

Population genetic structure of the Gulf of St. Lawrence aster, *Symphyotrichum laurentianum* (Asteraceae), a threatened coastal endemic

Stephen B. Heard, Linley K. Jesson, and Kirby Tulk

Abstract: The Gulf of St. Lawrence aster (*Symphyotrichum laurentianum* (Fernald) G.L. Nesom) is an endemic annual of saline habitats in the southern Gulf of St. Lawrence. It is listed as a threatened species, and has recently experienced population declines in much of its range. We used 11 allozyme markers to assay population genetic variation in six wild populations of *S. laurentianum* from the Magdalen Islands, Quebec (QC), the only remaining wild population from Prince Edward Island National Park (PEI), and a greenhouse population founded in 1999 with seed collected from PEI. *Symphyotrichum laurentianum* harbours moderate genetic diversity ($P_s = 0.36$, $A_s = 1.54$), with only modest spatial genetic structure (pairwise $F_{ST} < 0.15$) and no significant isolation by distance. The PEI population had greatly reduced allelic diversity compared with the populations from the Magdalen Islands, which likely act as a reservoir of genetic variation in *S. laurentianum*. Recent loss of alleles during population decline in PEI is suggested by the retention of greater allelic diversity in the greenhouse population. Estimates of breeding structure suggest small but nonzero rates of outcross pollination ($F_{IS} = 0.73$, 95% CI = 0.48–0.97; outcrossing rate ~16%). Population genetic structure in *S. laurentianum* can inform those forming and carrying out conservation and recovery plans for this threatened species.

Key words: population genetics, conservation, genetic diversity, Gulf of St. Lawrence aster, endemic.

Résumé : L'aster du Golfe St-Laurent (*Symphyotrichum laurentianum* (Fernald) G.L. Nesom) est une endémique annuelle d'habitats salins, dans le sud du Golfe St-Laurent. On la retrouve sur la liste des espèces menacées, et l'on observe récemment un déclin de ses populations dans plusieurs régions de son aire de distribution. Les auteurs ont utilisé 11 marqueurs allozymiques pour évaluer la variation génétique des populations dans six populations indigènes du *S. laurentianum* provenant des Îles de la Madeleine (QC), de la seule population indigène résiduelle du Parc National de l'Île du Prince-Édouard (PEI) ainsi que d'une population établie en serre en 1999, à partir de semences venant de PEI. Le *S. laurentianum* présente une variation génétique modérée ($P_s = 0,36$; $A_s = 1,54$), avec seulement une modeste structure génétique spatiale ($F_{st} < 0,15$ par paire) et aucun isolement significatif par la distance. La population de PEI montre une diversité allélique plus réduite comparativement à celles des Îles de la Madeleine, qui agissent vraisemblablement comme réservoir de variation génétique pour le *S. laurentianum*. La conservation d'une plus grande diversité allélique dans la population maintenue en serre suggère une perte récente d'allèles au cours du déclin de la population de PEI. Une estimation des structures de fécondation suggère des taux de pollinisation croisée faibles mais supérieurs zéro ($F_{is} = 0,73$; 95 % CI = 0,48–0,97; taux de croisement ~16 %). La compréhension de la structure génétique des populations du *S. laurentianum* peut servir de base pour la conservation et la réhabilitation de cette espèce menacée.

Mots-clés : génétique des populations, conservation, diversité génétique, aster du Golfe St-Laurent, endémique.

[Traduit par la Rédaction]

Introduction

The Gulf of St. Lawrence aster (*Symphyotrichum laurentianum* (Fernald) G.L. Nesom) is a halophytic annual endemic to the southern Gulf of St. Lawrence, with

populations in the Magdalen Islands (=Îles-de-la-Madeleine) of Quebec (QC); in Prince Edward Island (PEI); and in New Brunswick (NB) (Fig. 1). It is currently listed as “threatened” under Canada’s Species at Risk Act, but since receiving that status it has experienced marked population declines in much of its range. Threats to *S. laurentianum* populations include habitat loss, both excessively high and excessively low rates of natural and anthropogenic disturbance (populations often thrive on microhabitats opened by recent disturbance, such as ice scour or all-terrain vehicle traffic, but once established do not tolerate further disturbance well), and locally high rates of seed predation (e.g., Houle and Belleau 2000; Reynolds and Houle 2003; COSEWIC 2004; Steeves et al. 2008). A recovery plan is in place for New Brunswick and a national recovery plan is

Received 15 March 2009. Published on the NRC Research Press Web site at botany.nrc.ca on 3 November 2009.

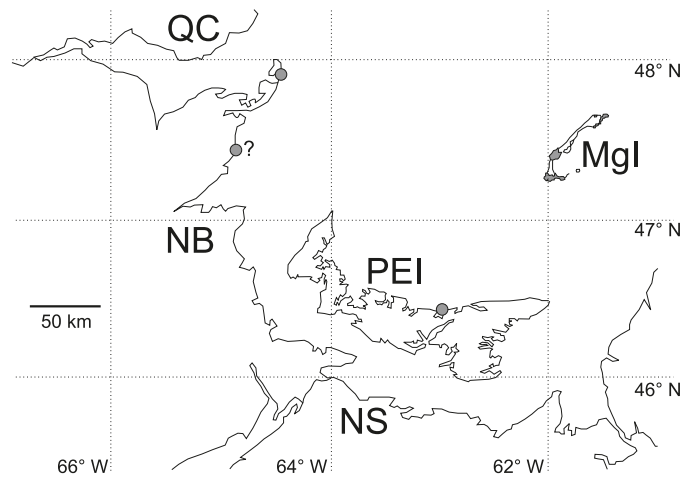
S.B. Heard¹ and L.K. Jesson. Dept. of Biology, University of New Brunswick, Fredericton, NB E3B 5A3, Canada.

