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Conservation Genetics of Remnant Lilium philadelphicum Populations in the Midwestern United States

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ABSTRACT.-In recent decades, an increasing number of plant species have been negatively affected by anthropogenic habitat fragmentation and disturbance. In many cases, the habitat matrix between populations has been converted from a natural to an urban environment, One such species, *Lilium philadelphicwm* (Liliaceae) a showy perennial with a naturally patchy distribution, currently has populations in parts of its range in North America that persist on highly urbanized and fragmented landscapes. In this study, we used six nuclear microsatellite loci to characterize the amount and apportionment of genetic diversity among 12 remnant populations in the Midwest United States. Genetic diversity was high (7-31 alleles per locus, mean H_O = 0.44-0.70). An analysis of molecular variance (AMOVA) detected a low level of genetic structure (F_{ST} = 0.06, $P < 0.001$), and no effect of isolation by distance among sites (r^2 = 0.02, P = 0.28). Principle coordinate analysis (PCoA) of inter-individual generic distances revealed essentially no structuring with PC axes one and two explaining only 22.5 and 19.7% of the observed variation respectively. Moreover, Bayesian exploration of population structure supported this observed lack of structure with a low optimum number of estimated genetic "clusters" *(e.g.,* populations; $K = 1$). If habitat fragmentation does affect gene flow among populations we cannot yet detect a strong genetic signature of this process; most likely due to the recency of landscape disturbance relative to the long generation time of this species. These results suggest that the genetic composition of these remnant populations is relatively homogenous and as such, provides land managers with a large potential germplasll1 source with a broad genetic base for use ill local restoration activities.

INTRODUCTION

Habitat destruction has become a leading threat to North American flora and fauna in the last 100 y (Noss and Csuti, 1994). Although many important habitats and ecosystems have been successfully protected, biological refugia are becoming increasingly isolated *(i.e.,* "insularized") as the land around them becomes highly modified and/or urbanized by human activities (Van Rossum, 2007; DiBattista, 2008). In some cases, widespread continuous natural areas have become a network of widely distributed island refugia separated by large tracts of land not suitable for native organisms (Pickett *et al.,* 2001; Wang and Moskovits, 2001).

The potential demographic (Lande, 1988; Oosterrneijer, Luijten and Nijs, 2003) and genetic effects of habitat fragmentation on plant and animal populations have been well documented (Couvet, 2002; DiBattista, 2008 and references therein). But, even though the potential effects of habitat fragmentation are generally well understood, not all plant species respond similarly (Aguilar *et al.,* 2006; Honnay andJacquemyn, 2007). First, life stages *(e.g.,* juveniles versus adults) may be affected differently by fragmentation, and processes such as demographic bottlenecks and historical isolation may confound analyses of con temporary gene flow (Aldrich *el. al.,* 1998; Sork *et al.,* 1999;Jacquemyn *et al.,* 2004). The ecological and life history characteristics of a plant species-such as mating system, pollinator in teractions

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and local abundance-strongly influence how it will be affected by habitat fragmentation (Young, Boyle and Brown, 1996; Young, Brown and Zich, 1999; Bacles, Lowe and En nos, 2004; Lienert, 2004; Aguilar *et al.,* 2006; Honnay and Jacquemyn, 2007; Steffan-Dewenter and Westphal, 2008). For example, habitat fragmentation may not decrease g-ene flow among patches of individuals for wind pollinated tree species (Berge, Nordal and Hestmark, 1998; Bacles *et al.,* 2005; Craft and Ashley, 2007). Even so, habitat alteration may lead to pronounced inbreeding under some circumstances (McKay *et al.,* 2005). For example, habitat alteration can have profound genetic consequences for patchily distributed plants if the matrix surrounding existing patches affects movement of pollinators (or seed dispersers) among them; although as with plants, not all pollinators may be affected similarly (Didam *et al.,* 1996; Kocher and Williams, 2000; Wood and Pullin, 2002; Steffan-Dewenter and Westphal, 2008). It is a modification of the habitat between populations or patches of individuals that is especially relevant for highly vagile or patchily distributed species.

