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CONTRASTING PATTERNS OF GENETIC VARIATION IN CENTRAL AND PERIPHERAL POPULATIONS OF *DRYOPTERIS FRAGRANS* (FRAGRANT WOOD FERN) AND IMPLICATIONS FOR COLONIZATION DYNAMICS AND CONSERVATION

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Premise of research. Ferns are vital components of temperate and tropical ecosystems, but they have not been examined in the context of a central-peripheral hypothesis. *Dryopteris fragrans* is an ideal species to examine the genetic variation between central and peripheral populations because of its arctic north to temperate south distribution pattern. In addition to understanding colonization dynamics, our study also addresses the issue regarding the conservation value of peripheral plant populations.

Methodology. We examined 82 individuals from 22 populations from northern Canada (N-CA) and the northeastern United States (NE-US), which represent central and peripheral populations of *D. fragrans*, respectively. Two-hundred two loci were resolved using inter–simple sequence repeat markers, allowing analyses of genetic diversity and population structure, insights into gene flow and mating system, and correlations of genetic diversity with geographical distance, population size, and air temperature.

Pivotal results. *Dryopteris fragrans* exhibits high genetic diversity at the species level, with most of its genetic variation due to differences between populations. At the regional level, however, there is a sharp contrast in the patterns of genetic variation between N-CA and NE-US populations, with the latter exhibiting low genetic diversity, high population differentiation, low gene flow, and a predominantly inbreeding mating system. The NE-US populations also exhibit several unique loci that indicate that they are not merely a reduced representative of the overall genetic diversity of the species.

Conclusions. *Dryopteris fragrans* in the NE-US are genetically distinct from those in N-CA, and this result may serve as justification for the species' conservation in the NE-US. Our results also indicate that *D. fragrans* in the NE-US may have originated from the Canadian populations through several instances of single-spore founding events facilitated by long-distance spore dispersal and self-fertilization.

Keywords: climate change, ecological genetics, reproductive ecology, mixed-mating system, range expansion.

Online enhancements: appendix table.

Introduction

Dryopteris fragrans (L.) Schott (fragrant wood fern) is a suitable fern species to examine the pattern of genetic variation between central and peripheral populations. It has an interrupted arctic to subarctic distribution spanning Asia and North America but only one European population in Finland (Flora of North America Editorial Committee 2008). Its southernmost distribution reaches the temperate forests of the northeastern United States (NE-US), where it is listed as rare in all four states in which it occurs in this peripheral portion of its range. In contrast, *D. fragrans* is broadly distributed in boreal to arctic portions of eastern Canada (Payette et al. 2013). Nunavik of northern Quebec and adjacent Nunavut may be described as the central portion of its eastern North American range, as the species is broadly distributed to the south in temperate portions of Quebec, to the east in Greenland, to the north in the Canadian Arctic Archipelago, and further west in Canada and Alaska (Aiken et al. 2007; Flora of North America Editorial Committee 2008; Payette et al. 2013). Thus, *D. fragrans* is a globally secure species but is rare in the NE-US. At the center of its range, *D. fragrans* is widespread but locally uncommon, since it inhabits spatially restricted rocky surfaces, most commonly cliffs, screes, and rocky banks (Aiken et al. 2007). At the southern periphery of its range, *D. fragrans*'s habitat is more restricted, as it is solely epipetric, occurring in crevices on cliff faces (Aiken et al. 2007).

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Analysis of genetic variation between central and peripheral populations elucidates evolutionary processes, range expansion or contraction, and colonization history and also provides insights into conservation values of populations (Hampe and Petit 2005; Angert 2009). In a meta-analysis of 67 publications on plants by Eckert et al. (2008), approximately 30% of the studies reported genetic diversity at range edges to be equal to that at the center, while environmental stress experienced by peripheral populations of some species may influence the generation of genetic variation, resulting in higher genetic diversity. Higher genetic diversity in peripheral populations than in central populations has also been reported in several other publications (e.g., Beatty and Provan 2011; Provan and Maggs 2012; Pauls et al. 2013). In about 60% of the reports reviewed by Eckert et al. (2008), however, peripheral populations are characterized by lower genetic diversity coupled with increased genetic differentiation among populations. Eckert et al. (2008) pointed out that their report is biased toward angiosperms and conifers, and studies of these two groups of plants continue to accumulate (see Beatty and Provan 2011; Conord et al. 2012; Provan and Maggs 2012; Pauls et al. 2013; Chhatre and Rajora 2014). In a more recent related meta-analysis by Abeli et al. (2014), ferns remain absent from the literature on central-peripheral populations despite being vital components of many temperate and tropical ecosystems. Although some articles on ferns have examined the genetic structure of central (e.g., Jiménez et al. 2010) or peripheral (e.g., de Groot et al. 2012) populations, none of these articles examined both types of populations within a single study and focused directly on the genetic differences of central and peripheral populations.

