RHODORA, Vol. 115, No. 962, pp. 133–157, 2013

DOI: 10.3119/12-08; first published online: xxx, 2013.

QUATERNARY DIVERGENCE AND HOLOCENE SECONDARY CONTACT VIA THE NORTHWEST PASSAGE IN THE CIRCUMPOLAR *LATHYRUS JAPONICUS* (LEGUMINOSAE)

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ABSTRACT. We used the geographic distribution of genetic diversity in the beach pea, *Lathyrus japonicus* (Leguminosae) to reconstruct the location of a Pleistocene refugium and Holocene range expansion. DNA sequence data for the chloroplast ndhF-trnH spacer from a global sample of 22 populations in Asia, North America, and Europe were used to assess broad-scale, circumpolar patterns. The cpDNA data revealed five haplotypes including two largely allopatric, widespread haplotypes (one Pacific, the other Atlantic and inland in the Great Lakes). Three geographically restricted haplotypes were also recovered: one each in New Jersey, Lake Champlain, and the Pacific Northwest. The distribution of isozyme variation at 14 loci for 38 populations of beach peas from the Atlantic and Pacific coasts of North America, Lake Champlain, and the Great Lakes was used to reconstruct a North American history. One subset of allozymes was found on the Atlantic Coast and in the Great Lakes; a second subset was widespread from northwestern North America to Newfoundland. There was a nested pattern to the localization of allozymes along the Atlantic Coast; Cape Cod had the greatest number of allozymes. Pacific and Atlantic isozymes and cpDNA haplotypes occurred together along the coast of Newfoundland and adjacent Labrador. The pattern of genetic variation as assessed from isozymes and cpDNA allow the inferences that: 1) an early divergence yielded Atlantic and Pacific lineages and 2) the Atlantic lineage occupied a Wisconsinan refugium on the now-submerged coast of northeastern North America. Our evidence is consistent with there being Holocene secondary contact between the Atlantic and Pacific lineages in the area where they now overlap. We also suggest that an early post-glacial vicariance event is implicated in the history of the Lake Champlain populations.

Key Words: Holocene, *Lathyrus japonicus*, Leguminosae, biogeography, refugia, isozymes, *ndhF-trnH*

Recent work incorporating genetic data has provided the basis for strong inferences about the biogeography of Pleistocene refugia and Holocene range expansions for a diversity of species (Hewitt 2000; Hewitt and Ibrahim 2001). Restricted regions of high genetic diversity are evidence of refugia that have since given rise to relatively less diverse outlier populations during Holocene migrations (Leberg 1992). Reconstruction of Quaternary biogeography is especially advanced in Europe, where an array of species has been subjected to genetic analysis, yielding well-substantiated refugia and migration patterns (e.g., Hewitt 2004; Schmitt 2007). Of particular interest to us is the work on the Quaternary phylogeography of seven species of coastal plants from Europe (Kadereit and Westberg 2007), which reveals the historical impact of Pleistocene isolation on genetic divergence in the Mediterranean and Black Sea basins. Biogeographic patterns of traits such as plant habit, propagule tolerance of seawater, and cold tolerance reflect differences in population differentiation between species.

Past studies have emphasized refugia south of glaciation in eastern North America. For instance, Griffin and Barrett (2004) present genetic evidence that *Trillium grandiflorum* (Michx.) Salisb. weathered the last glaciation in south-central United States, and similar work suggests that *Asclepias exaltata* (L.) Muhl. also occupied a refugium there (Broyles 1998). Earlier work based on pollen-core records strongly suggests that many eastern North American forest tree species occupied refugia in the southeastern or south-central United States and migrated northward with climatic warming in the Holocene (Davis 1983).

However, there is evidence for northern refugia harboring North American species during the Pleistocene (Abbott and Brochmann 2003; Barrington and Paris 2007; Pielou 1991). Using cpDNA restriction-fragment analysis and fossil remains, Tremblay and Schoen (1999) found support for two Pleistocene refugia, one in Beringia and another in the high Arctic, for the common Arctic herb *Dryas integrifolia* M. Vahl. The genetic structure also suggested a mixed contribution of these glacial refugia to the establishment of present-day populations. By contrast, analysis of ITS sequence variation in North American *Ammophila breviligulata* Fernald (beach-grass) revealed that diversity was highest in the northern Atlantic region, suggesting a Pleistocene beach-grass refugium located on the exposed, unglaciated coastal plain in the area of Sable Island (Walker 1998).

The beach pea (*Lathyrus japonicus* Willd.), as profiled by Brightmore and White (1963), is a prostrate to scandent pioneer on shingle beaches and sand dunes. Violent wave action may result in exposure and fragmentation of its rhizomes; these fragments have been documented to function as propagules. *Lathyrus japonicus* is an outcrossing diploid; its principal pollinators are long-tongued bumblebees. Seed dispersal is by sea (Ridley 1930). In seawater, the seeds retain their buoyancy and viability up to five years. Migrant doves may also be dispersers, as they have been seen feeding on the seeds. A prominent member of sand-dune communities north of ~35° north latitude, the species is unusually hardy. Its northern limit is the −26.7°C January isotherm, and seedlings survive freezing to −80°C (Brightmore and White 1963).

The beach pea is a circumboreal species (Figure 1) widespread in the northern Pacific and Atlantic. It has a broad, but interrupted distribution at high latitudes; in the Arctic it is absent only from the Canadian Arctic Archipelago, the polar coast of Siberia, and the Svalbard Archipelago. There are also disjunct populations on the shores of Hudson Bay (including James Bay) and the mouth of the Mackenzie River, as well as inland along the sandy lakeshores of Lake Winnipeg, Lake Saskatchewan, the Great Lakes, and Lake Champlain.

Fernald (1932) and Hitchcock (1952) recognized two pairs of varieties within *Lathyrus japonicus*. Two of these varieties are common in the north temperate zone, especially on the Atlantic Ocean but also on the Great Lakes; they have larger leaflets but smaller flowers and are either pubescent (var. *pellitus* Fernald) or glabrous (var. *glaber* Fernald). In the Arctic and Pacific, two different varieties are prominent; these have smaller leaflets but larger flowers and again are either pubescent (var. *aleuticus* Fernald) or glabrous (var. *japonicus*). Hultén (1971) hypothesized that the temperate plants represent the range of the species during the Pleistocene, whereas the Arctic plants are the result of postglacial migration. Hultén argued that the extensive overlap in the ranges of the small-flowered and large-flowered plants was evidence for this postglacial migration. On the other hand, Fernald (1932) maintained that the widespread northern var. *aleuticus* represents the primitive or ancestral type, which gave rise to several more localized, north temperate lineages. Fernald may have assumed that the most widely distributed taxon was ancestral.

