# Population genomics of *Vaccinium vitis-idaea* L. and links to environmental conditions, total phenolics, and antioxidant capacity in Newfoundland and Southern Labrador


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# ABSTRACT

Lingonberry, also called Partridgeberry in Newfoundland (*Vaccinium vitis-idaea* L.) is native to Eurasia, Greenland, Iceland and North America. Partridgeberry is well known for its nutritional benefits, making it increasingly important for cultivation. While cultivation in Europe is widespread, it is only in developmental stages in North America. Knowledge of the genetic structure of wild populations, and its relationship with environmental conditions, phenolic content (TPC), and antioxidant capacity (AC) is necessary for the selection of desirable genotypes, but this knowledge is incomplete globally. Therefore, the genetic structure of 56 wild partridgeberry populations distributed across nine ecoregions of Newfoundland and Southern Labrador in Canada were investigated in the present experimental study. This thesis also evaluated the effects of environmental factors on the TPC and AC of partridgeberry leaves. By testing different variable levels, significantly higher TPC on leaves was found in individuals growing under elevated levels of surface water pH (>7). Significantly higher AC was found in individuals from the Central Newfoundland, North Shore Forest, and Maritime Barrens ecoregions and in individuals with low surface water pH (<6.6). AC was significantly lower for individuals with low sensitivity to acid rain (alkalinity of >200 µeq/L). Temperature and precipitation had no effect on TPC or AC. Contrary to expectations, no correlation between TPC and AC was found. Individuals formed three genetic groups, which showed some geographic structure according to ecoregion and temperature. Individuals collected in areas with the coldest mean annual and summer temperatures were clustered within one genetic group. As expected, geographically closer individuals were also genetically closer and contained similar quantities of TPC. However, I did not find any correlation between genetic distance and TPC or AC, suggesting that these desired biochemical traits for plant breeding programs are very much influenced by the environment. Future research should focus on the environmental effects I found over a longer period of time, under controlled field or greenhouse conditions, and the expression of genes in the phenyl-propanoid metabolism.

***Keywords: antioxidant capacity, ecoregions, environmental factors, genetic distance, genotyping-by-sequencing, phylogenetic tree, total phenolic content, Vaccinium vitis-idaea, lingonberry, partridgeberry.***

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# List of Abbreviations

| | |
|---|---|
| ABA | Abscisic Acid |
| AC | Antioxidant Capacity |
| AFLPs | Amplified fragment length polymorphisms |
| ANOVA | Analysis of variance |
| BA | Bayesian Analysis |
| BLOSUM | Blocks Substitution Matrix |
| DNA | Deoxyribonucleic Acid |
| DPPH | 2,2-diphenyl-1-picrylhydrazyl |
| EST | Expressed sequence tag |
| FASTA | Fast Nucleotide text based format representing sequences |
| FW | Fresh Weight |
| GBS | Genotyping by Sequencing |
| GE | Gallic Acid Equivalents |
| IBS | Identity-by-State |
| IL | Illinois |
| ISSR | Inter Simple Sequence Repeat Markers |
| K-W | Kruskal- Wallis analysis of variance |
| L | Litters |
| LSD | Least Significant Difference |
| MAFFT | Multiple sequence alignment program for unix-like operating systems |
| MCMC | Montecarlo Montecarlo |
| mEQ | mili-Equivalents |
| ML | Maximum Likelihood |
| mm | milimeters |
| mRNA | Messenger RNA |
| NGS | Next Generation Sequencing |
| NL | Newfoundland and Labrador |
| NY | New York |
| ORAC | Oxygen Radical Absorbance Capacity |
| PCR | Polymerase chain reaction |
| pH | potential of Hidrogen |
| RADseq | Restriction site associated DNA markers |
| RAxML | Randomized Axelerated Maximum Likelihood |
| RNA | Ribonucleic Acid |
| SNPs | Single nucleotide polymorphisms |
| TASSEL | Traits associations, evolutionary patterns, and linkage disequilibrium software |

| | |
|---|---|
| TPC | Total Phenolic Content |
| TROLOX | 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid |
| UPGMA | Unweighted Pair Group Method with Arithmetic Mean |
| UV | Ultraviolet |

## CO-AUTHORSHIP STATEMENT:

Chapter 2: This part of the project involved the collaboration between my lab-partner Zobayer Alam (ZA) and my supervisor Dr. Julissa Roncal (JR). Research questions and study design were conducted by JR, fieldwork for the collection of partridgeberries in Newfoundland was conducted by myself, ZA, JR and others as described in the acknowledgements. Laboratory work was conducted by myself with the help of Dr. Shahidi's research group in the Biochemistry Department at MUN. I conducted data analyses with insight from JR, and committee members. I wrote this thesis chapter with contributions from JR. Also, results from this thesis chapter were published in the journal "Botany" in 2016.

Chapter 3: Research questions and study design were conducted by JR. DNA extractions were conducted by myself and ZA. DNA sequencing and library construction as indicated in the acknowledgements. I conducted data analyses with insight from JR. I wrote the thesis chapter with input from JR and my committee members.

# CHAPTER 1: INTRODUCTION

The taxonomically complex genus *Vaccinium* is comprised of 450 species of shrubs in the heath family (Ericaceae) (Vander, 2005). Some species of the genus have commercial significance, including bilberries (*V. myrtillus*), blueberries (*V. angustifolium*), cranberries (*V. macrocarpon*), red huckleberries (*V. parvifolium*), and more recently lingonberry or partridgeberry (*V. vitis-idaea*) (Vander, 2005). Plant habit in this genus varies from species to species. Some species prostrate, while some are shrubs ranging from 0.2 to 1.2 meters tall. *Vaccinium* is globally distributed and usually develops its fruits from an inferior ovary, which develops into a berry, usually bright in color (Vander, 2005).

*Vaccinium vitis-idaea* L., most commonly known as partridgeberry in Newfoundland, foxberry or cowberry in Labrador, redberry in Northern Labrador, mountain cranberry in the United States, and lingonberry internationally, is an evergreen shrub source of various health benefits (Ho *et al.,* 1999). It is distributed throughout the Northern Hemisphere (Circumboreal distribution) and has spread toward the south along tropical mountain ranges (Persson, 1983). These shrubs are erect or prostrate (Eriksson, 2002). The flowers are white to pale pink and 3–8 millimeters in length, and bloom in late May and early June in more southern latitudes. Flowers are single, grouped, or arranged in long spikes in the leaf axil (Su, 2012). Fruits are carmine red (Su, 2012), 6 mm–10 mm, with a particular acidic taste, and their usual maturity time is from mid-August to September (Rupashinge, 2014). Similar to other Ericaceae species, partridgeberry grows generally well in acidic soil (Rupashinge, 2014). *Vaccinium vitis-idaea* spreads by a stolon to form thick clonal patches. Ranging from 10 cm to 40 cm in length. Leaves are oval, 5–30 mm long with an

entire leaf margin. *Vaccinium vitis-idaea* is evergreen, even in the coldest years. *Vaccinium vitis-idaea* grows inadequately when summers are hot, as seedlings that lack a well-developed rhizome system are often killed due to extreme dryness (Rupashinge, 2014). There are two subspecies of *Vaccinium vitis-idaea: vitis-idaea* (widespread in Europe and Asia predominantly) and the subspecies *minus* located in North America, plant height constitutes the main difference between the two subspecies, 25-30 cm for *vitis-idaea* and 18-20 cm for the subspecies *minus* (Hendrickson *et al*., 1996; Rupashinge, 2014). In this research I use plant material from the subspecies *minus*.