The recent application of a variety of molecular genetic techniques to address questions regarding natural populations has been particularly fruitful in provided insights into the genetic consequences of insularization for many plant species (Tecic *et al.,* 1998; Gustafson, Gibson, and Nickrent, 1999,2002; Ge *et al.,* 2003; Rottenberg and Parker, 2003; Gustafson, Gibson and Nickrent, 2004; Honnay and Jacquemyn, 2007). In particular, nuclear microsatellite loci have been successfully applied to address the potential population genetic consequences of habitat fragmentation on a variety of spatial and temporal scales (Aldrich *et al.,* 1998; Friar *et al.,* 2001; England *et al.,* 2002; Craft and Ashley, 2007). A common predicted effect of habitat fragmentation is that as large contiguous populations become divided into smaller isolated sub populations and that levels of dispersal *(i.e.,* gene flow) may be reduced among them (Honnay and Jacquemyn, 2007; DiBattista, 2008). This reduced gene flow may lead to decreased genetic diversity within subpopulations (dele to genetic drift and inbreeding), increased genetic divergence among subpopulations (Soule, 1980; Templeton *et al.,* 1990; Ellstrand and Elarn, 1993), possible deleterious fitness consequences and extirpation (Keller and Waller, 2002; Lienert, 2004 and references therein).

The goals of this study were to characterize the amount and apportionment of genetic diversity among remnant populations of *Lilium philadelphicum* (Wood or Prairie lily) distributed on a highly disturbed urban landscape the Midwest USA *(e.g.,* the Chicago metropolitan region). *Lilium philadelphicum* is a long-lived insect pollinated perennial and has a naturally patchy distribution, and thus offers a unique opportunity to investigate the potential effects of anthropogenic disturbance on patterns of genetic structure and gene flow among populations on a local spatial scale. Because this species is butterfly pollinated it is possible that this high degree of urbanization has reduced pollinator movemen t among habitat patches and indirectly reduced gene flow among *Lilium* populations. This study is unique because although many studies of the potential genetic consequences of habitat fragmentation have focused on populations distributed on relatively natural and/or agricultural habitats (Tecic *et al.,* 1998; Custafson, Gibson and Nickrent, 1999), few have been conducted on urban landscapes (Craft and Ashley, 2007; Culley, Sbita and Wick, 2007; Van Rossurn, 2007). Due to catastrophic habitat loss and urban development, *L. philadelphicum* is imperiled in the Midwest and many other parts of its range in North America (USDA - NRCS, 2008). The results of this study will provide baseline genetic data for Midwest populations that, because of their rarity (Swink and Wilhelm, 1994; USDA - NRCS, 2008), have important conservation priorities.

MATERIALS AND METHODS

STUDY SPECIES

Lilium philadelphicum L. is distributed across much of the United States and Canada and across a large portion of its range it is listed as threatened or endangered (USDA - NRCS, *2008). lilium philadelphicum* is a long-lived perennial restricted to mesic soils of relatively undisturbed tallgrass prairies and oak savannas in the Midwest, U.S.A. and the Prairie Provinces of Canada. It produces 1-4 flowers per individual and blooms throughout June and July, while vegetative reproduction is limited to the formation of bulbs. Seeds have a small wing-like structure and are most likely wind dispersed over very short distances (on the order of meters). It has been characterized as a butterfly pollinated obligate outcrosser (Edwards and Jordan, 1992). There are two commonly recognized varieties of this lily (Fernald, 1950; Barkley, 1986; USDA - NRCS, 2008) variety *andinum* (Nutt.) Ker Gawl is generally described as the western form and variety *philadelphicuan* is described as the eastern form, however there is significant overlap of the ranges of both forms. In this study, our research efforts are focused on *L. philadelphicum* var. *andinum* populations in northeastern Illinois and northwestern Indiana (Fig. 1).

TISSUE SAMPLE COLLECTION, SAlvIPLE SITES AND POPULATION DESCRIPTIONS

During the summers of 1999-2001 we collected leaf tissue samples from 13 *Lilium philadelphicum* populations in northeastern Illinois and northwestern Indiana (Fig. 1). Leaves (1 to 2 per individual) were either immediately frozen on dry ice and stored at *-80* C or dehydrated and stored in silica gel desiccant until needed for the genetic analyses. We collected voucher specimens at a subset of the sample sites and deposited them in the. Marion Ownbey Herbarium at Washington State University, Pullman (Accession numbers 356559-356586) .

