Genetic diversity and distribution of *Sarracenia purpurea* (Sarraceniaceae) in the western Lake Superior basin

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Abstract: Restoring plant populations requires an understanding of plant morphological adaptation to site locations and population genetic diversity and relatedness. This study examined the genetic and morphological diversity of *Sarracenia purpurea* L. within the natural fragmentation of western Lake Superior. Populations of *S. purpurea* were compared among three locations: Isle Royale National Park, the Keweenaw Peninsula, Michigan, USA, and Sleeping Giant Provincial Park, Ontario, Canada. Analysis of genetic and demographic data showed Canadian populations to be less robust with smaller plant sizes. Canadian populations were also slightly distinct genetically. Overall genetic diversity appears moderate (H = 0.30–0.36) and populations genetically similar. Analysis of molecular variance showed only 3.83% of variation among the three locations (p = 0.0049). Fragmentation did not have a distinguishable effect on genetic diversity and morphological characters but the limestone bedrock geology of the Canadian region may be starting to influence plant morphology and genetic differentiation. This indicates that restoration can take place within the western basin of Lake Superior using a variety of seed sources but regional geology may influence observed plant morphology.

Key words: genetic diversity, intersimple sequence repeats, Isle Royale National Park, Sleeping Giant Provincial Park, *Sarracenia purpurea*, restoration.

Résumé : La restauration des populations végétales nécessite une compréhension des adaptations morphologiques à la localisation des sites, ainsi que de la diversité de la génétique des populations et de leur parenté. Les auteurs ont examiné la diversité génétique et morphologique du *Sarracenia purpurea* L., dans le cadre de la fragmentation naturelle de l'ouest du lac Supérieur. Ils ont comparé les populations du *S. purpurea* entre trois localités; le parc national de l'Île Royale et la péninsule de Keweenaw, au Michigan en Ètats-Unis et le parc provincial de Sleeping Giant, en Ontario au Canada. L'analyse des données génétiques et démographiques montre que les populations canadiennes sont moins robustes, avec des plantes de plus petite dimension. De plus les populations canadiennes diffèrent légèrement, génétiquement. Dans l'ensemble, la diversité génétique semble modeste (H = 0,30–0,36) et les populations génétiquement similaires. L'analyse AMOVA ne montre que 3,83 % de variation parmi les trois localités (p = 0,0049). La fragmentation n'a pas eu d'effet apparent sur la diversité génétique et les caractères morphologiques, mais la géologie, avec sa roche mère calcaire dans la région canadienne, pourrait commencer à influencer la morphologie des plantes et la différenciation génétique. Ceci indique que la restauration peut s'effectuer dans le bassin ouest du lac Supérieur, en utilisant une variété de sources de semences, mais aussi que la géologie peut influencer la morphologie des plantes observées.

Mots clés : diversité génétique, séquences inter-simples répétées, parc national de l'Île Royale, parc provincial de Sleeping Giant, *Sarracenia purpurea*, restauration.

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Introduction

Wetland habitats around the world are experiencing severe ecological pressure through destruction, fragmentation, and degradation owing to human activities; more than half of all US wetlands have been lost since the 1800*s* (Mitsch and Gosselink 2000). The valuable nature of wetland ecosystems and the current threats they face place them near the top of the list for conservation and restoration (Zedler 2003).

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In an increasingly fragmented landscape, wetlands, both created and remnant, are becoming isolated ecosystems, a unique challenge for restorationizts. Genetic variation is an important conservation factor, providing the necessary materials to allow populations to respond to environmental pressures. Low variation can potentially reduce a species' ability to respond to environmental change and increase its susceptibility to outside pressures (Barrett and Kohn 1991; Huenneke 1991). Isolated and island populations tend to experience reduced genetic variability for a variety of reasons: small population sizes, founder effects, and low dispersal rates from neighboring populations (Frankham 1997). The distance between an island population and corresponding mainland, and the potential dispersal distance of a plant, affect genetic variation within these isolated populations. Island populations tend to have lower levels of genetic variation, although very few studies exist that focus particularly

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on plant species (Frankham 1997). Restored populations often exist as isolated in the landscape. To guarantee the long-term survival of a restored population, it is imperative to maintain natural levels of genetic variation (Barrett and Kohn 1991; Huenneke 1991). Aspects of a target restoration species' genetic variation need to be analyzed: how are individual populations differentiated, is there an associated adaptive value to that differentiation, and what is the potential for inbreeding and outbreeding depression in restored populations (Barrett and Kohn 1991). Understanding plant populations' genetic variation will help maximize genotype choice in the selection of material for restoration while minimizing the potential for inbreeding depression and lower genetic variation.