These plants have been harvested from the wild for centuries in Scandinavian countries and North America. In Atlantic Canada, the fruits are used as preserves and baked goods, and its leaves are used to make infusions or tea (Rupashinge, 2014). They are utilized to make Lillehammer berry alcohol, and in Eastern European nations partridgeberry vodka is sold (Rupashinge, 2014). The berries of partridgeberry are an important source of food for birds, bears and foxes (Penhallegon, 2006). Caterpillars of the case-bearer moths *Coleophora glitzella*, *C. idaeella*, and *C. vitisella* are feeders on *V. vitis-idaea* leaves (Debnath, 2007) which contain ample natural acids, vitamin C, vitamin A as beta-carotene, B vitamins including B1, B2, B3, potassium, calcium, magnesium, and phosphorus (Debnath and McRae, 2001). Seeds of these berries are rich in omega-3 fatty acids (Stewart and Nilsen, 1995).

Production of phytochemicals has made this plant medicinally important, and has become an attractive target for studies on conventional and biotechnological breeding (Goyali *et al.,* 2013; Vyas *et al.,* 2015). *Vaccinium vitis-idaea* has been utilized as an

aperitif, astringent, antihemorrhagic, and depurative agent, and it works as an antiseptic agent, especially for the urethra (Wang and Ballington, 2007). It is also used as a diuretic, and in different approaches to treat diabetes, fibrocystic breast changes, and urogenital conditions, nevertheless information is incomplete about which and how phytochemicals are associated to these medicinal properties (Wang and Ballington, 2007).

Phenolics and high antioxidant activity have been a recent target for future partridgeberry breeding programs (Kalt *et al.*, 2007) Phenolics are a class of chemical compounds consisting of a hydroxyl group (-OH) bonded directly to an aromatic hydrocarbon group. These are a large group of phytochemicals (secondary metabolites), showing a diversity of structures, from rather simple (e.g. quercetin), through polyphenols such as flavonoids and anthocyanins, to polymeric compounds such as tannic acid (Cheynier, 2012). Phenolic compounds are responsible for the colour of fruit, leaves, and other plant organs, and are substrates for plant enzymatic activities in the Shikimate pathway and accumulate with oxidative stress as a result of abiotic and biotic environmental stresses (Hernández, 2009; Cheynier, 2012). Phenolic compound activity comprises a small part of the antioxidant activity in plants. Radical scavenging is the main mechanism by which antioxidants act in plants, and several methods have been developed to measure antioxidant capacity. Antioxidants in plants are assessed by the scavenging of synthetic radicals in polar organic solvents, e.g. methanol, at room temperature. Those used include 2,2- diphenyl-1-picrylhydrazyl (DPPH) and other substances (Gordon, 2001), these methods that have been tested in *Vaccinium vitis idaea* (Wang *et al.,* 2005).

Epidemiological data as well as *in vitro* studies strongly suggest that plants within the commercially important *Vaccinium* genus contains phytochemicals that have strong protective effects against major disease risks including cancer and cardiovascular diseases. Species with commercialization potential such as partridgeberry need sufficient germplasm representation before selecting specific traits such as its high phenolic content. Genotyping by sequencing (GBS) is a next generation sequencing technique which can be used to obtain a DNA sequencing coverage of large plant genomes by reducing their genome complexity with the help of restriction enzymes (Elshire *et al.,* 2011). The application of GBS to plant breeding and conservation may allow agronomists to conduct marker-assisted selection in future breeding programs of promising species such as the ones in the genus *Vaccinium* (Elshire *et al.,* 2011).

The development of a successful nutrition-oriented breeding program in *Vaccinium* depends on two main factors: sufficient knowledge on the environmental factors affecting the antioxidant capacity and phenolic content, and the study of the genetic diversity and its interaction with environmental factors (Stang *et al*., 1990). In the last few years, *Vaccinium* has gained international interest due to the high antioxidant content and higher phenolic content. Of the secondary plant metabolites found in partridgeberries, phenolics have received the most attention, providing a proxy for antioxidant capacity. However, only a handful of studies measuring the phenolic content and their antioxidant capacity have been conducted on wild *V. vitis idaea* ssp. *minus* (Debnath and Sion, 2009; Vyas *et al*., 2015; Isaak *et al*., 2015).

In the following chapter of this thesis, I analyze the effects of eight environmental variables on the total phenolic content (TPC) of leaves in wild *V. vitis-idaea ssp. minus* individuals across Newfoundland and Southern Labrador. Their antioxidant capacity (AC) was also measured by their ability to capture free radicals using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. In order to understand the total phenolic and antioxidant capacity of this circumboreal species, I need to connect it with its environment where just a few studies have evaluated (Semerdjieva *et al*., 2003; Ek *et al*., 2006; Kalt *et al*. 2008; Roy and Mulder 2014), thus my study adds to the much-needed literature on this topic.

Subsequently in this thesis, I aimed at evaluating the genetic relatedness among partridgeberries individuals in Newfoundland and Southern Labrador, and their links to the environmental conditions that may promote differential synthesis of phenolic compounds and antioxidant capacity in leaves. Very few studies have characterized the genetic diversity of wild partridgeberry in North America and here I aim to fill this knowledge gap (Debnath, 2007a; Debnath, 2007b; Balsdon *et al*., 2011).

**References:**


- Balsdon, J. L., Smith, T. W., & Lundholm, J. T. (2011). Phenotypic and genotypic differentiation of *Vaccinium vitis-idaea* between coastal barrens and forests in Nova Scotia, Canada. *Botany*, *89*(3), 147-155.

- Cheynier, V. (2012). Phenolic compounds: from plants to foods. *Phytochemistry reviews*, *11*(2-3), 153-177.

- Debnath, S. C., & McRae, K. B. (2001). *In Vitro* Culture of Lingonberry (*Vaccinium vitis-idaea* L.) The Influence of Cytokinins and Media Types on Propagation. *Small Fruits Review*, *1*(3), 3-19.

- Debnath, S. C. (2007a). Strategies to propagate *Vaccinium* nuclear stocks for the Canadian berry industry. *Canadian Journal of Plant Science*, *87*(4), 911-922.

- Debnath, S.C. (2007b) Inter simple sequence repeat (ISSR) to assess genetic diversity within a collection of wild lingonberry (*Vaccinium vitis-idaea* L.) clones. *The Canadian Journal of Plant Science*, 87(2), 337-344.

- Debnath, S. C., & Sion, M. (2009). Genetic diversity, antioxidant activities, and anthocyanin contents in lingonberry. *International Journal of Fruit Science*, *9*(2), 185-199.

- Ek, S., Kartimo, H., Mattila, S., & Tolonen, A. (2006). Characterization of phenolic compounds from lingonberry (*Vaccinium vitis-idaea*). *Journal of Agricultural and Food Chemistry*, *54*(26), 9834-9842.

- Elshire, R. J., Glaubitz, J. C., Sun, Q., Poland, J. A., Kawamoto, K., Buckler, E. S., & Mitchell, S. E. (2011). A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PloS one*, *6*(5), e19379.

- Eriksson, O. (2002). Ontogenetic niche shifts and their implications for recruitment in three clonal *Vaccinium* shrubs: *Vaccinium myrtillus, Vaccinium vitis-idaea*, and *Vaccinium oxycoccos*. *Canadian Journal of Botany*, *80*(6), 635-641.

- Gordon, M. H. (2001). Measuring antioxidant activity. *Antioxidant in food: Practical applications Woodhead Publishing Ltd, Cambridge*, 71-84.

- Goyali, J. C., Igamberdiev, A. U., & Debnath, S. C. (2013). Morphology, phenolic content and antioxidant capacity of lowbush blueberry (*Vaccinium angustifolium* Ait.) plants as affected by in vitro and ex vitro propagation methods. *Canadian Journal of Plant Science*, *93*(6), 1001-1008.

- Hendrickson, P. A., Churchill, R. A., & Butt, E. (1996). The wild partridgeberry (*Vaccinium vitis-idaea* L. var. minus Lodd) industry in Newfoundland and Labrador and the potential for expansion utilizing European cultivars. In *VI International Symposium on Vaccinium Culture 446* (pp. 139-142).

