PHENOTYPIC AND GENOTYPIC DIFFERENTIATION OF PLANT POPULATIONS BETWEEN COASTAL BARRENS AND FORESTS IN NOVA SCOTIA, CANADA

By

Jennifer H.T. Lau

A Thesis submitted to
Saint Mary's University, Halifax, Nova Scotia
in Partial Fulfillment of the Requirements for
the Degree of Master of Science in Applied Science.

March, 2009, Halifax, Nova Scotia

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February 5, 2009, Halifax, Nova Scotia

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ABSTRACT

Distinct environmental differences between coastal barrens and forests suggest plant species that occur in both habitats may show phenotypic and/or genotypic differences. *Maianthemum canadense, Cornus canadensis, Kalmia angustifolia, Vaccinium vitis-idaea,* and *Gaultheria procumbens* showed no consistent differences in leaf thickness, stem thickness or plant height between coastal barrens, nearby forests and inland forests. The lack of relationship was likely because different plant species respond differently to environmental stressors. Soil moisture, average vegetation height and percent illumination were not good predictors of the three phenotypic traits for the five plant species. Amplified fragment length polymorphism method was used to assess the genetic diversity of 85 *V. vitis-idaea* specimens between the three habitat types. AMOVA revealed that most of the variation (87.8%) was within populations, suggesting gene flow occurs between the three habitat types. Conservation management in Nova Scotia should consider both coastal barrens and forests if development occurs on either habitat.

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Chapter 1

Phenotypic and genotypic differentiation between coastal barrens and forests plant populations in Nova Scotia, Canada: Introduction

Coastal barrens in Nova Scotia are rocky heathlands characterized by dwarf shrubs (predominately ericaceous vegetation), sparse tree cover, and harsh climatic conditions (Nova Scotia Museum 1997a). This shrub-dominated habitat often occurs at the extreme ends of the soil moisture gradient, at high elevations as well as in areas of exposure to marine salt (Latham 2003). In Europe and the United States, coastal barrens are known to contain rare plant species and have become of conservation concern (*e.g.*, Mitchell *et al.* 1997; Motzkin & Foster, 2002; Foster & Motzkin 2003; Latham 2003; Manning 2007). In Nova Scotia, coastal barren habitats occur along the Atlantic coast (*e.g.*, Peggy's Cove) and for the most part, are largely unprotected; they are accessible by foot and all-terrain vehicle (ATV), and are targeted areas for coastal development. Despite the importance of coastal barren habitats (Nova Scotia Museum 1997a), only one major study has been done on the coastal barrens along the Atlantic coast of Nova Scotia (*i.e.*, Oberndorfer 2006). One hundred and seventy-three species (vascular plants, macrolichen and moss species) including 6 provincially rare vascular plant species were identified on the coastal barrens of Nova Scotia (Oberndorfer & Lundholm 2009).

Coastal barrens support unique plant communities but also share many plant species with adjacent forest habitat. Forest understory species have been observed on the coastal barrens of Nova Scotia including Canada mayflower (*Maianthemum canadense*) and bunchberry (*Cornus canadensis*: Oberndorfer 2006). Pollen studies in New England have shown that some of the heathlands were once forested and vice versa (Motzkin & Foster 2002). In trying to direct conservation efforts in Nova Scotia, it is necessary to understand the interactions between the two ecosystems (forests and coastal barrens). Environmental differences between the coastal barrens and forests include light exposure, salt spray and wind intensity raising the question of how are these plant species surviving

in both habitats? One known trait of plant species is the ability to change their phenotypes in response to changes in the environment (phenotypic plasticity). Gene-flow between the coastal barrens and forests may be low for plant species that favour clonal propagation or are poor dispersers creating the potential for genotypic differentiation to occur between plant populations. With the potential for genotypic differentiation between the coastal barren and forest populations, both ecosystems may warrant conservation attention.

Study Species

Oberndorfer (2006) identified five plant species that grow in abundance on coastal barrens and in forests: Canada mayflower (*Maianthemum canadense*), bunchberry (*Cornus canadensis*), lambskill (*Kalmia angustifolia*), mountain cranberry (*Vaccinium vitis-idaea*) and wintergreen (*Gaultheria procumbens*). All five plant species reproduce clonally (Zinck 1998). *Kalmia angustifolia, V. vitis-idaea,* and *G. procumbens* are evergreen shrubs in the Ericaceae family, which is the dominant family on the coastal barrens. *Maianthemum canadense* and *C. canadensis* are deciduous perennial plants that grow in full shade to semi-shaded areas, which are conditions found in forests.

Maianthemum canadense

Maianthemum canadense reproduces vegetatively through rhizomes and sexually by seeds. Seedling recruitment and self-fertilization are thought to be rare (Silva *et al.* 1982; Worthern & Stiles 1998), suggesting that populations in different habitats will be sexually isolated and genetic diversity may be low.

Cornus canadensis

Cornus canadensis reproduces vegetatively through rhizomes and sexually by seeds. The seeds are dispersed primarily by birds (Burger 1987), suggesting no gene flow barrier between coastal barrens and nearby forest populations. Genetic diversity will likely be high in both populations with phenotypic plasticity occurring in response to the environmental differences.

Kalmia angustifolia

Kalmia angustifolia reproduces vegetatively through rhizomes and sexually by seeds. Its extensive rhizomatous growth aids in its quick vegetative spread into newly disturbed territories (Mallik 1995). Evidence suggests that failure to control *Kalmia* after timber harvest may shift vegetation from a forest plant community to a heathland plant community (Mallik 1995). Given the potential that the coastal barrens were once forested areas and disturbed, this suggests that *K. angustifolia* growth on the coastal barrens may be largely clonal spread which may lead to low genetic variability. If sexual reproduction is limited, physical obstacles are likely the largest barriers to clonal growth between the forests and coastal barrens, creating isolated populations.

Vaccinium vitis-idaea

Vaccinium vitis-idaea reproduces vegetatively through rhizomes and sexually by seeds. The subspecies *minus* is found in North America, Eastern Asia and along the northern coast of Siberia with another variety found in Sweden (Hulten 1949). *Vaccinium vitis-idaea*'s known method of pollination is by honey-bees (Persson & Gustavsson 2001, Trehane 2004) that can forage up to 650 m (Osborn *et al.* 1999). Populations of *V. vitis-*

idaea between the coastal barrens and forests in Nova Scotia are greater than 650 m apart suggesting a potential for sexual reproduction range limitation which may lead to genotypic differentiation between habitats.

Gaultheria procumbens

Gaultheria procumbens reproduces vegetatively through rhizomes and sexually by seeds. Populations of *G. procumbens* are known to be limited in disturbed areas due to its slow intrinsic growth rate and limited seedling establishment (Motzkin *et al.* 1996). Limited seedling establishment in favour of vegetative growth leads to low genetic diversity and potential for plant populations to have separated genetically between the coastal barrens and forests.

Research Rationale

The distribution of plant species depends on habitat suitability and dispersal of seeds (Ehrlen 2006). The distinct environmental differences between coastal barrens and nearby forests suggest plant species that occur in both habitats may show phenotypic differences due to plasticity. Furthermore, gene-flow between the two habitats may be low for plant species that favour clonal propagation creating a potential for genotypic differentiation to occur between clonal plant populations. In New England, coastal barrens were forests while forests were coastal barrens in the past (Motzin & Foster, 2002). Given the environmental differences observed between the coastal barrens and forests, to better direct conservation efforts in Nova Scotia it is necessary to determine whether the forests and coastal barrens should be treated as one or separate habitats. For example, barrens may simply represent early-successional forests. Typical barrens

vegetation may eventually be replaced by trees, which would require more research to determine the extent of coastal barrens that would need to be protected for the survival of the rare plant species. Conversely, genetic differentiation between the habitat types may lead to isolated subpopulations, which necessitates different conservation and protection management. The first step towards understanding both habitats is to determine which environmental variables plant species are responding to on the coastal barrens and in the forests. The potential phenotypic and genotypic differentiation occurring between the habitats or whether the habitats are interacting with one another can be studied using the five plant species that grow in both habitats. Preliminary analysis of the phenotypic variations of all the five plant species indicated that *V. vitis-idaea* had the greatest differentiation between habitats, so it was chosen for genetic analysis.

The objectives of this research are 1) to determine if there are consistent phenotypic and environmental differences for *M. canadense, C. canadensis, K. angustifolia, V. vitis-idaea* and *G. procumbent* between the coastal barrens and forest habitats, and 2) to determine if *V. vitis-idaea* has genetically differentiated between the coastal barrens and forest habitats.

The research is divided into two chapters in this thesis for the intent of future publications of each chapter separately.

Chapter 2

Phenotypic differentiation of five plant species between coastal barrens and forests in Nova Scotia, Canada

Abstract

General knowledge about plant-environment interactions can be studied from phenotypic differentiation of plant species in response to different habitats. To learn more about the interaction between coastal barrens and forests in Nova Scotia, five plant species that grow in both habitat types were studied for consistent phenotypic differences that may correlate with the differing environments. Interaction between habitat and site for all plant species indicated the study sites were not similar enough to combine for analyses. The lack of relationship between leaf thickness, stem thickness and plant height at both sites was likely due to different species responding differently to environmental stressors. Soil moisture, average vegetation height and percent illumination were not good predictors of phenotypes for these plant species. This study is a good foundation for future research on the interaction between coastal barrens and forest habitats and how different plants species respond to different environments.

Key words: leaf thickness, stem thickness, plant height, average vegetation height, soil moisture, percent illumination, coastal barrens, forests, clonality, Nova Scotia

Introduction

Many plant species inhabit a wide range of environments (Clausen *et al.*1941). Each environment can produce different phenotypic variations (plasticity) within plant species in response to its surroundings. Plant species with a greater ability to produce different phenotypes can populate a greater range of environments and potentially have a competitive advantage over other species (Givnish 2002). The study of phenotypic plasticity has become fundamental in research to determine the mechanisms of morphological responses to environmental stimuli (Pigliucci 2005). Research on phenotypic differentiation typically leads to the study of adaptation and natural selection; however, the basic understanding of how the phenotypes of plant species are responding between different habitats is still an important question for general knowledge about plant-environment interactions.

Coastal barrens (also called heathlands) are an understudied habitat in Nova Scotia. They are known to contain many provincially rare plant species (Oberndorfer & Lundholm 2009). Several common plant species on the coastal barrens are also abundant in the forests of Nova Scotia. Five plant species that grow in abundance on both the coastal barrens (Obendorfer 2006) and in the forests are Canada mayflower (*Maianthemum canadense*), bunchberry (*Cornus canadensis*), lambskill (*Kalmia angustifolia*), mountain cranberry (*Vaccinium vitis-idaea*) and wintergreen (*Gaultheria procumbens*). Each habitat imposes different external pressures on the plant species. Two main categories of external pressure on plant species are stress (*e.g.*, shortage of light, water/mineral nutrients and suboptimal temperature) and disturbance (*e.g.*, herbivory, pathogens, trampling: Grime 1977). Habitats varying in either of those factors may affect the phenotype of a plant species. Several studies have examined the effects of external

factors on phenotypes of specific or closely related plant species. For example, studies have demonstrated the effects of water deficit on leaf angle of *Aspidosperma quebracho-blanco* (Barchuk & Valiente-Banuet 2006), light and planting density on three *Laminum* species (Barisic *et al.* 2006), light quality on floral traits in *Arabidopsis thaliana* (Brock & Weinig 2007), heterogeneous environments on plant biomass and distribution on *Paris quadrifolia* (Jacquemyn *et al.* 2006), and different altitudes (and associated environmental differences) on plant growth on *Vaccinium myrtillus* (Fernandez-Calvo & Obeso 2004). The most similar study found to my research investigated the phenotypic range of several unrelated plant species (12 salt marsh plants) within marsh habitat and determined a lack of relationship between a single phenotypic trait and single environmental variables (Richards *et al.* 2005). No study I found examined how several unrelated plant species respond to different habitats in nature.

Phenotypic differentiation is likely to occur between the coastal barrens and forests with environmental differences such as salt spray, wind and light exposure. Personal observation of plants on the coastal barrens and in the forest noted thicker leaves, thicker stems and shorter plants on the coastal barrens. A preliminary study in 2006 on *M. canadense* and *K. angustifolia* showed significant differences between leaf thickness (both plant species) and plant height (only *K. angustifolia*) between plants growing on the coastal barrens and in the forests (Lau unpublished data).

The main questions that I asked in this study are: 1) Are there consistent phenotypic differences (leaf thickness, stem thickness and plant height) among the five plant species between the coastal barrens and forests? 2) Are there consistent environmental variables (average vegetation height, soil moisture and percent

illumination) that affect the phenotypes of the five plant species? 3) Are there consistent differences in soil nutrients between the coastal barrens and forests?

Materials and Methods

Study Sites and Habitats

Six locations (referred to as PEC, PEN, PEI, TAC, TAN, TAI) were chosen in Nova Scotia. The first two letters refer to the site and the last letter is the habitat type. Three habitat types (coastal barren (C), nearby forest (N), inland forest (I)) were located within each of the two study sites: Peggy's Cove (PE) and Taylor's Head Provincial Park (TA). Peggy's Cove and Taylor's Head are approximately 110 km apart. The coastal barren field locations were chosen within 100 m of the Atlantic Ocean to ensure maximal environmental influences from the ocean (*e.g.*, salt spray). The nearby forests were the closer of the two forest types to the coastal barrens towards the interior of the province. The inland forests were the farthest locations from the coastal barrens towards the interior of the province. The nearby and inland forest habitats were selected for closed canopy characteristics and permission to access onto private land. Distances from PEC to PEN and PEN to PEI are both approximately 3 km. Distances from TAC to TAN to TAI are approximately 1 km and 2.5 km respectively.

Selection criteria for habitats were the presence and abundance of the five plant species (*M. canadense, C. canadensis, K. angustifolia, V. vitis-idea* and *G. procumbens*).

Peggy's Cove coastal barren is one of the largest coastal barrens in Nova Scotia extending approximately 5 km in length and 4 km inland. The area is currently crown land with no formal protection. Disturbances to the coastal barrens arise from the recreational use of numerous informal foot-trails and ATV tracks, and areas for rock-

climbing. The coastal barren field site was 2.5 km east of Peggy's Cove lighthouse and 50 m from the ocean. The area is representative of the surrounding coastal barren vegetation, trails and areas of bare granite rock. The nearby forest at Peggy's Cove site is dominated by spruce and balsam fir trees. The forest floor is mostly soil with mossy areas. An unpaved road 10 m from the edge of the plots is occasionally used by the landowners. The inland forest is a mixed forest with a frequently used foot trail five meters from the edge of the plots. The forest floor is mostly soil covered in leaf litter. Both forests are closed canopy.

Taylor Head Provincial Park is a provincially protected peninsula that juts 6 km into the Atlantic Ocean. The peninsula is dominated by balsam fir and spruce forest stands with coastal barrens fringing the southwest coast. Adjacent to the ocean are large exposed areas of bedrock (meta-greywacke). The coastal barren field site was approximately 100 m from the coastline, 10 m away from a foot trail (not frequently used) and encompassed coastal barren vegetation representative of the area. Both my nearby and inland forests were within the provincial park. The nearby forest (approximately 10 m from a foot trail not frequently used) is characterized by closely grown spruce trees and mossy forest floor. The inland forest was approximately 20 m from a dirt road (near park entrance, frequently used). The canopy is dominated by spruce trees with stunted balsam fir trees (< 1 m high) scattered throughout the understory layer and moss covering the ground. Both forests are closed canopy.

Sampling

Fifty 3 m x 3 m plots were set up in a rectangular grid oriented north-south at each habitat. The rectangular grid was set up to capture areas that included all five plant

species. At Taylor's head, *G. procumbens* was not found in abundance in the forests and was not sampled at this site. Each plot was marked with a stainless steel rod or metal pin with a red flag. The plots on the coastal barren at Peggy's Cove were set up in three separate grids to avoid including walking trails in the plots.