K. Tulk.² Prince Edward Island National Park, 2 Palmer’s Lane, Charlottetown, PEI C1A 5V6, Canada.

¹Corresponding author (e-mail: sheard@unb.ca).

²Present address: Terra Nova National Park, Glovertown, NL A0G 2L0, Canada.

Fig. 1. Recent known (as of 2007) worldwide distribution of *Symphotrichum laurentianum*. QC, Quebec; NB, New Brunswick; NS, Nova Scotia; PEI, Prince Edward Island; Mgl, Magdalen Islands (part of the province of Quebec). The question mark indicates a New Brunswick population (Val Comeau) that has not recently been surveyed; the other NB population (Île Miscou) was extant in 2007 but too small for sampling. Sampled populations include the only extant PEI population and six populations spanning most of the Magdalen Islands distribution.



currently under development, but efforts to protect extant populations and to establish new populations remain handicapped by our lack of knowledge of population genetic structure within and among populations of *S. laurentianum*. Houle (1988) assayed allozyme variation for *S. laurentianum* from the Magdalen Islands, reporting population-level banding patterns consistent with polymorphism at some loci and the presence of some heterozygotes, but she did not interpret individual genotypes, and her data therefore do not allow estimates of standard population-genetic parameters.

Rare plant species often display low levels of within-population genetic variation (Cole 2003), at least at neutral marker loci, with the lowest levels of variation typically found for self-compatible endemics like *S. laurentianum*. However, the association between rarity and reduced genetic variation is far from absolute (Cole 2003; Sharma et al. 2003; González-Astorga and Castillo-Campos 2004). Low levels of genetic variation may exacerbate vulnerability to extinction when they result in microhabitat restriction or enhanced vulnerability to pathogens and herbivores (Huenneke 1991), and they may also be symptomatic of past population bottlenecks or inbreeding that suggest populations are more vulnerable than census population sizes might otherwise imply (Barrett and Kohn 1991; Huenneke 1991).

Among-population genetic structure is also of considerable importance for rare plant species. When such structure is strong, it represents both a target for conservation efforts (Avice and Hamrick 1996) and also an important consideration in the design of those efforts. For example, ex situ conservation efforts and reintroduction programs should be informed by spatial genetic variation (Huenneke 1991), especially when such variation might reflect local adaptation (Hufford and Mazer 2003). Finally, patterns in among-population genetic variation can support inferences about dispersal history and historical demography, which can be critical in choosing populations, habitats or sites targeted for conservation efforts.

We quantified within- and among-population genetic var-

iation in *S. laurentianum*, using allozyme markers, with five major goals. First, we estimated the equilibrium inbreeding coefficient F_{IS} as an indicator of breeding structure in natural *S. laurentianum* populations. Second, we sought to assay overall genetic variation, to allow comparison with other similarly rare and endemic plant species. Third, we tested the hypothesis that *S. laurentianum* populations in the Magdalen Islands act as a source, founding populations elsewhere in the range (which would therefore exhibit reduced genetic variation with a subset of source-population alleles). Fourth, we sought to measure among-population genetic variation (and determine its spatial structure) to ascertain the likely importance of using locally sourced plants for future reintroduction efforts. Finally, we compared genetic variation in a greenhouse population of *S. laurentianum* to variation in wild plants, to judge its suitability as a source for experimental and reintroduction explants (Stewart and Lacroix 2001).

Materials and methods

Study species

Symphotrichum laurentianum (Asteraceae: Astereae) is an annual halophytic aster endemic to salt marsh edges and dune slacks of the southern Gulf of St. Lawrence, often occurring in a narrow band between the high tide line and denser vegetation that forms where saline influence is less pervasive (Houle and Valéry 2003; Semple and Cook 2006). Flowering occurs August–September, with seed dispersal by wind and perhaps water (Houle et al. 2001; Lacroix et al. 2007) around October. Plants are gynomonocious (both female and bisexual florets occur in an inflorescence), with rayless, inconspicuous flowers. *Symphotrichum laurentianum* is non-apomictic but self-compatible (Houle 1988), although no estimates of field outcrossing rates have been available.

The largest populations of *S. laurentianum* are in the Magdalen Islands. Surveys in 2004 and 2005 revealed considerable fluctuation in population sizes, but at least eight

populations, totalling many thousands of plants, were present (de Lafontaine 2005; Anonymous 2006). While we did not make formal population estimates during our collection visit in 2007, some historical populations appeared to have disappeared, and others had declined in size. Outside the Magdalen Islands, *S. laurentianum* has recently undergone dramatic declines, with (as of 2007) a single population of about 400 individuals remaining in Prince Edward Island National Park, and at most two even smaller populations remaining in northeastern New Brunswick. *Symphotrichum laurentianum* is currently listed as “threatened” under Canada’s Species at Risk Act.

