Can assisted cross pollination be used to increase fitness in isolated plant populations?

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Original Project Objectives

The original objectives of the proposal were to 1) Determine the prevalence and extent of inbreeding depression in isolated populations of *Asclepias*, 2) Determine whether assisted pollination would be an effective tool in restoring reproductive vigor to isolated populations 3) Determine the effective isolation of the population based on the vagility of common pollinators. This study focused on the pollination of three species of *Asclepias* in three nature preserves, Capel Hill Prairie, Prospect Cemetery Prairie, and Loda Cemetery Prairie as well as a remnant railroad prairie along Highway 45 north of Champaign.

Prevalence and extent of inbreeding depression in *Asclepias* species could not be determined because sufficient seed could not be collected from the three species used in the study. Cross pollination of the first species, *Asclepias sulvantii*, was not successful. In addition, natural fruit set was low to non-existent in this species. Cross pollination was not successful in *Asclepias tuberosa*. Natural fruit set was also low in this species. Cross pollination was successful in *Asclepias syriaca* but Japanese beetles ate through the nylon mesh pollination exclusion bags. In some cases the beetles cross pollinated flowers in the bag and in others, ate fruit from this cross pollination study.

Because the original project failed to generate successful offspring, we adjusted out methodology to address the basic question of reproduction in isolated remnants in a different way. The attached report explains the new methodology and our results to date.

Does Population Size Affect Fitness Performance of Common Species?

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Introduction

Prior to European settlement, central North America was a vast grassland that stretched from Indiana west to Nebraska, south to Texas, and north to the Canadian provinces of Alberta and Saskatchewan (Transeau, 1935). European settlers arrived in the prairie by the 1600's, but it was only within the last 100 years that most of the conversion to agriculture took place (Cully et al., 2003). Today, less than 1% of the original prairie remains (Sampson, 1994). A major consequence of this extensive habitat loss has been a significant increase in the degree of fragmentation of the remaining grasslands. Habitat fragmentation and loss due to human activities has been identified as the most important factors contributing to the decline and loss of species worldwide (Noss and Cooperrider, 1994).

There is a high risk of local extinction associated with small, isolated populations (Heschel & Paige, 1995; Groom, 1998; Paschke, 2002). Population size is important in maintaining genetic diversity (Heschel and Paige, 1995; Luijten et al., 2000; Montgomery et al., 2000; Paschke et al., 2002). Small, isolated populations experience genetic drift and inbreeding depression leading to a loss of genetic variation over time (Heschel & Paige, 1995; Paschke, 2002). These isolated populations are then vulnerable to local extinction because of demographic, genetic, and environmental stochasticity (Heschel & Paige, 1995; Newman & Tallmon, 2001; Paschke, 2002).

Genetic variation is regarded as important in maintaining high levels of fitness. (Reed & Frankham, 2003). Genetic drift and inbreeding depression reduce the heterozygocity of a population (Luijten et al., 2000; Paschke et al., 2002; Primack, 2002). This in turn typically reduces various fitness related traits (Fischer & Matthies, 1998; Fisher et al., 2000; Paschke et al., 2002; Van Rossum et al., 2002; Reed & Frankham, 2003). Reduced fitness is associated with reduced levels of growth, survival, and reproduction (Primack, 2002; Reed & Frankham, 2003) as well as increased susceptibility to stress (Heschel & Paige, 1995). Reduced genetic variation also reduces a population's ability to adapt to changing environmental conditions (Heschel & Paige, 1995) such as drought or flood. High genetic diversity is then crucial in maintaining healthy, viable, and sustainable populations of species.

Numerous studies have documented the effects of population size, fragmentation, and inbreeding depression on rare or endemic species (Herschel & Paige, 1995; Fischer & Matthies, 1998; Morgan, 1999; Eisto et al, 2000; Luijten et al, 2000; Paschke, et al, 2002). But, what is not known is the health of common species. This study investigates the effects of habitat fragmentation and isolation on common species which are usually not of conservation concern. Various fitness traits of six common prairie species were examined to determine whether common species suffer the effects of genetic drift and inbreeding depression. The objectives of this study were to determine whether plants in small isolated populations have reduced fitness as compared to species in a large population. If they do, then this means that land and resource managers must take steps to insure the health of common, as well as rare species in small isolated populations.