Lilium philadelphicum is of great interest to local land managers and therefore many populations in this region are closely monitored. Moreover given the extensive magnitude of disturbance and urban development, available habitat for this species is extremely limited; essentially almost all populations are found within various county forest preserves or state parks. Consequently, the collection sites analyzed here most likely represent the majority of known extant *L. philadelphicum* populations in the Chicago metropolitan area. Because of the local rarity of this species, some of the collection sites had very small populations. Consequently due to small sample size, one site was omitted from the current analysis (Peotone) although its location is documented in this study for reference (Fig. I). Rather than eliminate additional critical populations because of small sample sizes, in three cases we conservatively combined them into synthetic populations for a subset of statistical analyses *(e.g.,* AMOVA). Given their extremely close geographic proximity (distance between sites given in parentheses) and assumed historical population connectivity, collections were combined to provide larger sample sizes as follows; Somme and Chicago Botanic Garden (4.7 krn), Gibson Woods and Tolleston Ridges (l.2 km), DuPont and Ivanhoe (1.2 km). Critically, we conducted the Bayesian estimations of hierarchical population structure on all samples without *a priori* population designations; these analyses supported the AMOVA and PCoA results and demonstrated that the few synthetic populations did not affect our findings *(see* below).

DNA EXTRACTION

Genomic DNA was extracted hom dried or frozen (-80 C) leaf tissue following a modified hexadecyltrimethy ammonium bromide (CTAB) extraction protocol (Doyle and

FIG. 1.—Locations of the 13 remnant *Lilium philadelphicum* populations in the Midwest sampled for this study. The location "Peotone" was dropped from this current analysis because of insufficient sample size but is shown in this figure for reference (*see* text for details). Light grey polygons are the boundaries of municipalities of the Chicago metropolitan area (largest centralized polygon is the city of Chicago proper) and illustrate the extremely high magnitude of urbanization of this landscape

Doyle, 1987), Briefly, leaf tissue (~10 mg) was ground to a fine powder **in** liquid nitrogen and combined with 600 ul hot (~55 C) CTAB buffer and 5.0 ul Proteinase K (Roche; Nutley, NJ), After a 2 h incubation at 65 C, 600 ul of chloroform-isoamyl alcohol (24:1, Sigma; St. Louis, MO) was added and each sample was vortexed then centrifuged for 5 m at

12,000 rpm. The aqueous phase was collected then precipitated with 1 ml of cold 95% ethanol and washed once with 500 ul 75% ethanol. The pelle ted DNA samples were dried in a vacuum centrifuge and re-suspended in 50 ul 1 x TE buffer.

MICROSATELLTE GENOTYPING

Six polymorphic microsatellite loci previously characterized for this species (Horning, Maloney and Webster, 2003) were amplified from the *Lilium philadelphicum* samples via Polymerase Chain Reaction (PCR). PCR reactions (10 ul volume) were performed on a Perkin-Elmer 9700 DNA thermo cycler and consisted of approximately 50 ng genomic DNA, 0.50 uM each of forward and reverse primers, 0.15 mM dNTP (each), 1.5-3.0 mm $MgCl_2$ (see below), 50 mm KCL, 10 mm Tris-HCL and 1.5 U of *Taq* DNA polymerase. Cycle parameters were 94 C for 3 m followed by 30 cycles of 94 C for 1 m, annealing temperature *(see* below) for 1 m, 72 C for 45 s, followed by a final extension step of 72 C for 5 m. Specific amplification conditions varied among loci, consequently reaction conditions were established as reported in Horning *et al.* 2003. PCR products were combined with the GENESCAN-500 TAMRA (Applied Biosystems; Foster City, CAl internal size standard, loaded into nitrocellulose membrane combs (The Gel Company Inc.; San Francisco, *CA),* and electrophoresed in 4.5% (29:1 acrylarnide: Bis-acrylamide, 6 M Urea) polyacrylamide gels (AutoMatrix^{TM,} National Diagnostics Inc.; Atlanta, GA) on an ABI 377 automated sequencer. Gel images were collected and processed in Genescan® v3.l.2 (Applied Biosystems) and individual genotypes at each locus were determined using Genotyper® v2.5 (Applied Biosystems).