Collections

We collected leaf tissue from seven field populations of *S. laurentianum* during August–September 2007 (Table 1): six in the Magdalen Islands and one in Prince Edward Island National Park. In 2007, the Prince Edward Island National Park population was the only extant population in PEI, with ~400 plants. Populations in New Brunswick were not sampled, as one known population (Île Miscou) was reduced, in 2007, to a single plant (L. Richardson, Nature New Brunswick, personal communication) and the other (Val Comeau) is situated on private land and no access has been permitted for several years. We also sampled a greenhouse population of *S. laurentianum*, maintained in greenhouse facilities at the University of New Brunswick, Fredericton, N.B. This population was founded in 2006 using seed stock (>500 seeds) from a second greenhouse population at the University of Prince Edward Island, Charlottetown, PEI. This population was, in turn, founded from seeds collected in 1999 from 10 maternal parents in the “East Marsh” population of Prince Edward Island National Park. The original collection represents a bottleneck in the recent history of the University of New Brunswick (UNB) greenhouse population, and we therefore expected this population to display reduced genetic variation. The East Marsh population is no longer extant, but was located less than 0.5 km from the Dune Slack population sampled here, with continuous *S. laurentianum* habitat and no obvious barriers to dispersal between them. East Marsh and Dune Slack were once similar in extent (<0.1 ha each) and both densely populated (tens of thousands of plants in each), and experienced similar population fluctuations in the years leading up to our study ending with steep declines in 2005–2007 (COSEWIC 2004, Steeves et al. 2008, K. Tulk, personal observation).

For field populations, a single fully-expanded leaf was removed from each sampled individual, wrapped in aluminum foil, and immediately flash-frozen by immersion in liquid nitrogen. Samples were returned to the laboratory and then transferred to a –80 °C freezer for long-term storage. Allozyme analyses were performed on newly thawed material. For the greenhouse population, allozyme analyses were performed on fresh material.

Allozyme and population genetic analyses

We assayed 10 enzymes representing 11 allozyme loci for electrophoretic variation: alcohol dehydrogenase (ADH, EC 1.1.1.1), glyceraldehyde 3-phosphate dehydrogenase (G3PDH, EC 1.2.1.12), glucose 6-phosphate dehydrogenase

(G6PDH, EC 1.1.1.49), glycerol 3-phosphate dehydrogenase (GPDH, EC 1.1.1.8), isocitrate dehydrogenase (IDH, EC 1.1.1.42), lactose dehydrogenase (LDH, EC 1.1.1.27), malate dehydrogenase (MDH, EC 1.1.1.37, 2 loci), malic enzyme (ME, EC 1.1.1.40), phosphoglucose isomerase (PGI, EC 5.3.1.9), and superoxide dismutase (SOD, EC 1.15.1.1). A second PGI locus was present but difficult to score reliably, so we do not report data for it. We ground leaves in one drop of extraction buffer (20 mg DTT, 10 mg EDTA, 25 mg BSA, and one drop Tween80, dissolved in 20 mL 0.05 mol·L⁻¹ sodium phosphate pH 7.0), and applied the extract to cellulose acetate plates. Gels were run for 20 min at 250 V. We used a 0.1 mol·L⁻¹ Tris-maleate buffer with pH 7.8 (Richardson et al. 1986) for ADH, GPDH, G6PDH, G3PDH, and G3PDH; a Tris-glycine buffer with pH 8.5 (Hebert and Beaton 1993) to separate ME, IDH, and LDH; a 0.02 mol·L⁻¹ phosphate buffer with pH 7.0 (Richardson et al. 1986) for PGI; and a citric acid aminopropyl morpholine buffer with pH 7.0 (Hebert and Beaton 1993) to separate alleles of MDH. Staining protocols followed Hebert and Beaton (1993). A subset of samples was run several times to ensure consistency of scoring of alleles.

We used genotypic data for the 11 loci in a number of calculations. First, we calculated *P* (percent polymorphic loci, with a polymorphic locus defined as one with the most common allele frequency less than 99% at the species level) and *A* (average number of alleles per locus, counting all detected alleles and all loci, polymorphic or not). We calculated *P* and *A* for each sampled population (P_p , A_p) and for the species as a whole (P_s , A_s), and calculated expected Hardy–Weinberg heterozygosity (H_e) for each population using unweighted averages across loci and across wild populations using pooled allele frequencies. We calculated Wright’s fixation indices, F_{IS} , F_{SR} , and F_{RT} using GenePop 4.0 (Raymond and Rousset 1995). These calculations were made locus-by-locus, and also over all loci. For F_{SR} and F_{RT} , GenePop uses a weighted analysis of variance estimation (Weir and Cockerham 1984). These fixation indices are based on a hierarchical model quantifying the reduction in heterozygosity expected with random mating at different spatial scales: among the Magdalen Islands populations (F_{SR}) and between Magdalen Islands and PEI populations (F_{RT}). There is no within-PEI F_{SR} because there is only one PEI population. We also used FSTAT (Goudet 1995) to derive a single estimate of F_{IS} , based on all loci and all wild populations, along with a 95% confidence interval (obtained by bootstrapping over loci). We used this global F_{IS} to estimate the field rates of selfing [$S = 2F_{IS}/(1 + F_{IS})$] and outcrossing ($1 - S$). We tested for isolation by distance among Magdalen Islands populations using a Mantel test in GenePop 4.0. Finally, we compared allelic diversity among regions and between greenhouse and field populations by making separate calculations of alleles per locus for the greenhouse, the PEI population, and the (pooled) Magdalen Islands populations.