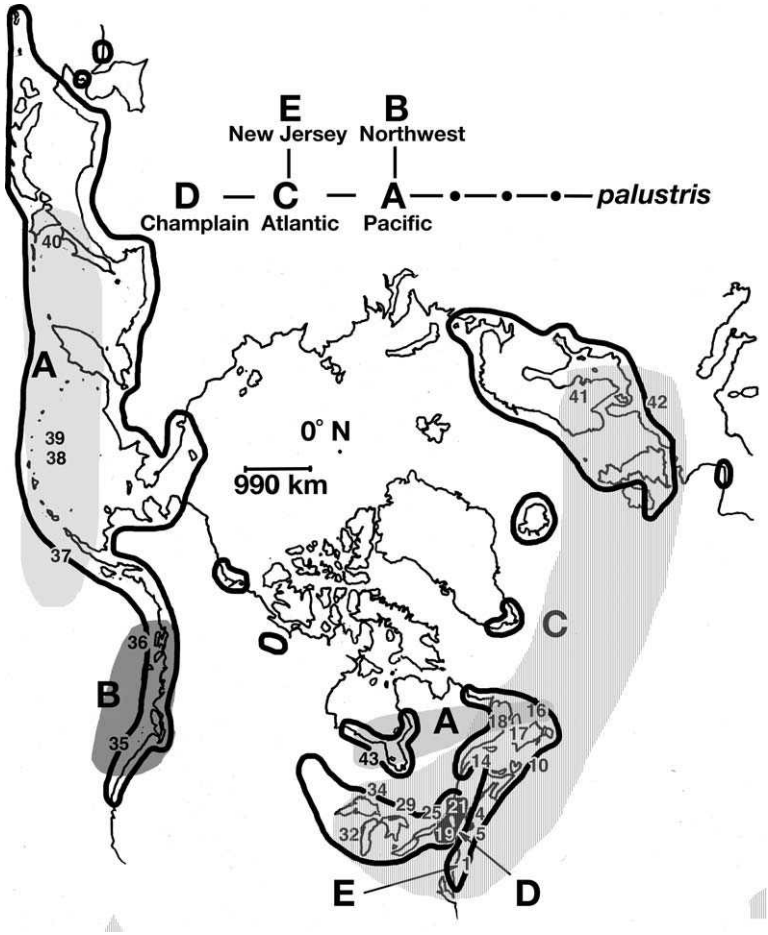

Figure 1. Distribution of *Lathyrus japonicus* (after Hultén, 1971), the five *ndhF-trnH* haplotypes encountered in *Lathyrus japonicus* (azimuthal equidistant projection centered on the North Pole) and haplotype network (see text for methods). Each solid line in the network represents a single nucleotide substitution or indel.

In this study, we used cpDNA-sequence and isozyme data to reconstruct the late Pleistocene and Holocene history of the beach pea and to test the alternative hypotheses of Fernald and Hultén. From a wide-ranging sample of North American plants, supplemented with samples from selected Asian and European populations, we sought to: 1) reconstruct the historical relationships

among populations from cpDNA haplotypes; 2) assess the pattern of genetic variation within and among beach pea populations in North America using isozyme data; and 3) reconstruct the biogeographic history of the species in North America in light of Pleistocene and Holocene events in the region. We were especially interested in the relationship of the Lake Champlain beach pea populations to those on the sea beaches of northeastern North America, which we hypothesized might have had a vicariant origin when Lake Champlain became isolated from the Gulf of St. Lawrence.

MATERIALS AND METHODS

Sites and sampling. We collected 963 individuals representing 39 *Lathyrus japonicus* populations from four regions of North America (Appendix 1) as follows: 1) Eighteen populations were sampled on the Atlantic coast from New Jersey to Labrador including the St. Lawrence River. 2) All six extant populations were collected from Lake Champlain. 3) Ten populations from the Great Lakes were sampled (1–3 per lake). 4) Four Pacific-coast populations from the Aleutian Islands to Oregon were collected by correspondents. The broad-ranging latitudinal coverage of the sample yielded accessions that included all of the combinations of indument and flower size typical of the varieties within *L. japonicus*.

Because *Lathyrus japonicus* reproduces vegetatively, adjacent ramets may derive from a single genetic individual. In order to minimize resampling of individuals, leaves were taken from plants spaced a minimum of 3.5 m apart. Where possible, collections were made on different dunes. Representative herbarium vouchers (indicated in Appendix 1) are deposited at the Pringle Herbarium, University of Vermont (VT).

cpDNA sequence variation. A total of 50 individuals representing 22 populations was used in the cpDNA sequence analysis (see Appendix 1). Isozyme genotypes were used to select genetically distinct individuals from geographically representative populations in North America. Sequence data alone were available for seeds received by correspondence from Japan, Oregon, James Bay, Denmark, and Sweden (10 individuals representing five populations); these materials extended representation of the species to

much of its circumboreal range. We also sequenced a single accession of *Lathyrus palustris* L. as an outgroup.

DNA extraction, amplification, and sequencing. Genomic DNA was isolated with a modified version of the Doyle and Doyle (1987) CTAB protocol, using procedures standard in our lab (Driscoll and Barrington 2007; Little and Barrington 2003).

The Polymerase Chain Reaction (PCR) was used to amplify *ndhF-trnH* and *trnH-psbA*, the two non-coding intergenic spacers flanking the chloroplast gene *trnH*. The primers used for amplification, which we designed, were *ndhF*1604: 5′-TTCGGGAGAGGA-CATATTCACATC-3′ and *psbA*50: 5′-CCGCGTTAGGTATCAG-CACT-3′. The 1170–1207 base-pair PCR fragment included 692 bases from the 3′ end of the chloroplast *ndhF* gene, 160–197 bases of intergenic spacer, *trnH* (40 bases), 225 bases of the other intergenic spacer, and 50 bases of the 5′ end of the *psbA* gene. PCR amplification took place in a 50 μL reaction mixture containing: 1× PCR Buffer II (Perkin-Elmer, Foster City, CA); 0.2 mmol/L of each dNTP; 0.5 μmol/L of each amplification primer; 2.0 mmol/L $MgCl_2$; 1 unit of AmpliTaq (Perkin-Elmer); and approximately 500 ng of genomic DNA. Reactions were incubated at 95°C for three min in an Amplitron II (0.2 mL well size; Thermolyne, Dubuque, IA), then cycled 35 times (95°C for 30 s, 56°C for 30 s, 72°C for 45 s), and finally incubated at 72°C for 10 min. The desired DNA fragments were extracted from 1% agarose gels and purified with the QIAquick PCR Purification Kit following the manufacturer's protocol (Qiagen Inc., Valencia, CA).

Automated dye-termination cycle sequencing followed the recommended protocol accompanying the ABI Prism ready-reaction kit (Perkin-Elmer, Foster City, CA). Each sequencing reaction utilized approximately 300 ng of purified PCR product and 3.2 pmol of primer. Sequencing primers, which we also designed, were *psb*A50, *trn*H1: 5′-CCAAGTGGATCAAGGCAGTG-3′, and *trn*H2: 5′-ATCCACTTGGCTACATCC-3′. Unbound dye terminators were removed from the reaction mixture with a Centrisep spin column (Princeton Separations, Adelphia, NJ) following the manufacturer's recommended protocol. Samples were analyzed with an ABI model 373A auto sequencer (Applied Biosystems, now Life Technologies, Carlsbad, CA) running software version 2.0.1S. Approximately 400–500 base pairs of data could be read with high

Table 1. Summary of variation in the *ndhF-trnH* region for *Lathyrus* accessions. Accessions are uniform within haplotype. Nucleotide and indel characters are reported by position in the aligned 225-nucleotide, 27-accession dataset. For indels: + = nucleotides present; − = nucleotides absent. (#) = Number of accessions; all are *L. japonicus* except where noted.