All samples were collected in the field between June 6th and August 2nd 2007. Plant samples were collected in the order of their species expected flowering time to obtain new leaf growth for future genetic testing, chronologically as follows: *M. canadense*, *C. canadensis*, *K. angustifolia*, *V. vitis-idaea*, and *G. procumbens*. One plant was randomly selected in each plot (if present) following the sampling method described by D. B. Ward (1974). Several plots were added to the sides of the rectangular grid of three habitats to increase sample size to $\geq$ 15 within a location for *V. vitis-idaea*: seven plots were added to Peggy's inland forest, 5 plots were added to Peggy's nearby forest, and 3 plots were added to Taylor's inland forest. At Peggy's Cove, I collected a total of 147 *M. canadense* (PEI=49, PEN=48, PEC=50), 120 *C. canadensis* (PEI=45, PEN=48, PEC=27), 90 *K. angustifolia* (PEI=30, PEN=30, PEC=30), 61 *V. vitis-idaea* (PEI=19, PEN=15, PEC=27) and 71 *G. procumbens* (PEI=20, PEN=20, PEC=31) samples. At Taylor's Head, I collected a total of 142 *M. canadense* (TAI=42, TAN=50, TAC=50), 132 *C. canadensis* (TAI=35, TAN=47, TAC=50), 84 *K. angustifolia* (TAI=25, TAN=29, TAC=30) and 78 *V. vitis-idaea* (TAI=20, TAN=30, TAC=28) samples. Spatial position of the plants within a population was measured with a measuring tape and compass. Each sample was comprised of an entire plant specimen, including roots and clonal offshoots (if present), and stored on silica gel. Fragments of the samples were used as herbarium vouchers.

Phenotypic data

Locations for measurement of each phenotypic trait for each plant species were chosen to keep measurements consistent in the field. Leaf thickness and stem thickness were measured for each plant using a digital caliper (mm). Leaf thickness was measured in the middle of the new leaf (if present) or in the middle of the most intact leaf present on the sampled plant. Stem thickness was measured on the stem under the lowest leaf except *K. angustifolia* where stem thickness was measured below the first branch. Plant height was measured differently for each plant species: *M. canadense* was measured from the ground to the tip of the leaf; *C. canadensis* was measured from the ground to the bottom of the leaves; *K. angustifolia* was measured from the ground to the highest vertical point of the plant without moving the plant; *V. vitis-idaea* was measured from the ground to the tip of the plant; and *G. procumbens* was measured from the ground to tip of the tallest leaf. When leaf litter was present, ground was considered from the top of the leaf litter.

Environmental data

Canopy height was estimated as the height of the tallest plant species above the sampled plant.

Average vegetation height was estimated as the average height of all the seedlings (dbh < 2.5 cm) and herbaceous plant species surrounding the sampled plant.

Soil moisture was measured from soil taken near the roots of the sampled plant (organic layer). Small glass vials were pre-weighed, weighed with wet substrate, and re-weighed after at least 72 hours in a drying oven, on an analytical balance (Mettler

Toledo® al54) to the 0.00001 g. Soil moisture was calculated from the water weight in the soil divided by the dry soil weight.

Percent illumination was measured using a LI-250A Quantum/Radiometer/Photometer light meter (LI-COR® Biosciences) at ground level after the removal of each selected plant sample. Percent illumination measurements were conducted between 10 am and 2 pm on days with clear skies. Control measurements of light in the forest were taken in areas with no canopy cover.

Soil data

Soil was collected in medium sized re-sealable plastic bags at 13 areas (encompassing 4 cornering plots) in the rectangular grid, except Peggy's Cove coastal barren where samples from 14 areas were collected, and sent to the Nova Scotia Agriculture College to be analysed for organic matter, pH level, phosphorous, K, Ca, Mg, Na, S, Fe, Mn, Cu, Zn, B, cation exchange capacity (CEC) and percent nitrogen.

Statistical analyses

Visual inspection of quantile normal graphs, scatterplots and boxplots were used to check for normality and homogeneity for phenotypic and environmental variables. D'Agostino and Bartlett's tests were used to quantify normality and homoscedasity (Zar 1999), respectively. Transformations of phenotypic and environmental variables were applied as necessary (Appendix A1). Bartlett's test is known to be overly sensitive to deviation (Zar 1999) and data that was not homogenous (according to Bartlett's test) was rechecked using visual graphs of homoscedasity.

In order to determine if there was a consistent effect of different habitats on leaf thickness, stem thickness and plant height, a two-way multivariate analysis of variance (two-way MANOVA) was performed on each plant species. Next, a linear discriminate analysis (LDA) determined the phenotypic variables with the greatest response to the different habitat types for each plant species. For all study species, if the MANOVA revealed a significant interaction effect between habitat and site on phenotypic traits (*i.e.*, effect on habitat is conditional on site), a one-way analysis of variance (ANOVA) was used to examine the effects of habitat on important discriminating phenotypic variables (from the LDA) within each site. If a significant difference was found between habitats within each site, a post-hoc two-sample t-test was done to compare each phenotype between each of the habitats (coastal barren and nearby forest, coastal barren and inland forest, nearby and inland forests) within each site. Group means were used to determine which habitat had the taller/shorter or thicker/thinner phenotypic characteristic(s).

To determine if there was a consistent effect of average vegetation height, soil moisture and percent illumination on leaf thickness, stem thickness and plant height of each plant species, each environmental variable was plotted in a linear regression against the phenotypic variables that responded the greatest to different habitat types. Significant relationship was determined at $\alpha \leq 0.05$.

To determine if soil properties differed consistently between the coastal barren and forests at both sites, a two-way MANOVA was performed. Next, a LDA determined which of the soil properties were the most important in distinguishing between different habitat types. If the MANOVA revealed a significant interaction effect between habitat and site on soil properties, a one-way analysis of variance (ANOVA) was used to examine the effects of habitat on the important discriminating soil properties (from the

LDA) within each site. If a significant difference was found between habitats within each site, a post-hoc two-sample t-test was done to compare each soil property between each of the habitats (coastal barren and nearby forest, coastal barren and inland forest, nearby and inland forests) within each site. Group means were used to determine which habitats had the higher/lower soil property value.

LDA, MANOVA, ANOVA, sample t-test and linear regressions were conducted using R statistical Computing Environment (version 2.5.1, R Development Core Team 2007) with multivariate functions provided in the vegan package (version 1.11-2, Oksanen et al. 2008). Interaction plots were created using SigmaPlot (Version 8.02, 2002)

Results

Phenotypic traits

The two-way MANOVA indicated a significant interaction effect between habitat and site for all plant species sampled at both sites (*G. procumbens* was only sampled at Peggy's Cove, thus was not analyzed) (Table 2.1).

Maianthemum canadense

Interaction between habitat and site was due to stem thickness, significantly increasing from the inland to the nearby forest at Peggy's Cove but decreasing between these habitat types at Taylor's Head (Figure 2.1A). Stem thickness increased significantly from the nearby forest to the coastal barren at Taylor's Head but no difference occurred between these habitat types at Peggy's Cove. Plants were taller at Taylor's Head than at Peggy's Cove; however, there was a habitat and site interaction for plant height (Figure 2.1B). The interaction was due to a significant decrease in plant height from the inland to

the nearby forests at Taylor's Head while plant height increased between these habitat types at Peggy's Cove. Plant height increased significantly from the nearby to the coastal barren at Taylor's head but no difference occurred at these habitat types at Peggy's Cove.

Cornus canadensis

Leaves were thicker in the inland forest and on the coastal barren at Peggy's Cove than between these habitat types at Taylor's; however, the interaction between habitat and site came from leaf thickness decreasing more in the nearby forest at Peggy's Cove, producing thicker leaves in the nearby forest of Taylor's Head than at Peggy's Cove (Figure 2.2).

Kalmia angustifolia

Interaction between habitat and site was due to leaf thickness between the nearby forests and coastal barrens. Leaf thickness was greater in the nearby forest at Taylor's Head than at Peggy's Cove but increased more from the nearby forest to the coastal barren at Peggy's Cove, which produced thicker leaves on the coastal barrens of Peggy's Cove than at Taylor's Head (Figure 2.3A). Plant height did not have a habitat site interaction (two-way ANOVA: $F_2 = 1.84$, $p = 0.16$; Figure 2.3B).

Vaccinium vitis-idaea

Interaction between habitat and site was due to leaf thickness between the inland and nearby forests. At Peggy's Cove, leaf thickness decreased significantly from the inland to nearby forest whereas leaf thickness was not significantly different between the forests at Taylor's Head (Figure 2.4).

Post-hoc results for phenotypic data

Post-hoc two sample t-tests indicated that none of the three phenotypic traits (leaf thickness, stem thickness and plant height) differed in a consistent pattern among the five plant species between the coastal barrens and forest habitats (Table 2.2). From the LDAs, the phenotype(s) that responded the greatest to habitat differences were different for each plant species.

Leaf thickness responded to habitat change for *C. canadensis, K. angustifolia, V. vitis-idaea* and *G. procumbens.* One consistency among all four species was that leaf thickness was significantly thicker on the coastal barrens than in the nearby forest at Peggy's Cove. All other significant differences for leaf thickness between habitats depended on species and site.

Stem thickness responded to habitat change for *M. canadense* and *G. procumbens.* No consistencies for significant difference between stem thicknesses among habitats occurred between the two species. Significant differences between habitats for stem thickness depended on plant species and site.

Plant height responded to habitat change for *M. canadense* and *K. angustifolia.* One consistency between the two species was that plant height was significantly taller in the inland forest than the nearby forest at Taylor's Head. All other significant differences for plant height between habitats depended on species and site.

Environmental variables

None of the three environmental variables (average vegetation height, soil moisture and percent illumination) affected any of the three phenotypes in a consistent pattern (Table 2.2). Canopy cover was removed as a covariate because it was highly

correlated with habitat type (coastal barrens have short shrubs and forests have tall trees) so habitat alone captured all the significant variation in vegetation height.

One environmental variable that had some consistency affecting a measured phenotype was average vegetation height. Average vegetation height had a positive significant relationship with plant height for *M. canadense* and *K. angustifolia* at Taylor's Head (Figure 2.5) and on the coastal barren at Peggy's Cove (Figure 2.6). No other consistencies occurred between average vegetation height and phenotypes among the five plant species.

Neither soil moisture nor percent illumination had consistent significant relationships between phenotypes among the five plant species.

Soil properties

There were consistent patterns seen between the coastal barrens and forest habitats for soil properties (Table 2.3). Of the 15 soil properties, pH, phosphorous and manganese varied the greatest between habitats (LDA) and were further analysed (Appendix A7).

The pH was significantly and consistently higher on the coastal barrens than in the forests at both sites (Figure 2.7A; Table 2.4). Phosphorous was significantly and consistently higher in the inland forest than in the nearby forest or on the coastal barrens at both sites (Figure 2.7B; Table 2.4). Manganese was significantly and consistently higher in the inland forest than in the nearby forest at both sites (Figure 2.7C; Table 2.4).

The two-way MANOVA indicated a significant interaction effect between habitat and site for all soil properties (Table 2.1); however, a two-way ANOVA determined there was no interaction between habitat and site for pH ($F_2 = 0.1183$, $p = 0.88$).

Interaction between habitat and site for phosphorous was due to greater values in the inland forest at Peggy's Cove than Taylor's Head but decreasing more between the inland to nearby forest at Peggy's Cove than Taylor's Head producing greater phosphorous in the nearby forest at Taylor's Head than at Peggy's Cove (Figure 2.7B). As well, phosphorous decreased significantly from the nearby forest to the coastal barren at Taylor's Head but did not differ significantly at Peggy's Cove. Interaction for manganese was due to the significant increase between the nearby forest and the coastal barren at Peggy's Cove, whereas no difference occurred at Taylor's Head (Figure 2.7C).

Discussion

Coastal barrens, with salt spray, higher wind and higher light exposure, are a harsher environment than nearby and inland forests for plant species. Interaction between habitats and the two sites for all plant species determined the study sites were not similar enough to combine for analyses and were analysed separately. Between the coastal barrens and forest habitats at both sites, none of the three phenotypic traits differed in a consistent pattern among the five plant species. The different phenotypic responses of each plant species to the changing environment show the complex interaction between plants and environment. Inconsistency between phenotypes and change in environment was likely because each plant species employs different morphological and physiological strategies in response to environmental stressors (Richards *et al.* 2005). Within species, the only consistent relationship between phenotypes and habitat changes at both sites was with one phenotype (leaf thickness) for *C. canadensis*, confirming again that the study sites were not similar to one another.

Cornus canadensis, *K. angustifolia, V. vitis-idaea* and *G. procumbens* all had greater leaf thickness on the coastal barrens than in the nearby forest at Peggy's Cove. Greater leaf thickness may have been a response to cope with the higher salinity levels found in coastal barren soils compared to surrounding forest habitats. Leaf thickness is known to be positively correlated with salinity levels in the substrate from salt spray (Alpha *et al.* 1996; Griffiths 2006). Although salinity did not change as much as other soil properties between habitats (from the LDA), salinity was significantly higher on the coastal barrens than in the forests; however, with the inconsistencies between changes in habitats among species and between sites, this study suggests that other potential variables (*e.g.*, age of plant, genetics, forest type) may also have an influence on leaf thickness. One possible explanation for the variation of leaf thicknesses between the forests at Peggy's Cove is that the inland and nearby forests are different forest types. At Peggy's Cove, the inland forest was a mixed forest type dominated by *Acer rubrum, Betula papyrifera*, *Betula alleghaniensis* and *Abies balsamea* while the nearby forest was a coniferous forest dominated with *Abies balsamea* and *Picea rubens*. A better parameter to measure than leaf thickness may have been greater leaf mass per area, which has been shown to correlate with plant species living under stressful conditions (Bussotti *et al.* 2000); however, given the intent for future genetic tests on these plant species, I preserved the plants in silica gel in the field, which changed the density parameter. Thus, mass per area of the leaves could not be measured.

Stem thickness was not a good measure of phenotypic differences between the coastal barrens and forest and showed no consistency in changes between the coastal barrens and forests for *M. canadense* and *G. procumbens*. Stem density would have been a better measure of plant support as density relates to thicker cell walls and more tightly

packed cells (Niinemets 1999); however, specimens were collected and stored in silica gel for future genetic tests, making it not possible to measure density.

Maianthemum canadense and *K. angustifolia* had taller plants in the inland than in the nearby forests at Taylor's Head; however, plant height showed more inconsistencies between all the other habitats for *M. canadense* and *K. angsutifolia.* Altitude has been shown to have a negative relationship with plant height (Fernandez-Calvao & Obeso, 2004); however, estimations of altitude using Google Earth™ showed that all habitats (barrens and forests) are at the same approximate altitude, eliminating altitude as a factor for decreased plant height of *K. angustifolia* on the coastal barrens. Although not measured, high winds and low substrate on the coastal barrens are likely factors in dwarfing the plant growth of *K. angustifolia.* Contrary to *K. angustifolia, M. canadense* was taller on the coastal barrens than in at least one of the forests at both sites. One reason for this may be that *M. canadense* is one of the shorter plants within a plant community; growing taller in shorter shrub communities, like the coastal barrens, may help the plant obtain more light, whereas growing taller in a forest may not help the plant capture more light. Another factor not considered in this study that may have affected plant height was the age of the plant samples (Rice & Bazzar 1989) with older plants being taller than younger plants. To control for this, plants would have needed to be grown from seed. Again, although salinity was not considered one of the major soil nutrients changing between habitats, it was still significantly higher on the coastal barrens than in the forests and may have played a role in the decreased plant height for *K. angustifolia* on the coastal barrens (Griffiths 2006). Limited phosphorous favours taller plants with their more extensive root system (Niinemet & Kull, 2003); however, phosphorous was highest in the

inland forests, suggesting it is not limited in this study's forests and not contributing to taller plants in the forests.

Contrary to Richards *et al.* (2005), plant-environment interaction in this study showed few significant relationships or consistencies across different plant species indicating plant phenotypes are not predictable with environmental variables. Alternatively, other environmental variables or factors that were not measured, such as salt spray (Griffiths 2006) and plant age (Rice & Bazzar 1989) may better explain leaf thickness, stem thickness and plant height for these five study species.

Average vegetation height showed a positive significant relationship with plant height for *M. canadense* and *K. angustifolia,* suggesting plant growth in the immediate vicinity has relation to plant height for these two species.

Soil moisture is known to positively affect plant height (Van Iersel & Nemali 2004; Xuma & Naidoo 2007) and affect oak leaves with thinner leaves in dryer soil (Long & Jones 1996); however, this study showed no consistent effects of soil moisture on any of the three phenotypes among all plant species. This suggests water is not limited or is equally limited to all the plant species at all of the habitats. Sclerophylly (thick hard leaves, like the ones on *G. procumbens* and *V. vitis idaea*), which is thought to be an adaptation against drought (*e.g.*, Poole & Miller 1975; Gullo & Salleo 1988), may show a relationship with soil moisture between habitats; however, the results from this study suggest soil moisture to be equally available in all the study habitats and the sclerophylly of the plant leaves will not likely show a relationship with soil moisture. Harsh climatic conditions, as on the coastal barrens, may create higher sclerophylly of the plant leaves. A quick method to test sclerophylly would be to cut a cross-section of the plant leaves and

measure the width of the cuticle under a microscope: thicker cuticles would imply the plant species was collected from a harsher environment (Dr. Evans, pers. comm.).