Sarracenia purpurea L. (Sarraceniaceae), the northern pitcher plant, is a primary component of northern peatlands. The wetland habitat of this plant species, bogs and poor fen peatlands, tends to exist as islands isolated in the landscape both spatially and hydrologically (Schwaegerle and Schaal 1979). The habitat type is rare in Illinois, Indiana, Ohio, and lower Michigan. *Sarracenia purpurea* is endangered in Illinois and there is interest in restoring its habitat and reintroducing this plant where it has been extirpated. To create a viable restoration plan, we need to understand relationships between individual populations, potential gene exchange, and inherent genetic variability. Previous studies using allozyme electrophoresis to examine founder effects on *S. purpurea* discovered moderate genetic diversity within populations (Schwaegerle and Schaal 1979). Schwaegerle and Schaal (1979) noted a high level of differentiation between populations unrelated to geographic distance. This would indicate both isolation with limited gene flow and, potentially, site specificity. High differentiation in this study could also potentially be due to sampling within two recognized subspecies of *S. purpurea* (Schnell 2002). A second study conducted on introduced *S. purpurea* populations in Switzerland showed low differentiation between populations, even at distances up to 30 km (Parisod et al. 2005). Given the discrepancies between these studies, this character should be examined for each potential restoration area. Strong founder effects have also been observed in this species from introductions of very small populations (one to three individuals) (Schwaegerle and Schaal 1979; Taggart et al. 1990). Populations of *S. purpurea* likely moved back into the Great Lakes region following the Pleistocene glaciation. Populations existed during the glaciation period in restricted areas around Maryland and in potential refugia throughout its historic range (Juniper et al. 1989). Historical reductions in *S. purpurea* genetic diversity in its more northern range have not been examined. Understanding local genetic diversity and gene flow patterns within the Great Lakes region will assist in creating successful and viable management plans.

The purpose of this study was to further explore the inherent variability of *S. purpurea* in relation to the isolation of populations within the Great Lakes region and to help develop a future restoration plan for extirpated species. We examined populations of *S. purpurea* on the Keweenaw Peninsula, Michigan, USA, Isle Royale National Park, USA, and Sleeping Giant Provincial Park, Ontario, Canada (Fig. 1). Populations on Isle Royale National Park, located within Lake Superior, are separated from mainland populations by approximately 26 km to the Canadian Peninsula and approximately 70 km to the Keweenaw Peninsula. We used a molecular genetics technique, intersimple sequence repeats (ISSRs), to examine within- and between-population genetic variability across this extreme isolation event. ISSRs have the potential to be highly variable and be useful in assessing phylogenetic relationships and to evaluate variation between populations (Zietkiewicz et al. 1994). ISSRs are neutral markers, which do not illustrate direct trait adaptation but are useful in examining overall genetic patterns between and within populations (Wolfe 1998; Wolfe and Liston 1998). To have a physical basis for comparison between populations, we also measured morphological characters such as leaf number and flower number in an attempt to define an index of individual plant fitness, potentially in relation to measured genetic variability.

We hypothesized that plant populations located on Isle Royale would exhibit greater genetic differentiation if gene flow does not occur across the barrier of Lake Superior. The populations on the Keweenaw Peninsula and the Canadian Peninsula would be more closely related owing to potential gene exchange through populations in northern Wisconsin and Minnesota. Also, we hypothesized reduced genetic variability on Isle Royale in response to any potential founder effect as seen in previous studies of *S. purpurea*.