Haplotype		Nucleotide and Indel Characters							
Letter (#)	Name	44–48	62–66	63	160–196	160	209	211	224
A (5)	Pacific	+	+	T	+	G	A	A	A
B (4)	Northwest	+	+	T	−	G	A	A	A
C (15)	Atlantic/Inland	+	+	T	+	G	C	A	A
D (1)	Champlain	+	+	A	+	G	C	A	A
E (1)	New Jersey	+	+	T	+	C	C	A	A
PAL (1)	*L. palustris*	−	−	T	+	G	A	G	G

confidence from each reaction. Forward and reverse sequences were obtained from independent PCR reactions.

Analysis of sequence data. Only the spacer between the *ndhF* gene and *trnH* (*ndhF-trnH*) yielded sequence variation. Relationships of the 50 beach pea haplotypes were inferred from a maximum-parsimony analysis of the aligned 225 bp *ndhF-trnH* sequences using PAUP 4.0b2 (Swofford 1999). Equally weighted parsimony analysis was conducted using the heuristic search option. We used 1000 random addition sequences with 10 trees held during tree bisection-reconnection (TBR) swapping of each addition sequence. The resulting trees were swapped to completion holding a maximum of 10, 000 trees. The *Lathyrus japonicus* haplotypes were rooted with respect to our collection of *L. palustris*, a near relative in *Lathyrus* section *Orobus* (Åsmussen and Liston 1998; Bässler 1966, 1973; Kenicer et al. 2005). Support for each node of the resulting tree was evaluated with 1000 bootstrap replicates (Felsenstein 1983) using the default parameters in PAUP. An unrooted tree arising from this analysis was also considered in inferring the historical biogeography of beach pea. Representative sequences of five of the encountered haplotypes have been deposited in GenBank (accession numbers are listed in Appendix 1). The Northwest sequence (B) was too short to be accepted by GenBank. It differs from Haplotype A by the deletion specified in Table 1.

Enzyme electrophoresis. Isozyme data were collected from all 963 individuals (Appendix 1). Standard methods for starch gel

electrophoresis were adapted from Soltis et al. (1983); their buffer system 2 was used to resolve phosphoglucose isomerase (PGI) and shikimic acid dehydrogenase (SKDH). Buffer system 8 was used for aspartate amino transferase (AAT), leucine amino peptidase (LAP), and malic enzyme (ME). Buffer system 11 resolved malate dehydrogenase (MDH), aldolase (ALD), isocitric acid dehydrogenase (IDH), and phosphoglucomutase (PGM). The modified tray buffer for system 6 of Godt and Hamrick (1991) was used to resolve triosephosphate isomerase (TPI); see Schmitz (2002) for further details. Bands were interpreted with reference to the typical number of loci, subunit structure, and subcellular compartmentalization known for angiosperms (Weeden and Wendel 1989). Inferred isozymes and their respective allozymes were coded in reference to their relative migration in the gel; the most anodal isozyme designated as 1, and the most anodal allozyme at each locus by the letter "A."

Analysis of isozyme variation. Assessment of genetic diversity using the isozyme data was carried out using BIOSYS-1 (Swofford and Selander 1981). Allozyme diversity was determined for the species as a whole and on a population level using three standard measures: percent polymorphic loci (P), alleles per locus (A), and expected genetic diversity (H_e). A locus was considered polymorphic if more than one allele was found at a frequency of at least one percent (99% criterion). At the species level, the percentage of polymorphic loci (P_s) was calculated by dividing the number of loci polymorphic in at least one population by the number of loci analyzed. Summing the alleles over all loci and dividing by the total number of loci yielded the mean number of alleles per locus (A_s). The mean genetic diversity (H_{es}) was obtained by averaging the H_e values over all loci. Diversity statistics at the population level were calculated as follows: the percent polymorphic loci (P_p) was determined from the percent polymorphic loci averaged over all populations, and the alleles per locus (A_p) also represents the mean over all populations. The genetic diversity (H_{ep}) was first calculated as the mean for each locus over all populations, and then averaged over all loci.

From an Unweighted Pair Group Method with Arithmetic Mean (UPGMA) analysis of the isozyme data (not shown), we retrieved a network that separated the Atlantic and Pacific accessions at the 0.90 similarity level, but did not yield informative patterns within these groups. This result led us to adopt an alternate approach, geographic mapping of allozyme presence/absence distribution

by population, which allowed much greater resolution of the distribution of genetic diversity.

RESULTS

cpDNA haplotypes. The aligned *ndhF-trnH* sequence was 225 nucleotides long. It included the last nucleotide of the *ndhF* gene, all of the *ndhF-trnH* spacer, and 27 nucleotides of *trnH*. Of the 225 nucleotides sampled, five were variable. In addition there were three indels. Treating the indels as single characters, the eight total varying characters (see Table 1 for details) comprised four (including two indels) that distinguished the study group from *Lathyrus palustris* and four (including one indel) that were variable in the study group. The variable characters together allowed the discrimination of five haplotypes, which showed strong geographic patterning (Table 2, Figure 1). Haplotype A (*Pacific*) was collected from the Pacific (Japan, the Aleutian Islands, the Alaskan Peninsula) and northern portions of North America including James Bay, Newfoundland, and adjacent southeastern Labrador. Haplotype B (*Northwest*) was restricted to samples from southern Alaska (Prince of Wales Island) and Oregon. Plants with haplotype C (*Atlantic-Inland*) were from the Atlantic coasts of North America and Europe, as well as from the Great Lakes and Lake Champlain. Haplotype D (*Champlain*) was unique to two populations on Lake Champlain, and Haplotype E (*New Jersey*) was unique to the New Jersey population. Though most populations had a single haplotype, four sampled populations had two of the five haplotypes. These were 1) Newfoundland populations 16 and 18, which yielded both the Pacific haplotype A and the Atlantic-Inland haplotype C; 2) Champlain population 19, which had both the Atlantic-Inland haplotype C and the endemic Champlain haplotype D; and 3) New Jersey population 1, which yielded both the Atlantic-Inland haplotype C and the endemic New Jersey haplotype E.

