Genetic, Phenotypic, and Habitat Analyses of *Dryopteris fragrans*, a Rare Fern in the Northern Forests: Implications for Management and Long-Term Survival

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The genetic and morphological markers used in this study reveal strong differentiation among populations of *Dryopteris fragrans* from the Northern Forests. Northern Forest populations occur at sites with a broad range of elevation, aspect and air temperature, but a narrow range of shading, moisture and pH.

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Project Summary

Dryopteris fragrans has a global interrupted circumpolar distribution in the Northern Hemisphere, and the temperate forests of the Northeastern United States represent the southern periphery of its natural geographic range. Genetic and phenotypic diversity of *D. fragrans* in the Northern Forests were investigated using DNA markers and morphometric analysis, respectively. Genetic diversity is low within and high among populations in the Northern Forests, whereas phenotypic diversity is high within and low among most of the populations. Although genetic and morphological traits are uncorrelated, both show considerable differentiation between the populations from the Northern Forests and in comparison to a representative population from Northern Canada.

In the Northern Forest region, *D. fragrans* occurs only on shaded to partly shaded cliffs. It is found at a broad range of elevation, aspect, and air temperature as well as on a large number of rock types. However, it is always rooted in fractures with a small amount of groundwater seepage. The chemistry of this water is enriched in calcium with a circumneutral pH. These results suggest that *D. fragrans* requires a narrow range of moisture and chemistry conditions.

Dryopteris fragrans provides an example of a plant species at the southern edge of its range that could be impacted by a changing climate. It appears to be more sensitive to moisture and chemical conditions rather than temperature, suggesting that changes in precipitation may be more important to its survival than warming. Genetic analyses suggest that these edge of the range populations provide valuable diversity that could be important to the species ability to respond to environmental change.

BACKGROUND AND JUSTIFICATIONS

- *Dryopteris fragrans* (L.) Schott (fragrant fern) occurs in arctic and subarctic regions in both hemispheres, with its southern populations reaching the temperate zone of the Northeastern United States.
- The New York, Vermont, New Hampshire and Maine populations are its southernmost populations in the Northern Hemisphere.
- This fern could serve as a model for many species that have their southern range limits in the Northern Forest.
- There is no published information on any aspect of population dynamics and ecology of fragrant fern.
- Fragrant fern is globally secure but rare across Northern Forest with State Heritage Program Ranks of S1 (NY), S2 (VT and NH), and S3 (ME), which represent about 5, 20, and 100 statewide occurrences, respectively.
- Fragrant ferns usually grow on remote cliffs, rooting in crevices on cliff faces and are often associated with other rare species of northern affinity (Bailey et al. unpublished results).
- Thus there is concern about maintenance of these edge-of-range populations in a changing climate.

Figure 1. *Dryopteris fragrans* growing on horizontal fractures on a cliff.

- Response of species to changing environments is likely to be determined largely by population responses at the periphery of their natural distribution range (Higgins and Richardson 1999, Hampe and Petit 2005).
- Edge-of-range populations have been found to be critical for accurately predicting the species' responses to climate change (Iverson et al. 2004, Thomas et al. 2004, Travis and Dytham 2004).
- They are also potentially valuable as sites of rapid diversification and speciation and excellent sources of restoration stocks (Lesica and Allendorf 1994, Coyne and Orr 2004, Yakimowski and Eckert 2007).
- They offer the opportunity to investigate adaptation, limits to adaptation, or niche conservatism (Hampe and Petit 2005, Crisp et al. 2009).
- As compared to the populations in the center of the distribution range, those at the periphery are often disproportionately important for the long-term conservation of genetic diversity, phylogenetic history and evolutionary potential of the species (Hampe and Petit 2005).
- Therefore, their investigation and conservation deserve high priority.
- The Northern Forest harbors many populations of species that are at the edge of their geographical range, but none of this type of population has yet been the subject of any NSRC investigation.