Materials and methods

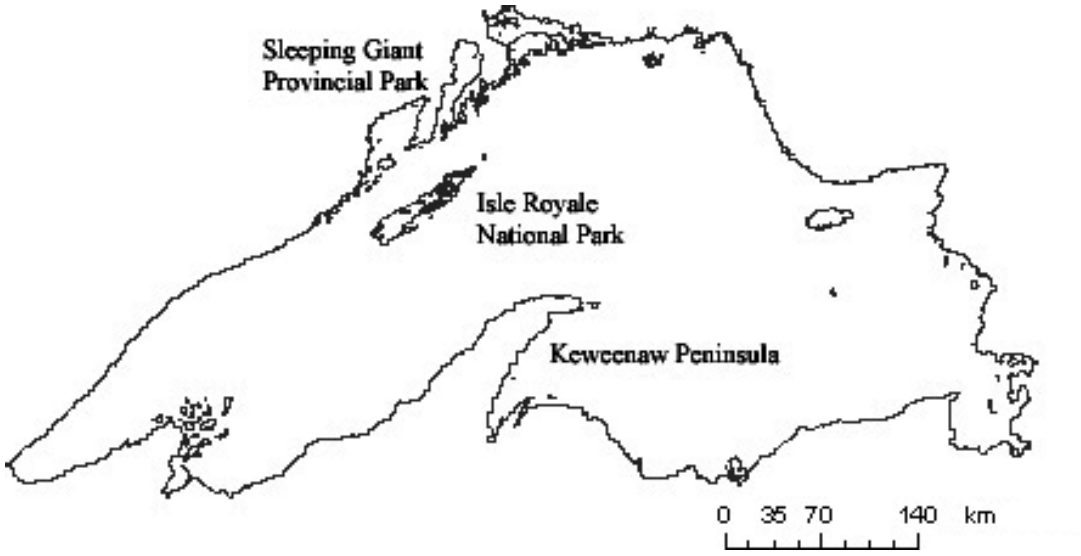
Study species

Sarracenia purpurea grows both in extremely acidic environments existing in floating bogs and poor fens and also in alkaline fen systems (Mandossian 1965) in the northern United States. Most of the nutrients needed to sustain plant growth are obtained from the digestion of certain insects within the plant pitcher. The interaction between the insect community and these plants is long established and a vital part of ecosystem function. *Sarracenia purpurea* primarily grows clonally via short rhizomes and sometimes sexually through seed production (Schwaegerle and Schaal 1979; Sheridan 1993; Godt and Hamrick 1996). Pollination is conducted mainly by the common queen green bee in early spring (Schnell 2002), but there appears to be a lack of seedlings establishing in most populations (Mandossian 1966*a*). Much past research has defined an individual *S. purpurea* plant as a rosette of 1–12 leaves. Plants are usually found in a clump of more than one plant, producing two or more flowers with the newest leaves emerging from the center of the plant (Mandossian 1965). Seed dispersal for this plant averages 5 cm from the parent plant (Ellison and Parker 2002), apparently limiting long-distance dispersal.

Data collection

Between May and June of 2003, five field sites were established in each of the three geographic study locations: Keweenaw Peninsula, Michigan, Isle Royale National Park, Michigan, and Sleeping Giant Provincial Park (total $n = 15$ sites). On each site, two 50 m transects were established, one running north–south through the center of the system and the other running east–west intersecting at the midpoint of the wetland. This distance typically encompassed the en-

Fig. 1. Lake Superior and the three study locations: Keweenaw Peninsula, Isle Royale National Park, and Sleeping Giant Provincial Park.



tire wetland from edge to edge. All *S. purpurea* plants within 1 m on either side of the transect line were tagged; a basal rosette clump was considered a single plant. We recorded number of leaves, number of flowers, and height of flower stalk for each tagged plant. Sites were revisited in August to record fruit number. During the first visit to each site, we randomly collected tissue samples from 30 of the surveyed plants at each site for genetic analysis (n = 450). Collected tissue samples consisted of the tip of a younger pitcher, smaller and greener than the older, rougher leaves. Tissue collected in the field was kept at –80 °C until DNA extraction could be performed.

DNA extraction and PCR protocol

DNA was extracted using the Qiagen DNeasy Plant Mini Kit (Qiagen, Germantown, Maryland). Samples were labeled and stored at –20 °C. ISSR primers were purchased from the University of British Columbia Biotechnology Laboratory and screened to choose eight polymorphic primers that showed reproducible and variable bands for analysis: primers 825, 836, 842, 854, 861, 868, 872, and 881 from SSR set No. 9. ISSRs were chosen for their ease of use, reliability, and reproducibility (Zietkiewicz et al. 1994). ISSRs are neutral markers and the strength of statistical analysis of neutral markers depends, in part, on reproducibility (Wolfe and Liston 1998). During primer screening, each primer was run through an independent PCR reaction twice with representatives of each population. Any bands only present in one of the repeats were deleted from analysis. Of the initial bands scored in the first primer screening, 80% were polymorphic, reproduced in the second screening.

