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Genetic diversity in isolated patches of the tallgrass prairie forb, *Lithospermum canescens* (Boraginaceae)[1]

Pamela M. Kittelson[2,3] and Stephen D. Handler[4]

Department of Biology, Gustavus Adolphus College, St. Peter, MN 56082

KITTELSON, P. M. AND S. D. HANDLER (Department of Biology, Gustavus Adolphus College, St. Peter, MN 56082). Genetic diversity in isolated patches of the tallgrass prairie forb, *Lithospermum canescens* (Boraginaceae). J. Torrey Bot. Soc. 133: 513–518. 2006.—Genetic diversity is not well understood for many species inhabiting isolated, fragmented systems. We evaluated genetic diversity for the mixed mating, native prairie forb, *Lithospermum canescens* (Michx) Lehm. (hoary puccoon), at an isolated tallgrass prairie site in southern Minnesota. Leaf tissue was collected from nearly all individuals inhabiting three disjunct patches and AFLP (amplified fragment length polymorphism) was performed on each individual. A high level of genetic diversity was maintained (Nei's Genetic Diversity, $h = 0.31$), and diversity was similar among the separate populations ($H_T = 0.31$, $H_S = .30$, $G_{ST} = 0.03$) based on 73 scoreable bands from five primer pairs. *Lithospermum canescens* has retained a considerable amount of diversity among the three patches despite existing in isolated patches; pollination by flying insects likely contributed to the genetic similarities among the patches. Our results suggest that seeds from any one patch could be used for restoration of other suitable habitat on the reserve.

Key words: AFLP, gene flow, genetic diversity, habitat fragmentation, *Lithospermum*, population genetic structure, tallgrass prairie.

Genetic diversity within and among plant populations is influenced by mating systems, pollination, population size and spatial distribution of individuals (Hamrick and Godt 1989, Ellstrand and Elam 1993). Generally, obligately outcrossing plants express less population differentiation compared to mixed mating or selfing species (Hamrick and Godt 1989, Gaudeul et al. 2000, Zawko et al. 2001, but see Auge et al. 2001), and insect pollinators tend to increase gene flow and can homogenize diversity among populations that are continuously distributed (Slatkin 1994, Hamrick et al. 1995). However, realized gene flow could be relatively low and/or localized selection strong, thus genetic neighborhoods could arise, especially in populations that experience some selfing or that are spatially isolated (Kerster and Levin 1968, Levin and Kerster 1974, Van Der Merwe et al. 2000, Kittelson and Maron 2001).

As a result of European settlement many natural habitats are small and fragmented, which potentially restricts gene flow, genetic diversity and a species' potential to respond to changing environments (Templeton et al. 1990, O'Brien 1994, Frankham et al. 2002). However, many small populations retain higher than expected heterozygosity and small reserves may play an important role in plant conservation (Lesica and Allendorf 1992). Thus, empirical data would help us better understand if variation declines or is preserved in small 'island' habitats and how genetic variation is structured; neutral markers such as AFLP provide one way to quantify diversity within and among patches of plants (Travis et al. 1996, Gaudeul et al. 2000, Zawko et al. 2001, Oostermeijer et al. 2003).

In the Midwestern U.S., less than 1% of tallgrass prairies remain (Tester and Keirstead 1995, Samson and Knopf 1994). Throughout southern Minnesota, small (< 50 ha), spatially isolated prairie remnants that contain relict plant populations have been set aside as reserves. Little is known about the genetic diversity of prairie plant populations that live in these small preserves. *Lithospermum canes-*

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[3] Author for correspondence. E-mail: pkittels@gac.edu

[4] Current address: University of Montana, College of Forestry and Conservation, Missoula, MT 59812.

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cens (Michx) Lehm., hoary puccoon, is a tallgrass prairie forb; it has a mixed mating system (Johnston 1952) and is patchily distributed so among-patch differentiation might be expected due to genetic drift and inbreeding. In this study we measure the level of genetic diversity within and among the isolated patches of *L. canescens* using AFLPs. Specifically, we ask if an isolated prairie remnant population retains genetic diversity and how this diversity is partitioned among patches.

Materials and methods. STUDY SITE, PLANT DESCRIPTION AND SAMPLING PROCEDURE. This study was conducted at Kasota Prairie in Le Sueur County, MN (T109N, R26W S06). Kasota Prairie is a ~36.5 ha remnant prairie owned by Unimin Corporation and managed by Save the Kasota Prairie. Most prairies in the area were converted to agriculture starting in 1850, but Kasota's shallow, rocky soils were never plowed. Instead, the site was grazed for 100 years. Domestic grazers were excluded in 1984 and active management ensued (e.g., controlled burns, mowing and seeding of a few select grasses and forbs). Kasota supports over 95 species of grasses and forbs, dominated by *Andropogon gerardii, Sorghastrum nutans, Dalea purpea* and *Monarda fistulosa* (Kittelson, unpubl. data). No *Lithospermum canescens* seeds have been introduced at any time during management, thus the patches of *L. canescens* are relictual.