References: Hampe A & RJ Petit. 2005. Conserving biodiversity under climate change: the rear edge matters. Eco Lett 8:461-467; Higgins SI & DM Richardson. 1999. Predicting plant migration rates in a changing world: the role of long-distance dispersal. The Amer Nat 153:464-475; Iverson LR, MW Schwartz and AM Prasad. 2004. How fast and far might tree species migrate in the eastern United States due to climate change? Global Ecol & Biogeo 13:209-219; Thomas D et al. 2004. Extinction risk from climate change. Nature 427:145-148; Travis JMJ & C Dytham. 2004. A method for simulating patterns of habitat availability at static and dynamic range margins. Oikos 104:410-416; Lesica P & FW Allendorf. 1995. When are peripheral populations valuable for conservation? Cons Bio 9:753-760; Coyne JA & HA Orr. 2004. Speciation. Sinauer Associates, MA; Yakimowski SB & CG Eckert. 2007. Threatened peripheral populations in context: geographical variation in population frequency and size and sexual reproduction in a clonal woody shrub. Cons Bio 21:811-822.

- The southern peripheral populations of fragrant ferns are well suited to analyze the climate-driven shifting of the species range and so their investigation directly aligns with the research agenda of NSRC.
- This research is based on the following aims:
  1) Determination and characterization of the levels of genetic diversity within and among populations.
  2) Determination and characterization of phenotypic diversity within and among populations.
  3) Characterization of the habitats within and among populations through analysis of vegetative, edaphic, and climatic factors.
  4) Correlation of genetic, phenotypic and habitat characteristics to obtain insights on how well the species is adapted to its warmer southern environment.
  5) Identification of populations that are at risk for long-term survival, could serve as most appropriate sources of restoration stocks, and might require a different management scheme.
- The results of the study provides insights on how well *D. fragrans* is adapted to its warmer southern environment and the potential of the populations in the Northern Forest to withstand future climate change.
- Based on these insights, plans for its management and long-term survival in the Northern Forest are proposed.
- This study will also help guide future research on other species in the Northern Forest that are rare and/or at the periphery of their natural distribution range.

METHODS

Study Sites: *Dryopteris fragrans* from four populations per state (NY, VT, NH and ME) were examined (Fig. 2). Four fronds (from four different individuals) were collected per population with a total of 16 fronds per state. For comparison, we obtained from colleagues archived samples of the same number of fronds and individuals that had been collected from Victoria Island, Nunavut, Canada, as representative population of the species' northern geographic range (Fig. 2).

Genetic Analysis: Inter-simple sequence repeat (ISSR) is a PCR-based technique that provides a large number of polymorphic loci and involves relatively little protocol development (Fig. 3). Banding patterns of 10 ISSR primers (Table 1) were compared across 66 individuals from 17 populations based on percentage polymorphic loci, number of unique loci, and number of genotypes per population. Nei's (1972) gene diversity and Shannon's information statistics (Lowentin 1900) were computed using POPGENE 3.1 (Yeh et al. 1999). Population structure was analyzed using AMOVA. Clustering based on genetic distance was visualized using DendroUPGMA.