Investigations of plants at their range margins are mostly on the northern limits of species, with relatively few studies focusing on populations in the southern part of the distribution range (Hampe and Petit 2005; Eckert et al. 2008; Bystriakova et al. 2014). Despite the few reports on southern peripheral populations, most consider these populations to harbor a disproportionate amount of the total genetic diversity across a species' range (Coyne and Orr 2004; Hampe and Petit 2005; Eckert et al. 2008; Provan and Maggs 2012; Pauls et al. 2013). Some southern peripheral populations have also been perceived as fast evolving and having greater evolutionary potential (Lesica and Allendorf 1995; Lammi et al. 1999; Chhatre and Rajora 2014). *Dryopteris fragrans* in the NE-US experiences warmer conditions than in the central and northern portions of its range, and under climate warming conditions it may experience environments that the species has not been exposed to in the recent past. These southern peripheral populations might diversify because of differing selection pressures from those in the center of the range, and their response to local conditions may involve development of genetic variation, phenotypic plasticity, and/or other life-history traits to support their persistence in this part of their range. They could also become a source of unique genetic variation within the species and may reveal specific populations with high levels of genetic diversity or differentiation, thereby providing support for their conservation in the NE-US. Knowledge of genetic diversity and population structure of central and peripheral populations of *D. fragrans* may provide insight into their colonization dynamics and conservation value, particularly in relation to the ensuing climate change. This study aimed to determine (1) levels of genetic diversity between and within central and peripheral populations of *D. fragrans*, (2) how genetic variation is partitioned between and within central and peripheral populations of *D. fragrans*, and (3) relationships between genetic and geographic distances and genetic diversity, population size, and air temperature. This study is the first to examine the pattern of genetic variation between central and peripheral populations in ferns.

Material and Methods

Study Sites and Population Sampling

A total of 82 fronds of *Dryopteris fragrans* (2n=82) were collected from 6 and 16 populations in northern Canada (N-CA) and the NE-US, respectively. The average geographic distance between these two regions is approximately 1850 km. Permission to collect fronds was obtained from all landowners and appropriate agencies where required. Documented populations in New York (NY), Vermont (VT), New Hampshire (NH), and Maine (ME), the four northeastern states where *D. fragrans* has been reported, were inventoried on the basis of herbarium records, Natural Heritage Program inventories in each state, and the authors' own field searches (fig. 1). We sampled from all four states to represent the entire NE-US where *D. fragrans* occurs and to examine for possible local differences in genetics and ecology. In each state, we attempted to sample from both more isolated populations and populations in closer proximity to other known populations. In all four states, the southernmost extant population was included in our study, including the NY populations that are the southernmost known populations in North America. In the NE-US, fronds from four individuals per population per state (fig. 1) were collected for genetic analyses (table 1) except for NY-HP, because no other *D. fragrans* individuals could be sampled safely. Fronds were collected no less than 1 m apart to avoid the sampling of possible clones or genetically closely related individuals and were collected only from individuals that had at least six fronds with a size range representative of other individuals at the site. Population size was based mostly on estimated counts of the individuals present at each site, supplemented with past inventories included in Natural Heritage Program databases. However, *D. fragrans* is very specific in its habitat choices, and it is unlikely that much unsearched suitable habitat was present at each site. For samples analyzed from N-CA, we sampled four plants per population for five populations in Nunavik, northern Quebec, following the same criteria used in the NE-US. However, only two plants were sampled at sites CA-LC and CA-PW. These populations were small, and individuals were clumped and possibly clonal; only two clumps at each population met our distance separation criteria. Herbarium sheets and silica gel desiccated tissue samples of four *D. fragrans* individuals from four locations within a 12-km radius on Victoria Island, Nunavut, Canada, were obtained from the Canadian Museum of Nature, Ontario, but no information was available on its population size (fig. 1; table 1). Air temperature at the NE-US populations was monitored with three iButton temperature loggers (Maxim Integrated, San Jose, CA) at each population, mounted on a nearby tree trunk and shielded from direct solar radiation by