STATISTICAL ANALYSES

Individual diploid genotypes were identified using Genotyper v2.5 (Applied Biosystems). For each locus we calculated the observed allele frequencies and the observed (Ho) and expected (HE) levels of heterozygosity with Fisher's exact test using GenePop (Raymond and Rousset, 1995). Deviations from Hardy-Weinberg equilibrium (HWE) were assessed using Markov Chain Monte Carlo simulations with the following parameters; dcmemoriza-tion = 1000, batches = 100, iterations = 1000. Genotypic linkage disequilibrium between loci was also assessed for single populations and across all populations using Fisher's exact test and the same chain parameters. Because preliminary analyses revealed significant deviations from HWE at several loci, we used the software program Micro-Checker (van-Oosterhout, 2004) to identify the possible presence of null alleles and other potential genotyping errors. By analyzing the distribution of homozygote excess among homozygote classes across loci, this program can differentiate between deviation from panmixia *(e.g.,* inbreeding) and other causes of deviations form HWE *(e.g.,* large allele drop out, null alleles). The presence of null alleles was detected at multiple loci in all but two populations. Consequently, following the inference key in van Oosterhout (2004) we used the Brookfield estimator (Brookfield, 1996); Brookfield equation 2 in van Oosterhout 2004) to estimate the frequencies of null alleles and to calculate adjusted allele frequencies and genotypes (to account for the downward allele size bias due to nulls) for subsequent analyses. In short, this procedure produces adjusted allele frequencies that take into account the presence of a null allele at a locus. Critically, we ran the statistical analyses with both the original and adjusted allele frequencies and our results were the same; this finding supports the use of the more appropriate adjusted allele frequencies. Additionally, this also indicates that the possible presence of a null allele at a given locus may not have a strong affect in our analyses *(see* below).

Estimates of differentiation across multi-locus genotypes (Wright's F_{ST}) were computed via analysis of molecular variation (AMOVA; (Peakall and Smouse, 2006) and references therein). To determine if genetic divergence between populations was associated with their geographic distance, we conducted a Mantel matrix correlation test using genetic (Nei's *D)* and geographic (km) distance matrices. Additionally, principal coordinate analysis (PCoA) was to explore multivariate relationships among inter-individual genetic distances and to identify a set of reduced-dimension traits *(e.g.,* PC eigen vectors): These analyses were conducted using GenAlex version 6 (Peakall and Smouse, 2006).

We used hierarchical Bayesian estimation of population structure as implemented in the software program Geneland 2.0.12 (Guillot, Mortier and Estoup, 2005) to further explore the data without *a priori* constraints *(e.g.,* population designations) that are associated with standard estimates of populat.ion structure. This approach is an individual-based iterative clustering analysis that employs a Markov-Chain Monte Carlo (MCMC) iteration to assign individuals to genetic clusters *(K* classes). We ran 12 independent *(K* = 1-12 where 12 is the maximum number of populations given our sampling) runs each with 10^6 iterations and a 20,000 iteration burn-in interval. Additionally we ran a second analysis that simultaneously explored *K* = 1-12 with the same MCMC parameters.

RESULTS

MICROSATELLITE VARIABILITY

The six microsatellite loci employed in this study were polymorphic in all eight populations (Table 1). There were significant heterozygote deficiencies at one or more loci in each of the eight populations (Table 1). In general, these heterozygote deficiencies may be due to null *(e.g.,* non-amplifying) alleles (Brookfield, 1996; van Oosterhout., 2004), or to inbreeding caused by restricted gene flow between patches or non-random mating within patches (Hartl and Clark, 1989). We detected the potential pl·esence of null alleles in all but three populations; nulls were not detected in Fermi Lab, Chicago Botanic Garden/Somme Prairie and Hoosier Prairie. No null alleles were detected at the Lpca5 locus. For the remaining loci, possible nulls were only detected in one to three populations. However, for locus Lpca20104 and locus Lpga4, nulls were detected in the majority of populations. Therefore, null alleles may in some instances contribute to significant heterozygote deficiencies at two loci (Lpca20104 and locus Lpga4). Consequently, we estimated the frequency of null alleles using the Brookfield estimator as described above and adjusted allele frequencies and genotypes as necessary. Critically, results obtained using the original allele frequencies and the adjusted frequencies were nearly identical in all analyses (data not shown). However, even though our results were not markedly different, we report the more conservative estimates that take the possible presence of null alleles into account (Pemberton *et al.,* 1995). Linkage disequilibrium was only detected between Lpga210 and Lpca870, however this estimate was considered non-significant after a sequential Bonferroni correction (Rice, 1989).