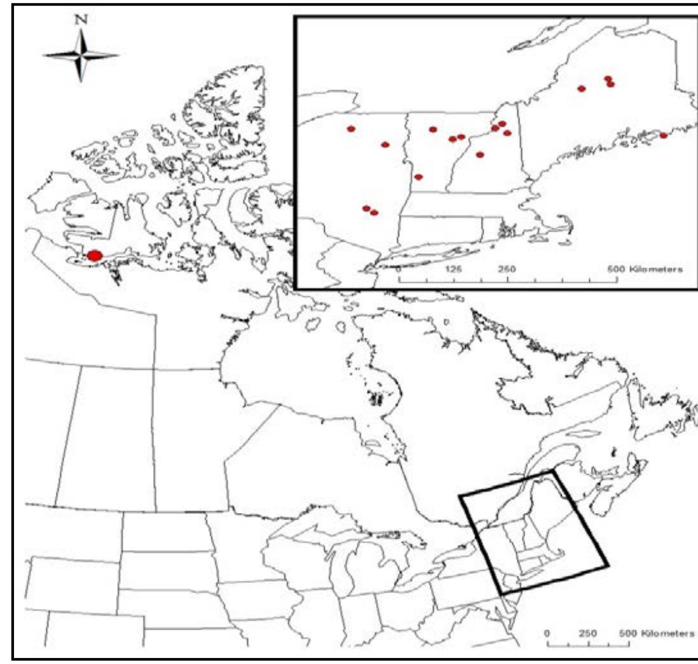

Figure 2. Locations of the 17 *D. fragrans* populations examined in this study.

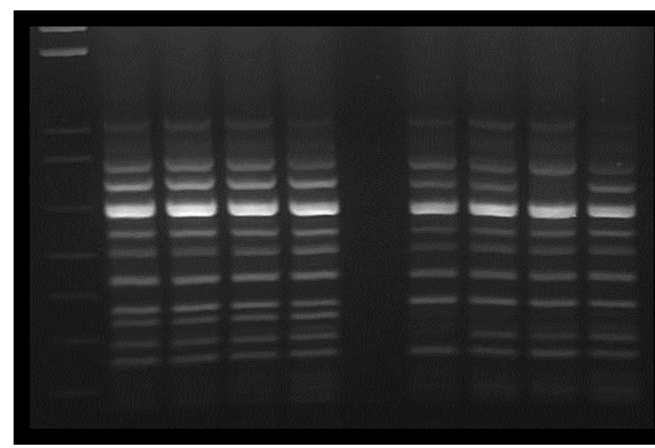

Figure 3. Representative multi-locus banding patterns from one ISSR primer.

Phenotypic Analysis: Morphological data were obtained from the same 66 individuals used for genetic analysis. Measurements were made from three fronds per individual for all traits examined (Fig. 4; Table 2). The morphological diversity among populations were analyzed using mean values from the various quantitative traits using SAS and Tukey-Kramer statistics. Hierarchical analysis was conducted. Dendrograms were constructed using UPGMA to depict morphological clustering patterns.

Figure 4. Representative frond of *D. fragrans* measured for various morphological traits (Table 2).

Habitat Analysis: Physical habitat was described at each site, including elevation, aspect, cliff height and fracturing patterns, and cover of vascular and non-vascular plants.

Rock samples were collected and prepared as thin-sections for examination under a petrographic microscope. Optical properties were used to identify mineral composition.

Table 1. ISSR primers used to analyze genetic diversity in *Dryopteris fragrans*.

Primer Code	Nucleotide Sequence (5' - 3')	Size (bp) Min-Max	T_a (°C)
UBC 815	$(CT)_8G$	320-1175	56°C
UBC 818	$(CA)_8G$	262-691	59°C
UBC 841	$(GA)_8YC$	283-818	58°C
UBC 844	$(CT)_8GC$	172-1240	59°C
UBC 847	$(CA)_9RC$	179-897	58°C
UBC 848	$(CA)_6RG$	198-788	59°C
UBC 850	$(GT)_8CC$	277-1284	56°C
UBC 857	$(AC)_9YG$	203-1204	59°C
SBS 1	$(CA)_6CC$	271-955	59°C
SBS 2	$(CA)_6CG$	257-1042	59°C

Table 2. Morphological traits of *D. fragrans*.

Traits	Abbreviation	Unit
Stipe length	SL	cm
Blade length	BL	cm
Blade width	BW	cm
Number of pinnae	NP	count
Pinnae length	PL	cm
Distance between pinnae	DP	cm
Number of scales	NS	count

As a chasmophytic species, *D. fragrans* does not root in soil. Thus water samples were collected to characterize the chemical environment experienced by these plants. As it was impossible to predict when fractures would be seeping, sites were visited multiple times if necessary in attempt to collect samples. Analyses included pH measured by potentiometric electrode at the field laboratory. Dissolved ions were analyzed at the US Forestry Sciences Lab in Durham, NH via inductively coupled plasma optical emission spectrometry and ion chromatography.