Illumination has been known to affect leaf thickness depending on the length of time leaves were exposed to the light (Immel *et al.* 1978) and plants are known to grow taller in light limiting conditions (Givnish 2002, Phares 1971); however, percent illumination showed no consistent relationship with any of the three phenotypes among all the five plant species. The minimal variation in percent illumination on the coastal barrens (always high illumination) was likely the reason no relationship was seen between any of the phenotypes and illumination on the coastal barrens.

Soil properties may have been a better predictor of the phenotypic differences seen in the plant species; however, substrate depth was low on the coastal barrens and required soil from four plots for analysis, while phenotypes were measured for each plant specimen. With the different sampling scale for soil properties and phenotypes, direct comparison could not be made. A relationship was determined for phosphorous, pH and manganese between the coastal barrens and forests. Bare-rock surfaces on the coastal barrens versus the thick organic soils in the forests were likely a contributing factor in the soil properties. Phosphorous was highest in the inland forests, pH was highest on the coastal barrens, and manganese varied between sites with the nearby forests being one of the lower values between habitats. Coastal barrens are described as having strongly acidic soils (Nova Scotia Museum 1997a) but our study showed that the soils in the nearby forests are more acidic than on the barrens. This is not surprising as spruce and pine trees are known to be found in acidic soils (Priha & Smolander 1999), which may have been caused by decaying coniferous needles (Dr. Evans, pers. comm..). Of all the soil properties tested, only organic matter and pH responded in the same pattern to changing

habitats at both sites. Differences in soil properties between sites may have been caused by the different proximity of the habitats to one another and to the ocean. At Taylor's Head, the coastal barren and nearby forest are approximately 1 km apart while at Peggy's Cove, the coastal barren and nearby forest are 3 km apart. All the locations at Taylor's Head are within 0.5 km of the coastline and will likely have stronger influences from the ocean than the forests at Peggy's Cove. Another contributing factor to differences in soil properties between sites may be in the type of bedrock: Peggy's Cove is dominated by granite and Taylor's Head is dominated by greywacke.

Future phenotypic studies on the coastal barrens should include root-to-shoot-growth ratio, which has been known to differ between habitats (Fernandez_Calvo & Obeso 2004), as well as fruit production which can be affected by habitat and herbivory. With the environmental variables affecting each phenotype differently depending on plant species, habitat and site, a common garden experiment may show more conclusive results about the relationship between phenotypes and environmental variables. Furthermore, genotypic fingerprinting studies are required to determine the relative contribution of plasticity and genetic differentiation to the phenotypic variation seen in these five study species.

Conclusion

The 15 soil properties showed different patterns among habitat types between Peggy's Cove and Taylor's head, but no consistent patterns were seen among soil properties between habitat types within site. These differences were likely affected by the type of bedrock at both sites and the amount of bedrock versus organic material present in the soil.

Analysing each site separately (as determined by the interaction effect between habitat and site for all plant species) showed leaf thickness, stem thickness and plant height phenotypes did not differ consistently between the habitats among the five species. The lack of relationship between phenotypes and habitat change in this study was likely due to each species responding differently to environmental stressors (Richards *et al.* 2005).

Environmental variables (*i.e.*, average vegetation height, soil moisture and percent illumination) did not affect the three measured phenotypes in a consistent pattern. Average vegetation height was the only variable that indicated some consistency between habitat changes for two plant species. With the majority of the plant-environment relationships not significant, the measured environmental variables for these five study species were not good predictors of leaf thickness, stem thickness and plant height. Future research should include genotypic fingerprinting and a common garden experiment.

Tables and Figures

Table 2.1: Two-way MANOVA of leaf thickness, stem thickness and plant height between three habitat types (coastal barren, nearby forest, inland forest) at two sites (Peggy's Cove, Taylors Head) for *Maianthemum canadense, Cornus canadensis, Kalmia angustifolia* and *Vaccinium vitis-idaea.* Two-way MANOVA of soil properties (organic matter, pH and soil nutrients) at three habitats (coastal barren, nearby forest, inland forest) and at two sites (Peggy's Cove, Taylors Head).

Plant species		Wilks	df	F	p-value
Maianthemum canadense	Habitat	0.807	2	15.97	< 0.001
	Site	0.865	1	22.08	< 0.001
	Habitat*Site	0.637	2	35.64	< 0.001
Cornus canadensis	Habitat	0.379	2	50.81	< 0.001
	Site	0.976	1	1.97	0.119
	Habitat*Site	0.763	2	11.77	< 0.001
Kalmia angustifolia	Habitat	0.311	2	43.92	< 0.001
	Site	0.821	1	12.02	< 0.001
	Habitat*Site	0.630	2	14.36	< 0.001
Vaccinium vitis-idaea	Habitat	0.828	2	4.32	< 0.001
	Site	0.969	1	1.41	0.242
	Habitat*Site	0.835	2	4.12	< 0.001
Gaultheria procumbens	Habitat	0.598	2	6.44	< 0.001
Soil properties	Habitat	0.021	2	23.52	< 0.001
	Site	0.207	1	15.06	< 0.001
	Habitat*Site	0.074	2	10.53	< 0.001

Table 2.2: Summary of significant results from two-sample t-tests for *Maianthemum canadense, Cornus canadensis, Kalmia angustifolia, Vaccinium vitis-idaea* and *Gaultheria procumbens.* Phenotypic traits measured were leaf thickness (Leaf), stem thickness (Stem) and plant height. Direction of significant results is indicated with greater than (>) and lesser than (<) symbols. Data was collected from three habitat types: coastal barrens (C), nearby forest (N) and inland forest (I). Positive and negative refer to relationships between the environmental variable and the phenotype and two sites: Peggy's Cove (PE) and Taylor's Head (TA). Environmental variables are average vegetation height (AVH), soil moisture (SM), and percent illumination (PI).

Species	Phenotype	Site		Environmental Variables					
		Between Habitats at Peggy's Cove	Between Habitats at Taylor's Head	AVH	Habitat	SM	Habitat	PI	Habitat
M. canadense	Stem	C < I, I < N	N < C < I			+	TA-C	+	TA-I
	Plant height	I < C, I < N	N < C, N < I	+	All habitats at TA and at PE-C				
C. canadensis	Leaf	N < C, N < I	N < C, N < I	+	TA-N	+	TA-C		
K. angustifolia	Leaf	N < C , I < C	C < N, C < I	+	TA-C				
	Plant height	C < N, C < I	C < N < I	+	All habitats at PE and TA	-	PE-N	-	PE-N
						+	PE-I		
V. vitis-idaea	Leaf	N < C , N < I	N < C , I < C					+	TA-N
G. procumbens	Leaf	N < C, N < I		-	PE-C				
	Stem	N < C, I < C		+	PE-C				

Table 2.3: Direction of organic matter, pH and soil nutrients (P, K, Ca, Mg, Na, S, Fe, Mn, Cu, Zn, B, CEC and percent N) between C = coastal barren, N = nearby forest, I = inland forest of group means. Significant difference between habitats is indicated with a directional significant greater than or lessen than sign when $p \leq 0.050$.

	Peggy's Cove	**Taylor's Head**
Organic matter	N C > I	N C > I
pH	C > I N	C > I N
P	I > C N	I > N > C
K	C > I N	N C > C I
Ca	C > I N	I C > C N
Mg	C > N > I	C > I N
Na	C > N > I	C > I N
S	C > I N	C N > N I
Fe	C I N	I C > C N
Mn	I C > N	I > N C
Cu	N > I C	C N > I
Zn	C N I	I > N > C
B	C > N I	C > I N
CEC	C > N > I	I > C N
Percent N	C N I	C > N I

Table 2.4: Means of coastal barrens, nearby forests and inland forests with standard error (SE), minimum and maximum value for pH, phosphorous and manganese of soil Peggy's Cove (PE) and Taylor's Head (TA).

Site	Variable	Habitat	mean	SE	min	max
PE	pH	Coastal barren	4.5	0.1	4.2	5.0
		Nearby Forest	3.9	0.0	3.7	4.1
		Inland Forest	4.1	0.1	3.8	5.0
PE	phosphorous	Coastal barren	46	6	17	108
		Nearby Forest	36	6	4	81
		Inland Forest	95	4	65	119
PE	Manganese	Coastal barren	2	3	1	38
		Nearby Forest	1	0	2	6
		Inland Forest	2	2	6	25
TA	pH	Coastal barren	4.7	0.0	4.5	5.0
		Nearby Forest	4.2	0.0	4	4.5
		Inland Forest	4.3	0.1	3.9	4.6
TA	phosphorous	Coastal barren	21	3	6	39
		Nearby Forest	49	5	18	78
		Inland Forest	80	6	49	112
TA	Manganese	Coastal barren	2	1	1	13
		Nearby Forest	2	1	2	19
		Inland Forest	3	2	6	31

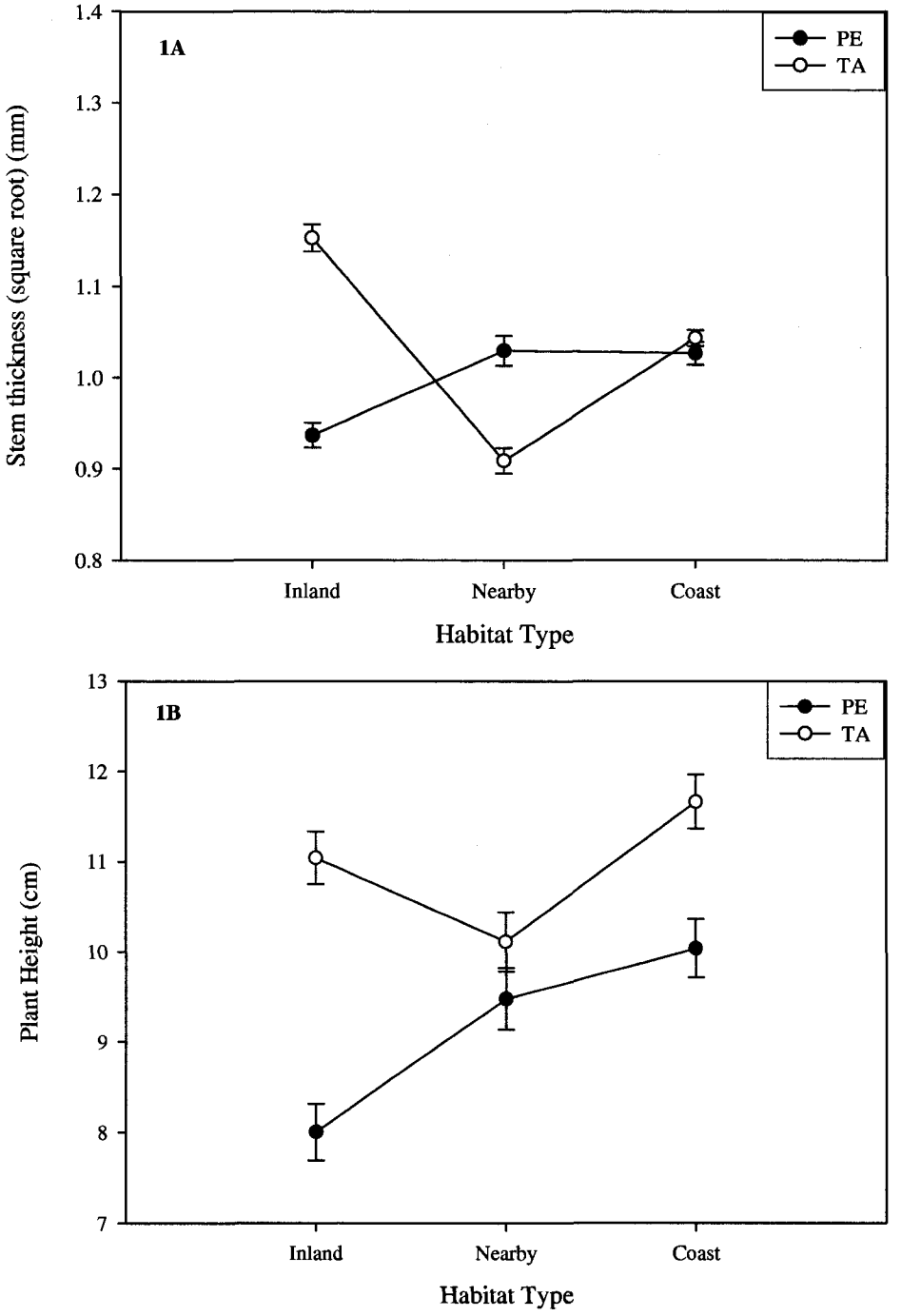

Figure 2.1: Interaction plot with mean ± standard error of 1A) square root stem thickness and 1B) plant height at different habitat types (Inland = inland forest, Nearby = nearby forest, Coast = coastal barren) at Peggy's Cove (PE) and Taylor's Head (TA). There were 147 samples at Peggy's Cove and 142 samples at Taylor's Head of *Maianthemum canadense.*

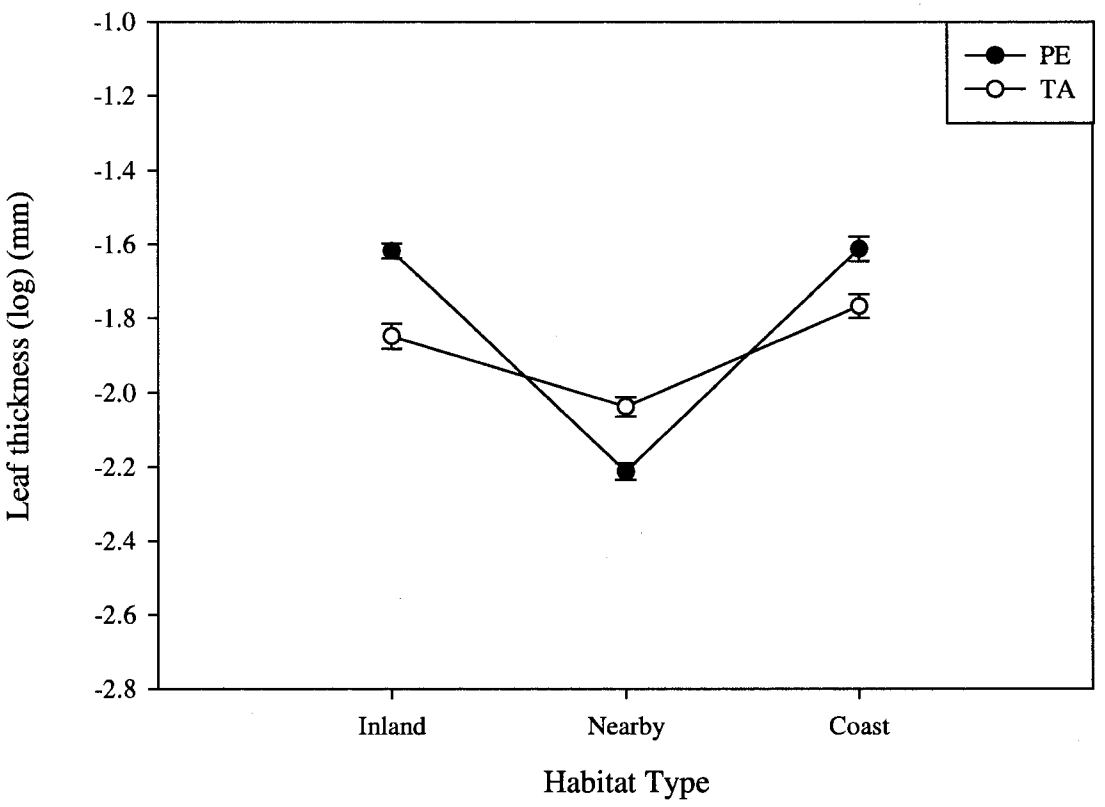

Figure 2.2: Interaction plot with mean ± standard error of leaf thickness (log) at different habitat types (Inland = inland forest, Nearby = nearby forest, Coast = coastal barren) at Peggy's Cove (PE) and Taylor's Head (TA). There were 120 samples at Peggy's Cove and 132 samples at Taylor's Head of *Cornus canadensis*.