POPULATION DIFFERENTIATION

AMOVA of multi-locus genotypes revealed that the majority of the observed genetic variation was partitioned within populations, with weak but statistically significant genetic differentiation among populations (F_{ST} = 0.06, $P < 0.01$). Pair-wise estimates of F_{ST} ranged between 0.01 and 0.16 (Table 2), and all but two pair-wise comparisons were statistically significant; both comparisons involved Censburg-Markham Prairie. Even though these inter-

TABLE 1.—Characteristics of six microsatellite loci in eight *Lilium philadelphicum* populations. Table includes: the number of genotypes (n), allelic diversity (A), expected level of heterozygosity under Hardy-Weinberg Equilibrium (H_E), observed level of heterozygosity (H_O). Statistically significant deviations from HWE are (* = $0.50 < P < 0.01$; ** = $P < 0.01$)

		Lpga210	Lpca870	Lpga9	Lpca20104	Lpga4	Lpca5
Howe's Prairie	n	29	25	29	16	28	29
	A	6	13	5	5	9	4
	H_O	0.66	0.68**	0.66	0.38**	0.71**	0.48
	H_E	0.75	0.85	0.63	0.68	0.82	0.47
Wolf Rd. Prairie	n	20	20	20	11	17	20
	A	5	8	8	6	8	4
	H_O	0.85	0.70	0.80	0.55**	0.53**	0.75
	H_E	0.70	0.76	0.82	0.77	0.72	0.58
Fermi Lab	n	5	5	5	4	3	5
	A	3	3	5	2	2	3
	H_O	0.60	0.20	1.00	1.00	0.00	0.40
	H_E	0.46	0.46	0.72	0.50	0.44	0.54
Somme/Chicago Botanic	n	11	11	11	10	11	11
Garden	A	4	5	4	5	4	3
	H_O	0.82	0.82	1.0**	0.60**	0.36**	0.91
	H_E	0.69	0.74	0.66	0.71	0.64	0.60
Gensburg-Markham	n	19	17	19	15	16	17
	A	7	14	7	6	10	5
	H_O	0.63	0.65*	0.58	0.67**	0.63**	0.71
	H_E	0.80	0.90	0.74	0.78	0.84	0.53
Gibson/Tolleston	n	22	22	21	17	17	20
	A	7	12	6	5	12	4
	H_O	0.59	0.73	0.57**	0.59**	0.59**	0.60
	H_E	0.73	0.80	0.73	0.75	0.80	0.55
Hoosier Prairie	n	9	9	9	9	9	9
	A	6	5	5	4	6	3
	H_O	0.89	0.78	0.56	0.78	0.33	0.22
	H_E	0.76	0.78	0.59	0.70	0.46	0.20
Illinois Beach State Park	n	17	19	17	21	17	19
	A	8	16	5	5	13	7
	H_O	0.53	0.68**	0.59	0.71**	0.47**	0.79
	H_E	0.68	0.87	0.75	0.78	0.89	0.67
Ivanhoe/DuPont	n	12	12	6	7	6	8
	A	4	13	4	3	7	4
	H_O	0.58	0.75**	0.50**	0.43	0.33**	0.50
	H_E	0.68	0.90	0.71	0.44	0.83	0.67
Mean	H_O	0.68	0.67	0.70	0.63	0.44	0.60
	H_E	0.69	0.78**	0.71	0.69**	0.72**	0.53

population comparisons are statistically significant, these sites are highly similar based on all other analyses and this result may be an artifact of microsatellite markers (Hedrick, 1999).

Analysis of inter-individual distances by Principal Coordinate Analysis confirmed there is little to no differentiation among populations *(e.g.,* the "clouds" of individuals almost

TABLE 2.—Pairwise F_{ST} estimates among *Lilium philadelphicum* populations (below diagonal), and interpopulation genetic distance; Nei's *D* (above diagonal). Based on 999 permutations, F_{ST} values were significant (* = 0.05 > P > 0.01; ** = P < 0.01) for all but two comparisons (denoted^{ns})