I-button temperature data-loggers were installed on tree trunks to characterize mesoclimate. Loggers recorded air temperature every 65 minutes for one full year. Data from the three loggers at each site were averaged to estimate mean monthly temperature. NOAA maps of regional air temperature, and data from specific meteorologic stations in the vicinity of study cliffs were used to compare mesoclimate with regional macroclimate.

Figure 5. A syringe was used to collect samples of seepage from fractures in which *D. fragrans* was rooted. Site VT-DM.

Figure 6. Installation of I-button temperature logger at site ME-SB.

RESULTS AND PROJECT OUTCOMES

Genetic Analysis

- Estimates of genetic diversity for all *D. fragrans* populations from NE-US were lower (H = 0 to 0.025 [mean = 0.017]; I = 0 to 0.037 [mean = 0.026]) in comparison to the NU-CAN population (H = 0.180; I = 0.263).
- NU-CAN population was the most genetically diverse population examined in the study. In NE-US, the most genetically diverse population was NH-CH (H = 0.025; I = 0.037), followed by NH-MM (H = 0.020; I = 0.029) and ME-SM and ME-RG.
- Four populations from NE-US (Table 2) were composed of individuals that exhibited the same banding patterns (0% polymorphism), but each population differed in banding patterns from each other.
- The rest of the NE-US populations had at least two individuals that were genotypically the same. All individuals from NU-CAN population were genotypically different from each other. A total of 45 genotypes were identified from the 66 individuals.
- There was no population-specific locus, but 78 loci differentiated the populations of NE-US from NU-CAN regions, each exhibited 39 specific loci. Both shared the same 106 loci .
- Combined genetic structure analysis (NE-US and NU-CAN) showed that there was higher genetic variation among population (78%) than within population (22%); the trend was the same when only NE-US populations were analyzed (86% and 14%, respectively).

- Combined Mantel test showed a slight significant correlation between genetic and geographic distances ($r = 0.98$; $p < 0.05$); analysis of only the NE-US populations showed no significant correlation between genetic and geographic distances ($r = 0.07$; $p > 0.05$).

Phenotypic Analysis

- UPGMA analysis grouped *D. fragrans* into two class sizes (small and large) which were both exhibited by individuals from NE-US, whereas individuals from NU-CAN only exhibited small class size.
- Individuals within NH-CH and NU-CAN populations generally exhibited the largest and lowest mean values, respectively, and for all the traits examined (Table 2).
- MANOVA analysis of NE-US and NU-CAN populations showed highly significant differences among populations for most of the morphological traits ($F = 2.03$; $p < 0.0001$). MANOVA analysis of NE-US only populations was also highly significant ($F = 2.11$; $p < 0.0001$).

Habitat Analysis

- *Dryopteris fragrans* in the Northern Forest was confined to cliff faces. Study sites included a broad range of elevation, aspect and types of rocks (Table 3). All cliffs were highly fractured and tended to be overhanging.

- Given this instability, talus slopes at cliff bases were common. At least the lower portions of the cliffs were shaded by talus forest.
- The upper sunny portions of cliffs, where present, were mostly devoid of ferns. Rather, *D. fragrans* tended to grow on lower, shaded portions of cliffs, particularly under the shelter of an overhang.
- Overhangs protect the plants from drying as well as direct atmospheric precipitation, perhaps contributing to both the narrow chemical and moisture regime required by this species.

Figure 7 (right). Two *D. fragrans (*arrows*)* near the base of cliff, protected by overhangs. Site ME-SB.

Figure 8 (below). Lower wooded cliffs occupied by *D. fragrans (*arrow*)*. Site NH-RS.

Table 3. Physical and chemical characteristics of *Dryopteris fragrans* in the Northern Forests.