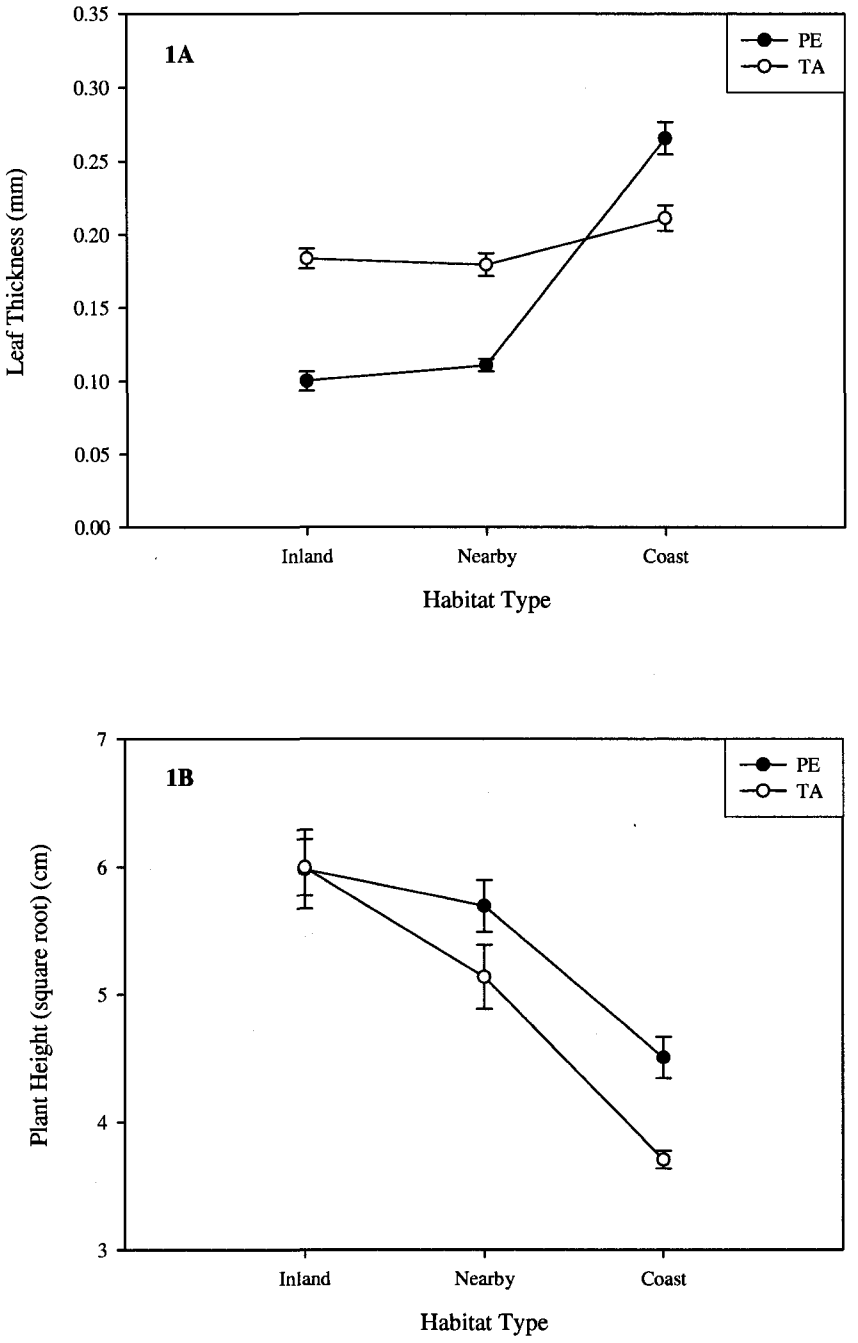

Figure 2.3: Interaction plot with mean ± standard error of 1A) leaf thickness and 1B) square root plant height at different habitat types (Inland = inland forest, Nearby = nearby forest, Coast = coastal barren) at Peggy's Cove (PE) and Taylor's Head (TA). There were 90 samples at Peggy's Cove and 84 samples at Taylor's Head of *Kalmia angustifolia.*

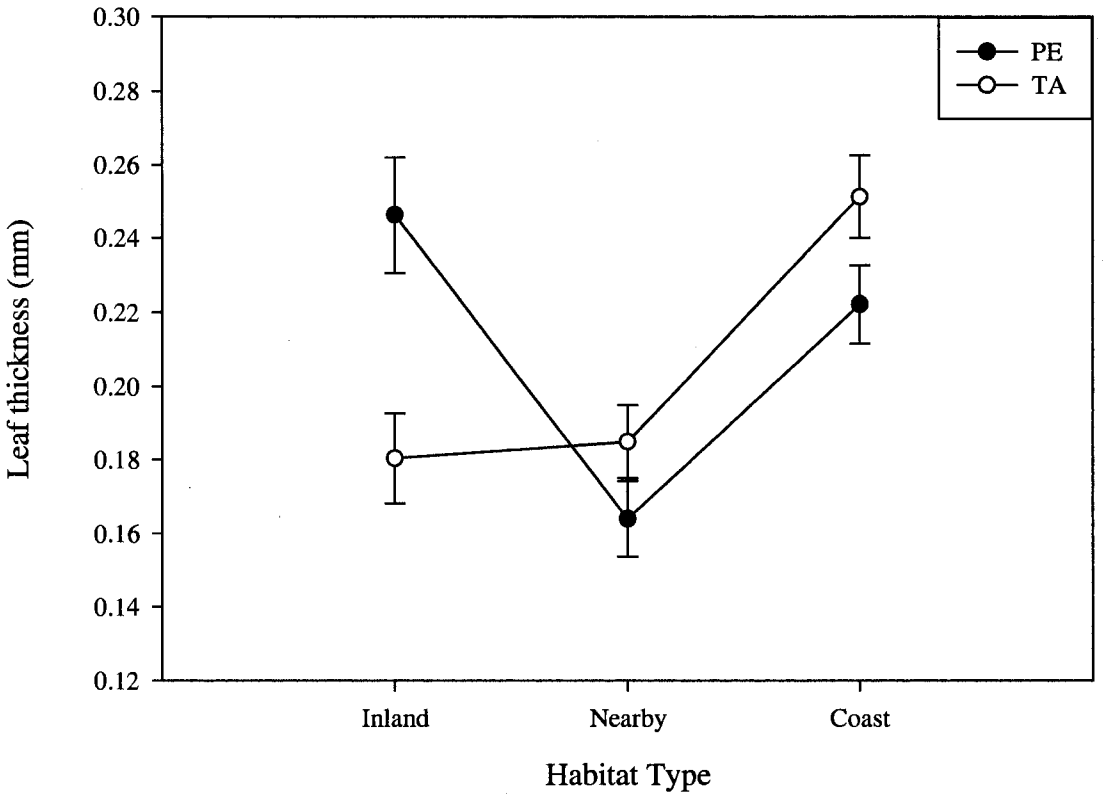

Figure 2.4: Interaction plot with mean ± standard error of leaf thickness at different habitat types (Inland = inland forest, Nearby = nearby forest, Coast = coastal barren) at Peggy's Cove (PE) and Taylor's Head (TA). There were 61 samples at Peggy's Cove and 78 samples at Taylor's Head of *Vaccinium vitis-idaea.*

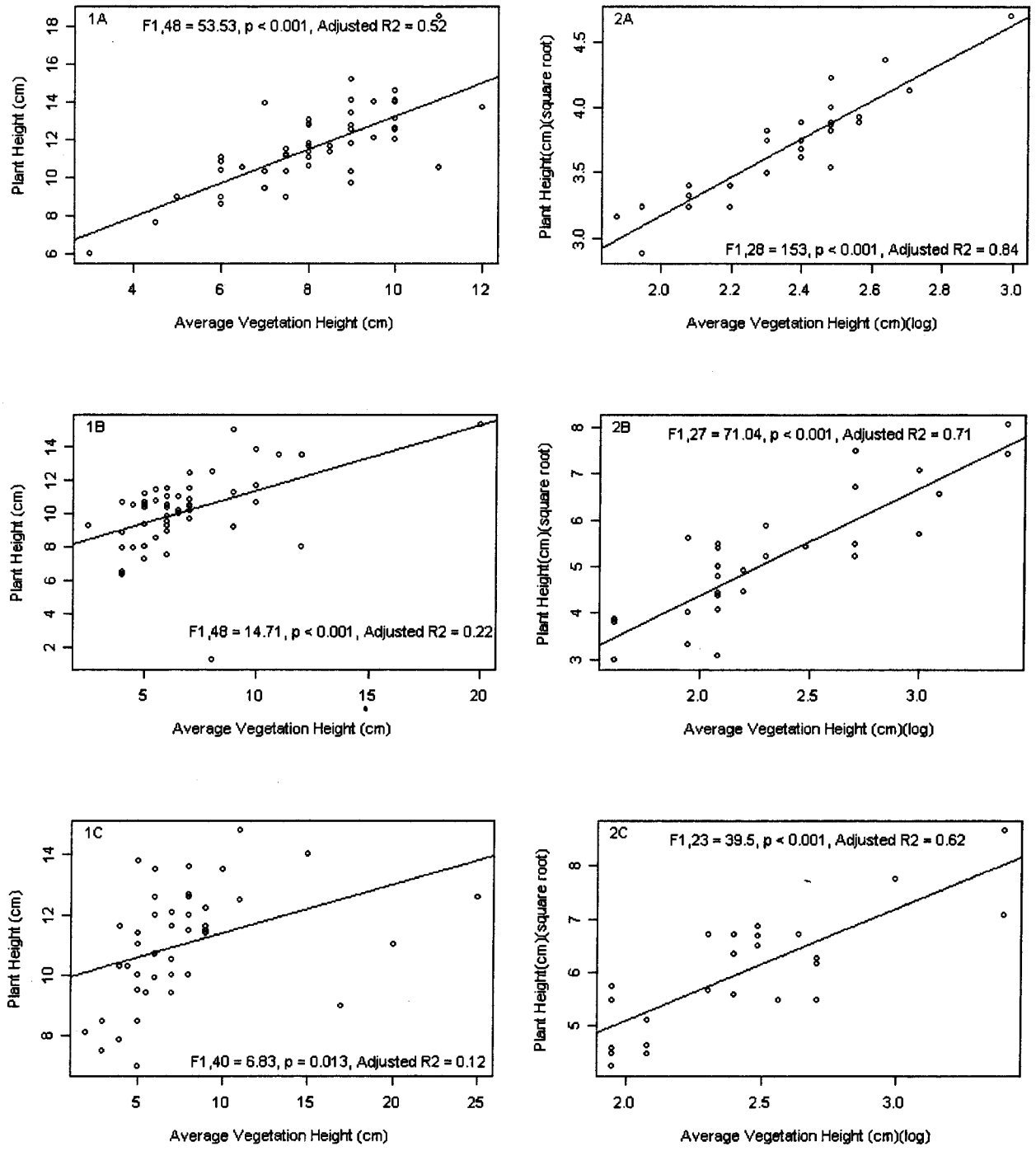

Figure 2.5: Relationship between average vegetation height and plant height at Taylor's Head Provincial Park: *Maianthemum canadense* 1A) coastal barren 1B) nearby forest 1C) inland forest, and *Kalmia angustifolia* 2A) coastal barren 2B) nearby forest 2C) inland forest.

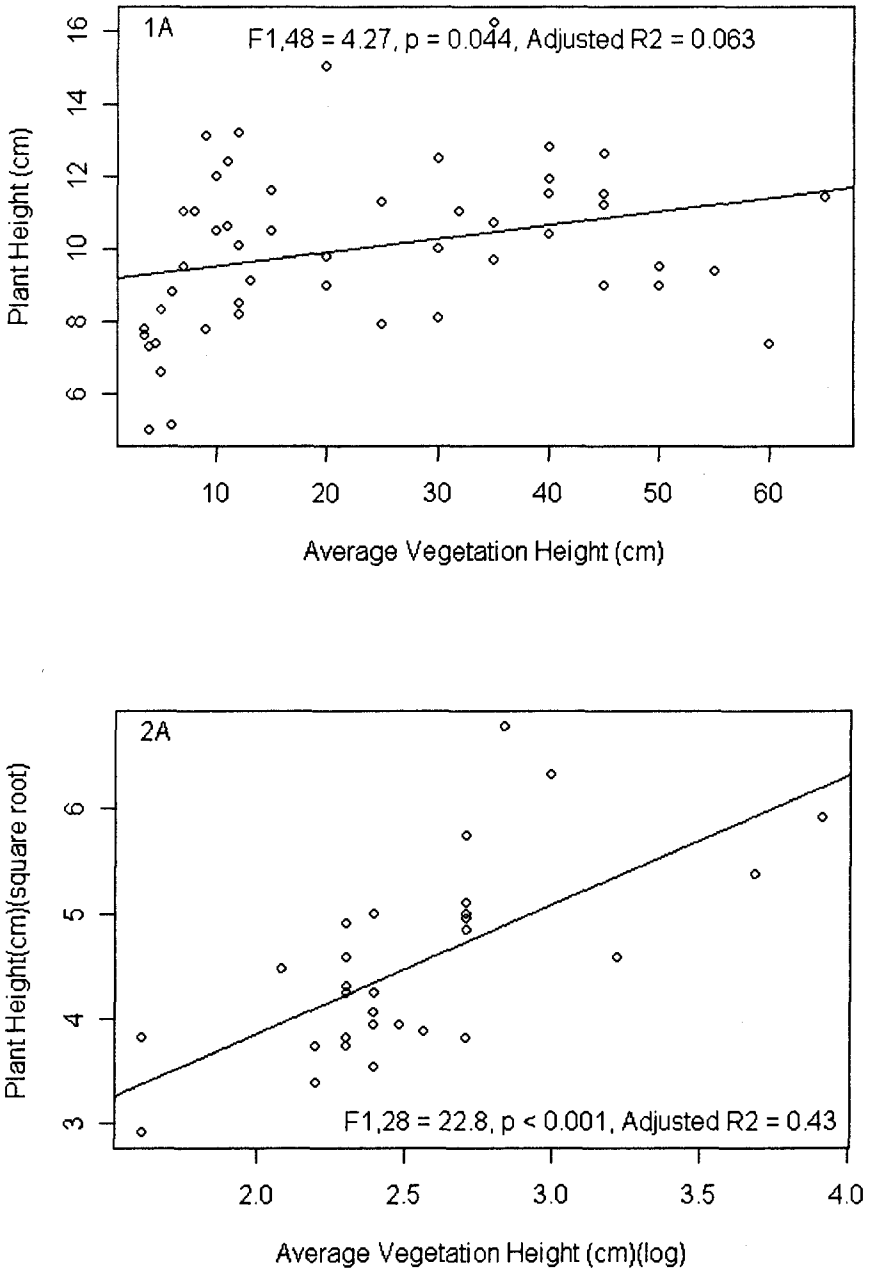

Figure 2.6: Relationship between average vegetation height and plant height at Peggy's Cove coastal barren for 1A) *Maianthemum canadense* and 2A) *Kalmia angustifolia.*

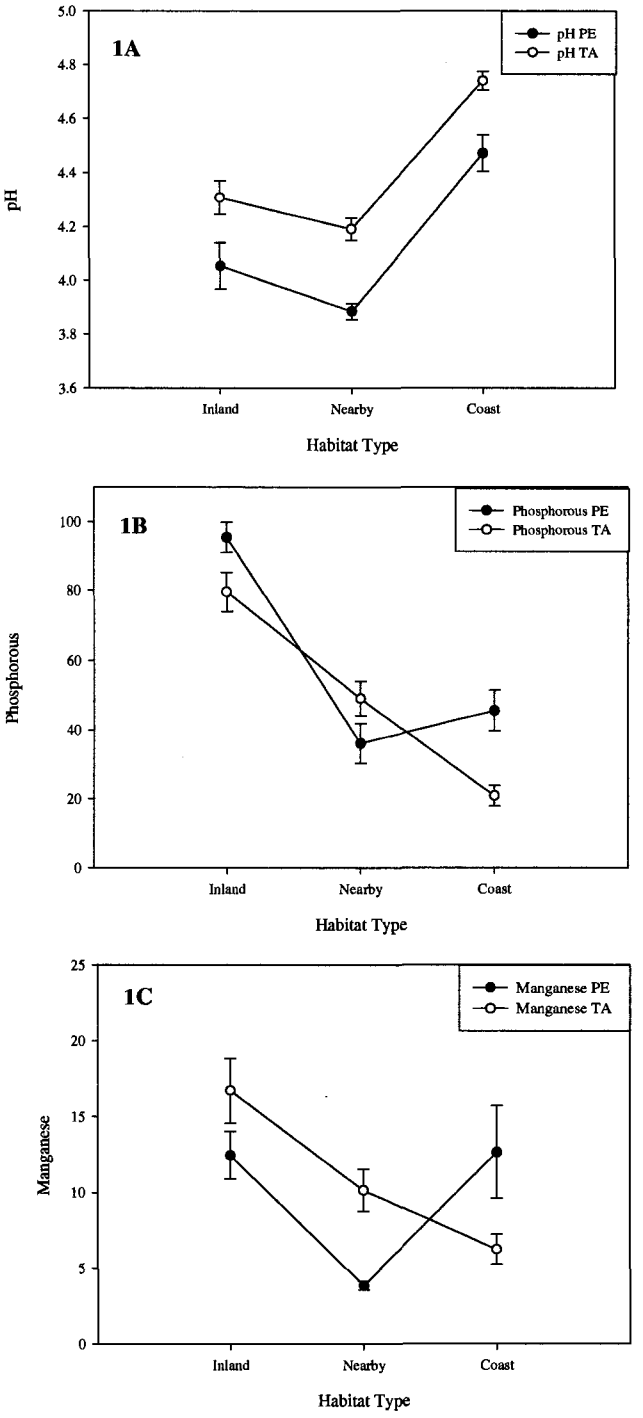

Figure 2.7: Interaction plots with mean ± standard error between habitat types for 1A) pH 1B) phosphorous 1C) manganese for 40 soil samples at Peggy's Cove and 39 soil samples at Taylor's Head.