	Howe's Prairie	Wolf Rd. Prairie	Fermi Lab	Somme/CBG	Gensburg-Markham	Gibson/ Tolleston	Hoosier Prairie	Illinois Beach S.P.	Ivanhoe/ DuPont
Howe's Prairie	–	0.29	0.52	0.29	0.19	0.29	0.41	0.33	0.51
Wolf Rd. Prairie	0.08**	–	0.26	0.18	0.12	0.12	0.22	0.16	0.35
Fermi Lab	0.15**	0.06**	–	0.32	0.34	0.26	0.48	0.36	0.27
Somme/CBG	0.08**	0.03*	0.09*	–	0.21	0.22	0.37	0.18	0.43
Gensburg-Markham	0.04**	0.01^{ns}	0.07**	0.04*	–	0.11	0.27	0.17	0.34
Gibson/Tolleston	0.08**	0.01*	0.06**	0.04**	0.01^{ns}	–	0.24	0.14	0.23
Hoosier Prairie	0.13**	0.06**	0.16**	0.11**	0.07**	0.07**	–	0.35	0.52
Illinois Beach S.P.	0.08**	0.02**	0.08**	0.03*	0.02**	0.02*	0.09**	–	0.36
Ivanhoe/DuPont	0.12**	0.08**	0.07**	0.10**	0.06**	0.05**	0.15**	0.07**	–

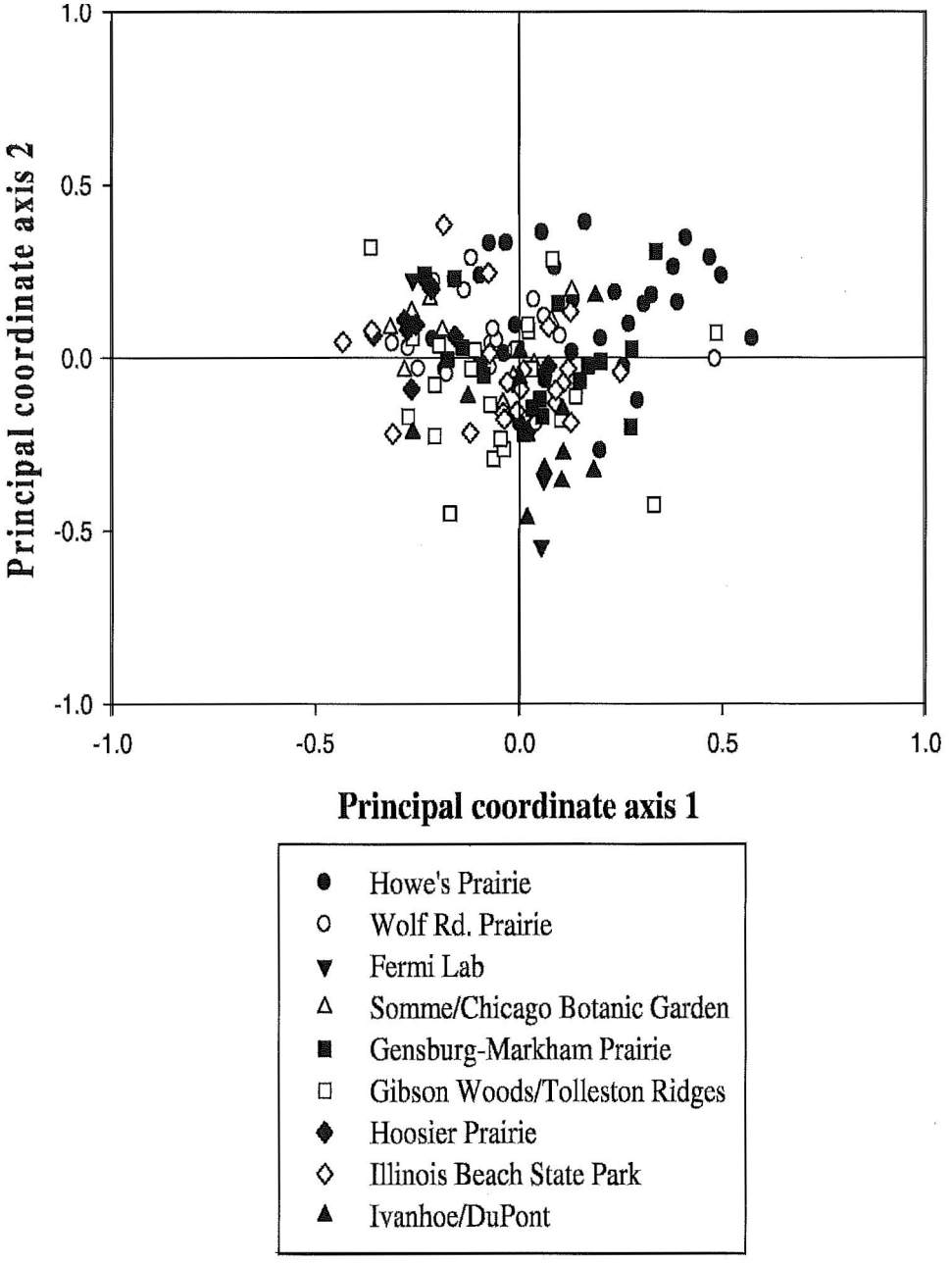

FIG. 2.-Results of Principal coordinate analysis (PCoA) of inter-individual genetic distances among the 149 individuals analyzed in this study. PC axes 1 and 2 explain 22.5% and 19.7% of the observed variation respectively