- Hernandez, I., Alegre, L., Van Breusegem, F., & Munné-Bosch, S. (2009). How relevant are flavonoids as antioxidants in plants?. *Trends in Plant Science*, *14*(3), 125-132.

- Ho, K. Y., Huang, J. S., Tsai, C. C., Lin, T. C., Hsu, Y. F., & Lin, C. C. (1999). Antioxidant Activity of Tannin Components from *Vaccinium vitis-idaea* L. *Journal of Pharmacy and Pharmacology*, *51*(9), 1075-1078.

- Isaak, C. K., Petkau, J. C., O, K., Debnath, S. C., & Siow, Y. L. (2015). Manitoba lingonberry (Vaccinium vitis-idaea) bioactivities in ischemia-reperfusion injury. *Journal of agricultural and food chemistry*, *63*(23), 5660-5669.

- Kalt, W., Howell, A. B., MacKinnon, S. L. and Goldman, I. L. (2007). Selected bioactivities of *Vaccinium* berries and other fruit crops in relation to their phenolic contents. *Journal of Science of Food and Agriculture*, 87(1) 2279–2285.

- Kalt, W., MacKinnon, S., McDonald, J., Vinqvist, M., Craft, C., & Howell, A. (2008). Phenolics of *Vaccinium* berries and other fruit crops. *Journal of the Science of Food and Agriculture*, *88*(1), 68-76.

- Penhallegon, R. (2006). Lingonberry Production Guide for the Pacific Northwest. Retrieved March 14, 2017, from OSU Extension Service OSU Extension Catalog: https://catalog.extension.oregonstate.edu/sites/catalog/files/project/pdf/pnw583.pdf

- Persson, H. Å. (1983). The distribution and productivity of fine roots in boreal forests. *Plant and Soil*, *71*(1-3), 87-101.

- Rupasinghe, H. V. (2014). Antioxidant, Cytoprotective and Neuroprotective Properties of Wild Partridgeberry of Southern Labrador. *Forestry and Agrifoods Agency. Government of NL*, *1*(1), 19-42.

- Stang, E. J., Weis, G. G., & Klueh, J. (1990). Lingonberry: potential new fruit for the northern United States. In *Advances in New Crops, Proceedings of the First National Symposium, New Crops: Research, Development, Economics* (pp. 321-323). Timber Press, Portland, Oregon.

- Stewart Jr, C. N., & Nilsen, E. T. (1995). Phenotypic plasticity and genetic variation of *Vaccinium macrocarpon*, the American cranberry. I. Reaction norms of clones from central and marginal populations in a common garden. *International Journal of Plant Sciences*, *1*(1), 687-697.

- Semerdjieva, S. I., Sheffield, E., Phoenix, G. K., Gwynn-Jones, D., Callaghan, T. V., & Johnson, G. N. (2003). Contrasting strategies for UV-B screening in sub-Arctic dwarf shrubs. *Plant, Cell & Environment*, *26*(6), 957-964.

- Su, Z. (2012). Anthocyanins and flavonoids of *Vaccinium* L. *Pharmaceutical Crops, 3*(1), 7-37.

- Vander Kloet, S. P. (2005). Longevity of experimentally buried seed in *Vaccinium*: relationship to climate, reproductive factors and natural seed banks. *Journal of Ecology*, *93*(6), 1167-1176.

- Vyas, P., Curran, N. H., Igamberdiev, A. U., & Debnath, S. C. (2015). Antioxidant properties of lingonberry (*Vaccinium vitis-idaea* L.) leaves within a set of wild clones and cultivars. *Canadian Journal of Plant Science*, *95*(4), 663-669.

- Wang, S. Y., Feng, R., Bowman, L., Penhallegon, R., Ding, M., & Lu, Y. (2005). Antioxidant activity in lingonberries (*Vaccinium vitis-idaea* L.) and its inhibitory effect on activator protein-1, nuclear factor-κB, and mitogen-activated protein kinases activation. *Journal of Agricultural and Food Chemistry*, *53*(8), 3156-3166.

- Wang, S. Y., & Ballington, J. R. (2007). Free radical scavenging capacity and antioxidant enzyme activity in deerberry (*Vaccinium stamineum* L.). *LWT-Food Science and Technology*, *40*(8), 1352-1361.

# CHAPTER 2: ENVIRONMENTAL FACTORS AFFECTING PHENOLIC CONTENT AND ANTIOXIDANT CAPACITY OF *VACCINIUM VITIS-IDAEA L.* IN NEWFOUNDLAND AND SOUTHERN LABRADOR

**Abstract**

Over the last two decades *Vaccinium* (Ericaceae) has gained international economic interest due to the presence of antioxidants and the presence of phenolics. Of the secondary plant metabolites found in partridgeberries, phenolics have received the most attention, providing a proxy for antioxidant capacity. In this study I analyze the effects of eight environmental variables on the total phenolic content (TPC) of leaves in wild *V. vitis-idaea ssp. minus* individuals across Newfoundland and Southern Labrador. Their antioxidant capacity (AC) was also measured by their ability to capture free radicals using a 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Significantly higher TPC was found in individuals that have elevated levels of surface water pH (>7). Significantly higher AC was found in individuals from the Central Newfoundland, North Shore Forest, and Maritime Barrens ecoregions and in individuals with low surface water pH (<6.6). AC was significantly lower for individuals with low sensitivity to acid rain (alkalinity of >200 µeq/L). Temperature and precipitation had no effect on TPC or AC. No correlation between TPC and AC was found. The results obtained here will have to be contrasted over a long period of time and against other plant tissues such as fruit, roots, or stems from *Vaccinium vitis-idaea* to examine whether different plant organs have a common response to environmental factors.

***Keywords: antioxidant capacity, DPPH, environmental factors, Newfoundland and Labrador, partridgeberries, Vaccinium vitis-idaea, total phenolic compounds.***

## Introduction

### Plant secondary compounds and their properties in *Vaccinium*

In the last twenty years, *Vaccinium,* has gained international interest due to the presence of antioxidants, which confer medicinal properties (Yuan *et al.,* 2011; Kraujalytė *et al.,* 2015). *Vaccinium* species are considered to be a good source of phenolic compounds, which are valued for their high antioxidant activity (Prior *et al.,* 1998). Due to raised health awareness and the evident relationship between phytochemicals in plant foods and the prevention of chronic diseases, the content and physiological activity of phenolic compounds in *Vaccinium* species have been studied. As a result, the phenolic profiles and quantitative composition of different species in the genus are well-documented (Taruscio *et al.,* 2004). Among the different *Vaccinium* species there are significant differences in phenolic content and antioxidant activity, as well as between the varieties within each species (Prior *et al.,* 1998; Taruscio *et al.,* 2004).

Phenolic compounds (e.g., flavonoids, tannins, lignin) are chemically diverse, carbon-based, secondary plant compounds that play numerous biological roles, such as insect-attractants for pollination and as part of the plant defense system against diseases (Haborne, 1997). Phenolics comprise a diverse group of phytochemicals that have one or more phenol group in their structure, which may be present in plants as simple molecules, oligomers, or polymers. The phenolic compounds in *Vaccinium* may be found in the form of simple phenolics, phenolic acids, flavonoids, stilbenes, condensed tannis, lignans, amongst other water-soluble vacuolar pigments (Penney *et al*., 1996; Naczk *et al*., 2004). These compounds are known to contribute to the astringency, bitterness, colour, flavour,

and odour of plants. The fruit of *Vaccinium* are appreciated for their specific aroma and taste, and for many years, they have comprised an important part of the Canadian and Newfoundland diet (Prior *et al.*, 1998, Debnath, 2007).