Chapter 3

Population differentiation of *Vaccinium vitis-idaea* (L.) between coastal barrens and forests in Nova Scotia, Canada

Abstract

As coastal barrens and forests are very different environments, it is surprising that some plant species grow in both habitats (*e.g.*, *Vaccinium vitis-idaea*). Harsh environments are thought to increase clonal reproduction, which may lead to isolated populations. AFLP's were used to assess the genetic diversity of 85 *V. vitis-idaea* plants between habitats. Three primer pairs produced 88 monomorphic and 26 polymorphic bands with 81 distinct fingerprints and 4 shared genotypes. The overall genotypic diversity (D = 0.99) and evenness (E = 0.77) were higher than found in other studies on *V. vitis-idaea* and was likely influenced by sampling methods. An AMOVA revealed that most of the variation (87.8%) was within populations, suggesting gene flow occurs between the habitats and *V. vitis-idaea* are reproducing through seeds. Future studies should include a long-term genetic study on *V. vitis-idaea* at a provincial scale to determine direction, extent and rate of genetic differentiation.

Keywords: clonal, *Vaccinium vitis-idaea*, AFLP, coastal barrens, forests, Nova Scotia, genetic diversity

Introduction

Coastal barrens are of conservation concern in parts of the world as many rare plant species have a high fidelity to these habitats (*e.g.,* Mitchell *et al.* 1997; Motzkin & Foster, 2002; Foster & Motzkin 2003; Latham 2003; Manning 2007). Despite their importance, only one major research study has been conducted on the coastal barrens of Nova Scotia (Oberndorfer 2006). Land use history in New England has indicated that coastal barrens may have once been forested (or vice versa) and the plant assemblages have changed since European settlement (Motzkin & Foster 2002). Coastal barrens in Nova Scotia have been noted to have forest understory plant species (Oberndorfer 2006). To better direct conservation efforts on the coastal barrens, it is necessary to study the interaction of plant populations between the coastal barrens and forests.

Several plant species inhabit both the coastal barrens and forests in Nova Scotia including Mayflower (*Maianthemum canadense*), bunchberry (*Cornus canadensis*), lambskill (*Kalmia angustifolia*), mountain cranberry (*Vaccinium vitis-idaea*) and wintergreen (*Gaultheria procumbens*; Oberndorfer 2006). All five plant species have the ability to reproduce sexually and clonally (*e.g.,* rhizomes). In harsh environments, such as the coastal barrens with high winds and salt spray, plant species that have the ability to reproduce sexually and clonally tend to favour clonal propagation (Persson & Gustavsson 2001). Low numbers of seedling plants were noted on the coastal barrens in Nova Scotia (O'Toole 2006) suggesting reduced sexual reproduction in favour of clonal reproduction (Eckert 2002). Plant populations that are high in clonal growth suggest genetic variation may be comparatively low (Persson & Gustavsson

2001). In combination with physical obstacles such as roads and housing development, clonal reproduction may restrict or eliminate gene-flow between the plant populations on the coastal barrens and in the nearby forests, potentially leading to reproductively isolated plant populations on the barrens. Regardless of natural history on the coastal barrens, the differing existing environmental conditions between the coastal barrens and forests may have caused population differentiation (Snaydon and Davies 1972; Levins 1962, 1963; Maynard-Smith 1966).

In the past, differentiation between plant populations was measured by phenotypic differences (*e.g.,* Clausen *et al.* 1941); however, advances in molecular techniques have made it possible to measure genetic variation quickly and at an affordable cost. Molecular techniques to examine variation in DNA sequences became available in the 1970s (Conner & Hartl 2004). Different DNA marker techniques are available with each containing its own strengths and weaknesses (Mueller & Wolfenbarger 1999, Box 3). Examples of widely used DNA marker techniques are randomly amplified polymorphic DNA (RAPD), and amplified fragment length polymorphism (AFLP; Figure 3.1). The primers used in the AFLP technique are twice as long as the primers used in RAPD so errors in binding to the template DNA are less frequent with the AFLP technique (Conner and Hartl 2004). As little is known about the genotypes of the plant species listed above, the AFLP technique is a prime candidate as it can generate DNA markers without prior sequence analysis (Mueller and Wolfenbarger 1999). The AFLP technique has been found to be ideal for assessing genetic differences among individuals, populations and independently evolving species (Mueller and Wolfenbarger 1999). In addition to

these benefits, this study employed AFLP markers as they are quick to develop (data can be generated in under a week), replicable, relatively inexpensive, versatile, and moderately easy to use giving high resolution data (Vos et al. 1995; Mueller and Wolfenbarger 1999; Bensch and Akesson 2005).

This study conducted a preliminary analysis of the five plant species and found that *V. vitis-idaea* exhibited the greatest phenotypic variation between the coastal barrens and forests in Nova Scotia (unpublished data). I examined the hypothesis that population differentiation is occurring between the coastal barrens and forest because of the low seedling count and the physical barriers potentially creating a gene flow barrier. I used the AFLP technique to determine the genetic variation of *V. vitis-idaea* on coastal barrens and nearby forests at two sites (Peggy's Cove and Taylor's Head) to address the following question: has *V. vitis-idaea* on the coastal barrens of Nova Scotia differentiated genetically from the *V. vitis-idaea* in the forests?

Materials and methods

Study Site and Habitats

The same method and description for sites (Peggy's Cove and Taylors Head) and habitat (coastal barrens, nearby forests, inland forests) selection applies as described in Chapter 2 (Phenotypic differentiation of five plant species between coastal barrens and forests in Nova Scotia, Canada) for *V. vitis-idaea.*

Study species

Vaccinium vitis-idaea is a low growing, clonal, evergreen, perennial shrub, which belongs to the Ericaceae family. It is commonly known as mountain cranberry, lingonberry or foxberry. *Vaccinium vitis-idaea* occurs in a variety of habitats such as wooded areas, heathlands, raised bogs, rocky exposed cliffs and mountain summits (Persson & Gustavsson 2001; Trehane 2004) with areas of acidic soils (Garkava-Gustavsson *et al.* 2005). *Vaccinium vitis-idaea* occurs only in cold climate areas of the Northern Hemisphere (Trehane 2004). In Nova Scotia, it is found in cooler regions such as exposed coastal headlands and barrens (Zinck 1998). *Vaccinium vitis-idaea* is common in the eastern area of Nova Scotia and in Cape Breton, and is scattered throughout inland areas (Zinck 1998). It ranges from Newfoundland to the northern United States and from subartic Alaska to Washington and Oregon, is native to northwestern Greenland, northern Europe, and mountainous regions of central and southern Europe and Asia (Trehane 2004). *Vaccinium vitis-idaea* is pollinated by honey-bees and bumble-bees (Persson & Gustavsson 2001, Trehane 2004). *Vaccinium vitis-idaea* has been cultivated for jam, juice, liqueur, and yoghurt (Persson & Gustavsson 2001; Trehane 2004). In Newfoundland, where *V. vitis-idaea* is regarded as a commercial crop, approximately 200,000 lbs are harvested per year; 1100-5500 lbs per year are harvested in Saskatchewan; however, these are far from the scale of production in Europe, where approximately 80 million lbs per year are produced (Trehane 2004).

Sampling

The same sampling method on coastal barrens, nearby forests and inland forests at Peggy's Cove and Taylor's Head as described in Chapter 2 (Phenotypic differentiation of five plant species between coastal barrens and forests in Nova Scotia, Canada) for *V. vitis-idaea.*

Amplified Fragment Length Polymorphism (AFLP)

I assayed 92 specimen of *V. vitis-idaea* for AFLP markers (15-17 randomly selected individuals per population). For each specimen, total DNA was extracted from approximately 20 mg of dried plant material using DNEasy® plant mini kit (QIAGEN). AFLP protocols followed Vos *et al.* (1995), with modifications described by Wolf *et al.* (2004) for the restriction, ligation and preamplification steps. Selective amplification followed LI-COR (Lincoln, Nebraska, USA) protocols, using labeled EcoRI + 3 primers from LI-COR and unlabeled *Mse* + 3 primers from Sigma-Genosys (Oakville, Ontario, Canada). Fragments were visualized using a LI-COR Longreadir 4200 and scoring was done manually. I selected three primer pairs that produced clear and readily scored bands: *Mse* + CTA x *Eco*RI + ACT, *Mse* + CAC x *Eco*RI + ACT and *Mse* + CAA x *Eco*RI + ACT. To assess the reliability of the bands, four samples were replicated twice. Bands that were inconsistent between replicates were excluded from the analysis.

Estimators of genotypic diversity

Three estimators of genotypic diversity were calculated based on number and frequency of individual genets (Ellstrand & Roose 1987, Persson & Gustavsson 2001):

1) PD = G / N, where PD is the proportion of distinguishable genets, G is the number of genotypes detected and N is the sample size;
2) $D = 1 - \{[\sum n_i(n_i-1)]/N(N-1)]\}$, where D is the complement of the Simpson index corrected for finite samples (as cited in Persson & Gustavsson 2001), n_i is the number of plants of genotype I, N is the total sample size. D is 0 in populations with one genotype and 1 in populations where every plant sampled is a different genotype; and
3) $E = (D_{obs} - D_{min}) / D_{max} - D_{min})$, where E is the measure of evenness (as cited in Persson & Gustavsson 2001), $D_{min} = [(G-1)(2N-G)]/[N(N-1)]$, $D_{max} = [N(G-1)/[G(N-1)]$. E is 0 in populations where every plant sampled is a different genotype and 1 in populations where all genotypes are represented by the same number of plants.

Statistical analysis

Genetic variation in the data was visualized using a Principal Co-ordinate Analysis (PCoA) from the AFLP data using a distance matrix calculated with $\sqrt{1}$ – Jaccard coefficient. The Jaccard similarity coefficient suits AFLP data as it ignores bands that are absent in both individuals. Bands of equal size are assumed to be homologous, and so, bands shared by two individuals are treated as evidence for

genetic similarity (Mueller & Wolfenbarger 1999); however, the loss of a band may be caused by different mutation events (*e.g.,* substitution, deletion, insertion; Hartwell *et al.* 2000), and so, the shared absence of a band in two individuals may not reflect common ancestry.

Partitioning of variance within individuals, among habitats and between sites was analyzed using analysis of molecular variance (AMOVA: Excoffier *et al.* 1992). Groups identified with PCoA were analyzed using AMOVA to determine if meaningful internal structure could be detected. To compare the genetic variation of *V. vitis-idaea* in each habitat across the two sites, I used a non-parametric multivariate analysis of variance (Anderson 2001). To summarize the variation, an average linkage cluster method was performed to visualize the relationships between habitats. The average linkage cluster method combines the two methods of single grouping and complete grouping. Other studies (Persson & Gustavsson 2001, Garkava-Gustavsson *et a*l. 2005) used an unweighted pair-group method with arithmetic averages (UPGMA), which is similar to a single cluster method; however, using the single, complete or average grouping did not qualitatively change the results; therefore, the average linkage cluster method was chosen as it employs both techniques.

PCoA, clustering and multivariate analysis of variance were conducted using R statistical Computing Environment (version 2.5.1, R Development Core Team 2007) with multivariate functions provided in the vegan package (version 1.11-2, Oksanen *et al.* 2008). AMOVA analysis was conducted using Arlequin (Excoffier *et al.* 2005).

Results

After exclusion of inconsistent fragments, the AFLP assays produced 114 readily scored bands (37, 26 and 51 bands per primer pair) with 85 samples clear enough for analyses. There were 88 monomorphic bands and 26 polymorphic bands. In total, there were 81 distinct fingerprints and 4 shared genotypes. All shared genotypes were within habitat (inland forest or nearby forest at Peggy's Cove) except one genotype that was shared between habitats (coastal barren and nearby forest at Taylor's Head). No bands were found to be unique to a habitat.

I calculated AMOVA, PCoA and pairwise comparison (cluster dendrogram) using both √Jaccard distance and Euclidean distance. While previous studies used the squared Euclidean distance to calculate AMOVA (*e.g.,* Persson & Gustavsson 2001, Albert *et al.* 2004, and Albert *et al.* 2005), I found that both the √Jaccard distance and Euclidean distance calculations produced qualitatively similar results. I chose to use results based on √Jaccard distance as it is more appropriate for AFLP data as indicated in the previous section. Only the results based on Jaccard distance coefficient are presented from here onwards; results using the Euclidean distance are provided in Appendix B, along with the non-parametric analysis of variance data comparing habitats within a site.

The AFLP Principal Co-ordinates Analysis (PCoA) for *V. vitis-idaea* calculated with √Jaccard distance showed a slight separation occurring by site and no clear separation by habitat. The PCoA captured 38% of the total variation on the first two axes (Figure 3.2).

The separation of data points into two groups (Figure 3.2) was manually checked to determine if gel trials and/or primer pair selection influenced the division. Neither gel trial and/or primer pair selection showed a preferential selection to either of the two groups. An analysis of molecular variance indicated a statistically significant ($p = 0.002$) difference between the two groups, explaining 10.85% of the variation (Table 3.1).

An AMOVA on the entire AFLP data revealed statistically significant variation among habitats within sites ($p < 0.001$, total variation = 8.92%), and among individuals within habitats ($p < 0.001$, total variation = 87.78%), but no significant differences between sites ($p = 0.098$, total variation = 3.31%: Table 3.2).

A non-parametric multivariate analysis of variance (Table 3.3) indicated that all habitats were significantly different from one another ($p < 0.001$), there were significant site differences ($p < 0.001$) and a significant interaction effect ($p = 0.012$). Comparisons of habitats within each site showed that Peggy's Cove (15-22%) habitats explained more of the genetic variation than the habitats at Taylor's Head (7-17%: Appendix B2).

Pairwise comparison of habitat similarity (Table 3.4) with a cluster dendrogram (Figure 3.3: $\sqrt{}$(Jaccard coefficient)) indicated grouping by sites. The same pairwise comparison was done using Euclidean distance (Appendix B3) with a cluster dendrogram (Appendix B4) giving the same qualitative results.

The genotypic diversity analyses showed the PD was 1.00 at Peggy's Cove and 0.93 at Taylor's Head. Values for D and E were 1.00 and 0 at Taylor's Head and 0.997 and 0.68 at Peggy's Cove. The PD, D and E were 0.87, 0.98, 0.54 at the inland

forest at Peggy's Cove and 0.92, 0.99 and 0 at the nearby forest at Peggy's Cove. The overall PD, D and E for *V. vitis-idaea* were 0.95, 0.999 and 0.77, respectively.

Discussion

Analyses of *V. vitis-idaea* revealed that most of the variation (87.8%) occurred within populations; however, there was evidence for genetic differentiation between the coastal barrens and nearby forests and a genetic separation between Taylor's Head and Peggy's Cove. The separation by site was visually shown in a PCoA (Figure 3.2) with Peggy's Cove population grouping slightly towards the top left side of the plot and Taylor Head's population grouping towards the bottom right side. Similarly, Persson & Gustavsson (2001) found two different populations of *V. vitis-idaea* grouped slightly to different sides of a metric multidimensional scaling (MDS) analysis, yet had a larger genetic variation (89.2%) within populations (RAPD-based AMOVA). Another study on *V. vitis-idaea* showed that geographical distribution between provinces (Newfoundland, Nova Scotia, New Brunswick and Quebec) had little effect on the genetic variation and more variation was found between individuals (ISSR-based AMOVA indicated 10 % of the total variance was attributed between populations and 90% of the total variation was among individuals within a population [Debnath 2007]). Studies performed on other *Vaccinium* species also found the greatest variation to be within population: 96.2% of the genetic variation found within *V. uliginosum* (RAPD-based AMOVA; Albert *et al.* 2005a), and 86.2% of the genetic variation found within *V. mytrillus* (RAPD-based AMOVA; Albert *et al.* 2004). Different molecular techniques were used (RAPD, ISSR or AFLP) on *V. vitis-idaea* but all gave the same qualitative results showing higher genetic diversity within

population, regardless of the geographical scale, indicating AFLP was a cost effective and efficient choice to use in this study.