Site	Elevation Above Sea Level M	Aspect ^{o}	Mean Annual Air Temperature ^{o}C	Lithology	pH	Ca mg/kg
NY-AZ	570	258	6.7	charnokitic gneiss	6.0	1.5
NY-HP	1006	340	3.5	anorthosite	6.5	4.0
NY-HR	607	355	7.4	sandstone	6.0	2.2
NY-WC	575	345	7.5	sandstone	6.2	1.2
VT-EQ	766	209	6.5	phyllite	7.4	12.7
VT-MN	1189	85	5.8	schist		
VT-OH	525	241	6.8	granite		
VT-DM	585	114	6.1	granite	7.0	2.4
NH-RS	265	247	8.3	schist and amphibolite	6.1	2.4
NH-CH	471	120	6.9	syenite	6.2	3.2
NH-MM	442	344	5.5	syenite	6.9	62.2
NH-IG	675	54	4.7	basalt	6.3	6.6
ME-KN	411	156	6.5	rhyolite	6.6	6.1
ME-RP	251	58	5.4	basalt	6.6	4.1
ME-SB	502	126	5.8	rhyolite	7.7	18.3
ME-SH	45	14	7.2	granite and basalt	6.7	1.7

Project Outcomes

- The level of genetic diversity of the NU-CAN population was higher than any of those from NE-US. The population in NE-US with the highest level of genetic diversity was NH-CH.
- NU-CAN and NE-US were genetically distinct, with 39 loci found only in the individuals from NU-CAN, and another 39 loci found only in the individuals from NE-US.
- Fronds in the NE-US were generally significantly larger than those in NU-CAN, with NH-CH representing the population with the largest fronds.
- Genetic and morphological markers were generally uncorrelated, but both markers revealed strong differentiation among populations from NE-US and in comparison to NU-CAN.
- *D. fragrans* sites occurred at a range of elevations, aspects and air temperatures, typical of the range of the Northern Forest region. Where study cliffs were in the vicinity of NOAA weather stations, mesoclimate at the cliff was slightly warmer than nearby weather stations, suggesting some warming influence of cliff faces.
- In the Northern Forest region, *D. fragrans* is a chasmophyte or only rooting in rock fractures with minimal soil. Rooting sites were partially shaded by overhanging rock and adjacent talus forests. Rooting crevices were moist, showing visible seepage during wet periods, but more frequently without visible seepage.
- While *D. fragrans* was found on rocks of a wide range of lithology, seepage from rocks was typically circumneutral with calcium concentrations elevated above what was typical for rooting zone of forest soils. Cliff overhangs, while protecting *D. fragrans* from sun and falling ice, may also be important in protecting plants from acidic surface runoff and direct atmospheric precipitation.

Outreach Efforts

- Two poster presentations have been presented to the public. The first was in SUNY-ESF campus as part of the "Spotlight on Student Research" on April 2013, and the other was in the Syracuse University campus as part of their "May Festival," which also included the showcase of student research (May 2013).
- Jessica Bouchard, the graduate student who primarily worked on the genetic and morphometric components of the project, gave two oral presentation on the findings of the research – one on "Eco-Lunch" a weekly group meeting of faculty and students in SUNY-ESF and SU who are interested in ecological studies, and the other as a "Capstone Seminar" presented to the faculty and students of SUNY-ESF and SU.
- An undergraduate student, Zachary Coty, has been trained on *in vitro* propagation of ferns, including *Dryopteris fragrans*. Gametophytes readily developed from *D. fragrans*, but sporophyte production had been very difficult. After several repeats and various protocol optimizations, a few sporelings were produced. More work is required to help increase the number of sporelings.
- Co-PI, Scott Bailey, made a presentation to a group of Inuits on this project and talked about vegetation and climate change when he was in northern Quebec last Aug (2014).
- There is a plan to present the results of the study to one of the scientific societies.
- A manuscript is under preparation and target journal for submission is PLOS ONE.