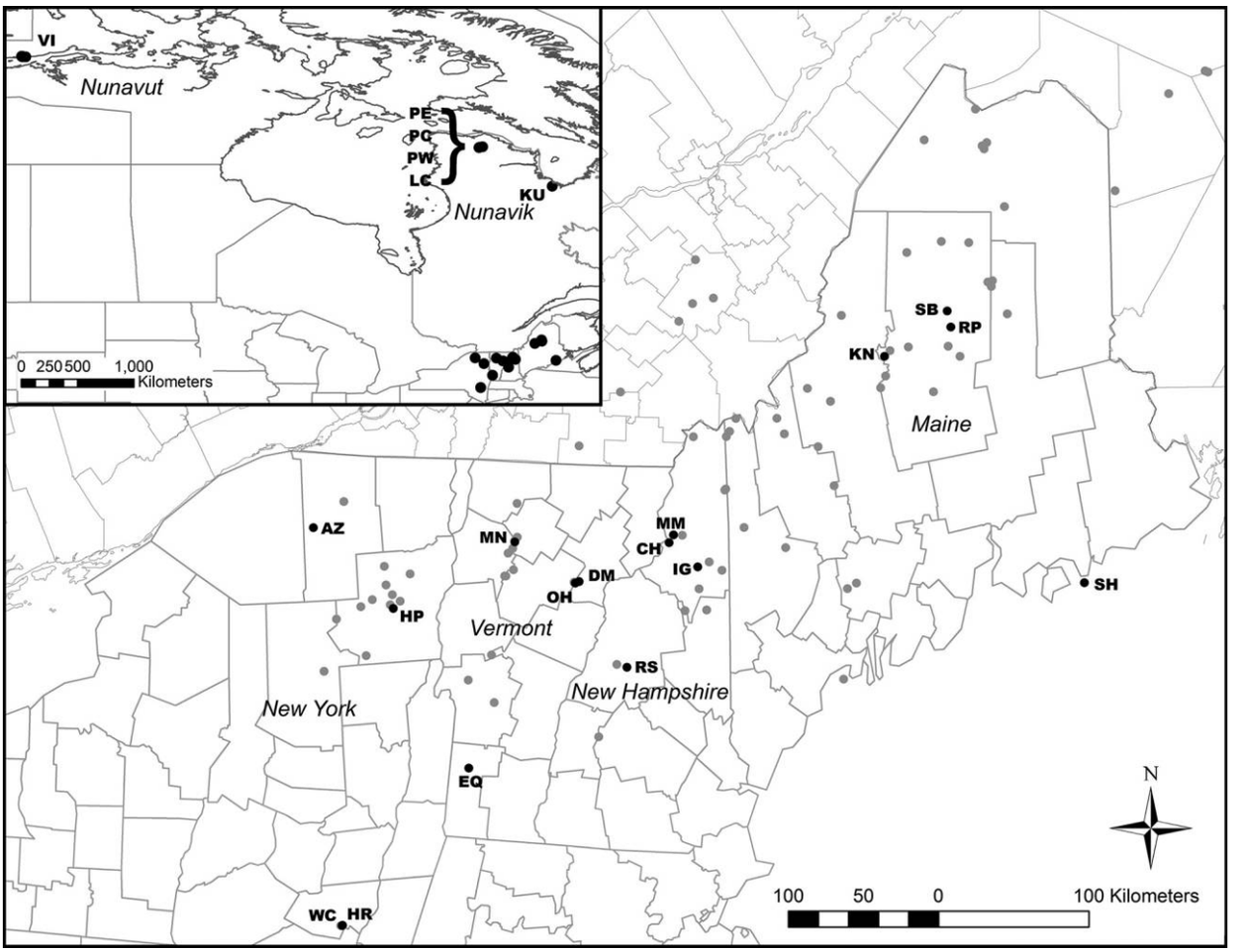

Fig. 1 Locations of *Dryopteris fragrans* populations examined in this study, as indicated by solid dark circles with two-letter site codes. Populations studied were in New York (NY), Vermont (VT), New Hampshire (NH), Maine (ME), and northern Canada (Nunavut and Nunavik; inset). All other known *D. fragrans* populations in the northeastern United States and adjacent portions of Canada are shown as gray circles.

a white PVC cap. Temperature was measured and recorded at 3-h intervals for 1 yr from July 2012 through June 2013. All measurements were used to calculate the mean annual air temperature. For the N-CA populations, air temperature at sampled sites was estimated for the same period on the basis of the closest established meteorological monitoring station.

DNA Extraction and Inter–Simple Sequence Repeat (ISSR) Polymerase Chain Reaction (PCR) Amplification

ISSR markers were used in this study, since this molecular marker system allows detection of even very low levels of genetic variation (Zietkiewicz et al. 1994; Fatemi and Gross 2009; Fernando et al. 2015). Genomic DNA isolation and quantitation were based on Bouchard (2013). Thirty-four ISSR primers were screened for use with *D. fragrans*, and 10 were selected on the basis of their consistent banding patterns following three independent PCR runs (Table 2). PCR conditions and gel electrophoreses were also based on Bouchard (2013).

Genetic Data Analysis

A binary matrix was constructed from ISSR-PCR amplified bands. Bands with the same mobility according to molecular weight were scored as 1 (denoting band presence) or 0 (denoting band absence). Gels were scored by manual inspection of the banding pattern images generated through Labworks image acquisition and analysis software (ver. 4; UVP Laboratory Products, Upland, CA). Bands that were consistent across replicates were scored, and each scored band was considered to be a locus present within the genome. A locus was considered polymorphic if it was absent in at least one *D. fragrans* individual.

Statistical analyses used to infer genetic diversity of *D. fragrans* were executed using POPGENE (ver. 1.3; Yeh et al. 1997). The indices of genetic diversity used were those that do not assume Hardy-Weinberg equilibrium, including percent polymorphic loci (PPL), Nei's (1973) gene diversity (h), Shannon's index of diversity (I; Lewontin 1972), and Nei's (1978) unbiased genetic distance and identity. PPL was calculated by

Table 1

Locations, Estimated Population Size, and Air Temperature for *Dryopteris fragrans* Populations

Population code	Collection site	State/territory	Country	Population size	Mean annual air temperature (°C)
CA-KU	Kuujjuaq	Nunavik	Canada	120	.0
CA-LC	Lamarche Canyon	Nunavik	Canada	7	−9.1
CA-PC	Puvirnituq Central	Nunavik	Canada	87	−9.1
CA-PE	Puvirnituq East	Nunavik	Canada	18	−9.1
CA-PW	Puvirnituq West	Nunavik	Canada	8	−9.1
CA-VI	Johansen Bay, Victoria Island	Nunavut	Canada	No data	−13.9
NY-AZ	Azure Mountain	New York	USA	20	6.7
NY-HP	Hunters Pass	New York	USA	20	3.5
NY-HR	Hillyer Ravine	New York	USA	30	7.4
NY-WC	Wildcat Ravine	New York	USA	200	7.5
VT-DM	Big Deer Mountain	Vermont	USA	150	6.1
VT-EQ	Mt. Equinox	Vermont	USA	250	6.5
VT-MN	Mt. Mansfield	Vermont	USA	25	5.8
VT-OH	Owl's Head Mountain	Vermont	USA	45	6.8
NH-CH	Cape Horn	New Hampshire	USA	200	6.9
NH-IG	Ice Gulch	New Hampshire	USA	32	4.7
NH-MM	Moore Mountain	New Hampshire	USA	61	5.5
NH-RS	Rattlesnake Mountain	New Hampshire	USA	108	8.3
ME-KN	Kineo Mountain	Maine	USA	40	6.5
ME-RP	Ripogenus Gorge	Maine	USA	500	5.4
ME-SB	Soubunge Mountain	Maine	USA	200	5.8
ME-SH	Schoodic Head	Maine	USA	10	7.2

dividing the number of polymorphic bands by the total number of bands surveyed at the population, region, or species levels. Genotype-frequency variation within and between populations was analyzed directly using Nei's gene diversity statistic (Nei 1973). Nei's unbiased genetic distance measures the accumulated polymorphisms per locus between populations, while Nei's unbiased genetic identity is a measure of the proportion of loci that are identical between populations (Nei 1972).