Maximum-parsimony analysis revealed that only one character (nucleotide 209, Table 1) was phylogenetically informative. The analysis resolved a single clade, an Atlantic-Inland clade comprising haplotypes C, D, and E with a 64% bootstrap value. However, the unrooted network arising from this analysis resolved relationships among all five haplotypes in a geographic context (Figure 1). In the single recovered network, the two broad-ranging haplotypes—A (Pacific) and C (Atlantic-Inland)—were interior and adjacent,

Table 2. Quantity (N) of each haplotype (A–E) in the sampled *Lathyrus japonicus* populations. No. = population identification number used on maps (see Figures 1–3). Population acronyms are detailed in Appendix 1.

Populations Sampled		Haplotype (N)				
No.	Location Acronym	A	B	C	D	E
1	NJ			1		1
4	PI			1		
5	CC			2		
8	CB			2		
14	GPB			8		
16	NFA	2		1		
17	NFB			2		
18	RBL	2		1		
19	AUS			1	2	
22	CP				4	
25	LOA			1		
29	LHA			1		
32	LM			1		
34	LSB			1		
35	PW		3			
36	SRH	1				
37	ATTU	2				
39	ORE		1			
40	JAP	3				
41	SWE			2		
42	DEN			2		
43	JB	2				

separated by one substitution. Haplotype B, from Oregon and Alaska, was geographically isolated from the southernmost Pacific populations of haplotype A, from which it differed by a unique indel. The localized New Jersey and Lake Champlain haplotypes D and E each differed by a single substitution from the widespread and geographically proximate haplotype C. *Lathyrus palustris* differed from all accessions of *L. japonicus* by two indels and two substitutions.

Isozymes. The ten enzyme systems resolved in this study were coded by 14 putative loci. Nine of the 14 loci resolved (64%) were polymorphic in at least one of the 38 populations. Allozyme frequencies for all variable loci are reported in Appendix 2.

The genetic structure of *Lathyrus japonicus* was comparable to other plant species with similar life-history characteristics (Table 3;

Table 3. Comparison of North American *Lathyrus japonicus* allozyme diversity statistics with values obtained for species with similar life-history characteristics. %P is percent polymorphic loci, A is alleles per locus, and H_e is expected genetic diversity. Data for other species from Hamrick and Godt (1990). $\%P_p$ is reported using 99% criterion.

	Allozyme Diversity Statistics					
	Species Level			Population Level		
Life History Characteristics	$\%P_s$	A_s	H_{es}	$\%P_p$	A_p	H_{ep}
long-lived herb	39.6	1.42	0.205	39.3	1.44	0.084
short-lived herb	41.3	1.72	0.116	28.0	1.40	0.096
widespread	58.9	2.29	0.202	43.0	1.72	0.159
boreal-temperate	79.7	2.64	0.186	64.5	2.08	0.184
self-pollinated	41.8	1.69	0.124	20.0	1.31	0.074
mixed animal pollination	40.0	1.68	0.120	29.2	1.43	0.090
sexual and vegetative	43.8	1.69	0.138	29.4	1.47	0.103
average plant species	51.0	1.97	0.150	34.2	1.53	0.113
Lathyrus japonicus	64.0	2.07	0.114	30.2	1.30	0.091

details in Appendix 3). The maximum number of alleles per locus was five, in TPI-2. At the population level, most of the genetic variation was found in the Atlantic and Pacific regions; inland regions were depauperate. Among the populations on the Atlantic, most of the genetic diversity was found in the Gulf of St. Lawrence (populations 10–18).

Of the 29 allozymes we encountered in the study set, 17 were geographically restricted in an informative way (the rest were ubiquitous in either monomorphic or polymorphic loci). There were six patterns of localization (Figures 2, 3).

1. Four allozymes were endemic to the northern Atlantic. These allozymes showed nested patterns of restriction in the east (Figure 2). MDH-2A and MDH-3B were found only in the Cape Cod population (region A, Figure 2). AAT-2B was localized in two groups of populations (region B, Figure 2), 3–6 (Rhode Island to Maine) and 9–10 (seaward Nova Scotia–Cape Breton Isle). MDH-3A was distributed from New Jersey to the St. Lawrence (region C, Figure 2).
2. Six allozymes were common in Pacific samples but restricted to populations 10–18 in the northeast. Penetration into the northeast was geographically nested (Figure 3), with population 18 having all six, populations 14 and 16 having three

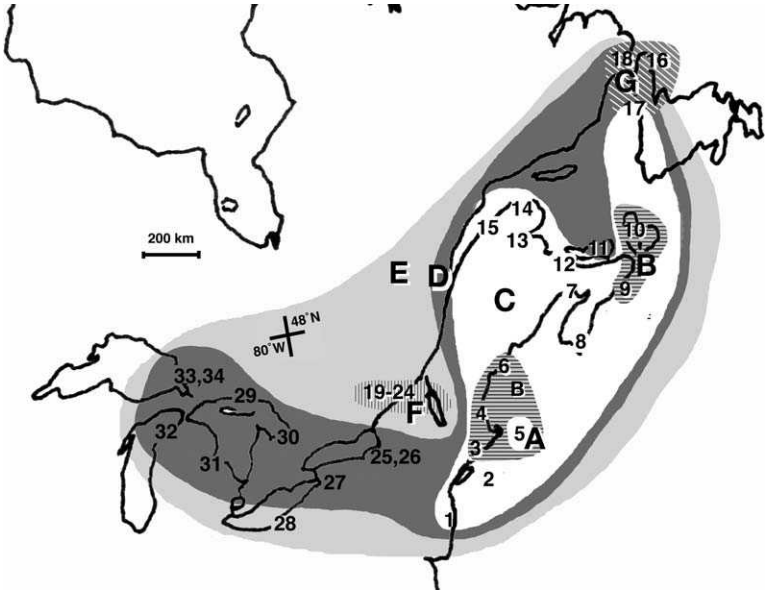

Figure 2. Distribution of *Lathyrus japonicus* allozymes endemic to eastern North America (azimuthal equidistant projection centered on the North Pole). Numbers are collection sites (see Appendix 1). Shaded areas labeled with letters are cumulative geographic patterns of allozyme *presence* as follows: A: E plus D plus C plus B plus MDH-2A, MDH-3B. B: E plus D plus C plus AAT-2B. C: E plus D plus MDH-3A. D: E plus TPI-2A. E: AAT-2C, IDH-B. F: E plus ALD-A. G (includes population 17): AAT-2D.

of the six, and the rest of the populations having one. Thus, in the Northeast, these common Pacific allozymes were commonest at the mouth of the St. Lawrence, especially in the area of Newfoundland.