Methods

Study Species

Six prairie species were chosen for this study based on their common occurrence in at least two of the four prairies used in this study: *Amorpha canescens* (Fabaceae), or lead plant is a perennial sub-shrub (Gleason & Cronquist, 1991). *Dalea purpurea* (Fabaceae) or purple prairie clover is a self compatible perennial herb (Molano-Flores, 2004; Gleason & Cronquist, 1991). *Eryngium yuccifolium* (Apiaceae), rattlesnake master, is a self compatible perennial herb (Molano-Flores, 2004; Gleason & Cronquist, 1991). *Parthenium integrifolium* (Asteraceae), wild quinine, is a self incompatible perennial herb (Molano-Flores, 2004; Gleason & Cronquist, 1991). *Physostegia virginiana* (Lamiaceae), or obedient plant is a perennial herb (Gleason & Cronquist, 1991). *Pycnanthemum virginianum* (Lamiaceae), is a perennial herb (Gleason & Cronquist, 1991).

Study Sites

Three small and one large site were chosen for this study. Capel Hill Prairie, a 0.5 hectare hill prairie, is a prairie opening in an oak hickory forest and completely isolated from any other prairie patches. It has been chronically isolated for at least a thousand years (I have no reference for this yet). Loda Cemetery Prairie is a 1.5 hectare black soil tall grass prairie remnant. It has been isolated for at least the last 150 years. It is isolated from the nearest tall grass prairie by 6 miles. Prospect Cemetery Prairie is a 3.5 hectare fragment of black soil prairie. It also has been isolated for at least 150 years. The nearest prairie is 2 miles from this fragment. Highway 45 Railroad Prairie is a long but narrow stretch of remnant prairie along a railroad right of way. An equivalent area of this site was chosen because the Highway 45 site is much larger than the others.

Experimental Design

Beginning in August, 2004, 20 to 30 plants of each species were located in each remnant population. The number of main stems, branches, and flowers/fruits per branch was recorded. Fruits with dehising capsules were bagged to prevent seed loss. Total reproductive biomass was collected as fruits matured. Biomass was air dried over a period of four weeks in paper bags, cleaned to remove stems and leaves, and weighed.

From each maternal plant, 25 seeds were weighed to determine average seed weight. Seeds from *Eryngium yuccifolium, Parthenium integrifolium, Physostegia virginiana,* and *Pycnanthemum virginianum* were stratified in moist perlite for 60 days at 0° C. Seeds from *Amorpha canescens* and *Dalea purpurea* were scarified with 200 grit sandpaper to break the seed coat. Seeds were then stratified in moist perlite for 14 days at 0° C. Seeds were placed on wet filter paper in petri dishes and exposed to a day night regime of 14 hours of light at 25° C and 10 hours of dark at 22.5° C. Two seedlings per maternal plant were transplanted into cell packs with Promix© as the potting medium. Seedling root to shoot ratio, percent survival, and total biomass will be measured after 45 days (results pending).

Data Analysis

Total reproductive biomass, seed weight, and germination rate (arcsine transformed) were analyzed for each species using one-way analysis of variance.

Results

One-way ANOVA showed that *Dalea purpurea*, *Parthenium integrifolium*, and *Physostegia virginiana* had significantly smaller seeds in at least one of the small populations (Figures 1-3). *Dalea purpurea* had smaller seeds at Capel Hill Prairie, the smallest isolated fragment, as well as at Prospect Cemetery Prairie ($F_{2,57}$= 8.784 ; P < .001; R^2 = .242) (Figure 1). *Parthenium integrifolium* had significantly smaller seeds in the smallest fragment where it was collected ($F_{2,58}$= 6.159 ; P = .004; R^2 = .178) (Figure 3). *Physostegia virginiana* also had significantly smaller seeds at Prospect Cemetery Prairie, the 3.5 hectare site ($F_{1,24}$= 8.265 ; P = .009; R^2 = .264) (Figure 3).