For *V. vitis-idaea*, AFLP markers were an effective tool to identify genotypic diversity. Overall, the genotypic diversity of *V. vitis-idaea* was relatively high for a clonal plant and was more similar to nonclonal, out-crossing plants (Persson & Gustavsson 2001); however, *V. vitis idaea* has been shown to have a high level of genetic diversity (Persson & Gustavsson 2001; Debnath 2007). In this study, the overall proportion of genotypes detected (PD = 0.95), the genotypic diversity (D = 0.999) and the evenness value (E = 0.77) were all higher than the values found for *V. vitis-idaea* by Persson and Gustavsson (2001; PD = 0.23, D = 0.84, E=0.81). The higher PD and D values observed as compared to those by Persson and Gustavsson (2001) were likely influenced by the scale of sampling. Persson and Gustavsson (2001) sampled plants closer together (8 plants for every $1m^2$), finding clones within their plots, while this study focused on genetic diversity between habitats and sampled plants at a larger scale (1 plant for every $3m^2$). The high PD found in this study suggests that if *V. vitis-idaea* in Nova Scotia spread clonally, individual clones cover less than 3 m^2. No potential clones were found on the coastal barrens (PD = 1, D = 1, E = 0), suggesting sexual reproduction has likely played a significant role in these populations despite the noted low seedling recruitment. Interestingly, the only potential clones found within a habitat were in the forests at Peggy's Cove, suggesting more vegetative reproduction in these forests than any of the other habitats that were sampled. Having one potential clone in the Peggy's nearby forest gave similar findings (PD = 0.92, D = 0.98, E < 0.001) as zero potential clones on the coastal

barrens; however, two potential clones in Peggy's inland forest (PD = 0.87, D = 0.981, E = 0.54) were enough to drop the PD and raise the E values. This suggests that had I sampled plants closer together and had found two potential clones at each habitat, I may have found results more similar to those of Persson and Gustavsson (2001). These findings are consistent with Eriksson's (1989) study showing *V. vitis-idaea* to have phalanx growth strategy (*i.e.,* clones tending to grow in close patches). Several other studies on clonal plants also showed a relatively high genetic diversity more commonly observed in nonclonal plants (Ellstrand & Roose 1987; Hamrick & Godt 1989; Jonsson *et al.* 1996; Kreher *et al.* 2000; Persson & Gustavsson 2001), implying that only a few seedlings per year are needed to maintain a population's genetic diversity (Watkinson & Powell 1993; Persson & Gustavsson 2001). Although low seedling numbers were noted on the coastal barrens (O'Toole 2006), the results suggest that the recruitment of *V. vitis-idaea* seedlings must be sufficiently high to maintain its genetic diversity.

A meta-analysis by Ellstrand and Roose (1987) of 27 studies involving 21 clonal plant species showed increasing primer pairs resulted in a higher number of detected genotypes; however, for each additional primer pair, the number of new genotypes detected rapidly decreased (Ayres & Ryan 1997, Persson & Gustavsson 2001). Although my study showed an increasing number of detected genotypes with increasing primer pairs, greater than three primer pairs in this experiment would likely have produced no qualitative differences in the results given only four potential clones were found in all the samples.

The sample size (85) and detected polymorphic markers (26) were low in this study compared to other studies: Escaravage (1998) had 400 samples that yielded 17 AFLP markers and Arens *et al.* (1998) had 143 samples that yielded 319 AFLP markers. However, more samples would not have likely changed the results qualitatively given the high genotypic overlap in populations.

Throughout the six locations, only four pairs of plants were determined to have the same genotype. One of these pairs occurred across the coastal barren and nearby forest at Taylor's Head (1 km apart). These four pairs of plants should be further investigated with a fourth and/or fifth primer pair to detect their relatedness. The farthest clone for *V. vitis-idaea* found in scientific literature was 20-30 m apart (Persson & Gustavsson 2001), which suggests that the pair found across habitats was not likely a clone.

The high genetic overlap in populations and limited potential clones in this study suggest that the coastal barrens are not a reproductively isolated habitat and are integrated with the surrounding habitats. The harsh environment and physical obstacles surrounding the coastal barrens have not stopped gene flow between habitats indicating sexual reproduction is likely occurring with vectors other than bees (*e.g.,* birds, mammals) to cross the large distance. Although never mentioned for *V. vitis-idaea*, other *Vaccinium* species are known to be dispersed through avian species (Crossland & Kloet 1996).

Comparison between habitats within and across sites showed that genetic variation occurred between all habitat types (coastal barrens, nearby forests and inland forests) at Peggy's Cove but only between the coastal barrens and inland forest

at Taylor's head. One factor that may have affected the differentiation at the Peggy's Cove locations versus Taylor's Head is the spatial arrangements of the locations. Peggy's Cove habitats are located farther from one another than are those at Taylor's Head. Contrary to spatial distance, the dendrogram indicated the coastal barren and inland forest at Peggy's Cove were more similar to one another than either were to the nearby forest.

It is still unknown whether the coastal barrens were once forested or vice versa in Nova Scotia; however, this study showed that, although there is gene flow between the forests and coastal barrens as indicated by high within population genetic variation, genetic differentiation is occurring between the habitats. Until the direction and extent of differentiation between plant species have been researched (plant species may be moving towards an isolated population), conservation efforts should focus on maintaining (1) species common to both habitat types, such as *V. vitis-idaea*, in coastal barrens and forest ecosystems, and (2) ensure no further loss of either habitat. The next step in research should analyse the genomic diversity of several other *V. vitis-idaea* populations as well as other clonal plants species that grow in both habitats within Peggy's Cove, Taylor Head and other sites along the Atlantic coast of Nova Scotia. These results will determine whether the coastal barrens and forests are genetically differentiating across Nova Scotia or are site specific. A genotypic study sampling *V. vitis-idaea* and other clonal plant specimens from the same region every few years will determine the direction and rate of habitat separation. This information will be crucial to properly conserve the coastal barrens and forests in Nova Scotia.

Conclusion

AFLP markers were an effective tool to identify genotypic diversity of *V. vitis-idaea.* Low seedling count and physical barriers between habitat types were thought to create population differentiation between *V. vitis-idaea* populations in Nova Scotia; however, analyses revealed that most of the variation (87.8%) occurred within populations. High variation within populations suggests that although the coastal barrens are harsh environments for plant growth, *V. vitis-idaea* is reproducing sexually and gene-flow is occurring between habitat types. Although most of the variation was within population and no bands were found unique to a habitat type, there was evidence for genetic differentiation between the coastal barrens and nearby forests (AMOVA) with a genetic separation between Taylor's Head and Peggy's Cove (PCoA). The overall genotypic diversity and evenness were higher than found in other studies on *V. vitis-idaea*, suggestion little clonal growth occurring in *V. vitis-idaea* in Nova Scotia, but was likely influenced by sampling methods.

Three primer pairs sufficed in this experiment and adding a fourth primer pair would not have likely produced qualitative differences in the results. Similarly, greater sample size would not have likely changed the results qualitatively given the high genotypic overlap in populations.

Future studies should include a long-term genetic study on *V. vitis-idaea* at a provincial scale to determine direction, extent and rate of genetic differentiation.

Tables and Figures

Table 3.1: Analysis of Molecular Variance (AMOVA) of *Vaccinium vitis-idaea* based on √(Jaccard coefficient) distances of AFLP markers. Variation was partitioned between the two groups visualized in the Principal Coordinate Analysis (PCoA; Figure 3.1A). Both groups contain data points for *Vaccinium vitis-idaea* from two sites (Peggy's Cove and Taylor's Head) and three habitats (coastal barren, nearby forest and inland forest). All cases based on 1023 permutations.

Source of Variation	d.f.	Sum Of Squares	Variance Components	% Total Variation	P-values
Between the two groups	1	0.851	0.01472	10.85	0.002
Among habitats within each of the two groups	10	1.940	0.01287	9.48	< 0.001
Among individuals within habitats	73	7.895	0.10814	79.67	< 0.001

Table 3.2: Analysis of Molecular Variance (AMOVA) of *Vaccinium vitis-idaea* based on √(Jaccard coefficient) distances of AFLP markers. Variation was partitioned between sites (Peggy's Cove and Taylor's Head, among populations within habitats (inland forest, nearby forest, coastal barren) and among individuals within populations for *Vaccinium vitis-idaea*. All cases based on 1023 permutations.

Source of Variation	d.f.	Sum Of Squares	Variance Components	% Total Variation	P-values
Between sites	1	0.466	0.00434	3.31	0.100
Among habitats within sites	4	1.122	0.01170	8.92	<0.001
Among individuals within habitats	79	9.098	0.11516	87.78	<0.001

Table 3.3: Non-parametric Multivariate Analysis of Variance comparing *Vaccinium vitis-idaea* AFLP data on the coastal barrens, nearby forests and inland forests across Peggy's Cove and Taylor's Head. Based on 1000 permutations.

	d.f.	Sums Of Square	Mean Square	F Model	R^2 (Pr(>F)	p-value
Habitat	2	0.0207	0.0104	5.48	0.0993	<0.001
Site	1	0.0204	0.0204	10.81	0.0979	<0.001
Habitat*Site	2	0.0182	0.0091	4.82	0.0873	0.012
Residuals	79	0.1492727	0.0018895		0.7156	

Table 3.4: Non-parametric Multivariate Analysis of Variance of *Vaccinium vitis-idaea* AFLP markers comparing coastal barrens to nearby forests, coastal barrens to inland forests, and nearby forests to inland forests at Peggy's Cove and Taylor's Head. C = Coastal Barren, N = Nearby Forest, I = Inland Forest. Based on 1000 permutations.

Habitats	Source of Variation	d.f.	Sum Of Squares	Mean Square	F Model	R^2 (Pr(>F)	p-value
C and N	Habitat	1	0.009	0.009	4.19	0.071	0.001
	Residuals	55	0.122	0.002		0.929	
C and I	Habitat	1	0.013	0.013	6.09	0.098	< 0.001
	Residuals	56	0.123	0.002		0.902	
N and I	Habitat	1	0.008	0.008	3.36	0.060	0.007
	Residuals	53	0.131	0.002		0.940	

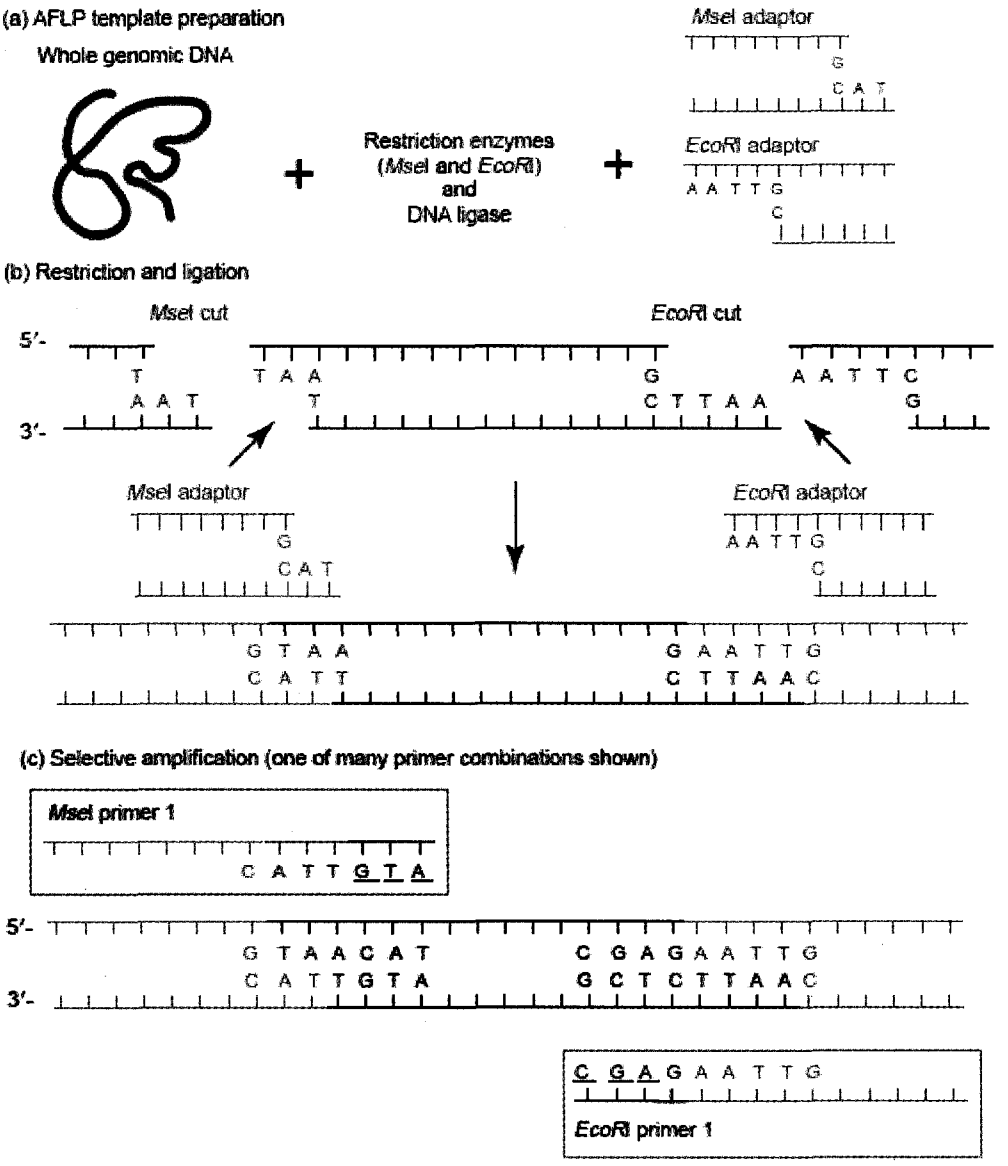

Figure 3.1: Amplified Fragment Length Polymorphism (AFLP) process taken from Mueller & Wolfenbarger (1999) Box 2.

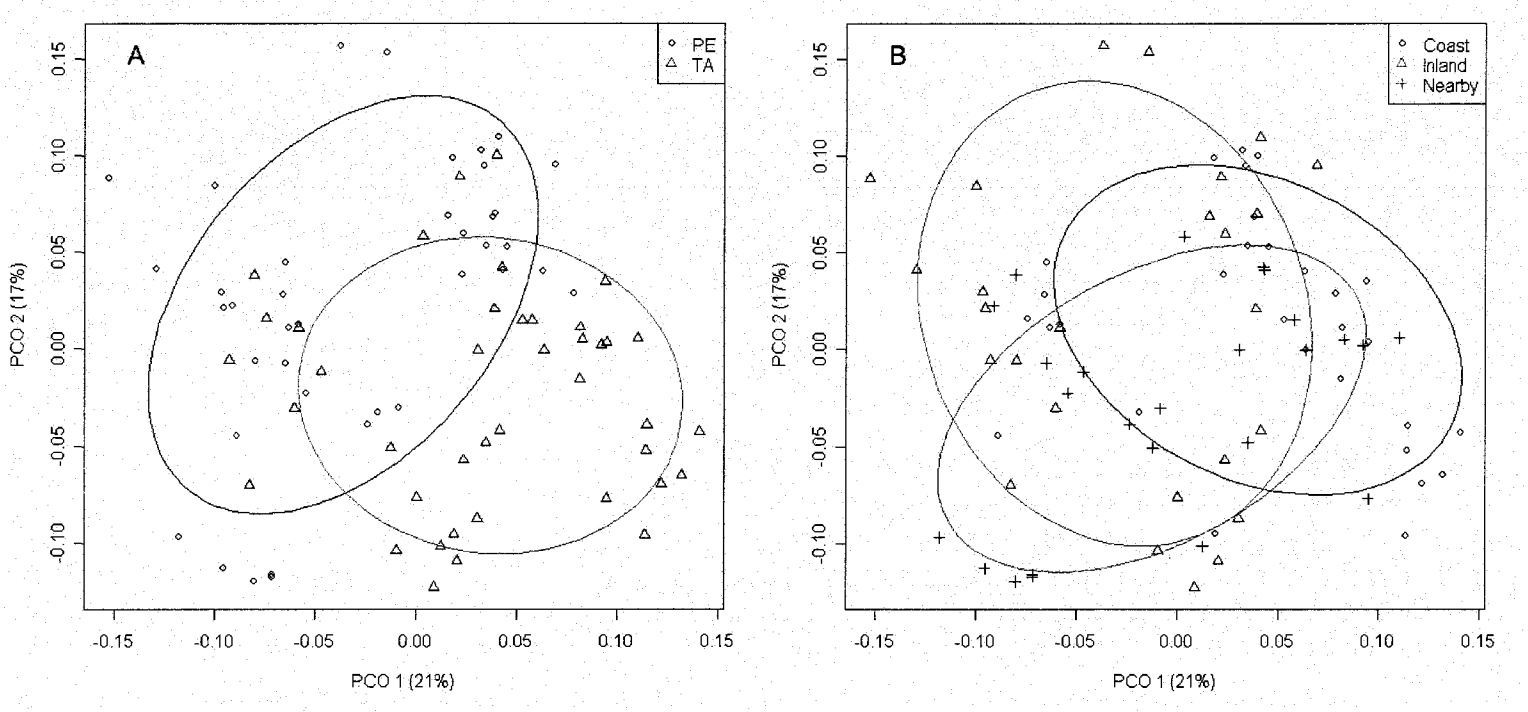

Figure 3.2: Principal Co-ordinates Analysis (PCoA) of *Vaccinium vitis-idaea*. Genetic distance ($\sqrt{}$(Jaccard coefficient) was calculated from 114 Amplified Fragment Length Polymorphism (AFLP) fragments and 85 samples. A. Individuals sampled by site showing the mean value of each site with a 68% confidence ellipse. B. Individuals sampled by habitat showing the mean value of each habitat types with a 68% confidence ellipse. Abbreviations in the graphs are as follows: PE = Peggy's Cove, TA = Taylor's Head, Coast = coastal barren, Inland = inland forest, Nearby = nearby forest.