3. There were three allozymes with a generalized distribution in the Atlantic, including the Great Lakes and Lake Champlain: TPI-2A (region D, Figure 2) plus AAT-2C and IDH-B (both in region E, Figure 2). These allozymes were absent from the Pacific and the Newfoundland population 16.
4. One allozyme, AAT-2D, was endemic to populations 16–18 in Newfoundland and adjacent Labrador (region G, Figures 2, 3).
5. Two allozymes were informative about the biogeography of populations from the Pacific Ocean (not the same as the area occupied by the Pacific haplotype A). TPI-2B was uniquely

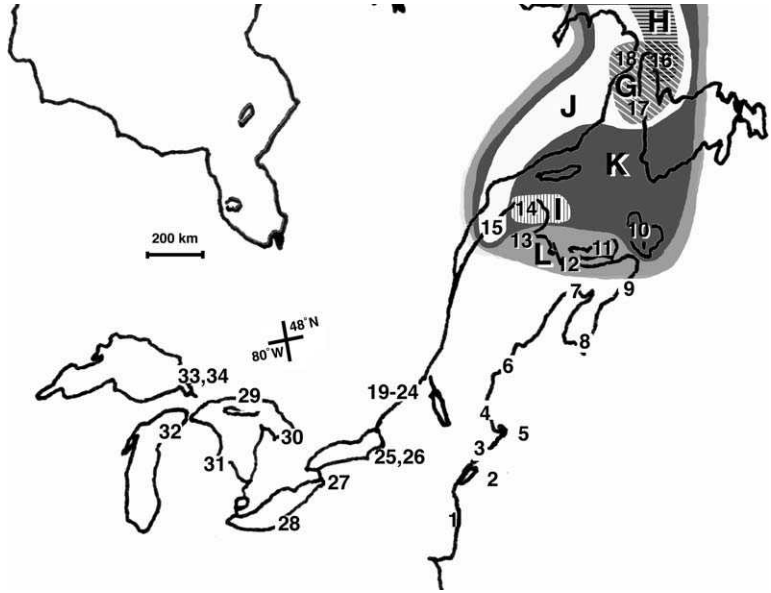

Figure 3. Eastern North American distribution of *Lathyrus japonicus* allozymes shared with Pacific North America (azimuthal equidistant projection centered on the North Pole). Numbers are collection sites (see Appendix 1). Shaded areas labeled with letters are cumulative geographic patterns of allozyme *presence* as follows: L: LAP-B. K: L plus IDH-A. J: L plus K plus TPI-2D. I: L plus K plus J plus AAT-2A. G: L plus K plus J plus AAT-2D. H: L plus K plus J plus AAT-1A and AAT-2A (and AAT-2D in population 16).

absent from the northwestern North America populations 35 and 39. A single allozyme, TPI-2E, was found only in the southern Alaska population 35. This was the only geographically localized allozyme found in the Pacific-Ocean isozyme sample.

6. Inland, ALD-A was endemic to three of the six populations sampled from Lake Champlain (region F, Figure 2). On the Great Lakes, TPI-2 and PGM-1 were the only polymorphic loci. Unique alleles were not detected in the Great Lakes region.

The geographic distributions of haplotypes and allozymes in North America were largely congruent. Plants with allozymes geographically restricted to the Atlantic usually also had the Atlantic-Inland cpDNA haplotype C, and plants with allozymes present in the Pacific populations usually also had the Pacific-Arctic

haplotype A. There were two areas of incongruence between these data sets. First, allozymes characteristic of the Pacific populations occurred not only in Newfoundland populations 16 and 18, which harbored the Pacific haplotype A, they also occurred to varying degrees in seven populations with Atlantic haplotype C (populations 10–15 and 17, distributed around the Gulf of St. Lawrence; Figures 1, 3). Second, on Lake Champlain, there was a complex co-occurrence of endemic and widespread haplotypes and allozymes. On the west side of the lake, the Ausable population lacked the endemic ALD-A allozyme (present in three of five Vermont populations), but two of three cpDNA samples yielded the endemic haplotype D (the third had the Atlantic-Inland haplotype C). On the east side of the lake, only the Colchester Point population had the endemic allozyme ALD-A, but the endemic haplotype D was retrieved in all four samples.

DISCUSSION

Both at the population and species level, *Lathyrus japonicus* harbors low to average levels of genetic diversity for plant species as measured by standard parameters (Table 1), in spite of its outcrossing reproductive biology and potential for wide dispersal of both rhizomes and seeds.

The strong historical signal in the geographic patterning of genetic diversity allows a series of historical inferences about the species. The interior and adjacent positions of the widespread haplotypes A and C in the network (Figure 1) indicate that *Lathyrus japonicus* diverged first into two lineages, which we call Arctic-Pacific and Boreal-Atlantic. The Arctic-Pacific lineage (haplotype A) now occurs in the northern Pacific and across the Canadian Arctic as far east as Newfoundland; the Boreal-Atlantic lineage (haplotype C) is found in the North Atlantic from Europe west to Newfoundland, New Jersey, Lake Champlain, and the Great Lakes. We tentatively suggest that these lineages diverged as they became isolated in different refugia (presumably Atlantic and Pacific) during the last glacial maximum. This pattern is reminiscent of the genetic differentiation of Atlantic and Mediterranean populations of the coastal plants *Cakile maritima* Scop. and *Salsola kali* L. subsp. *kali* (Kadereit and Westberg 2007).

The two widespread haplotypes each have satellite, localized haplotypes, suggesting that Holocene expansion of the beach pea

included peripheral fragmentation of the two ancestral lineages. Both have yielded unique haplotypes at their southern periphery: New Jersey haplotype E in the Atlantic and Northwest Coast haplotype B in the Pacific.

The incongruence between the isozyme and haplotype datasets suggests an interesting possibility. The Atlantic and Pacific population systems each have widespread, diagnostic allozymes—four in the Atlantic and six in the Pacific. However, the Pacific allozymes can be found in the northernmost Atlantic populations in North America, populations that have the Atlantic haplotype C. In contrast, the Atlantic allozymes are not found west of Newfoundland, and never outside of populations with the Atlantic haplotype C. Discordant pollen flow may be the explanation: pollen from the Pacific lineage may have recently been involved in mating with Atlantic-lineage populations southward in the Canadian maritimes.

The strong, consistently nested pattern of allozyme localization centered on Cape Cod signals that a Pleistocene refugium was nearby; we suggest that it was to the east of present-day Cape Cod on the exposed and unglaciated continental shelf, in the same area as proposed for *Ammophila breviligulata* (Walker 1998). In contrast, the limited allozyme diversity in the Great Lakes suggests that beach pea populations there were established only recently from marine populations. There is not enough signal to infer the location of a refugium for the Pacific lineage.

Lake Champlain beach pea populations are genetically differentiated from surrounding freshwater and salt-water populations in a complex way. A unique allozyme and haplotype are present in some of the Champlain populations, but some populations also have genetic variants widespread in the Great Lakes and Northern Atlantic. Thus, Lake Champlain evidently provided adequate peripheral isolation for genetic divergence, but the populations are polymorphic for a mix of ancestral and derived variants. The divergence leads us to suggest that the plants arrived in the Champlain Basin at the time of the incursion of the Atlantic into the basin in the latest Pleistocene (12,000–10,000 YBP) and have been isolated from the Atlantic only since that time.

The widespread haplotypes A and C occur together in Newfoundland populations 16 and 18, in the same area where widespread Pacific allozymes occur together with allozymes found throughout the Atlantic and Great Lakes populations. We suggest that populations of the two lineages expanded into the region and

made secondary contact as climate moderated in the Holocene, yielding a suture zone reminiscent of those seen in Europe at contact points between populations from different refugia (Hewitt 2000; Remington 1968). This history suggests that the Pacific lineage reached the Straits of Belle Isle via the Northwest Passage.