An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that can produce free radicals, leading to a chain of reactions that may damage cells. Antioxidants such as thiols or ascorbic acid (vitamin C) terminate these chain reactions. Antioxidants are phytochemicals, vitamins, and other substances that protect cells from the damage caused by free radicals (Prior *et al.,* 1998). Antioxidants naturally occurring secondary metabolites from plants that have protective or disease preventing properties (Prior *et al.,* 1998). There are more than ten thousand known phytochemicals produced by plant species.

Fruit from *Vaccinium* have been harvested from the wild for centuries (Prior *et al.,* 1998). Before the development of modern medicine, this genus was used for healing various infections, and it has been documented as a well-known remedy in European folk-medicine (Naczk *et al.*, 2004). Leaves and edible fruit in *Vaccinium* are rich in antioxidants, anthocyanins, and other phenolic compounds, which have been proven to reduce the risk of cancer (e.g., Skupien *et al.,* 2006; Neto 2007), hepatitis C (Takeshita *et al.,* 2009), cardiovascular disorders (e.g., McKay and Blumberg, 2007), diabetes (e.g., Martineau *et al.,* 2006; Wang *et al.,* 2010), and urinary-tract infections (Nowack and Schmitt, 2008, Perez-Lopez *et al.,* 2009). Extraction from leaves in this genus are rich in anthocyanins, which have been associated with improved night vision, the prevention of macular degeneration, anticancer activity, and reduced risk of heart disease (Kalt *et al.*,

2001). The compound resveratrol, which is found in *Vaccinium*, has also been associated with reduced risk of heart disease and cancer while another compound, pterostilbene, has been shown to lower cholesterol (Rimando *et al.,* 2004). Although the number of antioxidants is lower when the leaves or fruits are frozen or processed, they still have higher antioxidant levels than many other species such as strawberries, tomatoes, cucumbers, red berries and several species of grapes (Karlsson, 1985).

**Study species: *Vaccinium vitis-idaea***

*Vaccinium vitis-idaea* is an evergreen dwarf shrub with edible fruit, native to boreal forest and arctic tundra throughout the northern hemisphere from Eurasia to North America. The species is known by more than 25 English names, including lingonberry (international name), partridgeberry (in Newfoundland), alpine cranberry, cowberry or red berry (in Labrador and Northern Labrador respectively), dry ground cranberry, foxberry (in Nova Scotia), lowbush cranberry (in British Columbia and Alaska), moss cranberry, mountain cranberry, red berry, red bilberry, red whortleberry, and rock cranberry (Aiken *et al.*, 2011). The fruit are harvested from the wild, and leaves are traditionally used to make tea (Aiken *et al.*, 2011).

In Scandinavian countries, the *V. vitis-idaea* ssp. *vitis-idaea* L. industry is fairly well-developed from harvest to processing, with breeding programs to generate improved cultivars (Debnath, 2007; Aiken *et al.*, 2011). In Canada, the industry is at the initial stages of growth (Debnath, 2007). Currently, the market for partridgeberry (V. *vitis-idaea* ssp. *minus* (Lodd.) Hult) in the United States and Canada is very small compared to the European one, but has very good potential, harvesting near 35.7 tones/hectare in the US of

fruit per year (Penhallegon, 2006). There is also a good potential for exportation to other areas of the world, given the large potential of growth in Canada (Debnath, 2007).

While many *Vaccinium* species have been largely studied, such as blueberries (i.e. *V. corymbosum and V. angustifolium*) and cranberries (*V. oxycoccos* and V. *macrocarpon* Ait.); the wild partridgeberry (*V. vitis-idaea*) has not been studied as much as the ones in the genus. *Vaccinium vitis-idaea* subsp. *minus* is known to exhibit higher antioxidant activity and anthocyanin content than cranberry, blackberry, blueberry, raspberry, strawberry, and *V. vitis-idaea* subsp. *vitis-idaea* (Zheng and Wang, 2003; Bakowska-Barczak *et al.,* 2007; Debnath and Sion 2009; Vyas *et al.,* 2015) therefore raising the interest in North America to develop this wild fruit into a commercial crop suitable for exportation (Debnath *et al*., 2012).

Partridgeberry has been used therapeutically as a urinary antiseptic (Camire, 2002). Partridgeberries are extremely high in vitamin C, moderately high in vitamin A, and quite high in fiber and anthocyanins compared to apple, blueberries, grapes, plums, raspberries and strawberries (Zheng and Wang, 2003). Hippuric acid, an important medicinal constituent of partridgeberries' fruit and leaves, has been found to reduce the alkalinity levels in urine (Bomser *et al.,* 1996). Partridgeberry exhibits a potential anticarcinogenic activity, as evaluated by *in vitro* screening tests (Bomser *et al.,* 1996). With the increasing worldwide demand for healthy foods, crop breeding programs must select individuals with high concentrations of bioactive compounds, notably antioxidants. For years, breeding programs for partridgeberry were focused on creating varieties with improved commercial traits such as large fruit size, light-red colour (lighter coloured partridgeberries appear

fresher than darker ones), small wounds (large, wet wounds are susceptible to infections that cause post-harvest decay), firmness, and productivity (Castrejon *et al*., 2008). Recently, crop breeding programs are more focused on an aspect of plant quality extended to the improvement of the nutritional value (Vyas *et. al*., 2015). The phenolic content and antioxidant capacity of plants are targeted traits by plant breeders (Kalt *et al*., 2003). Breeding objectives for berry crops include investigating the germplasm of wild species to identify phenolic-rich clones or populations, and to breed cultivars with enhanced bioactivity (Scalzo *et al.,* 2005). The success of a plant breeding program depends not only on the knowledge of the physiological variations of a trait, but also on an understanding of how environmental factors affect these health-promoting characteristics, for future selection of the partridgeberry plants that grow in specific environmental conditions that have higher phenolic content and or higher antioxidant capacity.

**Factors affecting phenolic compounds and antioxidant capacity in *Vaccinium* and other plants**

The influence of biotic and abiotic factors on the biosynthesis of phenolic compounds is well-documented for many plants. The synthesis of secondary compounds in plants and their associated antioxidant levels change with stressors, such as drought, pathogens, pollutants, seasonal changes, temperature and ultraviolet radiation (Semerdjieva *et al.,* 2003a; Hansen *et al.,* 2006; Martinussen *et al.,* 2009; Nybakken *et al.,* 2012). Plant phenolic chemistry can respond to both the physical environment, such as light and temperature conditions (Semerdjieva *et al.,* 2003a; Hansen *et al.,* 2006; Martinussen *et al.,* 2009; Nybakken *et al.,* 2012), and to pathogens and herbivores (Mayer, 2004; Nagle *et al.,*

2011; Roy and Mulder, 2014). It has been hypothesized that higher temperatures will promote an increase in secondary plant compounds as a function of a temperature-regulated increased photosynthesis (Jonasson *et al.,* 1986). Plant exposure to increased UV-B light can increase the synthesis of flavones and flavonols, which are accumulated in the epidermis of leaves and stems, and offer a measure of protection against harmful UV-B radiation (Lake *et al.,* 2009). Semerdjieva *et al.,* (2003b) found that cell wall-bound phenolics in *V. vitis-idaea* leaves increase in response to UV-B light enhancement as a strategy to avoid the damaging effects of UV-B. High levels of polyphenolic compounds and anthocyanins could result from drought. In water deficit conditions, the drought inducible hormone abscisic acid (ABA) has been found to induce the accumulation of anthocyanins by increasing the expression of key genes involved in anthocyanin biosynthesis (Antolin *et al.,* 2008; Deluc *et al.,* 2009). The presence of insects can also induce the synthesis of phenolics as a mechanism of plant defense against herbivory (e.g., Pascual-Alvarado *et al.,* 2008). Plant secondary compounds are therefore expected to be higher in leaves than in fruit of partridgeberries, since the cost of replacement of damaged leaves is higher in conditions where resources are scarce. This hypothesis was corroborated in *Vaccinium corybosum*, where antioxidant activity and phenolic levels were 3 to 15 times higher in leaves compared to fruit, dependent as well on the development stage of fruit (Ehlenfeldt and Prior 2001; Yuan *et al.,* 2011).