Tissue from a random subsample (n = 30) of three populations from each of the three locations was used for analysis (n = 263 samples, one wetland contained only 26 plants): Threemile (TM), Perrault Bog (PB), and Boston Wetland (BW) on the Keweenaw, Kalmia Bog (KB), Lake Ojibwa (LO), and Wallace Lake (WL) on Isle Royale, and Rita Marsh (RM), Pickerel Lake North (PN), and Middleburn Fen (MF) on the Sibley Peninsula. DNA was amplified using PCR and each of the eight primers. The PCR Master Mix was a 15 μL reaction consisting of 2 μL of DNA, 1 μL of *Taq* DNA polymerase, 2.5 μL of reaction buffer, 1.9 μL of $MgCl_2$, 0.25 μL of dNTPs, 6.35 μL of double-distilled water, and 1 μL of primer. PCR was conducted with an initial denaturing of 4 min at 94 °C followed by 1 min at 72 °C, 1 min at 42 °C, and 1 min at 94 °C for 45 cycles. Resulting PCR product was then separated on a 1% agarose electrophoresis gel, stained in ethidium bromide, and visualized using a digital camera. The images were imported into a computer to analyze variable bands.

Data analysis

Multivariate analysis of variance was used to examine variability of morphological patterns among the three locations (Isle Royale, Keweenaw, and Canada) because of the likely interrelatedness of the four measured traits: leaf number, flower number, fruit number, and flower height. A Tukey analysis of means was then used to examine how variability was partitioned among the three locations through differences in means.

For the ISSR data, a total of nine populations were examined using eight primers. Polymorphic ISSR bands were scored as 1 (present) and 0 (absent) for purposes of data analysis. ISSRs are a dominant marker system where bands are identified as present or absent and determination of homozygosity versus heterozygosity is impossible. Owing to the nature of this marker system, all analyses are assumed to be dominant, diallelic markers in Hardy–Weinberg equilibrium (Lynch and Milligan 1994).

Using POPGENE version 1.31 (Yeh et al. 1997), we calculated two genetic diversity measures, Nei's measure of genetic diversity (H), $H = 1 - \Sigma p^2_i$, and Shannon's information index (I) $I = \Sigma p_i \log p_{i,}$ where p_i is the frequency of alleles in a population (Lewontin 1972) assuming Hardy–Weinberg equilibrium.

To assess variation in genetic diversity within individual populations and among the different locations, we used Arlequin version 2.00 (Schneider et al. 2000) to conduct an analysis of molecular variance.

We conducted a Mantel test using TFPGA version 1.3

Fig. 2. Means of flower number (*a*), leaf number (*b*), fruit number (*c*), and flower height (*d*) of *Sarracenia purpurea* in relation to location. Canadian populations are significantly different from Isle Royale and Keweenaw populations for all characters ($\alpha = 0.05$, $p < 0.001$).

