed dispersal distances, potential population seed production, and spatial dispersion of prairie rose gentian to better understand the both the ecology and genetics of these populations along the species range, but especially within the small populations found within Illinois.

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Table 1. Estimate of the number of seeds produced per fruit based upon baseline data from fruits collected from the Edgewood site approximately 2 miles North of Edgewood. Sample sizes refer to the minimum number of fruit produced within the population. Note that the Edgewood site data refer to only the 347 plants recorded during the 1996 growing season and not to the actual number of plants in the population (which may be as high as 500 individuals).

Site	N	Estimated Number of Seeds
Edgewood:		
Fruit Size		
Small	72	7,581.6
Medium	111	18,936.6
Large	195	79,696.5
Unknown Size	76	8,002.8
Total:	454	114,638.7
Farina (Population A):		
Fruit Size		
Small	15	1,579.5
Medium	34	5,800.4
Large	20	8174.0
Total:	69	15,553.9
Farina (Population B):		
Fruit Size		
Small	55	5,791.5
Medium	33	5,629.8
Large	30	12,261.0
Total:	118	23,682.3

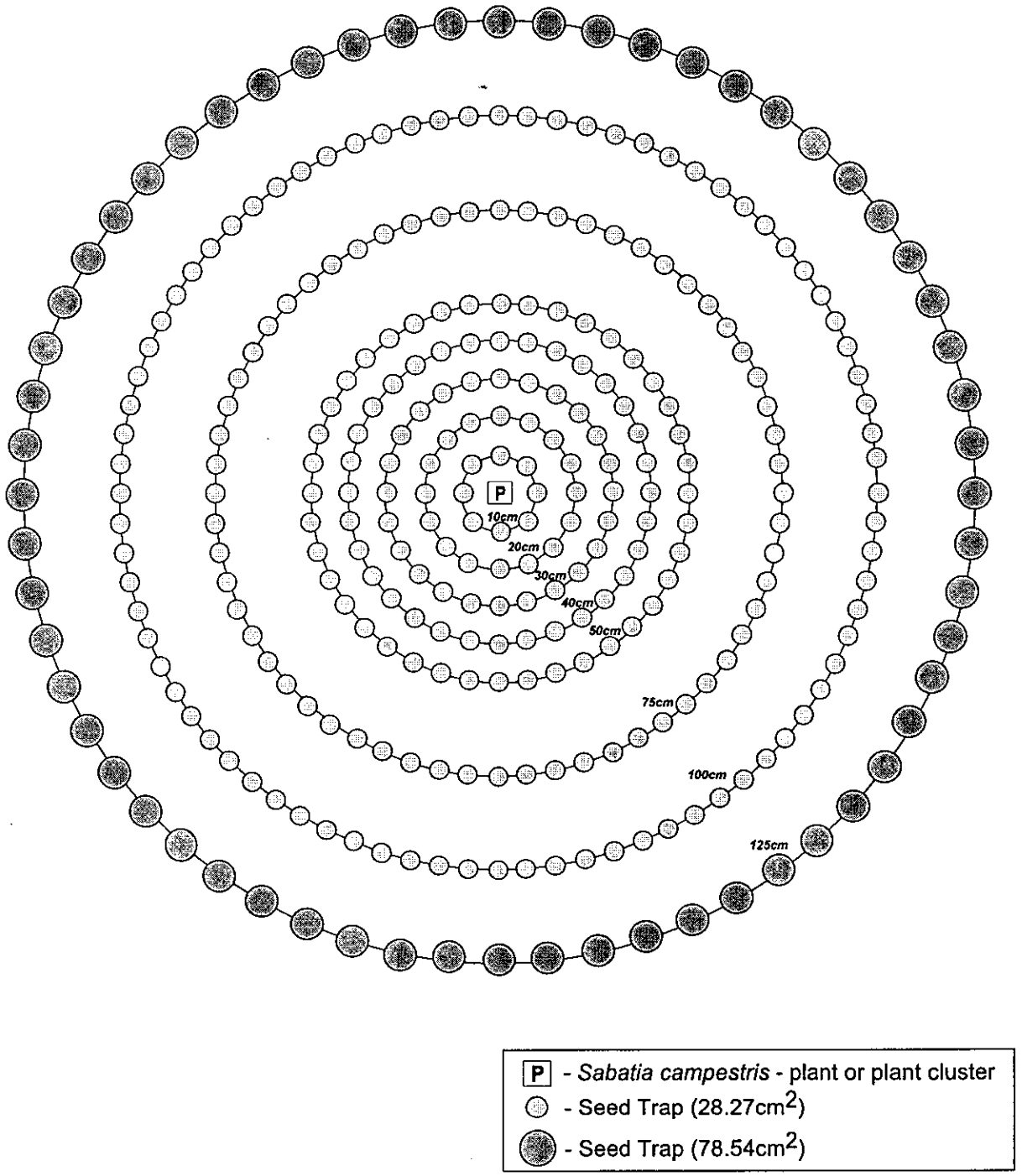

Figure 1. Illustration of large seed dispersal array. This set-up was used for arrays 1 and 2. The small arrays (3 and 4) only extended as far as 50 cm.