Total reproductive biomass was significantly smaller in at least one site for two of the six species. Mean reproductive biomass for *D. purpurescens* was significantly smaller at Capel Hill Prairie, the smallest and most isolated fragment ($F_{2,55}$= 37.417 ; P = .000; R^2 = .576) (Figure 5). *Pycnanthemum virginianum* had significantly less total reproductive biomass in Loda Cemetery Prairie, the1.5 hectare fragment ($F_{,2,67}$= 4.208 ; P = .019; R^2 = .115) (Figure 6). Other species were unaffected.

Data have been collected for germination rate for two of the six species so far. *Parthenium integrifolium* had a significantly lower rate of germination at the smallest site compared to the other two sites ($F_{2,55}$= 5.274 ; P = .008; R^2 = .161) (Figure 7). There was no significant difference in mean germination rate (Arcsine square root transformed) of *Amorpha canescens* between the two sites ($F_{1,25}$= 3.634 ; P = .068; R^2 = .127) (Figure 8). Nor was there significant differences in seed weight between the two sites ($F_{1,37}$= 0.457 ; P = .503; R^2 = .012) (Figure 4).

Table 1. One-way analysis of variance of various fitness related traits.

	Source	d.f.	M.S.	F.	P
Dalea purpurescens	Seed weight	2	1.384	8.784	0.000
	Total reproductive biomass	2	5.971	75.127	0.000
Parthenium integrifolium	Seed weight	2	1.651	6.159	0.004
	Germination percentage	2	0.365	5.274	0.008
Physostegia virginiana	Seed weight	1	0.012	8.265	0.009
Pycnanthemum virginianum	Total reproductive biomass	2	0.475	4.208	0.019
Amorpha canescens	Seed weight	1	0.161	0.457	0.503
	Germination percentage	1	0.358	3.634	0.068

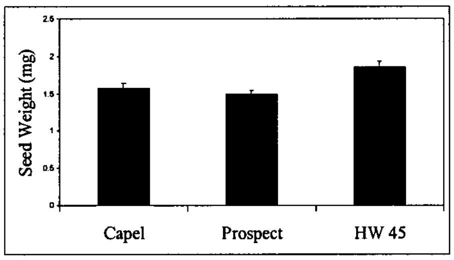

***Figure 1.** Average seed weight of* Dalea purpurea *($F_{2,57}$= 8.784 ;* P *=0 .000; R^2 =0 .242).*

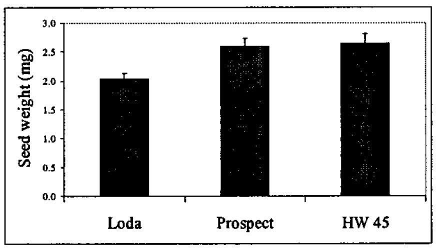

***Figure 2.** Average seed weight of* Parthenium integrifolium *($F_{2,58}$=6.159 ;* P *=0 .004; R^2 =0 .178).*

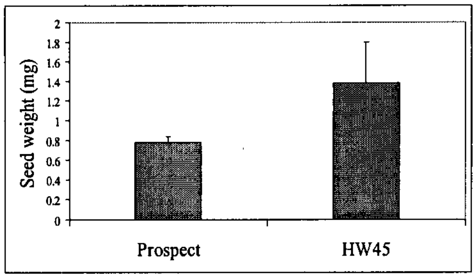

Figure 3. *Average seed weight of* Physostegia virginiana *($F_{1,24}$= 8.265 ; P = 0.009; R^2 = 0.264).*

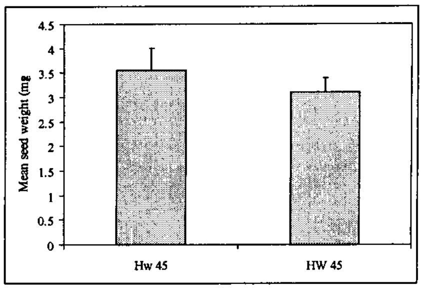

Figure 4. *Average seed weight of* Amorpha canescens *($F_{1,37}$= 0.457 ; P = 0.503; R^2 = 0.012).*