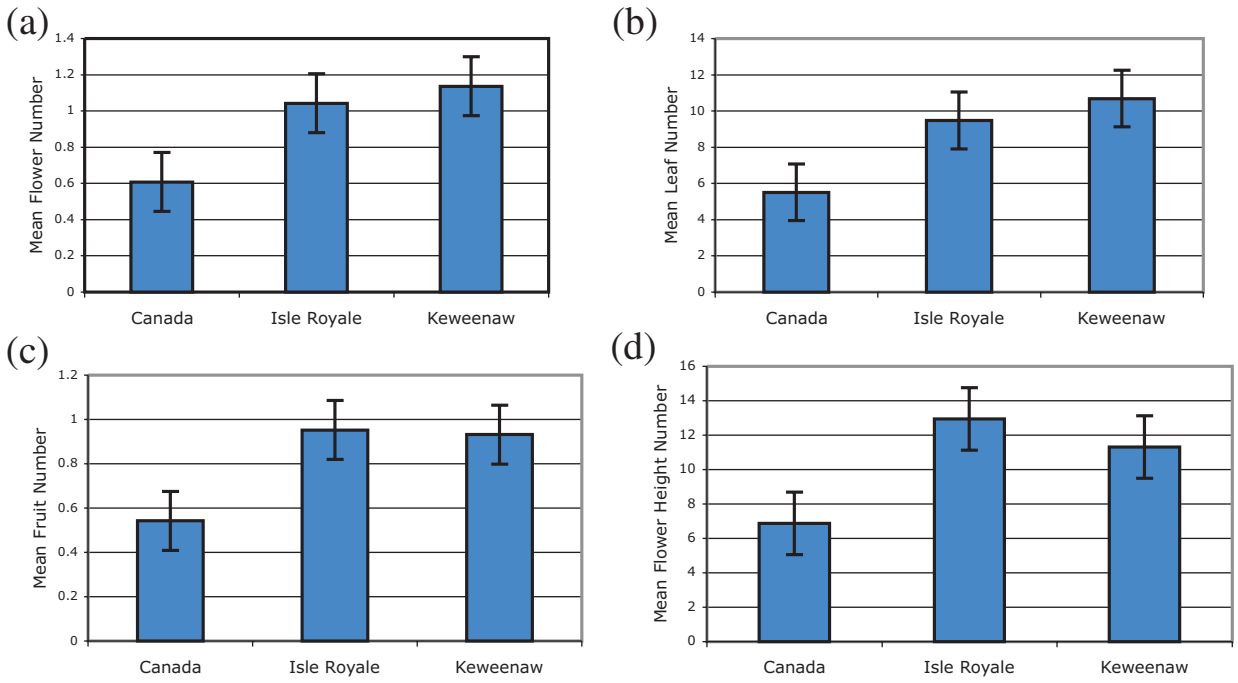


Table 1. Percent polymorphic loci, Shannon's index of diversity (*I*), and Nei's genetic diversity index (*H*) for individual populations and for each location for *Sarracenia purpurea*.

Location	Population	% polymorphic loci	Total % polymorphic loci	*I* (±SE)	Total *I* (±SE)	*H* (±SE)	Total *H* (±SE)
Isle Royale	Kalmia	88.24		0.47 (±0.21)		0.32 (±0.15)	
	Ojibwa	85.29	**97.06**	0.43 (±0.23)	**0.49 (±0.16)**	0.29 (±0.17)	**0.32 (±0.13)**
	Wallace	76.47		0.39 (±0.27)		0.27 (±0.19)	
Keweenaw	Boston	70.59		0.34 (±0.27)		0.23 (±0.19)	
	Perrault	82.35	**97.06**	0.42 (±0.26)	**0.46 (±0.20)**	0.28 (±0.17)	**0.3 (±0.16)**
	Threemile	79.41		0.42 (±0.24)		0.28 (±0.17)	
Canada	Pickerel	91.18		0.51 (±0.23)		0.35 (±0.17)	
	Rita	91.18	**97.06**	0.49 (±0.23)	**0.52 (±0.18)**	0.33 (±0.17)	**0.36 (±0.15)**
	Middlebrun	91.18		0.48 (±0.22)		0.32 (±0.16)	

Note: Values for each location (Isle Royale, Keweenaw, Canada) are in bold.

(Miller 1997) to examine the relationship between genetic and geographic distance. The dominant nature of ISSRs requires the use of band matching similarity coefficients to determine genetic distance. Genetic distance was calculated using Dice's (1945) band matching coefficient. These measures of genetic distance were visualized using UPGMA cluster analysis to create a neighbor-joining tree of all populations and of the three locations using NTSYSpc version 2.2 (Rolf 2005).

To examine possible clonality and clonal diversity in each population, we used GENOTYPE and GENODIVE (Merriman and VanTienderen 2004) to get the number of genotypes per population and calculate a Shannon–Wiener corrected diversity index.

Results

Analysis of the morphological data used a multivariate analysis of variance to compare the four character traits (leaf number, flower number, fruit number, and flower height) across locations (Isle Royale, Keweenaw, and Canada). Flower number, leaf number, fruit number, and flower height all showed a significant relationship to location ($p < 0.0001$). Using a Tukey analysis of means, we analyzed the variation in the demographic characters among the three locations. Examination of the Tukey statistics in relation to location showed the demographic characters of Isle Royale and the Keweenaw to be similar and both of these significantly different from the Canadian populations whose means were consistently lower ($\alpha = 0.05$) (Fig. 2). This pattern was observed for all morphological characters.