Geographically, the two widespread lineages, A and C, largely correspond respectively to the 1) Arctic-Pacific and 2) North-Temperate-Atlantic/Great-Lakes variety pairs recognized by Fernald (1932). Fernald may have been correct in suggesting that his Arctic variety *aleuticus* is the more ancient, because the Arctic-Pacific haplotype A is most similar genetically to our outgroup *Lathyrus palustris*; all populations of the Atlantic haplotype (C) share one additional divergent nucleotide. Hultén's and Fernald's inclusion of pubescence in delineating varieties is unwarranted, because morphometric analysis revealed that indument is highly labile within and between populations (Schmitz 2002). Consequently, the infraspecific taxonomy of Bässler (1966, 1973) best represents the history of *L. japonicus.*

ACKNOWLEDGMENTS. The authors thank Connie Åsmussen for generously sharing her work with *Lathyrus japonicus* and providing seeds; Sandy Talbot, Damon Little, Julie Dragon, and Cathy Paris provided beach pea population samples from the Aleutian Islands, Alaska; Prince of Wales Island, Alaska; James Bay, Canada; and Deer Creek, Lake Ontario, New York (respectively). Members of the University of Vermont Plant Systematics Group (Cathy Paris, Julie Dragon, and Heather Driscoll) made substantive contributions to the development of the analysis and text. This work was supported by USDA CSREES Hatch funding to D.S.B. through the Vermont Agricultural Experiment Station Project #VT-HA 594 and by a New England Botanical Club Graduate Student Research Award to S.A.S. It is based on the second author's Ph.D. thesis (Schmitz 2002).

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APPENDIX 1

Collection sites for *Lathyrus japonicus* (and one other species, as noted) population samples used for isozyme (N_1) and cpDNA (N_2) analysis. No. = population identification number used on maps. Location Acronym = population abbreviation used in tables. For representative accessions, herbarium vouchers—all at the Pringle Herbarium, University of Vermont (VT)—and Genbank Numbers (one for each haplotype encountered) follow population locations in parentheses.

Population Identification		Number of Samples			Compass Coordinates N, W (except where noted)	
No.	Location Acronym	N_1	N_2	Collection Sites Location (Voucher Collector, Genbank Number)	Lat.	Long.
ATLANTIC						
1	NJ	41	2	Island Beach State Park, New Jersey (*S.A. Schmitz*, GENBANK KC112558)	39°56′	74°04′
2	LI	34	0	Fire Island National Seashore, Long Island, New York	40°39′	73°07′
3	RI	14	0	Scarborough Beach, Rhode Island	41°25′	71°29′
4	PI	17	1	Parker River National Wildlife Refuge, Plum Island, Massachusetts	42°46′	70°48′
5	CC	40	2	Cape Cod National Seashore, Provincetown, Massachusetts (*S.A. Schmitz*, GENBANK KC112556)	42°03′	70°08′
6	ME	14	0	Morse Beach, Wiscasset, Maine	43°54′	69°43′
7	NSA	30	0	Parrsboro, Bay of Fundy, Nova Scotia	45°25′	64°20′
8	NSB	25	2	Blanche, Nova Scotia	43°38′	65°20′
9	NSC	30	0	Fancy's Point, Marie-Joseph, Nova Scotia	45°02′	61°59′
10	CB	30	0	Dominion Beach at Indian Bay, Cape Breton Island	46°08′	60°10′
11	PEI	28	0	Bothwell, Prince Edward Island	46°25′	62°08′
12	NB	30	0	Barachois, New Brunswick	46°12′	64°21′
13	GPA	30	0	Cap de Sable, Gaspé Peninsula, Québec	48°01′	65°28′
14	GPB	29	8	Grande Vallée, Gaspé Peninsula, Québec	49°11′	64°56′

Appendix. Continued.

Population Identification		Number of Samples		Collection Sites	Compass Coordinates N, W (except where noted)	
No.	Location Acronym	N_1	N_2	Location (Voucher Collector, Genbank Number)	Lat.	Long.
15	MQ	30	0	Matane, Québec	48°59′	66°55′
16	NFA	34	3	St. Anthony's, Newfoundland	51°22′	55°35′
17	NFB	30	2	Gros Morne Provincial Park, Newfoundland	49°46′	57°30′
18	RBL	14	3	Red Bay, Labrador (*S.A. Schmitz*)	51°47′	56°12′
LAKE CHAMPL	AIN					
19	AUS	48	3	Ausable Beach, New York	44°32′	73°25′
20	SAB	14	0	Alburg Dunes State Park, South Alburg, Vermont	44°52′	73°18′
21	DP	29	0	Delta Park, Colchester Point, Vermont	44°31′	73°15′
22	CP	25	4	Colchester Point, Vermont (*S.A. Schmitz*, GENBANK KC112557)	44°32′	73°16′
23	ATP	8	0	Strathmore at Appletree Point, Burlington, Vermont	44°30′	73°16′
24	SHE	31	0	South Hero Beach, South Hero, Vermont	44°36′	73°17′
GREAT LAKES						
25	LOA	35	1	Deer Creek, Lake Ontario, New York (*C.A. Paris*)	43°49′	76°15′
26	LOB	19	0	El Dorado Nature Preserve, Lake Ontario, New York	43°49′	76°15′
27	LENY	3	0	Evangola State Park, Lake Erie, New York	42°36′	79°06′
28	LEOH	7	0	Headwaters State Park, Lake Erie, Ohio	41°56′	80°39′
29	LHA	35	1	Providence Bay, Manitoulin Island, Lake Huron, Ontario	45°42′	82°24′
30	LHB	8	0	Wasaga Beach (Beach #2), Lake Huron, Ontario	44°27′	80°06′
31	LHC	32	0	Tawas State Park, East Tawas, Lake Huron, Michigan	44°15′	83°31′
32	LMC	40	1	Sleeping Bear Dunes National Lakeshore, Lake Michigan, Michigan	45°02′	85°41′

Appendix. Continued.