Results

Across field populations, F_{IS} estimates varied from 0.55 to 1.0 (Table 1), with an overall estimate of 0.73, indicating significantly nonzero rates of outcrossing (confidence inter-

Table 1. Populations of *Symphytotrichum laurentianum* represented in this study, and estimates of genetic diversity and equilibrium inbreeding coefficient F_{IS} . A_p , average number of alleles per loci; P_p , percent of polymorphic loci; and H_e , expected Hardy–Weinburg heterozygosity.

Site Name	Location ^a	N^b	F_{IS}	A_p	P_p	H_e
Dune Slack	Prince Edward Island National Park, PEI	48	— ^c	1.1	0.09	0.003
Étang des Caps	Magdalen Islands, QC	42	0.64	1.3	0.27	0.037
Barachois	Magdalen Islands, QC	51	0.55	1.5	0.46	0.09
Cap de l'Hôpital	Magdalen Islands, QC	20	1.0	1.1	0.09	0.037
Pointe aux Canots	Magdalen Islands, QC	44	0.73	1.5	0.27	0.086
Bassin aux Huîtres Ouest	Magdalen Islands, QC	41	0.65	1.4	0.36	0.055
Baie de Grosse-Île	Magdalen Islands, QC	36	1.0	1.2	0.18	0.033
Overall, wild	All sites, PEI + QC	282	0.73 (0.48, 0.97) ^d	1.54	0.36	0.054 ^e
UNB Greenhouse	Seed stock from East Marsh, Prince Edward Island National Park, PEI	44	1.0	1.3	0.18	0.039

^aExact locations are not provided for reasons of site security, but are available from site authorities (PEI National Park and QC Ministère des Ressources Naturelles et de la Faune).

^bReported sample size is the number of individual plants sampled. Although we scored 12 loci, we were not able to score every locus for every plant because we had only small amounts of leaf material and sometimes could not re-run questionable or failed assays. Total number of allozyme genotypes (loci × individuals) scored ranged from 181 (Cap de l'Hôpital) to 385 (Barachois).

^cAn estimate of F_{IS} would be based on just one individual heterozygous at just one locus.

^dCombined estimate across all sites excluding greenhouse. In parentheses: 95% confidence interval, based on bootstrapping over loci.

^eEstimate based on pooled allele frequencies.

val does not include 1.0). The greenhouse population had no heterozygosity at any locus ($F_{IS} = 1.0$).

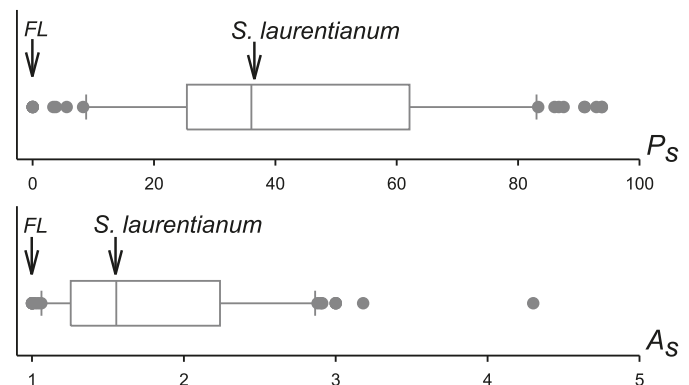
Allozyme allelic diversity in *S. laurentianum* is low, but nonzero (Fig. 2), with 4 of 11 loci being polymorphic ($P_s = 0.36$; a 5th locus has a rare allele that was ignored for this calculation) and 2 or 3 alleles detected at each of the polymorphic loci ($A_s = 1.54$ ignoring the same rare allele). At the population level, the lowest genetic diversity is found in the PEI field population (few polymorphic loci, few alleles per locus, and small expected heterozygosity; Table 1), with higher but variable genetic diversity in the Magdalen Islands (Table 1). The alleles we detected in the PEI field and UNB greenhouse populations are strict subsets of allelic diversity in the Magdalen Islands (Fig. 3), with four Magdalen Islands alleles undetected in the UNB greenhouse population, and six undetected in the PEI field population. Surprisingly, the UNB greenhouse population has considerably higher genetic diversity than the PEI field population (Table 1) and includes three alleles undetected in the PEI field population. One PEI allele was undetected in the greenhouse (Fig. 3).

The F_{SR} values among Magdalen Islands populations varied from small to moderate (all < 0.15), but did not show significant isolation by distance (Fig. 4). Differentiation between Magdalen Islands and PEI populations was modest ($F_{RT} = 0.04$) despite considerable loss of allelic diversity in PEI.