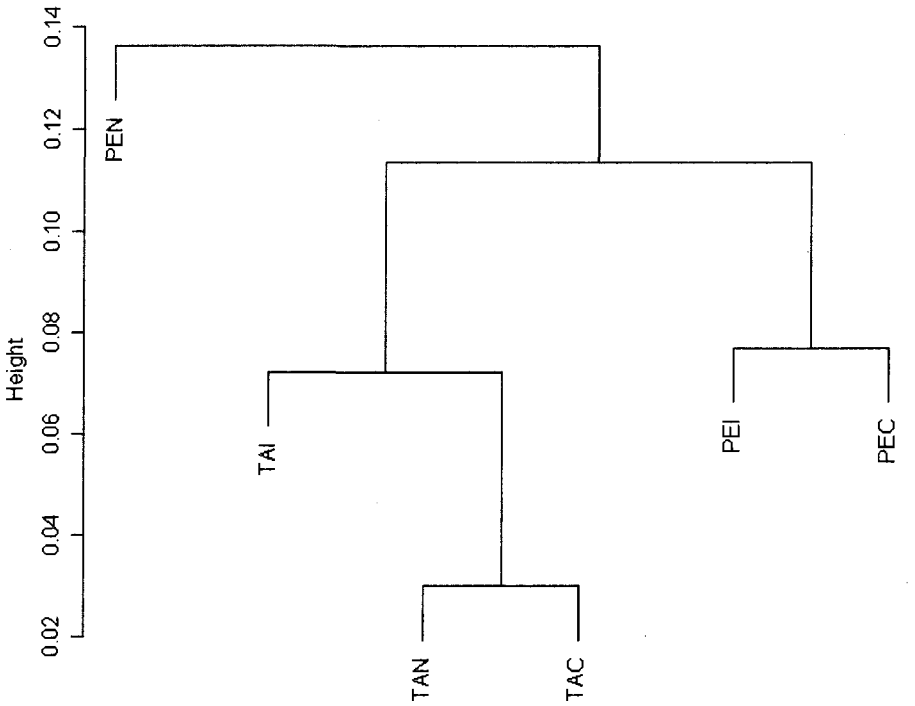

Figure 3.3: Dendrogram based on Average Cluster analysis of genetic similarity estimates of six *Vaccinium vitis-idaea* locations using √(Jaccard coefficient) pairwise distance matrix. Abbreviations in the figure are as follows: PEI = Peggy's Cove inland forest, PEN = Peggy's Cove nearby forest, PEC = Peggy's Cove coastal barren, TAI = Taylor Head inland forest, TAN = Taylor Head nearby forest, TAC = Taylor Head coastal barren.

Chapter 4

Phenotypic and genotypic differentiation of plant populations between coastal barrens and forests in Nova Scotia, Canada: Synthesis

It is essential to understand how phenotypes of plant species respond to different habitats for general knowledge about plant-environment interactions. This study demonstrates the lack of relationship between phenotypic traits and habitat differences that was likely due to different species responding differently to environmental stressors. Closely related species would be expected to respond with more similar mechanisms to environmental challenges, but no consistency (except for leaf thickness of *C. canadensis*) was seen in this study with the study sites dissimilar to one another. The measured environmental variables for the five study species were not good predictors of leaf thickness, stem thickness and plant height. Variables other than these studied (*e.g.,* plant age, salt spray and wind) may have shown a more consistent relationship with phenotypes. Bedrock type likely affected the soil properties between the forests and barrens. Furthermore, the pH level in soils indicated that the inland forests were more acidic than the coastal barrens. Soil properties may have been a direct link to phenotypic differences seen in the plant species; however, substrate depth was low on the coastal barrens and required soil from four plots for analysis, while phenotypes were measured for each plant specimen. With the different sampling scale for soil nutrients and phenotypes, direct comparison could not be made.

In this study, the sites were not similar enough to combine together for the phenotypic study (determined by the interaction between habitats and sites for all plant species) and each site was looked at separately. Differences in soil properties between Peggy's Cove and Taylor's Head may be partially correlated with the proximity of the habitats to the coastline and influences from the ocean. All the habitats at Taylor's Head were within 0.5 km of the coastline while the forests at Peggy's Cove were greater than 1 km from the coastline. Additionally, the different forest types at Peggy's Cove (inland

forest is a mixed forest type while the nearby forest was a coniferous forest) and different canopy heights in the forests at both sites (tree branches are lower in the nearby forests) likely influenced environmental variables (*e.g.*, wind and light) that may have influenced the phenotypes of the plant species. Furthermore, bedrock type is different between sites: Peggy's Cove was granite dominated and Taylor's Head was greywacke dominated.

Genotypic and phenotypic differentiation (only leaf thickness) of *V. vitis-idaea* occurred between habitats (coastal barrens and forests) and between sites (Taylor's Head and Peggy's Cove). Leaf thickness is potentially an adaptation to the habitats; however, to determine adaptation, a common garden experiment (grow all plant specimens in one environment) and/or reciprocal transplant experiment (transplant plant specimen from the coastal barrens to the forests and vice versa) would need to done to control for environmental effects. Genetic variation of *V. vitis-idaea* was very high within populations making *V. vitis-idaea* not ideal as an indicator species to distinguish between habitats. Other plant species from this study (*e.g., M. canadense, C. canadensis, K. angustifolia, G. procumbens*) may have been better to examine differentiation between habitats and could be used for future research. Since all plant species in this study showed phenotypic differentiation by habitat, reproductive strategy may be a better approach to look at habitat differentiation. *Maianthemum canadense, Cornus canadensis, Kalmia angustifolia* and *G. procumbens* are mostly clonal distributors with little seedling recruitment, therefore making them another candidate for local adaptation; however, from *V. vitis-idaea*, it is known that very little seedling recruitment is needed to keep a high genetic diversity, but whether this holds true for different plant species is unknown.

The information from this study on variation in phenotypes and genotypes demonstrates the need for long-termed studies. All the plant species in this study showed

some phenotypic differentiation and if grown in a common garden experiment, the observed phenotypic differences can be determined to be plastic or genetically linked. If variation persists in the common garden, it will suggest the phenotypes have adaptive qualities specific to the coastal barrens or forest. Another long-term study could include the reciprocal transplant experiments with clonal plant species from the coastal barren to the forests and vice versa to determine if the plants are locally adapted, if local adaptation depends on local plant communities and how this may vary between different habitats.

To further describe phenotypes that may have led to adaptation on the coastal barrens, future studies could measure leaf sclerophylly (hardness of leaf, usually measured in strength of leaf per unit leaf thickness). Heath plants are known to have a higher sclerophylly index than forest plants (Edwards *et al.* 2000; Table 1) suggesting that sclerophylly is an adaptation of plants species to allow growth in coastal barren conditions. Additionally, one known contributing factor to the phenotype of plant specimen that was not measured in this study was plant age (Rice & Bazzar 1989). I was unable to control for age in this study, but future work could address this issue by conducting a long-term study growing the plant specimen in a common garden from seed, which, in the case of the plant species from this study, may take several years.

Other studies determined costal barrens to be previously forested (or vice versa) through land use history (Motzkin & Foster 2002); therefore, it is important that the natural history of the coastal barrens in Nova Scotia be researched. Paleoecological studies (*e.g.*, pollen cores) can provide historical information about land-use, disturbances such as fire and changes in these and other factors over time (*e.g.,* Motzkin & Foster 2002). Such information would guide future research and allow for adaptive management strategies.

The importance of understanding the general principles of plant-environment relationship in order to predict changes in species occurrence and abundance is critical to conservation ecology (Kolb & Diekmann 2004). From this study, the genotypic differentiation and inconsistent phenotypic differentiation among plant species between habitat types have indicated that the best approach for conservation management of the coastal barrens and forests in Nova Scotia is (1) to consider the interaction between coastal barrens and forests prior to development in either habitat type and (2) when considering management of plant species on either habitat type, each species should be researched to determine environmental variables critical for its survival.

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Appendix A1

Transformations on all species and soil nutrients

Plant species	Variable	Transformation
Maianthemum canadense	Leaf thickness	log
Maianthemum canadense	Stem thickness	Square root
Maianthemum canadense	Soil moisture	log
Maianthemum canadense	Percent illumination	log
Cornus canadensis	Leaf thickness	log
Cornus canadensis	Stem thickness	log
Cornus canadensis	Plant height	log
Cornus canadensis	Average plant height	log
Cornus canadensis	Percent illumination	log
Kalmia angustifolia	Stem thickness	log
Kalmia angustifolia	Plant height	Square root
Kalmia angustifolia	Average plant height	log
Kalmia angustifolia	Soil moisture	log
Kalmia angustifolia	Percent illumination	log
Vacciniu vitis-idaea	Plant height	log
Vaccinium vitis-idaea	Average plant height	log
Vaccinium vitis-idaea	Soil moisture	log
Vaccinium vitis-idaea	Percent illumination	log
Gaultheria procumbens	Average plant height	log
Gaultheria procumbens	Percent illumination	log
	Ca	log
	Mg	log
	Na	log
	Mn	log
	Cu	log
	B	log
	CEC	log

Appendix A2

Means of coastal barrens, nearby forests and inland forests with standard error (SE), minimum and maximum value of *Maianthemum canadense* for phenotypes stem thickness and plant height from Peggy's Cove (PE) and Taylor's Head (TA).

Site	Phenotype	Habitat	mean	SE	min	max
PE	Stem Thickness(mm)	Coastal barren	1.06	0.03	0.81	1.61
		Nearby Forest	1.07	0.03	0.55	1.81
		Inland Forest	0.89	0.03	0.58	1.36
	Plant Height (cm)	Coastal barren	10.0	0.3	5.0	16.2
		Nearby Forest	9.5	0.3	4.5	15.4
		Inland Forest	8.0	0.3	3.8	13.1
TA	Stem Thickness(mm)	Coastal barren	1.09	0.02	0.87	1.41
		Nearby Forest	0.84	0.02	0.49	1.2
		Inland Forest	1.34	0.03	0.89	1.99
	Plant Height (cm)	Coastal barren	11.7	0.3	6.0	18.5
		Nearby Forest	10.1	0.3	1.2	15.3
		Inland Forest	11.0	0.3	7.0	14.8

Two Sample t-test of *Maianthemum canadense* for square root stem thickness and plant height. Data was collected from two sites (PE = Peggy's Cove and TA = Taylor's Head) and three habitat types (C = coastal barren, N = nearby forest and I = inland forest).

Site	**Habitat**	**Morphological characteristic**	**Df**	**t value**	**p-value**
PE	C and N	Stem thickness	96	-0.1461	0.884
		Plant Height	96	1.18	0.240
TA	C and N	Stem thickness	98	8.26	**<0.001**
		Plant Height	98	3.49	**<0.001**
PE	N and I	Stem thickness	95	-4.34	**<0.001**
		Plant Height	95	-3.17	**0.002**
TA	N and I	Stem thickness	90	12.09	**<0.001**
		Plant Height	90	2.08	**0.040**
PE	C and I	Stem thickness	97	4.84	**<0.001**
		Plant Height	97	4.53	**<0.001**
TA	C and I	Stem thickness	90	-6.67	**<0.001**
		Plant Height	90	1.47	0.14

Appendix A3

Means of coastal barrens, nearby forests and inland forests with standard error (SE), minimum and maximum value of *Cornus canadensis* for phenotype leaf thickness from Peggy's Cove (PE) and Taylor's Head (TA).

Site	Phenotype	Habitat	mean	SE	min	max
PE	Leaf Thickness(mm)	Coastal barren	0.20	0.006	0.14	0.27
		Nearby Forest	0.11	0.002	0.07	0.15
		Inland Forest	0.20	0.004	0.14	0.32
TA	Leaf Thickness(mm)	Coastal barren	0.18	0.005	0.11	0.25
		Nearby Forest	0.13	0.004	0.09	0.20
		Inland Forest	016	0.005	0.10	0.24

Two sample t-test of *Cornus canadensis* for log leaf thickness. Data was collected from two sites (PE = Peggy's Cove and TA = Taylor's Head) and three habitat types (C = coastal barren, N = nearby forest and I = inland forest).

Site	**Habitat**	**Df**	**t value**	**p-value**
PE	C and N	73	15.34	**<0.001**
TA	C and N	96	6.48	**<0.001**
PE	N and I	91	19.26	**<0.001**
TA	N and I	80	4.48	**<0.001**
PE	C and I	70	0.16	0.87
TA	C and I	82	1.71	0.09

Appendix A4

Means of coastal barrens, nearby forests and inland forests with standard error (SE), minimum and maximum value of *Kalmia angustifolia* for phenotypes leaf thickness and plant height from Peggy's Cove (PE) and Taylor's Head (TA).

Site	Phenotype	Habitat	mean	SE	min	max
PE	Leaf Thickness(mm)	Coastal barren	0.27	0.01	0.16	0.39
		Nearby Forest	0.11	0.004	0.07	0.16
		Inland Forest	0.10	0.006	0.04	0.16
	Plant Height (cm)	Coastal barren	21.1	1.6	8.5	46.0
		Nearby Forest	33.6	2.4	16.0	74.0
		Inland Forest	38.5	3.6	11.2	87.0
TA	Leaf Thickness(mm)	Coastal barren	0.21	0.009	0.11	0.33
		Nearby Forest	0.18	0.008	0.12	0.26
		Inland Forest	0.18	0.007	0.12	0.29
	Plant Height (cm)	Coastal barren	13.9	0.5	8.3	22
		Nearby Forest	28.2	2.8	8.9	65.0
		Inland Forest	37.1	2.7	18.0	75.0

Two sample t-test of *Kalmia angustifolia* for leaf thickness and square root (plant height). Data was collected from two sites (PE = Peggy's Cove and TA = Taylor's Head) and three habitat types (C = coastal barren, N = nearby forest and I = inland forest).

Site	**Habitat**	**Morphological characteristic**	**Df**	**t value**	**p-value**
PE	C and N	Leaf thickness	58	13.25	**<0.001**
		Plant Height	58	-4.59	**<0.001**
TA	C and N	Leaf thickness	57	2.72	**0.009**
		Plant Height	57	-5.54	**<0.001**
PE	N and I	Leaf thickness	58	-1.40	0.166
		Plant Height	58	0.78	0.437
TA	N and I	Leaf thickness	52	0.41	0.683
		Plant Height	52	2.53	**0.014**
PE	C and I	Leaf thickness	58	13.16	**<0.001**
		Plant Height	58	-4.26	**<0.001**
TA	C and I	Leaf thickness	53	2.44	**0.018**
		Plant Height	53	-10.78	**<0.001**

Appendix A5

Means of coastal barrens, nearby forests and inland forests with standard error (SE), minimum and maximum value of *Vaccinium vitis-idaea* for phenotype leaf thickness from Peggy's Cove (PE) and Taylor's Head (TA).

Site	Phenotype	Habitat	mean	SE	min	max
PE	Leaf Thickness(mm)	Coastal barren	0.22	0.01	0.10	0.31
		Nearby Forest	0.16	0.01	0.10	0.26
		Inland Forest	0.25	0.02	0.13	0.41
TA	Leaf Thickness(mm)	Coastal barren	0.25	0.01	0.13	0.40
		Nearby Forest	0.19	0.01	0.08	0.29
		Inland Forest	0.18	0.01	0.07	0.30

Two sample t-test of *Vaccinium-vitis-idaea* for leaf thickness at each site separately (PE = Peggy's Cove and TA = Taylor's Head) against all possible variations of the three habitat types (C = coastal barren, N = nearby forest, I = inland forest).