Mantel tests (Mantel 1967) were conducted in ARLEQUIN (ver. 3.5.1.3; Excoffier and Lischer 2010) to examine correlation between Nei's unbiased genetic distance and geographic distance among all *D. fragrans* populations and among populations from N-CA only and the NE-US only. Population latitude and longitude coordinates were used to build a pairwise Euclidean geographic distance matrix in the R package SpatialTools (tools for spatial data analysis; French 2015). Nonparametric permutational procedures were used to test for overall statistical significance based on 10,000 permutations. The correlations between Nei's gene diversity, population size, and air temperature were determined by Pearson correlation in R (ver. 3.2.3; R Core Team 2016).

Analysis of molecular variance (AMOVA) was performed in ARLEQUIN using the matrix of Euclidean squared distances. At the species level, AMOVA was performed to investigate the partitioning of genetic variation among populations and within populations. Two-level AMOVA tests were performed to examine genetic structure solely among and within the sampled N-CA and NE-US populations as well as combined samples from both regions. The coefficient of genetic variation (G_{ST}) and number of migrants successfully entering a popula-

Table 2

Inter–Simple Sequence Repeat (ISSR) Primer Codes and Their Nucleotide Sequences, Annealing Temperatures (TA), Product Size Ranges, Total Number of Loci (TNL), Total Number of Polymorphic Loci (TPL), and Percent Polymorphic Loci (PPL)

Primer code	Sequence (5′→3′)	TA (°C)	Min.-max. size (bp)	TNL	TPL	PPL
UBC 815	$(CT)_8GG$	56	320–1175	19	13	68.42
UBC 818	$(CA)_8GG$	59	262–691	17	12	70.59
UBC 841	$(GA)_8TC$	58	283–818	15	12	80.00
UBC 844	$(CT)_8GC$	59	172–1240	22	16	72.72
UBC 847	$(CA)_9AC$	58	179–897	18	16	88.89
UBC 848	$(CA)_8AG$	59	198–788	17	13	76.47
UBC 850	$(GT)_8CC$	56	277–1284	17	15	88.24
UBC 857	$(AC)_9TG$	59	203–1204	19	16	84.21
SBS 1	$(CA)_8CC$	59	271–955	20	14	70.00
SBS 2	$(CA)_8CG$	59	257–1042	20	15	75.00
Total			172–1284	184	142	774.54
Mean				18.4	14.2	77.45

tion per generation (N_m) were calculated through POPGENE on the basis of the null distribution of alleles. The presence/absence binary matrix was used to generate a distance matrix based on the coefficient for measuring pairwise band differences generated through the R package Poppr (population genetic analysis in R; Kamvar et al. 2014). An unweighted pair group method with arithmetic mean (UPGMA) dendrogram, a distance-based method of hierarchical cluster analysis used to group individuals and populations into clusters on the basis of molecular difference, was constructed from the distance matrix with 1000 bootstrap replications. We also used STRUCTURE (ver. 2.3.3; Pritchard et al. 2000), a Bayesian clustering method for using multilocus genotype data, to identify the number of distinct *K* (clusters or populations), assign individuals into one of the possible *K*, and identify admixed individuals. Ten independent simulations were performed for each of the possible *K* (1–22 for *D. fragrans* in our study) to obtain the maximum posterior probability associated with each *K*. Each run consisted of 250,000 burn-in iterations followed by 750,000 data iterations. The number of genetic clusters was visualized using STRUCTURE HARVESTER (Earl and vonHoldt 2012), based on the Evanno method (Evanno et al. 2005). CLUMP (Jacobsson and Rosenberg 2007) was then used to align membership coefficients on the basis of the 10 independent runs for the optimal number of clusters. The results were then graphically displayed using DISTRUCT (ver. 1.1; Rosenberg 2004).

Results

Genetic Diversity

The 10 ISSR-PCR primers used in this study provided 202 loci that were clear and reproducible across 82 *Dryopteris fragrans* individuals (table 2). At the species level, there were 176 polymorphic loci, PPL was 87%, and Nei's gene diversity index (*h*) was 0.213 (table 3). Shannon's information index (*I*) values were slightly higher than Nei's values but showed the same trend (table 3), and so only Nei's will be referred to from this point on. We also computed *I* values to allow comparison of our results with articles that used this statistic. Among the 202 loci, 122 (61%) were shared between populations from N-CA and the NE-US. Fifty-seven loci (28%) were exclusive to the N-CA populations, while 23 (11%) were exclusive to the NE-US populations. Population-specific loci were detected only in CA-VI from N-CA. Only 46 of the 176 species-level polymorphic loci could be attributed to NE-US individuals, and the rest of the polymorphic loci were from N-CA individuals (table 3). At the regional level, Nei's gene diversity for the N-CA and NE-US populations was 0.247 and 0.064, respectively (table 3).