Discussion

Symphytotrichum laurentianum has proven highly self-compatible in laboratory studies (Houle 1988) and propagation in the greenhouse does not require cross-pollination ($F_{IS} = 1.0$ for our greenhouse population). Houle (1988) argued that *S. laurentianum* could be cross-pollinated in the laboratory, but owing to floral anatomy was obligately self-

Fig. 2. Percent polymorphic loci (P_s) and alleles per locus (A_s) for *Symphytotrichum laurentianum* compared with Furbish's Lousewort ("FL"; data from Waller et al. 1987) and with 95 other rare and endemic plant species (data from Cole 2003). All estimates are based on allozyme data, although the loci surveyed vary. Boxes show 25th, 50th, and 75th percentiles; whiskers show 10th and 90th percentiles; and dots show the remaining range.



ing in the field, although no field data have been available to test this hypothesis. While we did not estimate outcrossing rates directly from seed families, our data indicate substantial, but far from complete, selfing (our overall estimate for natural populations was $F_{IS} = 0.73$, or $S = 0.84$). Thus, roughly 16% of the adult plants sampled were sired by outcross fathers, although we do not know what vectors effect cross-pollination of *S. laurentianum* in the field. The level of heterozygosity we detected in adult plants could have arisen through substantial outcrossing (~16%) with similar survival of selfed and outcross progeny, or of course through increased survival of a few outcrossed individuals if selfed progeny experience inbreeding depression.

Fig. 3. Alleles represented in Magdalen Islands, PEI, and greenhouse populations of *Symphotrichum laurentianum*. Allele numbering is arbitrary. Six additional loci are monomorphic everywhere, and are not represented here.

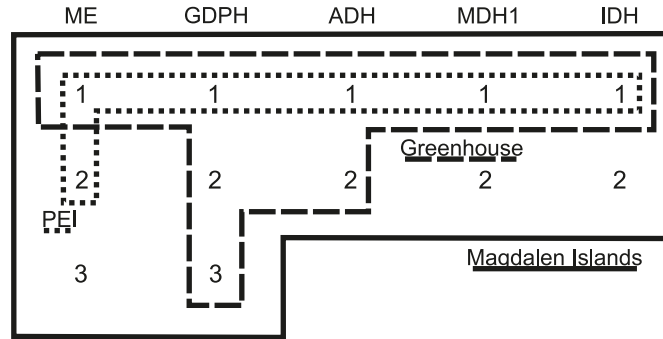
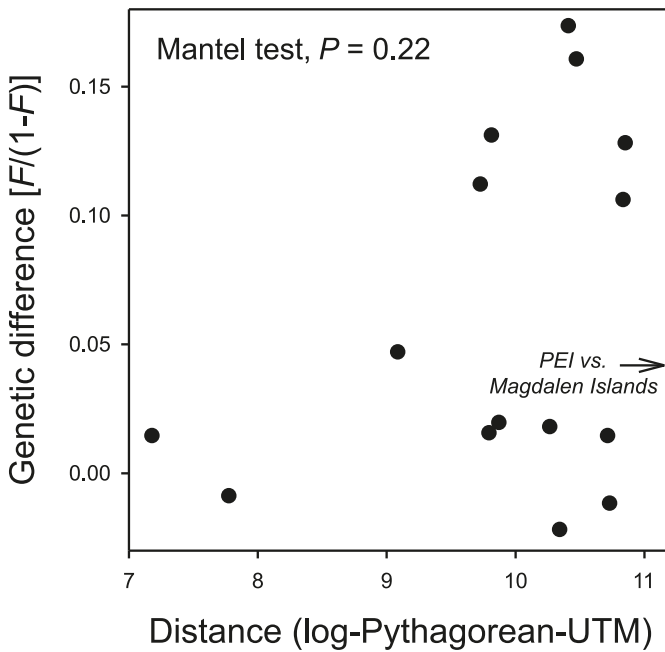


Fig. 4. Genetic differentiation vs. distance for *Symphotrichum laurentianum* populations in the Magdalen Islands, based on F_{SR} calculated between all (15) pairs of populations. The indicated value for PEI vs. the Magdalen Islands is based on $F_{RT} = 0.041$. UTM, universal transverse mercator co-ordinate system.



Beyond our estimates based on heterozygosity, there has been little field evidence for cross-fertilization in *S. laurentianum*. Plants are gynomonocious, with approximately 20% of florets bisexual and the rest pistillate (Lacroix et al. 2007). Despite this, fruit set in natural populations can be 60% of all florets (Houle 1988; Lacroix et al. 2007), indicating seed set in unisexual florets. There is, however, considerable evidence (Houle 1988) for pollen transfer among florets within capitula, likely as capitula are shaken by wind or other disturbance; emasculation experiments in natural populations would be needed to separate such geitonogamous selfing from outcrossing.

Symphotrichum laurentianum populations show modest levels of genetic variation, with both P_s and A_s values falling near the median for a recent compilation of values for rare and endemic plants (Cole 2003). Cole's (2003) compilation suggests very broad ranges for both P_s and A_s (Fig. 4), and

genetic variation is undoubtedly influenced by life-history traits that influence gene flow and the mating system (see for example Hamrick and Godt 1996), meaning that generalizations about expected levels of genetic variation in rare plants are difficult. Estimates of quantities like P_s and A_s are perhaps best seen as a way to screen threatened species to identify those for which low genetic variation might be an especially urgent conservation issue, exacerbating vulnerability to extinction via mechanisms such as vulnerability to pathogen attack (Huenneke 1991) or difficulty adapting to environmental change. Our data do not suggest that *S. laurentianum* is such a species — at least, not in the Magdalen Islands populations where we found it to be most genetically diverse. However, the PEI population, which had experienced a dramatic recent decline, retained much less genetic variation.