Few studies have evaluated the simultaneous effects of abiotic factors on the biochemical properties of *V. vitis-idaea*, a handful of studies have tested latitude and longitude, temperature and precipitation but rarely their combined effect (Åkerström *et al*., 2010).

Results obtained by Åkerström et al. (2010) suggest that cultivar, growing season, and growing location influence the composition and content of phenolic compounds in partridgeberries. Studies evaluating the natural variation of antioxidant activity in *Vaccinium* populations from different geographical areas have compared different environmental conditions, such as latitude and longitude (Debnath, 2007; Martinussen *et al.*, 2009). Latitude, through the variation in climatic regimes, appears to influence the accumulation of phenolics in partridgeberries. An increasing trend in antioxidant production through higher phenolic and anthocyanin content was observed in *Vaccinium myrtillus* from northern Europe compared to plants from southern latitudes in China and Southeastern Asia (Martz *et al.,* 2010; Wang *et al.,* 2010).

Evidence about changes in phenolic composition and antioxidant activity during post-harvest storage and handling, as well as processing is also available in the literature (Kalt *et al*. 2003). It is known that the content of phenolics in partridgeberries is affected not only by genetic differences and pre-harvest environmental conditions, but also by the degree of maturity at harvest (Zadernowski *et al.,* 2005). It has been observed that phenolic accumulation continues in overripe partridgeberries and other *Vaccinium* species, as maturity triggers the accumulations of natural pigments in plants (Debnath *et al*., 2012).

The literature reports contradicting effects of several abiotic environmental factors on the synthesis of phenolic compounds, with no common response across plant species, tissue types, and kind of secondary compound analyzed, making generalizations difficult to establish (Martinussen *et al.,* 2009 and Arslan and Ozcan 2011). For example, low temperatures produced a significantly higher content of sugars, acids, and total phenols in

bilberries (*V. myrtillus*, Martinussen *et al.,* 2009), whereas a higher mean temperature promoted the accumulation of phenolics in tomato (Dannehl *et al.,* 2014). Olives showed the highest phenolic content in a location with the highest average rainfall in Turkey (Arslan and Ozcan 2011), whereas in currant (*Ribes sp*.) cultivars, high precipitation was associated with lower contents of anthocyanins, flavonol glycosides, and hydroxycinnamic acid conjugates (Yang *et al.,* 2013). Environmental factors should therefore be evaluated on a species-specific basis and by measuring the desired biochemical profiles (i.e. a complete biochemical profile of phenolics, or a complete biochemical profile of tannins). Using a transplant experiment in Alaska, Roy and Mulder (2014) found increased levels of phenolic compounds in the leaves of *V. vitis-idaea* in colder soils, colder air temperatures, less canopy cover, and less nutrient availability. These findings contradicted those of Hansen *et al.,* (2006) in Sweden who found that the concentration of condensed tannins in leaves increased with temperature, shading, and nutrient addition. Roy and Mulder (2014) proposed that induction by insects might better explain the elevated levels of phenolics than the physical environment. In Atlantic Canada, higher levels of anthocyanins, proanthocyanidins, and total antioxidant activity in leaves were found at higher elevations and reduced temperatures, whereas tannins and flavonoids increased with precipitation (Vyas *et al.,* 2015). In my research, I extend the sampling from these earlier studies and evaluate additional environmental variables (i.e., ecoregion, coastal proximity, runoff, and surface water quality).

**Research hypotheses**

In this chapter, I analyze the effects of environmental variation on the total phenolic content (TPC) of leaves in wild *V. vitis-idaea ssp. minus* populations across Newfoundland and Southern Labrador. Their antioxidant capacity (AC) was also measured by their ability to capture free radicals using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. I had the following expectations about the chemical variation across sampling sites:

1) TPC and AC vary according to the ecoregion where plants grow.

2) TPC and AC are higher in areas with relatively higher temperatures and lower precipitation or water run-off.

3) Water quality has an effect on the TPC and AC of leaves.

4) TPC and AC are positively correlated because antioxidant properties are characteristic of common phenolics.

**Materials and Methods**

**Site selection and sample collection**

The province of Newfoundland and Labrador in Canada is divided into 19 ecoregions based on vegetation, topography, and soil characteristics, which are determined by the local climate and geological history (Meades 1990). Part of my research laboratory and I, conducted a field trip from August 15th to September 15th of 2014 to perform my sampling, where based on the presence of *V. vitis-idaea*, I selected a total of 56 sampling sites from nine ecoregions (see Table 2.1 and Fig. 2.1), with five to nine sites per ecoregion. I collected around 100-200 g of leaves in 50 ml centrifuge tubes from an approximate 2m perimeter patch of adult plants at each sampling site. The collected leaves

were fully green, and gathered both young and mature leaves. I avoided the collection of damaged or discolored leaves, leaves bitten by insects or dry leaves. I stored my samples in a cooler for two weeks in the field until I arrived in the laboratory, where I kept samples at -20$^0$ C for further analysis (Yuan *et al.,* 2011).

**Environmental variables**

Environmental data utilized in this research was obtained from the Water Resource Atlas of Newfoundland (1994). In order to evaluate how TPC and AC vary according to the different environmental conditions where wild plants grow, I grouped the 56 samples into categories using the following eight variables: ecoregion, mean annual temperature, mean summer temperature, coastal proximity, mean annual precipitation, mean annual runoff, pH of surface water, and alkalinity of surface water (Table S2.1). Using data from the Water Resources Division of the Government of Newfoundland and Labrador (1992), I partitioned the province into three mean annual temperature categories: low (1.0-2.9°C), medium (3.0-3.9°C), and high (4.0-7.9°C). The mean summer temperature was also of interest because this is the time when berries are produced. Samples were divided into three summer temperature categories: low (6-11.9°C), medium (12-13.9°C), and high (14-19.9°C). Samples were also categorized as coastal if they were located within a 2 km distance from the coast; otherwise, they were classified as being inland plants.

As well, using the Water Resources Division of the Government of Newfoundland and Labrador (1992), for mean annual precipitation, I separated the province into three areas: low (700-1100 mm), medium (1100-1300 mm) and high (1300-1500 mm). Runoff is defined as the portion of precipitation that flows into rivers, lakes, and oceans through

surface and ground drainage, an important growth factor for *Vaccinium* berries. This is of interest because it is a proxy for the amount of water available in an area. I divided the sampling sites into five mean annual runoff categories, distributed in the Island of Newfoundland and Southern Labrador: 500-800 mm, 800-1000 mm, 1000-1300 mm, 1300-1800 mm, and 1800-2200 mm. Among the several estimates for surface water quality provided by the provincial government, I selected two, pH and sensitivity to acid rain based on total alkalinity. I considered four pH categories: <6.1, 6.2-6.5, 6.6-7.0, and >7.0, as it has been reported to affect *Vaccinium* growth (Rosen et al., 1990). The ability of water to resist changes in pH, acidification, in particular, can be measured by its alkalinity. The sensitivity of water in the province to acid rain was categorized as extremely sensitive (<60 μeq/l), highly sensitive (60-100 μeq/l), moderate (100-200 μeq/l), and low (>200 μeq/l) (Government of Newfoundland and Labrador 1992).

**Chemicals**

DPPH (2,2-diphenyl-1-picrylhydrazyl radical), Folin-Ciocalteu's phenol reagent, Gallic acid, sodium carbonate, and 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) were purchased from the Sigma-Aldrich Canada Co. (Oakville, ON). Acetone and ethanol were purchased from Merck Canada Inc. (Kirkland, QC). All chemicals used in the study were of analytical grade.