completely overlap); this indicated a lack of structure with the first and second axes explaining nearly equivalent levels of variation (22.5% and 19.7%, respectively; Fig. 2). The overall relationship between among-population genetic distance (measured as Nei's *D,* Table 2) and geographic distance was not significant (Mantel $r^2 = 0.02$; $P = 0.28$).

Bayesian estimates of population structure supported the low level of differentiation as measured via AMOVA. For the 12 independent runs the average estimated posterior probabilities ranged from log -3303.68 ($K = 1$) to log -5328.27 ($K = 12$). The highest average estimated posterior probability was associated with $K = 1$, therefore the optimal number of populations was estimated to be one. Moreover, the simultaneous estimation also identified $K = 1$ as the optimum number of classes.

DICUSSION

GENETIC VARIABILITY AND POPULATION DIFFERENTIATION OF LILIUM PHILADELPHICUM

For these remnant lily populations, there was a weak but statistically significant estimate of genetic structure ($F_{ST} = 0.06$, $P < 0.01$). Our overall estimates were lower than those reported for other rare perennials analyzed using allozymes *(Rutidosis leptorrhynchoides,* FST = 0.17), nuclear microsatellites *(Grevillea macleasana,* FST = 0.218, and *Gymnadenia odoratissima,* $F_{ST} = 0.19$), and ISSRs *(Physaria bellii,* $F_{ST} = 0.24$) found on fragmented landscapes (Young, Brown, and Zich, 1999; England *et al.,* 2002; Gustafsson and Sjogren-Culve, 2002; Kothera, Richards and Carney, 2007). The multilocus F_{ST} estimate we report. is also much lower than those reported for other rare plant species analyzed on fragmented/ disturbed habitats with various molecular marker systems (Tecic *et al.,* 1998; Rottenberg and Parker, 2003; Tero, 2003). The small amount of observed variation explained by the PCoA further supports the pattern of weak or absent differentiation among sites as estimated using AMOVA (Fig. 2). Additionally, our Bayesian exploration that did not rely on *a priori* population designations supports these findings ($K = 1$). Taken together, these analyses indicate that these remnant *L.philadelphicum* populations in the Chicago metropolitan area are genetically similar as measured with these highly variable nuclear microsatellite loci.

In this study we identified a relatively high level of genetic variation at six SSR loci and demonstrated the utility of the markers we developed to address population genetic questions. In general, observed heterozygosities were lower than expected heterozygosities in the eight populations included in this study (Table 1). At two of the six loci (Lpca20104 and Lpga4), t.his reduction in observed heterozygosity may be due to a relatively high frequency of non-amplifying alleles. However, analyses with the original genotypic frequencies and those accounting for the possibility of null alleles did not differ significantly in magnitude and statistical significance. Critically, observed genetic diversity was high and this has important significance for potential restoration germplasm (Jones, 2005; Dolan, Marr, and Schnabel, 2007; Fant *et al.,* 2008).

Our results do not indicate a pronounced effect of habitat disturbance on the degree of genetic differentiation and subsequent gene flow among populations as our estimates of differentiation are moderate on an absolute scale (Hartl, 1980). It is possible that pollinators of *Lilium philadelphicum* have maintained their historical and potentially long-distance dispersal capabilities on this newly urbanized landscape and that these estimates reflect a contemporary level of population differentiation (Wood and Pullin, 2002; Steffan-Dewenter and Westphal, 2008). On the other hand, it is possible that, (a) pollinator dispersal may be limited by the extremely high degree of disturbance in such a way that would promote greater genetic differentiation among *L. philadelphicum* populations (Ries, 2001), and (b) existing native habitats are managed in ways that may alter pollinator

abundance (Panzer, 20(0) and that we have yet to indirectly measure this. Additionally, anthropogenic habitat disturbance/destruction in the Midwest has primarily occurred within the last ~ 150 y. This is a "recent" landscape disturbance relative to the longevity of *L. philadelphicum* that is typical of many herbaceous perennials. Consequently, there may be a lag in the detectable genetic signal and future characterizations of gene flow and population structure may more accurately reflect the effects of habitat modification on pollinator movement. This is similar to other genetic studies of plants distributed on disturbed landscapes where it appears that fragmentation has possibly led to genetic differentiation among populations, but the signal of historical processes *(e.g.,* connectivity via gene flow) has not been erased (Honnay *et al.,* 2(06).