The moderately high level of genetic variation in *S. laurentianum* is perhaps surprising given that many features of its life history might lead one to predict low levels of genetic variation within populations. Populations are founded by long-distance dispersal of likely small numbers of individuals, and once established, exhibit dramatic fluctuations in size. Bottlenecks that result from these processes will increase the loss of alleles through genetic drift (Husband and Barrett 1992; Ellstrand and Elam 1993; Barrett 1996). Nevertheless, *S. laurentianum* is far more genetically variable than many rare plants (Fig. 2), which underscores the complexity of ecological controls on genetic variation. An interesting comparison can be drawn with the well-studied Furbish's lousewort (*Pedicularis furbishiae* S. Watson, Scrophulariaceae), another eastern North American endemic with strict habitat requirements and vulnerability to disturbance (Waller et al. 1987), but one that displays extraordinarily low levels of genetic variation (Fig. 2). While there are many ecological differences between the two species, we speculate that the much higher level of genetic variation in *S. laurentianum* can be attributed to an important difference between the two species' population structures: for Furbish's lousewort, all known populations are very small, but for *S. laurentianum* the Magdalen Islands populations have historically been very large and could have acted as reservoirs of genetic variation.

Our data are consistent in several ways with a model in which the Magdalen Islands populations act both as a reservoir for genetic variation and as a source for the founding of

local populations in PEI. Such a model would likely apply to New Brunswick populations as well, although we were not able to sample either of the two recently extant NB populations (surveying these populations, and sampling them if they are still extant, is a high priority for future work). Most tellingly, the alleles detected in our PEI and PEI-origin greenhouse populations are strict subsets of Magdalen Islands allelic diversity, something unlikely under an alternative hypothesis in which Magdalen Islands, PEI, and NB populations represent remnants of a once broader distribution without significant long-distance dispersal. Further support for the Magdalen-source model comes from the fact that spatial genetic structure among *S. laurentianum* populations was modest (Fig. 4), with the most conspicuous spatial feature being the difference in allelic diversity between the Magdalen Islands and PEI populations. Our estimates of population differentiation are much lower than average for other primarily selfing species (Hamrick and Godt 1989, 1996; Heywood 1991; Morjan and Rieseberg 2004), suggesting that long-distance gene flow either through pollen or through seed has been more extensive than might otherwise be assumed (or that genetic variability was reduced prior to colonization of new populations). Neither seed nor pollen biology of *S. laurentianum* would seem, a priori, well suited to frequent long-distance dispersal: seeds are often retained in seed heads and wind dispersal is very limited (Houle et al. 2001; Lacroix et al. 2007), while the rayless flowers are inconspicuous and outcrossing rates are low. The occasional long-distance seed dispersal that must be required to establish new local populations is likely to be highly dependent on either strong storms or on ocean currents (and in either case is unlikely to produce a simple, monotonic relationship between geographic distance and genetic differentiation). Our estimates of spatial genetic structure are less compatible with the alternative “remnants” model: under such a model, we would expect stronger spatial genetic structure and probably a significant pattern of isolation by distance.

Conservation efforts for *S. laurentianum* are now moving beyond the simple protection of surviving populations, with a published recovery plan for New Brunswick (NBDNR 2007), and a national recovery plan that will cover the species' entire range currently in preparation (Jennifer Stewart, Canadian Wildlife Service, personal communication, 2009). Our results can help inform those forming and carrying out conservation and recovery plans for *S. laurentianum*. Whenever possible, conservation efforts should protect genetic diversity within the targeted species (Avisé and Hamrick 1996), and this reinforces the value of the Magdalen Islands populations: these are not just the largest populations, but also the most genetically diverse. Most of that variation, however, is within rather than between populations. While we have not tested directly for local adaptation of *S. laurentianum* populations, our data do not argue strongly for the importance of conserving each population separately (even the most geographically distinct population, in PEI, differs from the rest only by the absence of some alleles).

Because rapid population-size changes appear to be routine in *S. laurentianum* (Stewart and Lacroix 2001; COSEWIC 2004), and local extirpations are common (COSEWIC 2004; K. Tulk, unpublished data), ex situ prop-

agation followed by reintroductions is likely to be a candidate strategy in the development of recovery plans (Stewart and Lacroix 2001). Interestingly, the greenhouse population (founded from PEI seed in 1999) resembles, in its allelic makeup, Magdalen Islands populations more strongly than it does the extant PEI population. This is suggestive of recent erosion of genetic diversity in the PEI populations, although we do not know if this is a cause or a result of the marked demographic declines experienced by *S. laurentianum* in PEI. The greenhouse population, then, should be a suitable source for any reintroductions — better even than modern PEI-source seed. Such reintroductions were begun in 2008 in Prince Edward Island National Park, in habitats similar to those historically occupied by *S. laurentianum* populations, and early signs of success suggest that this is a promising management option. In *S. laurentianum*, as in other threatened plant species (e.g., Suárez-García et al. 2009), ex situ collections may prove of considerable value in preserving genetic variability that is lost or imperiled in the wild.