Population Identification		Number of Samples		Collection Sites	Compass Coordinates N, W (except where noted)	
No.	Location Acronym	N_1	N_2	Location (Voucher Collector, Genbank Number)	Lat.	Long.
33	LSA	27	0	Montreal River Harbor, Lake Superior, Ontario	47°18′	84°35′
34	LSB	28	1	Batchawana Bay, Lake Superior, Ontario	46°56′	84°37′
PACIFIC						
35	ORE	14	1	Newport, Oregon	44°37′	124°03′
36	PW	30	3	Prince of Wales Island, Alaska (*D.P. Little*)	55°32′	132°53′
37	SRH	7	1	Sandy River Harbor, Alaska Peninsula, Alaska	55°17′	162°27′
38	SHY	13	0	Shemya Island, Aleutian Islands, Alaska	52°43′	174°07′
39	ATU	10	2	Attu Island, Aleutian Islands, Alaska (*S. Talbot*, GENBANK KC112559)	52°55′	172°27′
40	JAP	0	3	Hokkaido, Japan	42°50′	140°80′ E
EUROPE (ATLANTIC)						
41	SWE	0	2	Västernorrland, Sweden	62°75′	17°09′ E
42	DEN	0	2	Copenhagen, Denmark	55°40′	12°34′ E
ARCTIC CANADA						
43	JB	0	2	James Bay, Canada (*J.A. Dragon*)	53°12′	85°47′
–	out-group	0	1	*Lathyrus palustris* — Barachois, New Brunswick, Canada (*S.A. Schmitz*, GENBANK KC112555)	46°14′	64°25′

APPENDIX 2

Allozyme genotype counts for the sampled isozymes of North American *Lathyrus japonicus*. Population numbers and abbreviations as in Appendix 1. Order of isozymes, each separated by commas, is AAT-1, AAT-2, ALD, IDH, MDH-1, MDH-2, MDH-3, ME, PGI-2, PGM-1, SKDH, TPI-1, TPI-2, LAP. Different inferred genotypes within a single population are separated only by a space.

ATLANTIC

1 NJ. BB:41, CC:41, BB:41, BB:41, AA:41, BB:41, AA:02 AC:07 CC:32, AA:41, AA:41, AA:07 AB:15 BB:19, AA:41, AA:41, AA:19 AB:21, BB:41

2 LI. BB:34, CC:34, AA:34, BB:34, AA:34, BB:34, AC:34 CC:30, AA:34, AA:34, AA:05 BB:29, AA:34, AA:34, AA:20 AB:08 BB:06, AA:34

3 RI. BB:17, BC:03 CC:14, BB:17, BB:17, AA:17, BB:17, AA:05 AC:08 CC:04, AA:17, AA:17, AB:06 BB:11, AA:17, AA:17, AA:07 AB:07 BB:03, AA:17

4 PI. BB:20, BC:03 CC:17, BB:20, BB:20, AA:20, BB:20, AC:04 CC:16, AA:20, AA:20, AB:05 BB:15, AA:20, AA:20, AA:05 AB:12 BB:03, AA:20

5 CC. BB:40, BB:01 BC:02 CC:37, BB:40, BB:40, AA:40, AA:16 BB:25, AB:17 CC:23, AA:40, AA:40, BB:40, AA:40, AA:40, AA:36 AB:04, AA:40

6 ME. BB:14, BB:01 BC:02 CC:11, BB:10, BB:14, AA:14, BB:14, AC:01 CC:13, AA:14, AA:14, AB:03 BBB:11, AA:10, AA:14, AA:01 AB:08 BBB:05, AA:14

7 NSA. BB:08, CC:32, BB:32, BB:32, AA:08, BB:08, AA:01 AC:02 CC:29, AA:08, AA:32, BB:24, AA:28, AA:32, AA:10 AB:09 BB:13, AA:32

8 NSB. BB:10, CC:25, BB:25, BB:25, AA:10, BB:10, AC:07 CC:18, AA:10, AA:25, BB:01, AA:29, AA:25, AA:01 AB:17 BB:07, AA:25

9 NSC. BB:30, BC:01 CC:29, BB:30, BB:30, AA:10, BB:10, AC:07 CC:23, AA:10, AA:30, AA:03 BB:06, AA:30, AA:30, AA:05 AB:20 BB:04, AA:30

10 CB. BB:30, BC:01 CC:21, BB:30, AB:01, BB:29, AA:30, BB:30, AA:03 AC:12 CC:14, AA:30, AA:30, AA:05 AB:03 BB:21, AA:30, AA:30, AA:08 AB:12 BB:08, AA:27 AB:02

11 PEI. BB:30, CC:15, BB:30, BB:30, AA:30, BB:30, CC:30, AA:30, AA:30, BB:15, AA:30, AA:30, AA:05 AB:23 BB:01, AA:22 AB:04 BB:03

12 NB. BB:30, CC:30, BB:30, BB:30, AA:30, BB:30, AC:13 CC:17, AA:30, AA:30, AA:03 AB:02 BB:25, AA:30, AA:30, AA:18 AB:10 BB:02, AA:15 AB:13 BB:02

13 GPA. BB:30, BC:02 CC:28, BB:30, BB:30, AA:30, BB:30, AC:05 CC:25, AA:30, AA:30, AA:16 AB:04 BB:09, AA:30, AA:30, AA:06 AB:15 BB:09, AA:27 AB:03

14 GPB. BB:29, AC:03 BC:02 CC:25, BB:29, AB:09 BB:20, AA:29, BB:29, AA:01 AC:01 CC:28, AA:29, AA:29, AA:05 AB:05 BB:09, AA:29, AA:29, AA:08 AB:10 BB:09 BD:01 AD:01 DD:01, A:27 AB:02

15 MQ. BB:12, BC:06 CC:23, BB:30, AB:07 BB:23, AA:30, BB:30, AC:02 CC:28, AA:30, AA:30, AA:03 AB:04, BB:05, AA:30, AA:24, AA:07 AB:16 BB:04 AD:03, AA:24 AB:06

16 NFA. AB:02 BB:23, AA:21 DD:04, BB:34, AA:21 AB:13, AA:34, BB:34, CC:34, AA:34, AA:13, AB:03 BB:30, AA:12, AA:31, BD:07 B C:02 CD:14 DD:11, AA:25 AB:06 BB:03

17 NFB. BB:23, BC:03 CC:17 DD:03, BB:26, AA:03 AB:10 BB:17, AA:26, BB:26, AC:02 CC:24, AA:26, AA:10, AA:05 AB:05 BB:16, AA:10, AA:30, AA:06 AB:05 BB:06 AD:03 BD:08, AA:22 AB:05 BB:03

18 RBL. BB:11, CC:01 DD:10, BB:14, AA:07 AB:03 BB:02, AA:14, BB:14, CC:14, AA:14, AA:14, AB:02 BB:10, AA:14, AA:14, AD:03 BD:06 DD:05, AA:09 AB:05

LAKE CHAMPLAIN

19 AUS. BB:50, CC:50, BB:50, BB:50, AA:50, BB:50, CC:50, AA:50, AA:50, AA:03 AB: 06 BB:41, AA:50, AA:50, AB:07 BB:43, AA:50

20 SAB. BB:14, CC:14, BB:14, BB:14, AA:14, BB:14, CC:14, AA:14, AA:14, AA:14, AA:14, AA:14, BB:14, AA:14

21 DP. BB:30, CC:30, AB:06 BB:24, BB:30, AA:30, BB:30, CC:30, AA:30, AA:30, BB:30, AA:30, AA:30, BB:30, AA:30

22 CP. BB:31, CC:31, AB:17 BB:14, BB:31, AA:31, BB:31, CC:31, AA:31, AA:31, BB:31, AA:31, AA:31, BB:31, AA:31

23 ATP. BB:08, CC:08, BB:08, BB:08, AA:08, BB:08, CC:08, AA:08, AA:08, BB:08, AA:08, AA:08, BB:08, AA:08

GREAT LAKES

24 SHE. BB:30, CC:30, AB:24 BB:07, BB:30, AA:30, BB:30, CC:30, AA:30, AA:30, AB:01 BB:30, AA:30, AA:30, BB:30, AA:30

25 LOA. BB:10, CC:35, BB:35, BB:35, AA:10, BB:10, CC:35, AA:08, AA:35, AA:02 BB:33, AA:35, AA:35, AA:01 AB:04 BB:30, AA:35