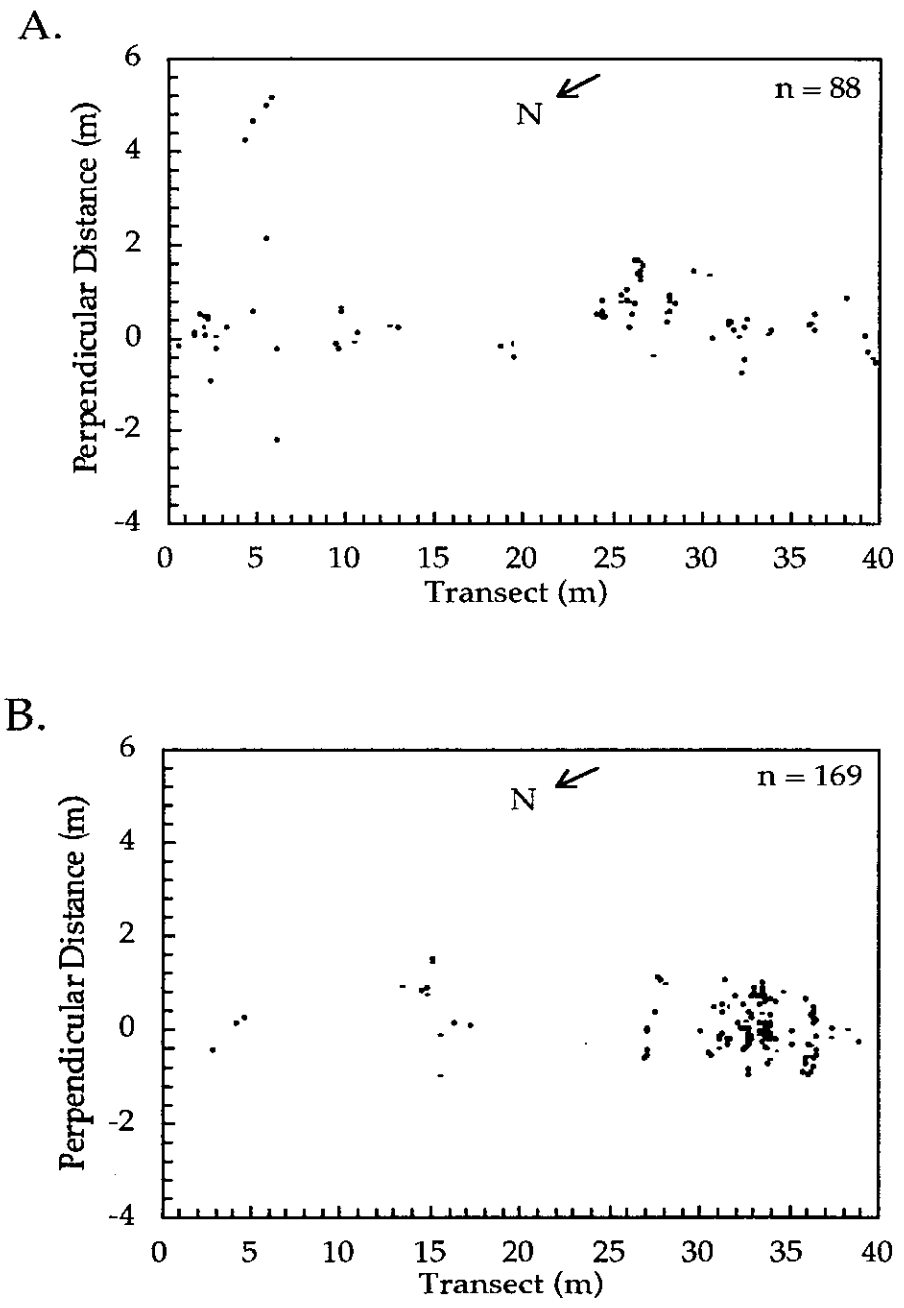

Figure 2. Map locations of individual prairie rose gentian plants from both sites located southwest of Farina, IL. Site A is separated from site B by 170 m of tallgrass prairie vegetation. Each circle represents an individual plant. Note that both populations exhibit a patchy and linear distribution.