Our population-genetics data are consistent with a view of *S. laurentianum* population biology with a central set of populations (in the Magdalen Islands) serving both as a source supplying founders for populations at the range margins (PEI and likely NB) and also as a reservoir of genetic variation. However, given the restricted area occupied by the central set of populations (the entire Magdalen Islands archipelago has an area of only 205 km², with suitable habitat for *S. laurentianum* representing only a very small fraction of that), *S. laurentianum* appears to have a somewhat tenuous grasp on existence. Electrophoretic and experimental crossing data suggest a recent origin of *S. laurentianum* as an eastern endemic from the similar western species *S. ciliatum* (Houle 1988) (although relatively high genetic variation in *S. laurentianum* might suggest a large founding propagule size, multiple founding events, or ongoing gene flow from *S. ciliatum*). Local endemics like *S. laurentianum* may often arise from more broadly distributed ancestors, but when they are highly restricted and habitat specialist like *S. laurentianum*, it would not be surprising if they were ephemeral on evolutionary timescales. From a conservation perspective, our data point to the long-term importance of protecting the Magdalen Islands populations and the necessity of coordinating recovery plans across political jurisdictions.

Acknowledgements

We thank Justin Ancheta, Paul Ayres, Lori-Anne Duffy, and Alain Richard for help in the field; and Amanda Cavanagh, Katie Friars, Will Huttges, Liz Mills, Vanessa Paesani, and Danielle Perley for help in the greenhouse and the lab. Two anonymous reviewers provided valuable comments on the manuscript. We are grateful to Parks Canada, the Ministère du Développement Durable, de l'Environnement et des Parcs du Québec and Attention Fragiles for cooperation and for collection permits and to the New Brunswick Department of Natural Resources for possession permits. Christian Lacroix kindly provided seed stock to establish the UNB greenhouse population. This work was funded by Parks Canada and by the Natural Sciences and Engineering Research Council of Canada Discovery grants to S.B.H. and L.K.J.

References

Anonymous. 2006. *Symphotrichum laurentianum* (Aster du Saint-Laurent): Sommaire pour l'ensemble des occurrences du Québec. Centre de Données sur le Patrimoine Naturel du Québec (www.cdpnq.gouv.qc.ca).

Avise, J.C., and Hamrick, J.L. 1996. Conservation genetics: case histories from nature. Chapman and Hall, London, UK.

Barrett, S.C.H. 1996. The reproductive biology and genetics of island plants. *Philos. Trans. R. Soc. Lond., B*, **351**: 725–733. doi:10.1098/rstb.1996.0067.

Barrett, S.C.H., and Kohn, J.R. 1991. Genetic and evolutionary consequences of small population size in plants: Implications for conservation. *In* Genetics and conservation of rare plants. Edited by D.A. Falk. Oxford University Press, New York, N.Y. pp. 3–30.

Cole, C.T. 2003. Genetic variation in rare and common plants. *Annu. Rev. Ecol. Evol. Syst.* **34**: 213–237. doi:10.1146/annurev.ecolsys.34.030102.151717.

COSEWIC. 2004. COSEWIC Assessment and update status report on the Gulf of St. Lawrence Aster, *Symphotrichum laurentianum* in Canada. Committee on the Status of Endangered Wildlife in Canada, Ottawa, Ont.

de Lafontaine, G. 2005. Protocole de suivi des populations d'Aster du Saint-Laurent, *Symphotrichum laurentianum*, aux Îles-de-la-Madeleine. *Can. Field Nat.* **119**: 556–568.

Ellstrand, N.C., and Elam, D.R. 1993. Populations genetic consequences of small population size: implications for plant conservation. *Annu. Rev. Ecol. Syst.* **24**: 217–242. doi:10.1146/annurev.es.24.110193.001245.

González-Astorga, J., and Castillo-Campos, G. 2004. Genetic variability of the narrow endemic tree *Antirhea aromatica* Castillo-Campos & Lorence (Rubiaceae: Guettardeae) in a tropical forest of Mexico. *Ann. Bot. (Lond.)*, **93**: 521–528. doi:10.1093/aob/mch070. PMID:15056561.

Goudet, J. 1995. Fstat version 1.2: A computer program to calculate *F* statistics. *J. Hered.* **86**: 485–486.

Hamrick, J.L., and Godt, M.J. 1989. Allozyme diversity in plant species. *In* Plant population genetics, breeding, and genetic resources. Edited by A.H.D. Brown, M.T. Clegg, A.L. Kahler and B.S. Weir. Sinauer, Sunderland, Mass. pp. 43–63.

Hamrick, J.L., and Godt, M.J.W. 1996. Effects of life-history traits on genetic diversity in plant species. *Philos. Trans. R. Soc. Lond., B*, **351**: 1291–1298. doi:10.1098/rstb.1996.0112.

Hebert, P.D.N., and Beaton, M.J. 1993. Methodologies for allozyme analysis using cellulose acetate electrophoresis: A practical handbook. Helena Laboratories, Beaumont, Tex.