Eight ISSR primers were used to examine the nine populations (total $n = 263$). From the eight primers, 34 reproducible bands were scored with 97.1% of bands showing polymorphism. Levels of polymorphism varied between individual populations from 70.6% up to 91.2% (Table 1).

Table 2. Analysis of molecular variance table of genetic diversity for three *Sarracenia purpurea* population groups: Isle Royale, Keweenaw, and Canada.

Source of variation	df	Variance	Percentage of variation	*p*
Among groups	2	0.21937	3.83	0.0049
Among populations within groups	6	0.76753	13.39	<0.0000
Within populations	254	4.7432	82.78	<0.0000
Total	262	5.7301		

Genetic diversity statistics were examined at three levels: within populations, within groups of populations based on location (i.e., Isle Royale, Keweenaw, and Canada), and among all populations. The nine individual populations showed a very narrow range of both diversity indices examined, Shannon's and Nei's. The narrow range of these indices prompted examination of pooled populations based on location. Nei's genetic diversity ranged from 0.23 to 0.35 and Shannon's ranged from 0.34 to 0.51 (Table 1). Both indices are still very close in range, indicating little difference in genetic diversity between these locations. The level of observed diversity was moderate to moderately high for a clonal species in all populations.

The analysis of molecular variance showed that only 3.83% of the observed variance was due to genetic variation among the three locations, indicating that the populations are fairly similar to each other ($p = 0.0049$) (Table 2). The majority of the variation (82.8%) was represented in differences among individuals within populations ($p = 0.0000$).

The identity of genetic individuals was analyzed using GENOTYPE and GENODIVE. The programs identify unique genotypes in each population and thus indicate the level of clonality in each population. We sampled a large number of individuals in each population; the number of genotypes ranged between 20 and 29 for an average sample size of 30 plants (Table 3). Only one population, Boston on the Keweenaw Peninsula, showed a high level of clonality with only eight genetic individuals. This indicates that we sampled a wide range of genotypes and saw a high diversity of genotypes in most populations.

A Mantel test was conducted to examine possible relationships between genetic distance and geographic distance among all nine populations. Genetic distance did not correlate significantly with geographic distance ($r^2 = 0.061$, $p = 0.075$), so as geographic distance increased, there was not a correlative increase in genetic dissimilarity.

A UPGMA neighbor-joining tree based on Dice's (1945) genetic distance coefficients was created for individual populations and for the three locations (Fig. 3). Using all populations, no distinct patterns of separation of populations occurred. Overall confidence in this tree was low because genetic distances were so close. A tree created of just the three locations broke Canada out with a distance of 0.03 and Keweenaw and Isle Royale were closely related (Fig. 3).

Discussion

Considering all three locations (Isle Royale, Keweenaw, and Canada), we observed relatively moderate to high levels of genetic diversity for a potentially clonal species using Shannon's index of diversity (Table 1). An examination of genotypes showed a wide range of individuals in each population (Table 3). We also saw very low amounts of genetic differentiation between populations (3.83%, $p = 0.0049$), indicating that overall populations are fairly similar to each other but are genetically diverse within each population. These numbers are similar to those discovered between two introduced populations of *S. purpurea* in Switzerland (Parisod et al. 2005). Distant populations show great similarity even though seed dispersal appears to be very low (Ellison and Parker 2002). This pattern does not necessarily suggest gene flow. Populations are diverse but each individual population contains the same diversity seen in other populations regardless of geographic distance. This indicates that genetic drift has not significantly altered the genetic diversity of populations. Clonal reproduction is likely still very important but has also not reduced the diversity of genotypes within the populations.

Table 3. Shannon–Wiener's corrected diversity index and number of genotypes observed in each population.