**Sample extraction for biochemical tests**

Frozen partridgeberry leaves were ground into powder and disrupted using a coffee bean grinder (Think Kitchen 104), so the final powder had a diameter size smaller than 0.3 milimeters. A total of one gram of fine leaf powder was homogenized with 30 ml of 70%

aqueous acetone. To homogenize the plant material, I exposed samples to sonication using an ultrasonic water bath (Blackstone-NEY Ultrasonic, Jamestown, NY) at an amplitude of 60 Hz at 40°C for 30 minutes. Centrifugation at 4,000 rpm for 10 minutes at 4°C followed the sonication. The liquid supernatant was transferred to a new 50 ml centrifuge tube using a Whatman No. 1 90 mm filter paper, and the sample was diluted to a final volume of 70 w/vol (weight per volume). These plant extracts were stored at -20°C until further analyses (George, 2015).

**Determination of total phenolic content**

TPC was determined using the Folin-Ciocalteu method described by McDonald *et al.,* (2001), with minor modifications from the methodology described by George (2015). Briefly, 50 µl of the diluted plant extract was mixed with 50 µl of 2N Folin-Ciocalteu reagent for three minutes. 100 µl of 5% $Na_2CO_3$ was added to this mixture, and the volume was adjusted to 1 ml with distilled water. The mixture was allowed to stand for 30 minutes in the dark, followed by a one-minute vortexing. The absorbance of the mixture was read at 760 nm using a UV–VIS spectrophotometer (Ultrospec 4300 pro, Jasco Corporation, Tokyo, Japan). A calibration curve was established using Gallic acid solutions (0-5 mg/ml). The results are expressed in milligrams of Gallic acid per 100 grams of fresh leaves (GE mg/100g). Measurements were conducted in triplicate, and the values were averaged, with its respective standard deviation value.

**Determination of antioxidant capacity**

The antioxidant capacity of extracts was measured using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, following that of Kedare and Singh (2011) with modifications. I

prepared a stock solution of 300 µM DPPH in ethanol and mixed 750 µl of this freshly prepared solution with 250 µl of plant extract. I incubated this mixture in the dark for 30 minutes at room temperature. In the radical form, DPPH has an absorbance at 517 nm, which disappears with the acceptance of an electron from an antioxidant compound. I measured the decrease in absorbance at 517 nm against a blank without extract using a UV–VIS spectrophotometer (Ultrospec 4300 pro, Jasco Corporation, Tokyo, Japan). The scavenging capacity was expressed as the percentage of inhibition of DPPH consumption, which was calculated using the following formula: % inhibition=(*Acontrol*–*Asample*)×100/*Acontrol*, where *Acontrol* is the absorbance of the DPPH solution without extract, and *Asample* is the absorbance of the sample with DPPH. Measurements were performed in triplicate, and the average was plotted against a Trolox standard curve. I expressed the results as milligrams of Trolox per 100 grams of fresh leaves (TE mg/100g).

**Statistical analyses**

We compared the effects of ecoregion, mean annual temperature, mean summer temperature, coastal proximity, mean annual precipitation, mean annual runoff, pH of surface water, and alkalinity of surface water on the variation of TPC and AC of leaves. Statistical comparisons of TPC and AC among plants from the eight environmental factors described above were performed using a one-way analysis of variance (ANOVA) or a t-test, with a standard significance threshold of $p < 0.05$. To control the family wise error rate while conducting the ANOVAs, a Bonferroni correction was applied to correct the p-value given the number of tests presented, and only significant results are shown. The one-

way ANOVA compared the means between the groups I was interested in and determines whether any of those means are statistically significantly different from each other.

To determine which specific groups differed from each other, I used a *post hoc* test. Post-hoc pairwise comparisons used Fisher's Least Significant Difference (LSD) method. Non-parametric Kruskal-Wallis tests were conducted when samples violated the normal distribution and equality of variances assumptions.

I conducted a Pearson correlation between TPC and AC to test the hypothesis that phenolic content positively influenced the antioxidant capacity of leaves collected in the wild. The Pearson correlation test provided a measure of the strength and direction of the association that may exist between two variables measured on an interval scale. This test attempted to draw a line of best fit through the data of two variables, and the correlation coefficient, r, indicates how far away each data points was from the best fit line (i.e., how well the biochemical data fits this model). I also conducted Pearson correlations between TPC, AC, latitude and longitude to elucidate how did the biochemical properties vary geographically. I conducted these analyses in SPSS 23.0 for Mac (SPSS Inc., Chicago, IL, USA).

**Results**

**TPC and AC variation according to ecoregion**

TPC in leaves were not found to be significantly different among ecoregions (Table 2.1). The highest mean amount of TPC was found in leaves taken from the Maritime Barrens (mean=362.83 GE mg/100g FW), and the lowest from the Strait of Belle Isle Barrens (mean= 275.86 GE mg/100g). On the contrary, I found that ecoregion had an impact on

AC. The Maritime Barrens, North Shore Forest, and Central Newfoundland Forest had the highest ACs (302, 297, and 298 TE mg/100g FW, respectively), while the Western Newfoundland Forest and the Northern Peninsula Forest had the lowest ACs (243 and 244 TE mg/100g FW, respectively). Raw data are disclosed in the supplementary Table S2.2

**TPC and AC variation according to climatic factors and surface water quality**

Temperature had no effect on TPC nor AC (Table 2.2). TPC and AC were not significantly different among the three mean annual or the three mean summer temperature categories. Mean annual precipitation and mean annual runoff also showed no significant effects on TPC or AC (Tables 2.3, 2.4). Surface water pH was found to have an effect on TPC and AC (Table 2.4). The highest TPC values were achieved at pH>7.0 (mean=404 GE mg/100g) while AC increased with decreasing pH, with the highest values achieved at pH<6.5. As with the ecoregion variable, surface water sensitivity to acid rain was found to have an effect on AC but not on TPC. AC was highest in the extremely, highly, and moderate sensitivity categories, with <200 μeq/l, and lowest in the category of areas with low sensitivity to acid rain (>200 μeq/l; Tables 2.4 and Table S2.2), but not for TPC.

**TPC and AC variation according to coastal proximity and correlations among TPC, AC, latitude and longitude**

Coastal and inland populations did not show significantly different TPC and AC values (Table 2.5). I found a 15% correlation between the TPC and AC of leaves; however, this correlation was not significant at a significance level of $p<0.05$. Longitude was found to have a significant positive correlation with AC ($R^2=0.48$). All other correlations with geographical coordinates were not significant (Table 2.6).

## Discussion

The antioxidant capacity presented for partridgeberry leaves is a multi-factor concept, resulting from an intricate chain of biological processes under the influence of certain environmental conditions in the Island of Newfoundland and Labrador in the specific timeframe of this study. This work measured the variation of TPC and AC in leaves of *V. vitis-idaea* according to ecoregion, temperature, precipitation, runoff, soil water quality, and coastal proximity. A parallel study measured the variability of TPC and AC on fruits, those results are not disclosed in this thesis.

### TPC and AC variation according to ecoregion

Although not significant, the highest mean amount of TPC was found in leaves taken from the Maritime Barrens. Summers in this ecoregion are generally cooler compared to the other ecoregions in the Island of Newfoundland (Meades, 1990). The mean annual temperature for this ecoregion is approximately 5.5°C. The mean summer temperature is 11.5°C and the mean winter temperature is -1°C (Meades, 1990). The mean annual precipitation for this ecoregion ranges 1200 to over 1600 mm. The Maritime Barrens are classified as having an oceanic mid-boreal eco-climate (Meades, 1990). Other conditions that are contrasting against the rest of the ecoregions is the poor drainage soil, strong winds, lack of protective snow cover and exposure to light, that has allowed competitive dwarf shrub species, such as partridgeberry to dominate in these areas and thrive (Meades, 1990).