Sixty-one genotypes comprised the 82 *D. fragrans* individuals (table 3), and whereas each of the 20 N-CA individuals had a unique genotype, the 62 NE-US individuals comprised

Table 3

Population, Regional, and Species-Level Genetic Diversity of *Dryopteris fragrans,* Based on 10 Inter–Simple Sequence Repeat Primers

Population code	Sample size	No. multilocus genotypes	Total no. loci	No. polymorphic loci	PPL	*h*	*I*
CA-KU	4	4 (1 + 1 + 1 + 1)	134	60	30.24	.117 (.19)	.171 (.27)
CA-LC	2	2 (1 + 1)	106	17	8.42	.035 (.12)	.051 (.17)
CA-PC	4	4 (1 + 1 + 1 + 1)	138	69	34.15	.128 (.19)	.19 (.27)
CA-PE	4	4 (1 + 1 + 1 + 1)	135	66	32.2	.117 (.18)	.176 (.26)
CA-PW	2	4 (1 + 1 + 1 + 1)	117	21	10.4	.043 (.13)	.063 (.19)
CA-VI	4	4 (1 + 1 + 1 + 1)	145	83	41.49	.164 (.21)	.239 (.3)
N-CA	20	20	179	147	72.77	.247 (.19)	.375 (.27)
NY-AZ	4	2 (1 + 3)	122	1	.49	.001 (.02)	.002 (.03)
NY-HP	2	1 (2)	125	0	0	0 (0)	0 (0)
NY-HR	4	3 (1 + 1 + 2)	128	4	1.95	.007 (.06)	.011 (.08)
NY-WC	4	1 (4)	127	0	0	0 (0)	0 (0)
VT-DM	4	3 (1 + 1 + 2)	125	2	.98	.003 (.03)	.005 (.05)
VT-EQ	4	2 (2 + 2)	124	2	.98	.004 (.04)	.006 (.06)
VT-MN	4	2 (1 + 3)	129	1	.49	.003 (.04)	.003 (.05)
VT-OH	4	3 (1 + 1 + 2)	125	5	2.44	.009 (.06)	.014 (.09)
NH-CH	4	4 (1 + 1 + 1 + 1)	132	12	5.85	.023 (.1)	.034 (.14)
NH-IG	4	3 (1 + 1 + 2)	125	2	.98	.005 (.05)	.006 (.07)
NH-MM	4	4 (1 + 1 + 1 + 1)	129	9	4.39	.018 (.09)	.026 (.12)
NH-RS	4	1 (4)	122	0	0	0 (0)	0 (0)
ME-KN	4	3 (1 + 1 + 2)	126	5	2.44	.01 (.07)	.014 (.09)
ME-RP	4	4 (1 + 1 + 1 + 1)	128	6	2.93	.012 (.07)	.017 (.1)
ME-SB	4	4 (1 + 1 + 1 + 1)	126	6	2.93	.013 (.08)	.019 (.11)
ME-SH	4	1 (4)	124	0	0	0 (0)	0 (0)
NE-US	62	41	145	46	22.77	.064 (.14)	.099 (.21)
Species	82	61	202	176	87.13	.213 (.15)	.343 (.21)

Note. Values in parentheses are standard deviations. *h* = Nei's gene diversity; *I* = Shannon's information index; N-CA = northern Canada; NE-US = northeastern United States; PPL = percent polymorphic loci.

41 different genotypes, which indicates that 21 individuals shared a genotype with at least one other individual (table 3). No genotype was shared between any of the 22 populations examined. On the basis of PPL, *h*, *I*, and number of genotypes, the N-CA populations exhibited higher values than any of the NE-US populations. All individuals sampled from four populations in the NE-US (NY-HP, NY-WC, NH-RS, and ME-SH) showed identical banding patterns within each of the populations and thus exhibited no genetic polymorphism or diversity. However, the genotypes differed between these four populations.

Nei's unbiased pairwise genetic identities between N-CA populations ranged from 0.737 to 0.882, while genetic distance ranged from 0.126 to 0.305. CA-PC and CA-PE were the two most genetically similar populations, followed by CA-PE and CA-KU and by CA-PC and CA-KU (table A1, available online). CA-VI and CA-PW were the two populations that were most genetically distant, followed by CA-VI and CA-LC and by CA-PW and CA-LC (table A1). For the NE-US *D. fragrans* populations, genetic identities ranged between 0.897 and 0.981, and accordingly Nei's unbiased pairwise genetic distances were low between NE-US populations, ranging from 0.020 to 0.108 (table A1). NH-MM and ME-RG were the two most genetically similar populations, followed by VT-OH and NH-CH and by NH-MM and NH-CH (table A1). VT-ME and NH-IG were the two most genetically distant populations, followed by NY-WR and NH-RM and by VT-ME and NH-RM (table A1).

Correlation Analysis

The Mantel test for all 22 *D. fragrans* populations showed significant correlation between Nei's unbiased pairwise genetic distance and geographic distance ($P = 0.000$). However, separate Mantel tests for N-CA ($P = 0.147$) and NE-US ($P = 0.238$) populations showed no significant correlation between genetic and geographic distances. Pearson correlation indicated no significant relationship between genetic diversity and estimated population size at the species level ($P = 0.638$) as well as when N-CA and NE-US samples were analyzed separately ($P = 0.172$ and 0.238, respectively). Correlation between genetic diversity and air temperature was significant at the species level ($P < 0.001$) but not at the regional level ($P = 0.736$). There was no significant correlation between population size and air temperature at both the species ($P = 0.974$) and the regional ($P = 0.974$) level. Correlation between geographic distance and air temperature was significant at the species level ($P = 0.001$) but not at the regional level ($P = 0.482$).

Population Structure

A two-level AMOVA at the species level indicated that about 78% of the total genetic variation partitioned among populations and 22% partitioned within populations (table 4). However, a two-level AMOVA of N-CA populations showed that only 36% and 64% of the total genetic variation was due to differences among and within populations, respectively. For NE-US populations only, about 86% of the total genetic variation was found among populations, while 14% was found within populations. Three-way AMOVA (table 5) indicated that most (69%) of the genetic variation was due to differences between the two regions. Genetic variation among populations within regions and within all populations was 18% and 13%, respectively. Table 4 shows that population differentiation at the species level was high ($G_{ST} = 0.779$). When analyzed separately, the G_{ST} value for NE-US populations was very high ($G_{ST} = 0.855$) compared with that for N-CA populations only ($G_{ST} = 0.384$). The gene flow estimate based on the number of migrants per generation at species level was low ($N_m = 0.082$), particularly for NE-US populations only ($N_m = 0.058$), whereas it was higher for N-CA populations only ($N_m = 0.345$).