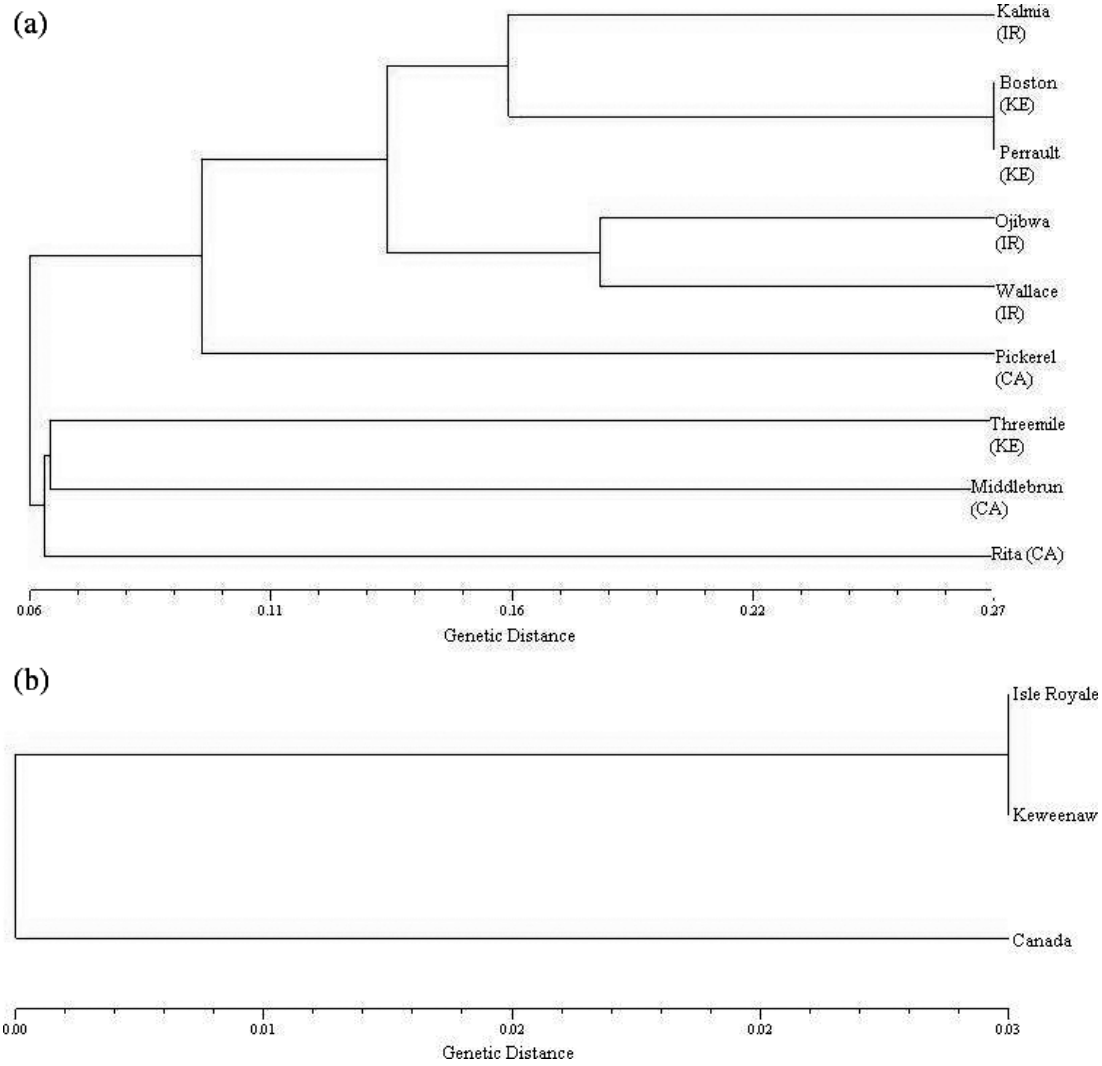
Population	Sample size	No. of genotypes	Shannon–Wiener's corrected diversity index
Kalmia	30	29	2.640617
Ojibwa	30	23	1.764375
Wallace	30	25	1.960506
Boston	30	8	0.689949
Perrault	30	22	1.699266
Threemile	29	20	1.574762
Pickerel	30	27	2.186221
Rita	29	23	1.856454
Middlebrun	25	24	2.479304

Note: Most populations had low sampled clonality and high diversity of genotypes observed.