AC varied significantly according to the ecoregion where individuals grow, corroborating my first expectation. The Central Newfoundland Forest, The North Shore Forest, and the Maritime Barrens exhibited higher AC (Table 2.1). The area comprised by these three ecoregions is the driest on the island, despite its proximity to the ocean (Meades, 1990), following a general rule that dryness increases as moving towards the interior of the island northwards (Meades, 1990). Low precipitation in this area, is common as well as moisture deficiencies compared to the rest of the ecoregions. As Reddy et al. (2004) suggested, drought conditions will lead to generally higher AC, as moisture deficiencies lead to overall less rainfall, allowing the expression of different metabolites to protect the plant against drought conditions. The North Shore Forest and the Central Newfoundland Forest are some of the most geologically diverse regions in the province, with underlying rock formations belonging to four geologic zones. These ecoregions in Newfoundland are characterized by exposed bedrock with little soil development, and gravelly soil composed of unconsolidated rock fragments (Meades, 1990). This would support the conclusions of Debnath (2009, 2012) and Vyas et al. (2015) that generally a good composition of different rocky soils leads to higher AC in leaves of *V. vitis idaea*. There are numerous factors that separate the vegetation in the North Shore Forest and the Central Newfoundland Forest with its neighboring ecoregions. Forest fires occur regularly in these ecoregions and are often extensive, allowing small shrubs to colonize faster in these areas (Meades, 1990), this would imply that dryer conditions will be an indicator of high AC levels in leaves. My results were similar to other studies where the antioxidant activities increased when dry soil conditions were present (Fu and Huang, 2001).

**TPC and AC variation according to climatic factors and surface water quality**

Contrary to my expectation, while testing one by one climatic variables like precipitation and temperature, did not affect the TPC or AC of leaves. Temperature and precipitation affect the synthesis or inhibition of plant secondary products as shown in Kujala *et al*., (2000) and Vega-Galvez *et al*., (2009).

UV-B (Semerdjieva *et al.,* 2003a), shade (Hansen *et al.,* 2006), increased temperature and nutrients (Hansen *et al.,* 2006) have been experimentally shown to induce phenolics in *V. vitis-idaea*. Contrasting evidence also exists, with increased phenolics concentrations in colder soil and air temperatures, and lower nutrient availability (Roy and Mulder, 2014). In this study, induction by insects was proposed to better explain the elevated levels of phenolics than the physical environment. Several studies have shown that phenolics can be induced by herbivores (e.g., Boege 2004, Stevens and Lindroth, 2005; Kaplan *et al.,* 2008; Pascual-Alvarado *et al.,* 2008).

Water quantity can affect the production of antioxidants in plants. My results, however, did not show any significant variation with respect to mean annual precipitation. I consequently rejected the hypothesis of higher TPC and AC in regions of lower precipitation. My results were similar to those of Vyas *et al.,* (2015), with no correlation between phenolic content and rainfall. To my knowledge this is the first study to evaluate a correlation between runoff and phenolic variation. Although low precipitation and runoff do not necessarily indicate water limitation, water stress has been shown to affect the synthesis of secondary compounds. For example, the increase and accumulation of polyphenols in response to drought has been demonstrated in various crops, including

peach and grape (Rahmati *et al.,* 2015). The direction and strength of specific phenolic compound responses to water stress may also depend on the intraspecific genetic composition. For example, two different grape (*Vitis vinifera)* cultivars yielded different responses to water deficit under controlled greenhouse conditions. In one cultivar, water limitation reduced total anthocyanins and flavonols, and increased hydroxycinnamic acids, whereas in the other cultivar, water deficit resulted in increased flavonols and reduced catechins (Niculcea *et al.,* 2015).

In this analysis, I evaluated the effects of surface water quality using pH and sensitivity to acid rain. These two variables were the only ones with significant effects on TPC and AC in leaves of *V. vitis-idaea*. Leaves with higher AC were found at pH < 6.5, whereas the highest TPC content was found at pH > 7.0, although specific site testing need to be made to conclude that pH is biologically important in the development of AC or TPC. To my knowledge, no other study has tested the effect of water pH on the synthesis of secondary compounds, and the direction of the effect needs further support. Only soil pH has received some attention, but not water pH. In Mulberry (*Morus alba*) an increase of salt concentration in soil and pH augmented antioxidant enzyme activities, namely superoxide dismutase, catalase, peroxidase, and glutathione reductase (Ahmad *et al.,* 2014).

**TPC and AC variation according to coastal proximity and correlations among TPC, AC, latitude and longitude**

TPC and AC levels have been tested in coastal proximal species such as oak and mangrove (*Phytophtora ramorum* and *Avicennia officinialis*) among others (Nagle *et al.,* 2011;

Thirunavukkarasu *et al.,* 2011). The concept of coastal proximity involves the development of salt tolerant plants, and as a general rule, salt tolerant plants have a higher AC and TPC, because as soil salinity levels increase, the stress on germinating seedlings also increases (Thirunavukkarasu *et al.,* 2011). Perennial plants, such as *V. vitis idaea* seem to handle salinity better than annual plants since they adapt to the improper balance of nutrients they require for healthy growth, exhibiting a defense mechanism with higher TPC and AC (Nagle *et al.,* 2011; Thirunavukkarasu *et al.,* 2011).

It has been reported that the AC of *Vaccinium* is primarily due to total phenolics (Sellappan *et al.,* 2002; Moyer *et al.,* 2002; Yuan *et al.,* 2011). A linear relationship existed between ORAC (Oxygen Radical Absorbance Capacity) and anthocyanin (r(xy) = 0.77) or total phenolic (r(xy) = 0.92) content in several *Vaccinium* species (Prior *et al.,* 1998). I thus expected to find that differences in TPC reflect changes in AC, in a similar direction and magnitude. The non-significant correlation between TPC and AC, however, was only 15%, and I provide potential reasons for this. Plant phenolic compounds do not all have the same antioxidant capacity *in vitro* (Rice-Evans *et al.,* 1996; Cao *et al.,* 1997; Wang *et al.,* 1998). Variations in the specific profiles of phenolic compounds that are synthesized from population to population can have different overall AC values. The little overall variation in TPC and AC measurements might also mask a higher correlation between TPC and AC in leaves. An analysis of five *Vaccinium* species was concordant with my results since it proved that antioxidant activity decreased during fruit ripening, while total phenolic content tended to increase with maturity (Yuan *et al.,* 2011). Overall, variation in the activity of plant phenolics arises from three major sources: (1) genetic differences in

tolerance among plants, (2) variation in phenolic structure and concentration, and (3) variation in conditions that influence the mode of phenolic action, especially oxidative activation (Debnath and Sion 2009; Vyas *et al.,* 2015). Therefore, there might not be a simple correlation between the number of phenolic compounds and the antioxidant activity of plant extracts.

In 2012, Curran showed that the environment is a determining factor in the variation of phenolic content in partridgeberry, where latitude and longitude play an important role in the differences in antioxidant activity (Curran, 2012). Jaakola and Hohtola (2010) reviewed the changes in flavonoid content with respect to increasing latitude and found a positive relationship. In a study that evaluated the inhibition of advanced glycation end products by leaf extracts from six tropical *Vaccinium* species, a significant positive relationship was found between the degree of inhibition and latitude, which was attributed to the underlying climatic gradient affecting plant physiology (Ferrier *et al.,* 2012). Because the latitudinal gradient analyzed in my study was not large (46.7-51.6°N), I did not find any correlations between the two biochemical variables and latitude. However, AC was correlated with longitude, which might reflect the different ecoregion, climatic, and water quality characteristics along the longitudinal gradient I studied (52.8-59.4°W).