The urbanization of the landscape surrounding habitat patches may affect levels of genetic diversity and population structure in ways other than reducing pollinator movement. Similar to other *Lilium* species (Linhart, 1994), in many parts of its range on natural landscapes *L. philadelphicum* is freq uen tly found in small patches (< 10 individuals), although in some instances patches can be larger (100 + individuals). In a closely related species with very similar seed pod morphology *E1ythronium grandiflorum,* Weiblan and Thomson (1995) found that primary seed dispersal was highly restricted to within one meter of the parent plant. Therefore, it is possible seed dispersal is naturally limited in *L. philadelphicum* and populations will most likely only spread via seed within remnants rather then between them. Consequently, it is likely that genetic differentiation among populations will increase over time, albeit slowly in this perennial species with long generation times.

IMPLICATIONS FOR CONSERVATION AND RESTORATION

It is important to note that historically disturbance was integral in the Tallgrass Prairie and Oak Savanna ecosystems that *Liliuni philadeiphicum* inhabits, Both naturally occurring *(e.g.,* lighting strikes) and anthropogenic *(e.g.,* burning by Native Americans) fires, grazing by large mammals and burrowing by small mammals created the landscape that today is preserved in small remnants (Collins, 1987; Ewing and Engle, 1988; Gibson, 1989). Much of this disturbance is absent today (controlled burning is an important management tool) and there still are many anthropogenic threats to the persistence of *L. philadelphicum* in the Midwest even though most populations are protected from habitat destruction and/or loss in county nature preserves and state parks. Many existing populations are small and are endangered within these preserves by a variety of disturbances *(e.g.,* collection by visitors, herbivory) that may eliminate recruitment via sexual reproduction in any given flowering season. Moreover, urban deer populations are large and often concentrate in remnant natural habitats; subsequent grazing can cause considerable damage to these sites (Fletcher, 2001; Cote *et al.,* 2004; Anderson *et al.,* 2005). Consequently, the conservation and management of this species and other rare plants is of great interest to land managers throughout the region.

The genetic characterization of neutral genetic diversity and differentiation of remnant populations we present here is essential for identifying potential germ plasm for use in restoration practices (Lesica and Allendorf, 1999; Rogers and Montalvo, 2004; Jones, 2005; Dolan, Marr and Schnabel, 2007; Fant *et al.,* 2008). Our results indicate that none of the sampled populations are highly genetically divergent or unique in terms of the neutral genetic diversity contained within them. Therefore any existing sites, especially larger self-sustaining populations, could serve as *in situ* germplasm sources that could be used to augment other populations or used to re-introduce *Liliumphiladelphicum* to new or previously occupied sites.

Because these populations occupy such narrow latitude, elevational and climactic zone, and many populations exist on sites with similar ecological characteristics; germplasm could be utilized throughout this narrow geographic region. However, even at this small spatial scale local ecotypic variation could exist; additional common garden studies would be needed to confirm this (Hufford and Mazer, 2003; Holderegger, Kamm and Cugerli, 2006).

CONCLUSIONS

In this study we characterized the nuclear genetic diversity and degree of population differentiation of a long lived Lily species surviving in remnant prairie patches on a highly urbanized landscape. Our results suggest that genetic diversity is fairly high as measured by neutral nuclear microsatellite loci; however, population differentiation is weak over the small spatial scale sampled. Given the longevity of this species and the relatively recent- albeit dramatic-habitat loss, these markers are most likely providing estimates of historical levels of population connectivity, that apparently was high, and yield insight into past processes on a previously undisturbed landscape. Critically, because these remnant populations are essentially genetically homogenous and persist in ecologically similar locations, potential germplasm sources with a broad genetic base exist *in situ* for use in restoration throughout the Chicago metropolitan region.

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