These small differences observed between populations are contrary to those discovered in studies of *S. purpurea* in the United States and Ireland (Schwaegerle and Schaal 1979; Taggart et al. 1990). Studies ranging over the northeastern United States found that a large portion of genetic diversity existed between populations of *S. purpurea* (Schwaegerle and Schaal 1979). This study encompassed populations both north and south of the last glacial advance into the United States. Historically, it is thought that populations of *S. purpurea* recolonized from just south of the glacial extent following glacial retreat (Juniper et al. 1989). It has long been recognized that two subspecies of *S. purpurea* exist, a northern and a southern species, that intersect at the glacial maximum (Wherry 1933). Our study only examined the northern subspecies, *Sarracenia purpurea* subsp. *purpurea*. Differences observed in our study versus those encompassing a broader ranger of populations could be a reflection of a historical bottleneck and reintroduction from select species.

The small differences that do occur between populations seem to be primarily separating the Canadian populations from the Keweenaw and Isle Royale populations as observed in the UGPMA neighbor-joining tree (Fig. 3). Interestingly, this separation of Canadian populations corresponds to observed morphological differences between populations. Overall, Canadian populations appear to be

Fig. 3. UPGMA dendrograms based on genetic distance calculated with Dice's (1945) band matching coefficients for all nine sampled *Sarracenia purpurea* populations (*a*) and the three locations (*b*).



morphologically smaller than Keweenaw and Isle Royale populations with smaller leaf number, flower number, flower height, and fruit number (Fig. 2). These differences between populations in terms of plant development might well be due to differences in physical location. Previous studies of *S. purpurea* have shown it to be fairly plastic, exhibiting different leaf and flower characteristics depending on the pH of the substrate (Mandossian 1966*b*). The Sibley Peninsula in Canada is underlain by a mixture of limestone, sandstone, and other calcareous geologic material of the Sibley Group (Pye 1969), contributing to a more basic soil. *Sarracenia purpurea*, in its more northern ranges, tends to grow in fairly acidic areas, but occasionally, it inhabits alkaline fens in the Great Lakes region (Mandossian 1965). Both the Keweenaw and Isle Royale are composed of similar sets of bedrock and quaternary geology, making their bog–fen ecosystems fairly similar. Morphological differences between Canadian and Keweenaw and Isle Royale populations are perhaps due in part to these different growing conditions. Measures of pH were not taken for this study but can be assumed to be more acidic in the Keweenaw and Isle Royale populations than in Canadian populations. Variations in both genetics and morphology are likely related back to the geologic setting of the species and their relation to their environment. The observed genetic differentiation between Canadian populations and Keweenaw and Isle Royale populations indicates some process creating genetic differences, but observed morphological differences cannot be directly correlated with morphological measures. Owing to the neutral nature of our marker system, we can only observe that both genetic and morphological differentiation exists between the Canadian and Keweenaw and Isle Royale populations of *S. purpurea*. Quaternary and bedrock geology have been shown to play an important role in wetland type development and often to control wetland type through limitation of certain landscape characters such as water input, pH, etc. (Bedford 1999).

We were unable to elucidate any actual patterns of genetic dispersal across the fragmentation barrier of Lake Superior owing to lower levels of genetic differentiation between populations. The lack of differentiation between Isle Royale and the Keweenaw is not necessarily due to current gene flow between populations. In light of the similarity of environments, populations in both regions are likely being

subjected to the same environmental stimuli. Examined populations perhaps have not had enough time or experienced enough pressure to force genetic differentiation since the last glaciation event (Ridley 1993; Young et al. 2000), and genetic drift owing to isolation of populations does not appear to be playing a big role in altering the genetic composition of individual populations.

Overall, *S. purpurea* populations in the western Lake Superior Basin appear to have moderate to high genetic diversity for a supposed clonal species and to be fairly similar genetically. Patterns seen in this region likely reflect the glacial history of the area and genetic and morphological differentiation is perhaps being observed as populations become adapted slowly over time to differing physical locations. Levels of genetic diversity and genetic similarity observed in this study indicate that preservation should occur on the individual level and that restoration can take place within the western basin of Lake Superior using a variety of seed sources. This is presented with caution to attempt to utilize seed sources from similar physical locations. Also, in terms of restoration outside the upper Great Lakes, more populations need to be examined to ensure successful restoration. A further step to evaluating this plant would be to examine genetic relatedness across a larger scale in the hopes of teasing out more patterns of genetic diversity than those observed here. Studies related to a variety of geologic settings would help to further illustrate patterns shown in this study and will help further understanding of the reproductive ecology of *S. purpurea*.

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