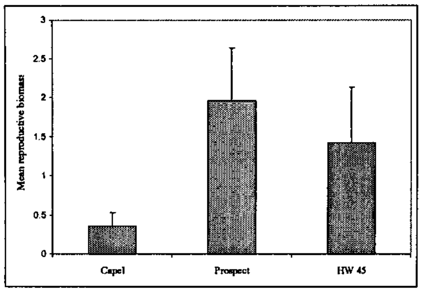

Figure 5. *Mean total reproductive biomass of* Dalea purpurea *($F_{2,55}$= 37.417 ; P = 0.000; R^2 = 0.576).*

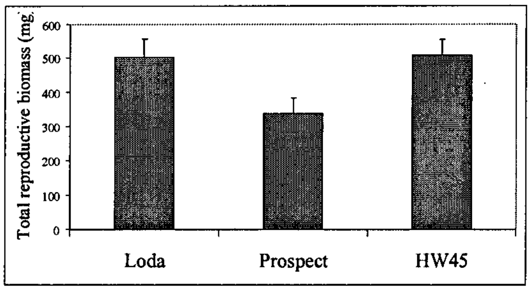

Figure 6. *Mean total reproductive biomass for* Pycnanthemum virginianum *($F_{2,67}$= 4.208 ; P =0 .019; R^2 = 0.115).*

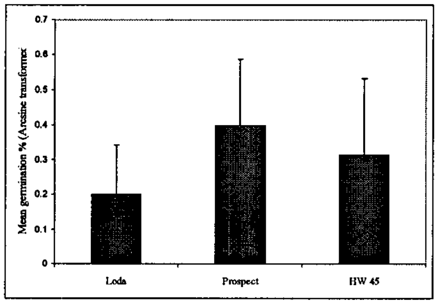

Figure 7. *Mean germination percentage (Arcsine square root transformed) of* Parthenium integrifolium *($F_{2,55}$= 5.274 ; P = 0.008; R^2 = 0.161).*

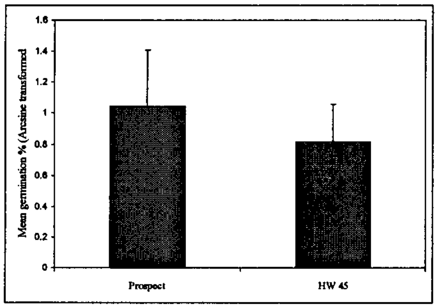

Figure 8. *Mean germination percentage (Arcsine square root transformed) of of* Amorpha canescens *($F_{1,25}$= 3.634 ; P = 0.068; R^2 = 0.127).*

Discussion

Results of investigations of plant fitness in six common species have suggested that common species have suffered the effects of genetic drift and inbreeding depression similar to threatened species. Half of the species in the study had a significant reduction in seed size in plants from smaller populations. One species had reduced total reproductive biomass, and one had reduced germination rate. These results are consistent with those of Heschel and Paige (1995) and Luijten et al (2000) who studied the effects of population size on the relative fitness of rare species. Heschel and Paige showed that mean seed mass was significantly reduced in populations with less than 100 individuals. Luijten et al (1999) found that population size was positively correlated with seed set, seedling size, number of flowering stems, and flower heads, adult survival, and total relative fitness.

Germination trials have not yet concluded so results for this fitness measure are incomplete but *Parthenium integrifolium* has shown reduced fitness in the smaller of the two isolated populations which is interesting. Eisto et al (2000) found that seed germination ability was independent of population size. Morgan (1998) found similar results in *Rutidosis leptorrhynchoides*, button wrinklewort. He found no differences in seed germinability between populations. It is significant then, that a common species like wild quinine had reduced germination rate in a small, isolated population. What this suggests is that land and resource managers must take steps to insure the health of common, as well as rare species in small isolated populations.

Summary

This study examined fitness traits including seed weight, total reproductive output, and germination rate of six common plant species, to determine whether plants in small fragmented populations had reduced fitness as compared to those in larger populations. Fitness traits are reduced in some plant species within fragmented populations suggesting that common species have suffered the effects of genetic drift and inbreeding depression. This suggests that it is no longer just rare species that are of conservation concern, but that all species in isolated habitats of conservation concern. .

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