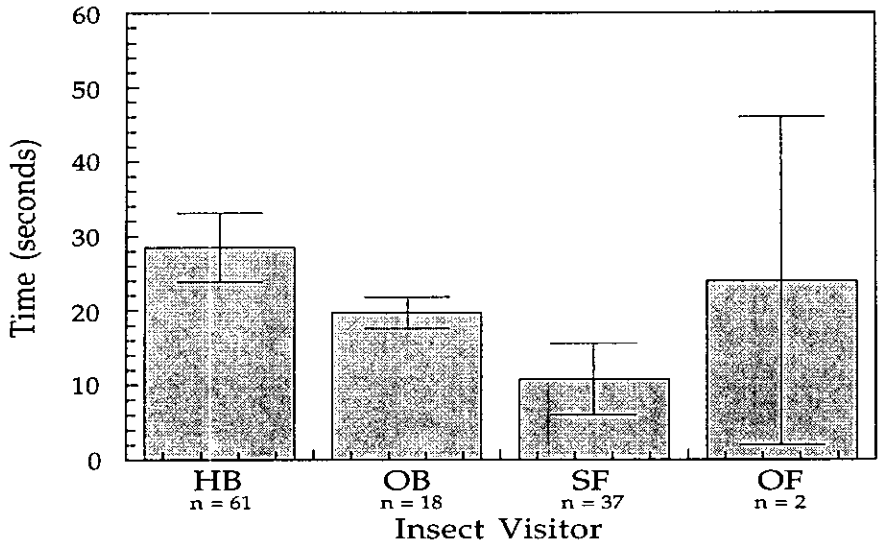

Figure 3. Duration of floral visitation by insect visitors. HB = halictid bees, OB = other bees, SF = syrphid flies, OF = other flies. No statistical significance was found for duration of visitation.

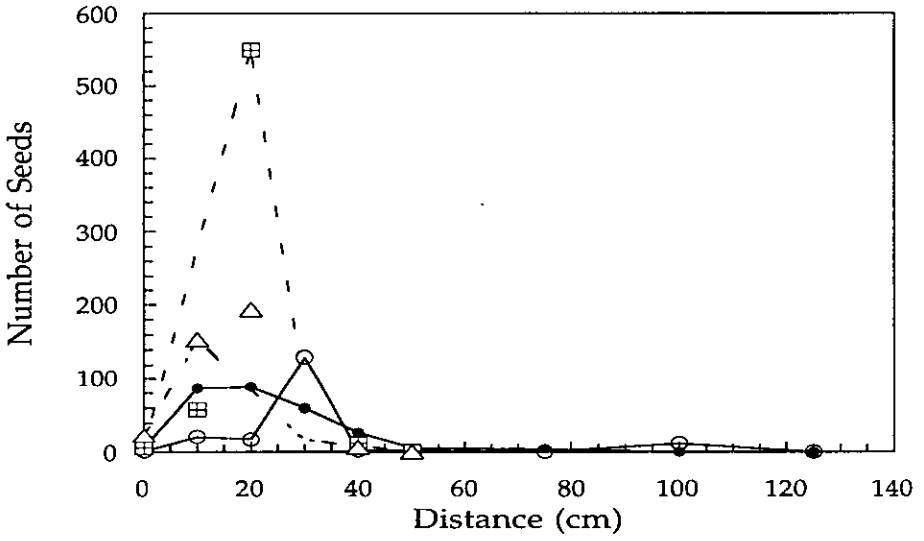

Figure 4. Seed shadows for 4 arrays: 2 small and 2 large. Dispersal peaks for arrays often reflect dispersal of an entire fruit.