Hoary puccoon, *Lithospermum canescens* (Michx) Lehm., is a native tallgrass prairie forb that grows in well-drained soils between Ontario and Texas (Gleason and Cronquist 1963). Plants at our site range in size from 0.3 to 0.9 m diameter. Individuals have six to 12 stems (each 15–45 cm in length) arising from a woody taproot. We saw no evidence of clonality in the field or in herbarium samples. Longevity of puccoon individuals is unknown, but the lifespan of perennials with similar life history traits and from the same habitat ranged between 5–25 years with most species not exceeding 16 years (Dietz and Schweingruber 2002; Ehrlén and Lehtilä 2002). *Lithospermum canescens* can reproduce as early as the second year (Kittelson pers. obs). Hoary puccoon's bright yellow-orange flowers contain a nectary that can attract insect pollinators including bees and butterflies (Hilty 2003). Plants are heterostylous, making long-styled (pin) or short-styled (thrum) flowers; outcrossed nutlets are produced from pollinations between pin and thrum plants. However, *L. canescens* also can produce nutlets from cleistogamous flowers and chasmogamous, selfed thrum flowers (Johnston 1952). Seed dispersal appears limited and fruits of other *Lithospermum* spp. fall close to the maternal parent (Kerster and Levin 1968, Weller et al. 2000).

Lithospermum canescens was uncommon at Kasota Prairie; we only found five isolated patches on dry, western-facing hillsides and two of these patches contained only one or two plants respectively. We collected tissue from individuals in the other three patches; approximately 200 m separated patches 1 and 2, and 2 from 3, while patches 1 and 3 were 400 m apart. We labeled and collected tissue from all plants that could be identified as a distinct individuals ($n_1 = 9$, $n_2 = 15$, $n_3 = 12$, total $n = 36$). An individual was identified by following all stems back to the root crown; there may have been a one to two additional individuals in patch 2 and 3, but we could not ascertain if they were distinct or connected to other sampled plants. For each tissue sample, we collected four to six apical leaves, stored them on ice and extracted DNA within five hours.

DNA EXTRACTION AND AFLP PROCEDURE. All leaf samples were rinsed with isopropanol and deionized water to kill and remove bacterial or fungal contaminates. We ground 50–80 mg of leaf tissue in liquid nitrogen. DNA was extracted from each sample ($\geq$ 10 ng/μL) using the DNEasy Plant Mini Kit (QIAGEN Inc., Valencia, CA).

We performed AFLP selective amplification of each DNA sample with the IRDye Fluorescent AFLP Kit for Large Plant Genome Analysis (Li-COR Inc., Lincoln, NE); a 1:10 dilution factor was used in the pre-amplification step. AFLP amplification products were loaded into a polyacrylamide gel (6.5%, 0.25 mm thickness). We visualized results using a Li-COR IR^2 Global Edition DNA sequencer and Saga^{MX} Automated AFLP Analysis Software (v. 3.0). We tested 20 primer combinations, but only five combinations yielded bands that were consistently clear and reproducible: M-CTG/E-AAC, M-CTG/E-ACT, M-CTG/E-ACC, M-CTG/E-AGC, and M-CTT/E-ACT.

Table 1. Number of samples collected per patch, percent polymorphic loci and genetic diversity (h) for each patch and the entire sample population of *Lithospermum canescens* at Kasota Prairie, MN. No significant differences in genetic diversity among patches existed ($H = 2.46$, df = 2, $P = 0.29$).

Patch	Number	% Polymorphic loci	Genetic diversity ($h \pm$ SD)
1	9	93.15	0.3110 (0.1535)
2	15	98.79	0.3085 (0.1298)
3	12	95.89	0.2794 (0.1449)
Total	36	99.82	0.3088 (0.1297)

Analyses. From the five primer combinations that provided reproducible bands, we scored a total of 73 clear, unambiguous bands between 80 and 500 bp; weakly stained or non-reproducible bands were not counted. We determined measures of genetic diversity and hierarchical genetic structure using POPGENE v. 1.31 (Yeh et al. 1997). We calculated gene diversity (h) for each band and then averaged h ($\pm$ SD) for the three patches and the whole site following Nei (1973); we tested for significant differences in h using ANOVA on ranks because data did not conform to parametric assumptions. We also calculated unbiased genetic distances (d) between patch pairs (Nei 1978). Total variation was partitioned within and among patches using H-statistics (Nei 1973); a measure of spatial genetic structure among the three patches (G_{ST}) was derived using, $G_{ST} = D_{ST}/H_T = (H_T - H_S)/H_T$, where H_s equals the mean gene diversity partitioned within patches, H_T is the gene diversity for all the individuals, and D_{ST} the average gene diversity between subpatchs; thus, G_{ST} measures the amount of genetic subdivision among patches and is the multi-allelic analogue of F_{ST} (Nei 1987). G_{ST} ranges between 0 and 1, with 0 equaling no genetic differentiation among patches. To test if markers differed significantly between the three patches, we generated bootstrapped confidence intervals for the scored markers by running 1000 permutations to test for significant deviations from $G_{ST} = 0$. We obtained indirect estimates of gene flow (Nm) from G_{ST} values, where $Nm = 0.5(1- G_{ST})/G_{ST}$ (Slatkin and Barton 1989). Nm less that 1 suggests that genetic drift is prevalent enough to fix alternative alleles in different patches (Wright 1969).