To show genetic relationships at the population level, a UPGMA cluster analysis was performed using Nei and Li's (1979) similarity coefficient. Two major clusters were formed, one by all the N-CA populations (cluster 1) and the other by all the NE-US populations (cluster 2; fig. 2). In the N-CA cluster, CA-PE and CA-PC populations were the most closely related, while the CA-VI population was the most genetically differentiated. In the NE-US cluster, UPGMA indicated three subclusters. Subcluster 2A is VT-EQ, which was the most genetically differentiated population. Subcluster 2B consisted of VT-OH, NH-RS, NY-HP, NY-AZ, and VT-DM, which indicated that these populations were genetically related. Subcluster 2C consisted of all other NE-US populations, with NY-HR and NH-IG as the two most genetically differentiated populations in the subcluster. NH-MM and ME-RP shared the shortest branch lengths and had the highest genetically simi-

Table 4

Two-Level Analyses of Molecular Variance on *Dryopteris fragrans* from 6 Populations from Northern Canada (N-CA) and 16 from the Northeastern United States (NE-US), Separately and Combined

Source of variation	df	Sum of squares	Variance components	Percentage variation	*P*	G_{ST}
N-CA only:						
Among populations	5	275.00	11.26 (V_A)	36.06	.000	
Within populations	14	252.75	18.05 (V_B)	63.94	.000	.855
NE-US only:						
Among populations	15	374.07	6.19 (V_A)	85.52	.000	
Within populations	46	45.00	.98 (V_B)	14.48	.000	.384
N-CA and NE-US:						
Among populations	21	1471.99	17.50 (V_A)	77.91	.000	
Within populations	60	297.75	4.92 (V_B)	22.09	.000	.779

Note. G_{ST} = coefficient of genetic variation.

Table 5

Three-Level Analyses of Molecular Variance on 82 *Dryopteris fragrans* Individuals from 6 Populations from Northern Canada and 16 from the Northeastern United States

Source of variation	df	Sum of squares	Variance components	Percent variation	*P*	G_{ST}
Among regions	1	846.93	26.98 (V_A)	69.17	.000	
Among populations within regions	20	625.07	7.06 (V_B)	18.10	.000	
Within populations	60	297.75	4.96 (V_C)	12.72	.000	.873

Note. G_{ST} = coefficient of genetic variation.

larity. Bootstrap support values were 100% for the two main clusters as well as for the differentiation of VT-EQ from other NE-US populations. The dendrogram that used individual-level data (not shown) clustered all individuals belonging only to the same population. STRUCTURE analysis indicated that the optimal number of genetic clusters was $K = 2$, which correlated with the groupings of all individuals based on their regional locations (i.e., N-CA and NE-US; fig. 2). Since the Evanno method showed only the highest level of genetic structure, each cluster was also analyzed separately. Analysis of only N-CA populations indicated that $K = 2$, with CA-VI as the first subcluster (1A) and the rest of the N-CA populations as the second subcluster (1B; fig. 2). NE-US populations showed $K = 6$, where the first two subclusters were the same as 2A and 2B of UPGMA analysis. The difference was that STRUCTURE analysis was able to differentiate subcluster 2C of UPGMA analysis into four subclusters: 2C1 (NY-HR), 2C2 (NH-IG), 2C3 (NY-WC and VT-MN), and 2C4 (ME-SB, ME-KN, ME-SH, NH-CH, ME-RP, and NH-MM; fig. 2).

Discussion

Genetic Variation in Dryopteris fragrans

We observed a high level of genetic diversity in *D. fragrans* at the species level ($h = 0.213$), and this value is higher than that observed in most ferns that have been analyzed using ISSR markers, including *Botrychium pumila* ($h = 0.14$; Camacho and Liston 2001), *Ceratopteris pteridoides* ($h = 0.14$; Dong et al. 2007), *Ceratopteris thalictroides* ($h = 0.15$; Dong et al. 2008), and *Asplenium scolopendrium* var. *americanum* ($h = 0.18$; Fernando et al. 2015). However, the high genetic diversity in *D. fragrans* at the species level is mostly due to the higher genetic diversity of the N-CA populations ($h =$

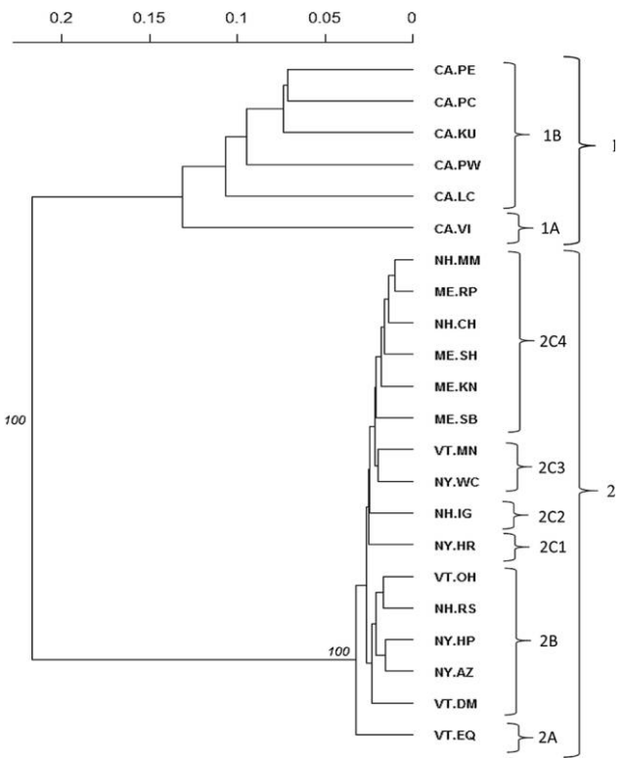

Fig. 2 Unweighted pair group method with arithmetic mean (UPGMA) dendrogram of 22 populations of *Dryopteris fragrans* representing 6 populations from northern Canada and 16 from the northeastern United States. The bar represents the similarity coefficient. Brackets and numbers correspond to clusters based on UPGMA and STRUCTURE analyses.