IMPLICATIONS & APPLICATIONS IN THE NORTHERN FOREST REGION

- Considering that: a) most of the genetic variations in *Dryopteris fragrans* is due to the differences among populations; b) the NE-US populations exhibit 39 region-specific loci; and c) many of the individuals in the NE-US exhibit larger fronds, it is therefore necessary that all the populations examined be conserved so as not to risk losing any alleles that might be important in their long-term survival in the Northern Forest region.
- Conservation efforts should reflect the importance of all peripheral populations in the Northern Forests as valuable sources of conspecific genetic diversity.
- *Dryopteris fragrans* from NH and ME generally exhibit higher levels of genetic diversity as compared to those from VT and NY. Individuals with large fronds are mostly from NH and ME. Individuals from NY generally represent those with lowest levels of genetic diversity and smallest fronds. Our results generally correlate with the State Heritage Program Ranks based on population size, which is S1 for NY, S2 for VT and NH, and S3 for ME, which represent about 5, 20, and 100 statewide occurrences, respectively.
- The populations in NY are the most vulnerable due to their isolation, small size, and relative lack of genetic diversity.
- The NH-CH population appears to be the best source of propagules (spores or individuals produced via *in vitro* propagation) for augmentation, introduction or reintroduction.
- The highly differentiated NY and NH populations indicate that they require different management schemes, perhaps the NY populations will have more focused on protection and/or augmentation, whereas the NH populations will concentrate more on maintenance and propagation.

FUTURE DIRECTIONS

- The study of *Dryopteris fragrans* as a representative of a population at the edge of its natural distribution range requires a more in-depth analysis. In particular, the genetic basis of the morphological variation observed in the populations from the NE-US need to be established using DNA markers that directly correlate with adaptive traits, e.g., co-dominant markers like microsatellites. This genetic marker will also allow direct analysis of allele frequency and gene flow, and thus provide insights on the breeding system of *D. fragrans.*
- The ISSR primers that were used in the current project can serve as the tool to isolate microsatellite sequences and thus facilitate the methodological aspect of the next study.
- In vitro propagation should be developed to allow the production of young ferns that can be used to augment populations with small numbers or restore their occurrence in some of the historical sites. These ferns will also allow experimental manipulations in the laboratory to obtain insights in their growth requirements and other responses that are difficult to tease out from field data or determine during field work.
- Sampling for genetic, morphological and habitat analyses should be expanded to include more populations from the northern and central parts of the species range. During the summer of 2014, with partial support from an NSRC supplement, we collected a few samples for genetic analysis, and studied habitats in two portions of northern Quebec – one at arctic timberline (58^{o}N) and one well within the arctic tundra biome (61^{o}N). Data from the central part of the range will allow the examination of a possible north to south transitory pattern or gradient of genetic, morphological and habitat diversity in *D. fragrans*.

List of products

- Peer-Reviewed Publications:

  Bouchard JR, Bailey SW, Leopold DJ and Fernando DD. Genetic and phenotypic diversity of *Dryopteris fragrans*, a rare fern in the temperate forests of the Northeastern United States (in preparation).

  Bailey SW. Lessons from a fern: Reassessing the role of lithology in biogeography (in preparation).

- Other Publication:

  Jessica R. Bouchard. 2013. Genetic and phenotypic diversity of *Dryopteris fragrans*, a rare fern in the temperate forests of the Northeastern United States. Master of Science Thesis. Department of Environmental and Forest Biology, SUNY College of Environmental and Forestry, One Forestry Drive, Syracuse, NY.

- Conference Presentations:

  Bouchard JR. 2013. Genetic and phenotypic diversity of *Dryopteris fragrans*, a rare fern in the temperate forests of the Northeastern United States. Spotlight on Research (College-wide poster presentation for graduate and undergraduate students in SUNY-ESF) on April 10, 2013. The poster won 1st place, judged based on poster quality and presentation by the student.

  Bouchard JR. 2013. Genetic and phenotypic diversity of *Dryopteris fragrans*, a rare fern in the temperate forests of the Northeastern United States. Syracuse University's May Festival highlighting student research. Poster Presentation (April 2014).