26 LOB. BB:08, CC:17, BB:17, BB:17, AA:08, BB:08, CC:17, AA:08, AA:17, BB:17, AA:17, AA:17, AB:01 BB:16, AA:17

27 LENY. BB:03, CC:03, BB:03, BB:03, AA:03, BB:03, CC:03, AA:03, AA:03, AA:03, AA:03, AA:03, AA:03, AA:03

28 LEOH. BB:07, CC:07, BB:07, BB:07, AA:07, BB:07, CC:07, AA:07, AA:07, AA:04 AB:02 BB:01, AA:07, AA:07, BB:07, AA:07

29 LHA. BB:18, CC:34, BB:34, BB:34, AA:18, BB:18, CC:34, AA:34, AA:34, BB:34, AA:34, AA:34, AA:22 AB:04 BB:07, AA:34

30 LHB. BB:01, CC:08, BB:08, BB:08, AA:01, BB:01, CC:08, AA:01, AA:08, BB:08, AA:08, AA:08, AA:08, AA:08

31 LHC. BB:10, CC:32, BB:32, BB:32, AA:32, BB:01, CC:32, AA:32, AA:32, BB:09, AA:32, AA:32, AA:32, AA:32

32 LMC. BB:01, CC:40, BB:40, BB:40, AA:01, BB:01, CC:40, AA:01, AA:40, BB:12, AA:40, AA:35, AA:12 AB:16 BB:07, AA:36

33 LSA. BB:10, CC:21, BB:21, BB:21, AA:10, BB:10, CC:21, AA:08, AA:21, BB:21, AA:21, AA:21, AA:05 AB:08 BB:08, AA:21

34 LSB. BB:36, CC:36, BB:36, BB:36, AA:36, BB:36, CC:36, AA:36, AA:36, BB:36, AA:36, AA:36, AA:03 AB:10 BB:24, AA:36

PACIFIC

36 PW. AA:01 AB:29, AA:30, BB:30, AA:30, AA:30, BB:30, CC:30, AA:30, AA:30, BB:30, AA:30, AA:30, CE:09 DD:19 EE:02, AA:30

37 SRH. BB:07, AA:07, BB:03, AA:07, AA:03, BB:03, CC:03, AA:03, AA:03, BB:07, AA:03, AA:07, CC:03 CD:02 DD:01, AA:04 AB:02 BB:01

38 SHY. AB:01 BB:11, AA:10, BB:04, AA:10, AA:04, BB:04, CC:04, AA:10, AA:04, AB:02 BB:10, AA:04, AA:10, BD:04 CC:02 CD:04 DD:02, AA:10
39 ATU. AA:02 AB:01 BB:07, AA:10, BB:03, AA:10, AA:07, BB:07, CC:07, AA:10, AA:03, BB:10, AA:03, AA:10, BB:04 BC:03 BD:02 DD:01, AA:09 BB:01

APPENDIX 3

Genetic diversity statistics by population ($_p$) from isozyme data for North American *Lathyrus japonicus*. A is alleles per locus, %P is percent polymorphic loci reported using 99% criterion, and H is genetic diversity, observed ($_o$) and expected ($_e$).

Population	A_p	$\%P_p$	H_{op}	H_{ep}
ATLANTIC				
1 NJ (New Jersey)	1.2	21.4	0.076	0.078
2 LI (Long Island)	1.2	21.4	0.025	0.056
3 RI (Rhode Island)	1.3	28.6	0.101	0.105
4 PI (Plum Island)	1.3	28.6	0.086	0.076
5 CC (Cape Cod)	1.4	28.6	0.041	0.09
6 ME (Maine)	1.3	28.6	0.071	0.071
7 NSA (Nova Scotia)	1.1	14.3	0.025	0.044
8 NSB (Nova Scotia)	1.1	14.3	0.069	0.052
9 NSC (Nova Scotia)	1.3	28.6	0.068	0.087
10 CB (Cape Breton)	1.4	42.9	0.078	0.103
11 PEI (Prince Edward Is.)	1.1	14.3	0.067	0.056
12 NB (New Brunswick)	1.3	28.6	0.09	0.097
13 GPA (Gaspé)	1.4	35.7	0.069	0.093
14 GPB Gaspé)	1.6	42.9	0.089	0.118
15 MQ (Matane)	1.5	42.9	0.12	0.121
16 NFA (St Anthony)	1.5	42.9	0.1	0.113
17 NFB (Gros Morne)	1.6	42.9	0.102	0.157
18 RBL (Red Bay)	1.4	35.7	0.101	0.112
Mean	1.3	30.2	0.077	0.091
LAKE CHAMPLAIN				
19 AUS (Ausable NY)	1.1	14.3	0.019	0.025
20 SAB (S. Alburg, VT)	1.0	0.0	0	0
21 DP (Delta Park VT)	1.1	7.1	0.014	0.013
22 CP (Colchester Pt. VT)	1.1	7.1	0.039	0.029
23 ATP (Appletree Pt, VT)	1.0	0.0	0	0
24 SHE (South Hero, VT)	1.1	7.1	0.058	0.037
Mean	1.1	6.0	0.026	0.017
GREAT LAKES				
25 LOA (Ontario)	1.1	0.0	0.004	0.004
26 LOB (Ontario)	1.1	14.3	0.008	0.019
27 LENY (Erie)	1.0	0.0	0	0
28 LEOH (Erie)	1.1	7.1	0.02	0.031

Appendix 3. Continued.

Population	A_p	$\%P_p$	H_{op}	H_{ep}
29 LHA (Huron)	1.1	7.1	0.009	0.029
30 LHB (Huron)	1.0	0.0	0	0
31 LHC (Huron)	1.0	0.0	0	0
32 LM (Michigan)	1.1	7.1	0.033	0.035
33 LSA (Superior)	1.1	7.1	0.027	0.036
34 LSB (Superior)	1.1	7.1	0.019	0.025
Mean	1.1	5.0	0.014	0.018
PACIFIC				
36 PW (Prince of Wales I)	1.2	14.3	0.09	0.075
37 SRH (Sandy River Harbor)	1.1	14.3	0.044	0.066
38 SHY (Shemya)	1.3	14.3	0.065	0.063
39 ATU (Attu)	1.3	21.4	0.043	0.08
Mean	1.2	16.0	0.072	0.071
	A_s	P_s	H_{os}	H_{es}
Lathyrus japonicus	2.07	42.9	0.073	0.114