0.247), which sharply contrasts with the low genetic diversity of the NE-US populations ($h = 0.064$). The only published report available on the genetic analysis of *D. fragrans* is that of Huang et al. (2010), who analyzed 46 samples from six populations from China using amplified fragment length polymorphism markers and concluded that genetic diversity was high ($h = 0.292$). Although based on a different type of molecular marker, their report corroborates our results of high genetic diversity of *D. fragrans* in the center of its geographic range. Therefore, our results indicate that ferns can now be included in the general perception that peripheral populations, particularly those in the southern limit of their distribution range, have lower levels of genetic diversity than those in the range centers. On the other hand, more fern species should be examined in this context to establish whether the pattern in *D. fragrans* is common among ferns.

Genetic variation in *D. fragrans* at the species level is mostly due to differences among populations; concordantly, the estimate for gene flow is low. This same trend is exhibited by NE-US populations when analyzed separately from N-CA populations. These results are consistent with a predominance of inbreeding at the species level and for the populations of *D. fragrans* in the NE-US. On the other hand, genetic variation in *D. fragrans* from N-CA is mostly due to within-population differences, and this trend is supported by the high estimate for gene flow. The low genetic differentiation and high rate of gene flow between N-CA populations are consistent with a predominance of outcrossing among the populations. Therefore, there is also a contrast on how genetic variation is distributed among and within populations between the two regions as well as the implications for their rates of gene flow and types of mating systems.

The mean value for Nei's pairwise genetic identity in conspecific plant populations is often above 0.90 (Soltis and Soltis 1989; Chung et al. 2013). In *D. fragrans*, the mean genetic identity among NE-US populations is high (0.94), but it is lower (0.81) among N-CA populations and even lower (0.61) among combined N-CA and NE-US populations. The difference in the patterns of genetic similarity between the two regions correlates with their contrasting levels of genetic diversity. The Mantel test of combined N-CA and NE-US samples showed significant isolation by distance, which suggest that geographic distance is a factor in the genetic differentiation between the two regions. Concordantly, cluster analyses show distinct separation of the two regions, although populations within each region did not strictly group according to their geographic locations. At the regional level, the Mantel test indicates no significant correlation between genetic and geographic distances among the NE-US populations, implying the lack of isolation by distance, and the same applies for the N-CA populations.

The size of a population typically determines the ability to maintain a level of genetic variation from which selection can occur. Small population size has been correlated with reduced genetic variability, but the significance of this relationship depends on several factors, including population history and life-history traits (Ellstrand and Elam 1993). In widespread species, the correlation between population size and genetic diversity tends to be lower than those in rare species (Leimu et al. 2006). In *D. fragrans*, which is a widespread species, there is no significant correlation between genetic diversity and population size at the species and regional levels. If genetic drift mainly determines genetic variation of peripheral populations, low genetic diversity should be associated with small population size and display higher levels of interpopulation differentiation, but when population size and genetic diversity are not related, historical factors may also contribute to the current patterns of genetic diversity (Ellstrand and Elam 1993). In *D. fragrans*, historical factors likely include postglacial colonization coupled with a series of founding events involving long- and short-distance spore dispersals. The lack of a correlation between population size and genetic diversity and contrasting levels of genetic diversity also imply a recent origin of the peripheral relative to the central populations of *D. fragrans*. However, definition of discrete populations is problematic in N-CA, where large clumps of widely scattered individuals of *D. fragrans* are found on rocky knolls and slopes in the general tundra matrix while intervening unsuitable habitat is indistinct. In the NE-US, *D. fragrans* are confined to isolated habitats, where the plants are restricted to fractures in cliffs surrounded by primarily soil-covered, unsuitable habitat (S. W. Bailey, personal observations).

Colonization Dynamics of Dryopteris fragrans

In general, ferns in temperate regions are short in stature and located on forest floors, and so they tend to have lower potential for long-distance spore dispersal compared with tree ferns and ferns growing in trees canopies in the tropics (Peck et al. 1990; Flinn et al. 2014). Interestingly, such dispersal limitations could be ameliorated by a position high on a cliff face or the relatively open habitat in arctic tundra of *D. fragrans*. However, unlike the *D. fragrans* in N-CA, those in the NE-US occur only in crevices on cliff faces (Aiken et al. 2007). There is no evidence that air temperature is a limiting factor to *D. fragrans* dynamics in the NE-US. In fact, despite the warmer conditions of recent decades, new populations of *D. fragrans* continue to be discovered, some of which may represent recent colonizations, and repeated inventories have shown expansion of some populations based on databases of the Natural Heritage Programs from New York to Maine. Despite the higher air temperature in the NE-US, the availability of the preferred habitats of *D. fragrans* could be further limited by the inability to maintain moisture long enough to support successful establishment from spores. The ferns are rooted in crevices that seep water only intermittently, as supported by our observations of water seepage at our study sites. Taken together, these conditions may create fewer safe sites or periods of moisture long enough to sustain the entire reproductive process. Most reports on the high migration capacity of ferns take into account only the capacity of spores for long-distance dispersal and ignore other critical aspects of the fern reproductive process, including spore germination, formation and maturation of bisexual gametophytes, fertilization, and establishment of the young sporophyte. These stages are life-history traits that are highly dependent on the availability of moisture from start to finish (reviewed in Bystriakova et al. 2014; Fernando et al. 2015). Therefore, despite the high potential for gene flow in *D. fragrans*, its success via formation of new sporophytes would be limited in the NE-US. Populations that experience