Site	**Habitat Types**	**Df**	**t value**	**p-value**
PE	C and N	40	3.60	**<0.001**
TA	C and N	56	4.43	**<0.001**
PE	N and I	32	4.12	**<0.001**
TA	N and I	48	-0.29	0.777
PE	C and I	44	-1.32	0.194
TA	C and I	46	4.19	**<0.001**

Appendix A6

One-way ANOVA (Type 1) of *Gaultheria procumbens* for leaf thickness. Data was collected from Peggy's Cove and three habitat types (coastal barren, nearby forest and inland forest).

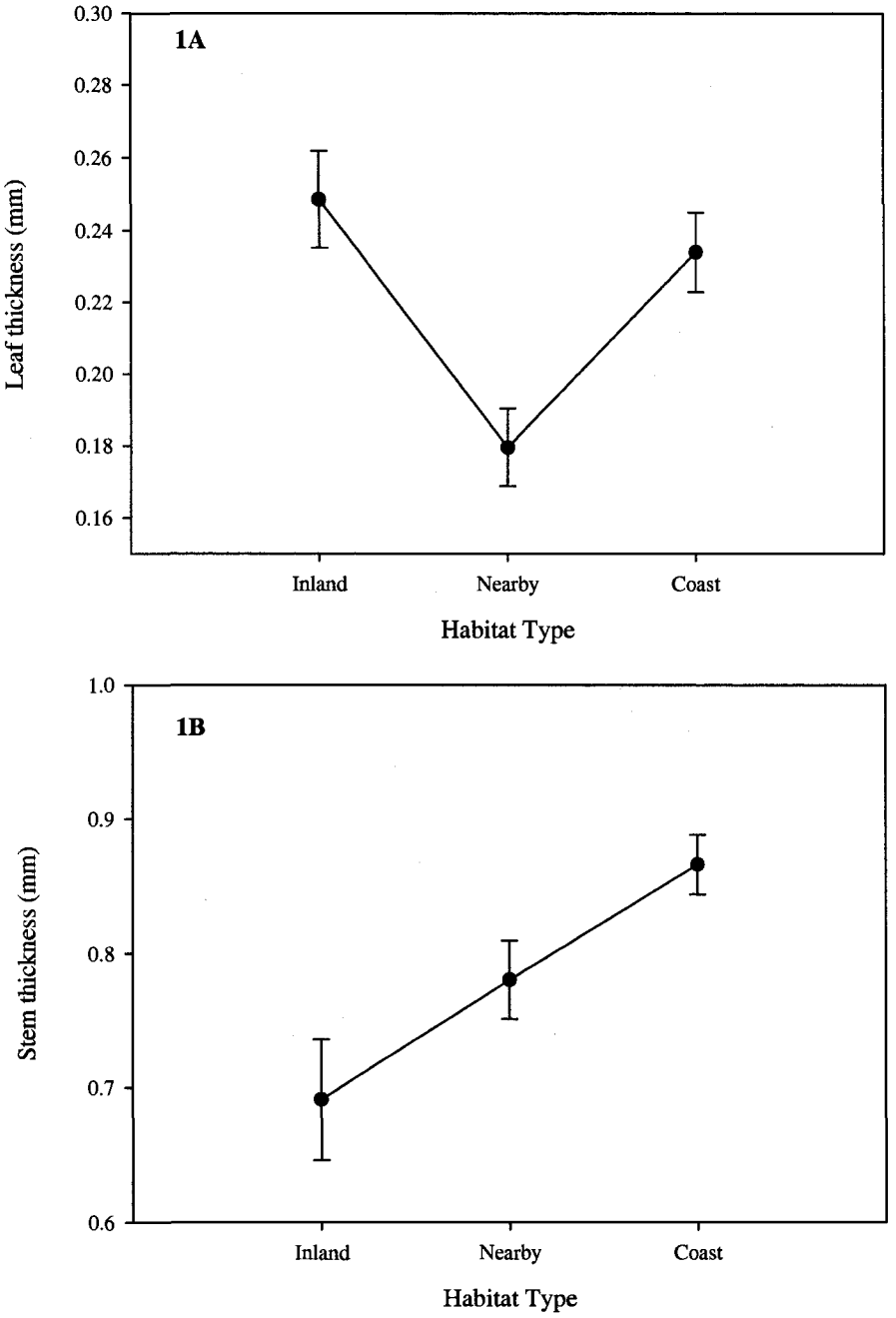
Morphological characteristic		Df	Sum squares	Mean square	F value	p-value
Leaf thickness	Habitat	2	0.055	0.027	8.23	**<0.001**
	Residuals	68	0.225	0.003		

Means of coastal barrens, nearby forests and inland forests with standard error (SE), minimum and maximum value of *Gaultheria procumbens* for phenotypes leaf thickness and stem thickness from Peggy's Cove (PE).

Site	Phenotype	Habitat	mean	SE	min	max
PE	Leaf Thickness(mm)	Coastal barren	0.23	0.06	0.13	0.37
		Nearby Forest	0.18	0.01	0.10	0.28
		Inland Forest	0.25	0.01	0.17	0.41
	Stem Thickness	Coastal barren	0.87	0.02	0.59	1.1
		Nearby Forest	0.78	0.03	0.46	1.0
		Inland Forest	0.69	0.04	0.44	1.32

Two sample t-test of *Gaultheria procumbens* for leaf thickness, stem thickness and plant height. Data was collected from Peggy's Cove and three habitat types (C = coastal barren, N = nearby forest and I = inland forest).

Habitat	Morphological characteristic	Df	t value	p-value
C and N	Leaf thickness	49	3.35	**0.002**
	Stem thickness	49	2.37	**0.0219**
N and I	Leaf thickness	38	4.02	**<0.001**
	Stem thickness	38	-1.67	0.103
C and I	Leaf thickness	49	-0.84	0.405
	Stem thickness	49	3.87	**<0.001**



Relationship between 1A) leaf thickness and 1B) stem thickness at different habitat types (Inland = inland forest, Nearby = nearby forest, Coast = coastal barren) at Peggy's Cove for 71 samples of *Gaultheria procumbens.*

Appendix A7

Two sample t-test of soil nutrients for pH, phosphorous and manganese. Data was collected from Peggy's Cove (PE) and Taylor's Head (TA) with three habitat types (C = coastal barren, N = nearby forest and I = inland forest).

Site	Habitat	Soil property	Df	t value	p-value
PE	C and N	pH	25	7.75	**<0.001**
		phosphorous	25	1.15	0.261
		manganese	25	2.28	**0.032**
TA	C and N	pH	24	10.06	**<0.001**
		phosphorous	24	-4.94	**<0.001**
		manganese	24	-1.97	0.060
PE	N and I	pH	24	1.85	0.077
		phosphorous	24	8.13	**<0.001**
		manganese	24	7.68	**<0.001**
TA	N and I	pH	24	1.57	0.129
		phosphorous	24	4.13	**<0.001**
		manganese	24	2.37	**0.026**
PE	C and I	pH	25	3.83	**<0.001**
		phosphorous	25	-6.69	**<0.001**
		manganese	25	-1.15	0.261
TA	C and I	pH	24	6.17	**<0.001**
		phosphorous	24	-9.38	**<0.001**
		manganese	24	-4.10	**<0.001**

Appendix B1

Analysis of Molecular Variance (AMOVA) of *Vaccinium vitis-idaea* using Euclidean distance. Variation was partitioned between sites (Peggy's Cove and Taylor's Head, among populations within habitats (inland forest, nearby forest, coastal barren) and among individuals within populations for *Vaccinium vitis-idaea*. All cases based on 1023 permutations.

Source of Variation	d.f.	Sum of Squares	Variance Components	% Total Variation	p-values
Between sites	1	21.443	0.24164	6.15	0.098
Among habitats within sites	4	44.519	0.56625	14.40	<0.001
Among individuals within habitats	79	246.744	3.12334	79.45	<0.001

Appendix B2

Non-parametric Multivariate Analysis of Variance comparing *Vaccinium vitis-idaea* AFLP data on the coastal barren to the nearby forest of at Peggy's Cove site. Based on 1000 permutations.

	d.f.	Sums Of Square	Mean Square	F Model	R^2 (Pr(>F)	p-value
Habitat	1	0.0157892	0.0157892	7.2508849	0.2181	<0.001
Residuals	26	0.0566166	0.0021776		0.7819	

Non-parametric Multivariate Analysis of Variance comparing *Vaccinium vitis-idaea* AFLP data on the coastal barren and the nearby forest of Taylor's Head site. Based on 1000 permutations.

	d.f.	Sums Of Square	Mean Square	F Model	R^2 (Pr(>F)	p-value
Habitat	1	0.00305	0.0030533	1.9563105	0.0676	0.094
Residuals	27	0.0421	0.0015608		0.9324	

Non-parametric Multivariate Analysis of Variance comparing *Vaccinium vitis-idaea* AFLP data on the coastal barren and the inland forest of Peggy's Cove site. Based on 1000 permutations.

	d.f.	Sums Of Square	Mean Square	F Model	R^2 (Pr(>F)	p-value
Habitat	1	0.0087224	0.0087224	5.0010915	0.1515	0.001
Residuals	28	0.0488348	0.0017441		0.8485	

Non-parametric Multivariate Analysis of Variance comparing *Vaccinium vitis-idaea* AFLP data on the coastal barren and the inland forest of Taylor's Head site. Based on 1000 permutations.

	d.f.	Sums Of Square	Mean Square	F Model	R^2 (Pr(>F)	p-value
Habitat	1	0.0095034	0.0095034	5.4088368	0.1722	0.002
Residuals	26	0.0456825	0.0017570		0.8278	

Non-parametric Multivariate Analysis of Variance comparing *Vaccinium vitis-idaea* AFLP data on the nearby and inland forest of Peggy's Cove site. Based on 1000 permuatations.

	d.f.	Sums Of Square	Mean Square	F Model	R^2 (Pr(>F)	p-value
Habitat	1	0.0169313	0.0169313	7.4699257	0.2232	< 0.001
Residuals	26	0.0589316	0.0022666		0.7768	

Non-parametric Multivariate Analysis of Variance comparing *Vaccinium vitis-idaea* AFLP data on the nearby and inland forest of Taylor's Head site. Based on 1000 permutations.

	d.f.	Sums Of Square	Mean Square	F Model	R^2 (Pr(>F)	p-value
Habitat	1	0.0037331	0.0037331	2.0139762	0.0746	0.087
Residuals	25	0.0463396	0.0018536		0.9254	

Non-parametric Multivariate Analysis of Variance comparing *Vaccinium vitis-idaea* AFLP data on the coastal barrens of Peggy's Cove to the coastal barrens of Taylor's Head. Based on 1000 permutations.

	d.f.	Sums Of Square	Mean Square	F Model	R^2 (Pr(>F)	p-value
Habitat	1	0.0129743	0.0129743	8.2561017	0.2277	< 0.001
Residuals	28	0.0440015	0.0015715		0.7723	

Non-parametric Multivariate Analysis of Variance comparing *Vaccinium vitis-idaea* AFLP data on the nearby forest of Peggy's Cove to the nearby forest of Taylor's Head. Based on 1000 permutations.

	d.f.	Sums Of Square	Mean Square	F Model	R^2 (Pr(>F)	p-value
Habitat	1	0.0104178	0.0104178	4.7565318	0.1598	0.005
Residuals	25	0.0547554	0.0021902		0.8402	

Non-parametric Multivariate Analysis of Variance comparing *Vaccinium vitis-idaea* AFLP data on the inland forest of Peggy's Cove to the inland forest of Taylor's Head. Based on 1000 permutations.

	d.f.	Sums Of Square	Mean Square	F Model	R^2 (Pr(>F)	p-value
Habitat	1	0.0152313	0.0152313	7.8394273	0.2317	< 0.001
Residuals	26	0.0505158	0.0019429		0.7683	

Appendix B3

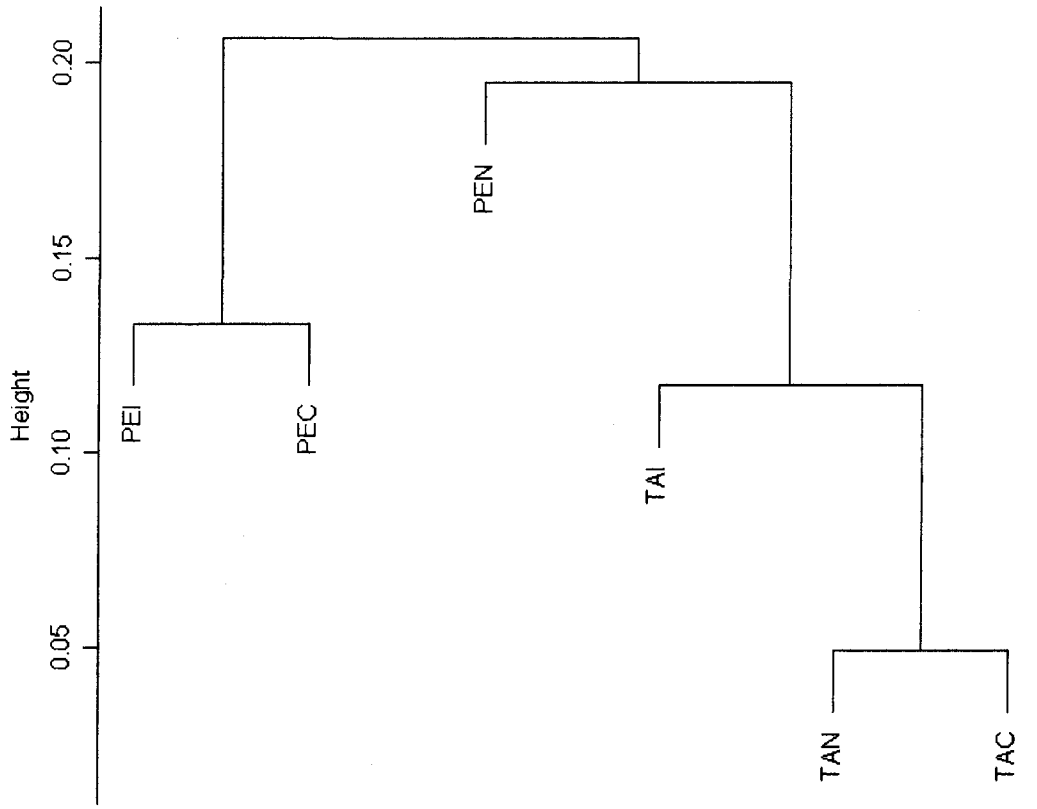
Pairwise differences of *Vaccinium vitis-idaea* AFLP markers based on Euclidean distances comparing pairs of population samples between habitats at Peggy's Cove and Taylor's Head. PEI = Peggy's Cove inland forest, PEN = Peggy's Cove nearby forest, PEC = Peggy's Cove coastal barren, TAI = Taylor Head inland forest, TAN = Taylor Head nearby forest, TAC = Taylor Head coastal barren.

	PEI	PEN	PEC	TAI	TAN	TAC
PEI	0					
PEN	0.23553	0				
PEC	0.13300	0.22423	0			
TAI	0.21764	0.11989	0.16353	0		
TAN	0.21684	0.18271	0.06045	0.06521	0	
TAC	0.32722	0.28228	0.20408	0.16968	0.04933	0

Pairwise differences of *Vaccinium vitis-idaea* AFLP markers based on √(Jaccard coefficient) comparing pairs of population samples between habitats at Peggy's Cove and Taylor's Head. PEI = Peggy's Cove inland forest, PEN = Peggy's Cove nearby forest, PEC = Peggy's Cove coastal barren, TAI = Taylor Head inland forest, TAN = Taylor Head nearby forest, TAC = Taylor Head coastal barren.

	PEI	PEN	PEC	TAN	TAI	TAC
PEI	0.00000					
PEN	0.15074	0.00000				
PEC	0.07690	0.13923	0.00000			
TAN	0.12418	0.12175	0.03447	0.00000		
TAI	0.12686	0.09019	0.08978	0.04259	0.00000	
TAC	0.19212	0.18036	0.11427	0.03003	0.10156	0.00000

Appendix B4



Dendrogram based on Average Cluster analysis of genetic similarity estimates of six *Vaccinium vitis-idaea* locations using Euclidean pairwise distance matrix. Abbreviations in the figure are as follows: PEI = Peggy's Cove inland forest, PEN = Peggy's Cove nearby forest, PEC = Peggy's Cove coastal barren, TAI = Taylor Head inland forest, TAN = Taylor Head nearby forest, TAC = Taylor Head coastal barren.