Heywood, J.S. 1991. Spatial analysis of genetic variation in plant populations. *Annu. Rev. Ecol. Syst.* **22**: 335–355. doi:10.1146/annurev.es.22.110191.002003.

Houle, F. 1988. Étude biosystématique de la section *Conyzopsis* du genre *Aster* (Asteraceae). Ph.D. thesis, Université de Montréal, Montréal, Que.

Houle, G., and Belleau, A. 2000. The effects of drought and waterlogging conditions on the performance of an endemic annual plant, *Aster laurentianus*. *Can. J. Bot.* **78**: 40–46. doi:10.1139/cjb-78-1-40.

Houle, G., and Valéry, S. 2003. A mixed strategy in the annual endemic *Aster laurentianus* (Asteraceae) — a stress-tolerant, yet opportunistic species. *Am. J. Bot.* **90**: 278–283. doi:10.3732/ajb.90.2.278.

Houle, G., Morel, L., Reynolds, C.E., and Siégl, J. 2001. The effect of salinity on different developmental stages of an endemic annual plant, *Aster laurentianus* (Asteraceae). *Am. J. Bot.* **88**: 62–67. doi:10.2307/2657127. PMID:11159127.

Huenneke, L.F. 1991. Ecological implications of genetic variation in plant populations. *In* Conservation of rare plants: biology and genetics. Edited by D.A. Falk and K.E. Holsinger. Oxford University Press, Oxford, UK. pp. 33–41.

Hufford, K.M., and Mazer, S.J. 2003. Plant ecotypes: genetic differentiation in the age of restoration. *Trends Ecol. Evol.* **18**: 147–155. doi:10.1016/S0169-5347(03)00002-8.

Husband, B.C., and Barrett, S.C.H. 1992. Effective population size and genetic drift in tristylous *Eichhornia paniculata* (Pontederiaceae). *Evolution*, **46**: 1875–1890. doi:10.2307/2410037.

Lacroix, C.R., Steeves, R., and Kemp, J.F. 2007. Floral development, fruit set, and dispersal of the Gulf of St. Lawrence Aster (*Symphotrichum laurentianum*) (Fernald) Nesom. *Can. J. Bot.* **85**: 331–341. doi:10.1139/B07-011.

Morjan, C.L., and Rieseberg, L.H. 2004. How species evolve collectively: implications of gene flow and selection for the spread of advantageous alleles. *Mol. Ecol.* **13**(6): 1341–1356. doi:10.1111/j.1365-294X.2004.02164.x. PMID:15140081.

NBDNR. 2007. Recovery strategy for the Gulf of St. Lawrence Aster (*Symphotrichum laurentianum*) in New Brunswick, Canada. New Brunswick Department of Natural Resources, Fredericton, N.B.

Raymond, M., and Rousset, F. 1995. GENEPOP (version 1.2): Population genetics software for exact tests and ecumenicism. *J. Hered.* **86**: 248–249.

Reynolds, C.E., and Houle, G. 2002. Mantel and partial Mantel tests suggest some factors that may control the local distribution of *Aster laurentianus* at Îles de la Madeleine, Québec. *Plant Ecol.* **164**: 19–27. doi:10.1023/A:1021213720355.

Richardson, B.J., Baverstock, P.R., and Adams, M. 1986. Allozyme electrophoresis: a handbook for animal systematics and population studies. Academic Press, Sydney, Australia.

Semple, J.C., and Cook, R.E. 2006. *Solidago*. *In* Flora of North America. Edited by Flora North America Editorial Committee. Oxford University Press, Oxford, UK. pp. 107–166.

Sharma, I.K., Jones, D.L., and French, C.J. 2003. Unusually high genetic variability revealed through allozyme polymorphism of an endemic and endangered Australian orchid, *Pterostylis aff. picta* (Orchidaceae). *Biochem. Syst. Ecol.* **31**: 513–526. doi:10.1016/S0305-1978(02)00186-2.

Steeves, R., Nazari, V., Landry, J.F., and Lacroix, C.R. 2008. Pre-dispersal seed predation by a coleophorid on the threatened Gulf of St. Lawrence Aster. *Can. Entomol.* **140**: 297–305. doi:10.4039/N07-040.

Stewart, S.S., and Lacroix, C.R. 2001. Germination potential, updated population surveys and floral, seed and seedling morphology of *Symphotrichum laurentianum*, the Gulf of St. Lawrence Aster, in the Prince Edward Island National Park. *Can. Field Nat.* **115**: 287–295.

Suárez-García, C., de Pas, J.P., Febles, R., and Caujapé-Castells, J. 2009. Genetic diversity and floral dimorphism in *Limonium dendroides* (Plumbaginaceae), a woody Canarian species on the way of extinction. *Plant Syst. Evol.* **280**: 105–117. doi:10.1007/s00606-009-0166-8.

Waller, D.M., O'Malley, D.M., and Gawler, S.C. 1987. Genetic variation in the extreme endemic *Pedicularis furbishiae* (Scrophulariaceae). *Conserv. Biol.* **1**: 335–340. doi:10.1111/j.1523-1739.1987.tb00053.x.

Weir, B.S., and Cockerham, C.C. 1984. Estimating *F*-statistics for the analysis of population structure. *Evolution*, **38**: 1358–1370. doi:10.2307/2408641.