reduced gene recruitment result in genetic drift and, thus, low genetic diversity (Ellstrand and Elam 1993; Booy et al. 2000; Hsu et al. 2000). On the other hand, habitats are not as restricted for the N-CA populations, and moisture likely lingers longer with lower air temperature, allowing spore germination to sporophyte production to occur. It seems that there are more chances for the effects of gene flow to be realized, which contributes to higher genetic diversity and differentiation of the populations.

The mating system of a species is commonly estimated from the genetic structure of its populations, where genetic variation partitions mainly within populations in outcrossers and among populations in selfers (Cao et al. 2006; Kang et al. 2008; Bucharová and Münzbergová 2012). Numerous reports have documented lower levels of genetic variation in selfing populations or species of plants (Soltis and Soltis 1989; Hamrick and Godt 1996; Dong et al. 2007). On the basis of the aforementioned estimation of mating systems, it appears that NE-US *D. fragrans* populations are predominantly selfing, whereas those in N-CA are predominantly outcrossing. This difference in mating systems helps explain the contrasting levels of genetic diversity between the central and peripheral populations. At the species level, these results indicate that *D. fragrans* exhibits a mixed-mating system that allows selfing when outcross partners are not available, a trait that likely contributed to its expansion into the NE-US. The mixed-mating reproductive strategy is becoming more commonly known in ferns (Fernando et al. 2015).

Long-distance spore dispersals from several hundred to several thousand kilometers have been reported in some species of ferns (Perrie et al. 2010; de Groot et al. 2012), which supports our statement of the occurrence of long-distance spore dispersal in *D. fragrans*. The occurrence of single-spore colonization and the ability to establish new populations from single spores through selfing is supported by the four populations of *D. fragrans* in the NE-US that are composed of single-multilocus genotypes. Colonization dynamics such as founder effects resulting in habitat patches established from at least a single spore followed by subsequent intragametophytic selfing typical for homosporous ferns are likely responsible for the low genetic diversity in peripheral *D. fragrans* populations. In the NE-US, the most genetically differentiated population of *D. fragrans* is VT-EQ. Visual comparison of the banding patterns revealed that all individuals in the VT-EQ population lack two loci that are present in all of the other NE-US populations and individuals. Interestingly, many individuals from several N-CA populations also do not have these two loci. In addition, another individual in VT-EQ lacks a locus that is also lacking in three individuals in CA-VI but that is present in all other NE-US and N-CA individuals. These and a few other genetic similarities of VT-EQ with individuals from N-CA indicate possible close relationships and from where they may have been derived. In addition to long-distance dispersal and founder effects (Perrie et al. 2010; de Groot et al. 2012), postglacial recolonization events are also recognized as causes for low levels of genetic diversity in populations (Qiu et al. 2011; Chung et al. 2013). The higher genetic diversity in central populations of *D. fragrans* implies closer position to the possible glacial refugia of the species. Thus, our results suggest that *D. fragrans* in the NE-US may have originated through long-distance dispersal events via the Canadian populations rather than recolonization from unglaciated refugia to the south of the limit of glaciation. The ability of *D. fragrans* to undergo continued selfing may also buffer NE-US populations with time to evolve adaptations suited for their new environment. Although generally rare events, mutations and/or arrival of spores from other N-CA populations into the NE-US, followed by gametophyte establishment, cross-fertilization, and genetic recombination, may allow the generation of more genetic variation within NE-US populations. These processes might have contributed to the genetic differentiation among peripheral populations and between central and peripheral populations. In fact, the contribution of just one effective migrant per generation may be sufficient to alleviate the effects of drift and reduce inbreeding depression (Wang 2004). In addition, the mating system during colonization (e.g., selfing) may differ from that in established populations (e.g., cross- or mixed-mating system; Pannell 2015).

Conservation of Dryopteris fragrans *in the NE-US*

Despite the low level of genetic diversity of *D. fragrans* in the NE-US, these southern peripheral populations can be highly valuable for the long-term conservation of genetic variation and phylogenetic history of the species. In relation to the significant genetic differentiation between N-CA and NE-US populations, the NE-US populations are characterized by the presence of 23 unique loci. Presence of a locus in a population has been ascribed to genetic recombination and/or mutation (Ellstrand and Elam 1993; Camacho and Liston 2001), but since the distribution of the unique loci in *D. fragrans* is random and not correlated with specific populations or set of individuals, they are likely due to random mutations. Although not directly examined in our study, it has been reported that peripheral populations may also possess unique loci or a combination of loci that may be related to responses to their new environment (Hunter and Hutchinson 1994; Hampe and Petit 2005; Pauls et al. 2013). Therefore, our results provide support for the conservation of *D. fragrans* in the NE-US even if it is not apparent from our data what the adaptations are. It is interesting to note that Fernald (1923) described the populations of *D. fragrans* in the NE-US as a distinct variety on the basis of greater length of fronds, more scattered and longer pinnae and pinnules, and fewer and smaller scales on the stipes and back of fronds. Our preliminary morphological analysis supports Fernald's report (see Bouchard 2013). Overall, our results add credence to Fernald's hypothesis (1923) that a distinct southern *D. fragrans* "variety" exists.

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