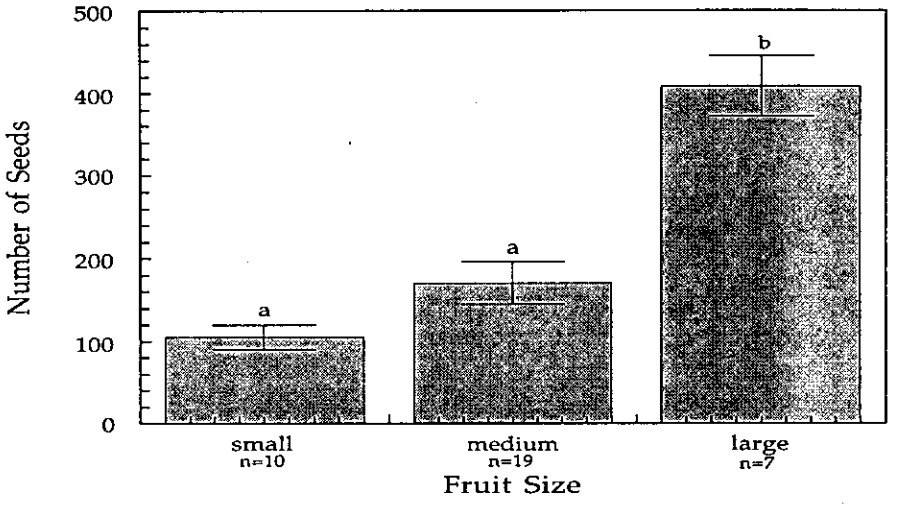

Figure 5. Graph illustrates the relationship between fruit size and number of total seeds. Different letters above standard error bars indicate significant differences between different fruit sizes.

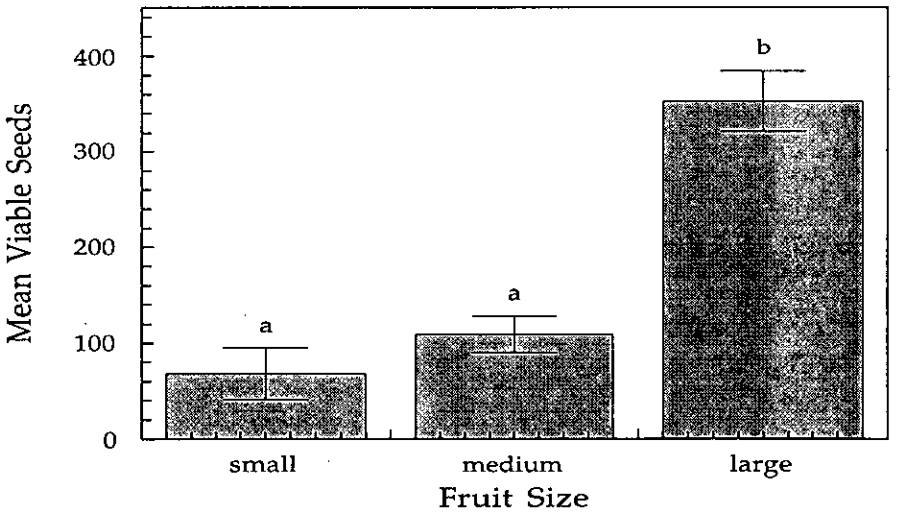

Figure 6. Graph illustrates the relationship between fruit size and estimated number of viable seeds. Different letters above standard error bars indicate significant differences between different fruit sizes.

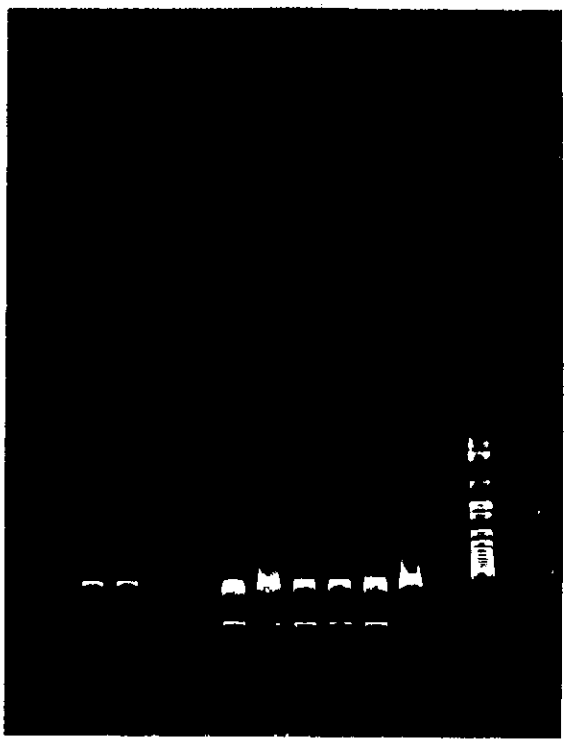

Figure 7. Photograph of agarose gel to test quality of DNA extraction. Lanes 1 and 2 are prairie rose gentian from Bond County, IL, lanes 3 and 4 represent prairie rose gentian from Oklahoma, lanes 5 and 6 represent prairie rose gentian from Texas, lanes 7 and 8 represent prairie rose gentian from Edgewood, IL, and lanes 9 and 10 represent marsh pink, *Sabatia angularis*, a related species which was run for comparison.