Results. Percent polymorphic loci was over 93% in each patch (Table 1). Gene diversity (h) ranged from 0.297 to 0.311 and was similar in each patch, in fact there were no significant difference in h among the three patches (df = 2, $H = 2.46$, $P = 0.29$; Table 1). Also no individuals or patches possessed unique bands. H_T and H_S also were very similar, 0.3093 and 0.2996 respectively, showing that the majority of the variation resided among individuals within patches (H_S). Accordingly, the global G_{ST} value was very low; overall only 3% of the total genetic variation resided among the patches. However, some spatial genetic structure among the patches existed because our 95% confidence interval for G_{ST} did not overlap with zero ($G_{ST} = 0.0314$ with 95% confidence interval = 0.0066). The indirect estimate of the average rate of migration (Nm) between patches was 15.4 individuals/generation. Overall genetic similarity among the patches was reflected in very low values for genetic distance. Pair-wise distances indicated that the three patches were virtually identical; none of the patch pairs differ by more than 1% and no clear relationship between geographic distance and genetic similarity of the three patches was detected (Table 2).

Discussion. Our analysis of neutral AFLP markers from *Lithospermum canescens* found high levels of gene diversity (h; Hamrick and Godt 1989) across Kasota Prairie, which is an isolated prairie remnant where inbreeding and drift might be expected to reduce genetic diversity (Templeton et al. 1990, Travis et al. 1996, Schmidt and Jensen 2000). Similarly, other studies report that geographically isolated or remnant populations are not genetically depauperate or more differentiated (Friar

Table 2. Pair-wise comparisons of Nei's unbiased (1978) measure of genetic distance for the three isolated patches of *Lithospermum canescens* at Kasota Prairie, MN.

Patch	1	2	3
1	—	—	—
2	0.0005	—	—
3	0.0001	0.0025	—

et al. 2000; Muir et al. 2004, Leimu and Mutikainen 2005, Zaghloul et al. 2006). If we assume *L. canescens* longevity is 5–25 years (Ehrlén and Lehtilä 2002) and age of first reproduction is about 3 years, then genetic diversity has likely been preserved in this fragmented, relict population for 6–40 generations. Selection favoring heterozygosity, seed banks and overlapping generations may counteract drift and inbreeding in small populations, and may explain why small and/or fragmented populations retain more diversity than expected (Lesica and Allendorf 1992, Cabin et al. 1998, McCue and Holtsford 1998, Bahulikar et al. 2004).

Our results indicate that patches of *L. canescens* at Kasota Prairie should be considered one panmictic population. Nearly identical values for H_S and H_T, a G_{ST} of 0.031 and low genetic distances all imply that very little genetic differentiation exists among the patches (Hartl and Clark 1997). In this case, the small patches maintained diversity, indicating that global genetic diversity can be maintained in fragmented populations relative to a continuous one of equal size (Maruyama 1972). Our indirect estimate of gene flow was high, $Nm = 15.4$ suggesting that effective outcrossing occurs among the *L. canescens* patches and there is a very low probability that genetic drift fixes alleles in any one patch (Slatkin and Barton 1989, Hamrick et al. 1995). Even though *L. canescens* can produce selfed seeds our genetic data imply that few selfed seeds are produced and/or inbreeding depression may exist. Outcrossing is likely mediated by insect pollinators because *L. canescens* seeds are not animal dispersed, the nutlets fall close to the maternal plant, and in the field we spotted bees and butterflies amid the puccoon patches. These insect pollinators can potentially (Kerster and Levin 1968) and apparently do travel the distances separating our patches.

It is important to keep the spatial scale of this genetic study in mind; while interpatch genetic differentiation was low, interpopulation genetic structure across a larger geographic scale is likely to be higher (Berge et al. 1998; Lammi et al. 1999), thus determining G_{ST} across a larger geographic scale should also be done. Accordingly, it is interesting that G_{ST} is significant even though it is low; small differences among patches could indicate incipient divergence and suggests that continued monitoring of among patch genetic diversity should be maintained.

Even though global genetic diversity would not decrease significantly if one patch went locally extinct, human alteration to tallgrass prairie habitats often occurs across the geographical scale of our whole site and is known to destroy all patches rather than one or two (pers. obs). Moreover, the similarity of neutral markers among the patches does not necessarily mean there are no differences in *L. canescens* life history traits that could affect fitness. While little overall genetic diversity would be lost if one patch of hoary puccoon went locally extinct, it is unknown if any unique morphological or physiological traits differ among the patches.

Our data suggest that small remnant populations of some plants can maintain high levels of genetic diversity and are valuable for conservation. It is important to preserve the small patches of *L. canescens* because they retain the diversity of the whole population. Managers could take seed from any patch and use it to establish new patches on or near the reserve. Additionally, it appears that insects play an important role maintaining genetic diversity of *L. canescens*, thus managers should continue to focus on maintaining pollinator abundance and diversity.

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