**Impact of this study and limitations**

There are numerous products from *Vaccinium* leaf extracts sold in the world market as dietary supplements. The demand for these extracts for the production of food supplements, nutraceuticals, and cosmetic products is growing. Crude extracts of

*Vaccinium* fruit and leaves rich in phenolics are of increasing interest to the food industry because they hinder oxidative degradation of lipids and, thereby, improve food quality and nutritional value. This study has filled a knowledge gap on the variation of antioxidant capacity throughout an environmental gradient in Newfoundland with the goal of contributing to a crop improvement program in this Canadian province.

I acknowledge the limitation of evaluating biochemical properties of crops from a single year and using a single plant without replication and thus could not be able to see the difference among the genotypes statistically, so these conclusions should be corroborated by long-term studies. In addition, the evaluation of these and other environmental factors such as the ones in the specific soils in Newfoundland, as well as specific phenolic compound profiles (e.g anthocyanins, tannins) across the entire distribution range of *V. vitis-idaea* awaits further investigation. I opted for an approach of measuring TPC and AC from wild plants instead of plants growing in a closed greenhouse because *V. vitis-idaea* does not currently grow in cultivation and berries currently exported from Newfoundland are from wild populations.

**Table 2.1** Total phenolic content (TPC in mg GAE/100g FW) and antioxidant capacity (AC in mg TE/100g FW) of leaves in *V. vitis-idaea* sites grouped by ecoregion. N=sample site size.
Different letters within the same column (in bold) indicate significant differences at $p<0.05$ (one-way ANOVA and LSD test)

| ECOREGION | **N** | **TPC** | **AC** |
|---|---|---|---|
| ***WESTERN NFLD FOREST*** | 7 | 340.75 ± 21.63 a | 243.37 ± 7.71 **a** |
| ***CENTRAL NFLD FOREST*** | 9 | 362.36 ± 15.37 a | 297.84 ± 18.30 **b** |
| ***NORTH SHORE FOREST*** | 6 | 321.77 ± 18.79 a | 297.47 ± 17.79 **b** |
| ***NORTHERN PENINSULA FOREST*** | 6 | 320.97 ± 34.72 a | 244.21 ± 14.03 **a** |
| ***MARITIME BARRENS*** | 7 | 362.83 ± 35.89 a | 302.20 ± 10.14 **b** |
| ***EASTERN HYPER OCEANIC BARRENS*** | 6 | 313.12 ± 37.82 a | 280.54 ± 7.79 **ab** |
| ***LONG RANGE BARRENS*** | 5 | 306.58 ± 24.23 a | 262.40 ± 8.95 **ab** |
| ***STRAIT OF BELLE ISLE BARRENS*** | 5 | 275.86 ± 33.96 a | 271.97 ± 17.08 **ab** |
| ***FORTEAU BARRENS*** | 5 | 288.25 ± 24.32 a | 280.23 ± 8.76 **ab** |
| ***F-STATISTIC (P-VALUE)*** | | 0.7 (0.69) | 2.73 (0.01) |

**Table 2.2** Total phenolic content (TPC in mg GAE/100g FW) and antioxidant capacity (AC in mg TE/100g FW) of leaves in *V. vitis-idaea* sites grouped by mean summer temperature and mean annual temperature. N=sample site size. Same letter "a" within the same column indicates lack of significant difference at $p<0.05$ (one way ANOVA and LSD test)

| | N | TPC | AC |
|---|---|---|---|
| **MEAN SUMMER TEMPERATURE (°C)** | | | |
| 6-11.9 | 10 | 302.96±21.31 a | 279.19±6.77 a |
| 12-13.9 | 23 | 322.82±15.50 a | 272.81±7.04 a |
| 14-19.9 | 23 | 340.02±14.07 a | 279.97±10.31a |
| F-STATISTIC (P-VALUE) | | 1.01 (0.37) | 0.21 (0.81) |
| **MEAN ANNUAL TEMPERATURE (°C)** | | | |
| 1.0-2.9 | 11 | 294.78±18.79 a | 276.14±8.29 a |
| 3.0-3.9 | 12 | 309.50±14.94 a | 260.41±9.53 a |
| 4.0-7.9 | 33 | 342.98±13.09 a | 283.13±5.21 a |
| F-STATISTIC (P-VALUE) | | 2.50 (0.09) | 1.52 (0.23) |

**Table 2.3** Total phenolic content (TPC in mg GAE/100g FW) and antioxidant capacity (AC in mg TE/100g FW) of leaves in *V. vitis-idaea* sites grouped by mean annual precipitation. N=sample site size.
Same letter "a" within the same column indicates lack of significant difference at $p<0.05$ (one-way ANOVA and LSD test)

| | N | TPC | AC |
|---|---|---|---|
| **MEAN ANNUAL PRECIPITATION (MM)** | | | |
| 700-1100 | 26 | 308.58±12.64 a | 277.84±7.95 a |
| 1100-1300 | 17 | 358.51±59.83 a | 269.38±10.35 a |
| 1300-1500 | 13 | 319.79±23.38 a | 284.81±8.64 a |
| F-STATISTIC (P-VALUE) | | 2.83 (0.07) | 0.58 (0.56) |

**Table 2.4** Total phenolic content (TPC in mg GAE/100g FW) and antioxidant capacity (AC in mg TE/100g FW) of leaves in *V. vitis-idaea* sites grouped by mean annual runoff, surface water pH, and surface water sensitivity to acid rain. N=sample site size.
Different letters within the same column (in bold) indicate significant differences at $p<0.05$ (one-way ANOVA or Kruskal-Wallis, LSD test and corrected Bonferroni P value).

| | N | TPC | AC |
|---|---|---|---|
| **MEAN ANNUAL RUNOFF (MM)** | | | |
| 500-800 | 17 | 313.55±15.76 a | 286.23±10.35 a |
| *800-1000* | 11 | 327.07±18.60 a | 289.64±11.81 a |
| *1000-1300* | 8 | 325.25±31.23 a | 251.17±14.88 a |
| *1300-1800* | 12 | 340.24±21.12 a | 276.61±9.44 a |
| *1800-2200* | 8 | 332.77±29.38 a | 265.68±9.90 a |
| *F-STATISTIC (P-VALUE)* | | 0.26 (0.90) | 1.646 (0.18) |
| ***SURFACE WATER PH*** | | | |
| *<6.1* | 15 | 330.81±23.02 **ab** | 288.02±8.28 **a** |
| *6.2-6.5* | 10 | 336.14±17.62 **ab** | 308.06±10.90 **a** |
| *6.6-7.0* | 25 | 301.16±10.64 **a** | 263.49±6.72 **b** |
| *>7.0* | 6 | 403.78±20.49 **b** | 252.98±20.03 **b** |
| *F STATISTIC (BONFERRONI CORRECTED P-VALUE)* | | 9.67 (0.023) | 5.26 (0.009) |
| ***SURFACE WATER SENSITIVITY TO ACID RAIN (MEQ/L)*** | | | |
| *EXTREMELY SENSITIVE (<60)* | 13 | 319.49±24.53 a | 279.99±6.88 **a** |
| *HIGHLY SENSITIVE (60-100)* | 10 | 333.40±20.01 a | 300.43±12.38 **a** |
| *MODERATE (100-200)* | 15 | 325.07±15.30 a | 289.04±9.38 **a** |
| *LOW (>200)* | 18 | 328.43±17.40 a | 251.47±8.95 **b** |
| *F-STATISTIC (BONFERRONI CORRECTED P-VALUE)* | | 0.08 (0.972) | 5.27 (0.020) |

**Table 2.5** Total phenolic content (TPC in mg GAE/100g FW) and antioxidant capacity (AC in mg TE/100g FW) of leaves in *V. vitis-idaea* sites located in coastal areas versus inland. N=sample site size.
Same letter "a" within the same column indicates lack of significant difference at $p<0.05$

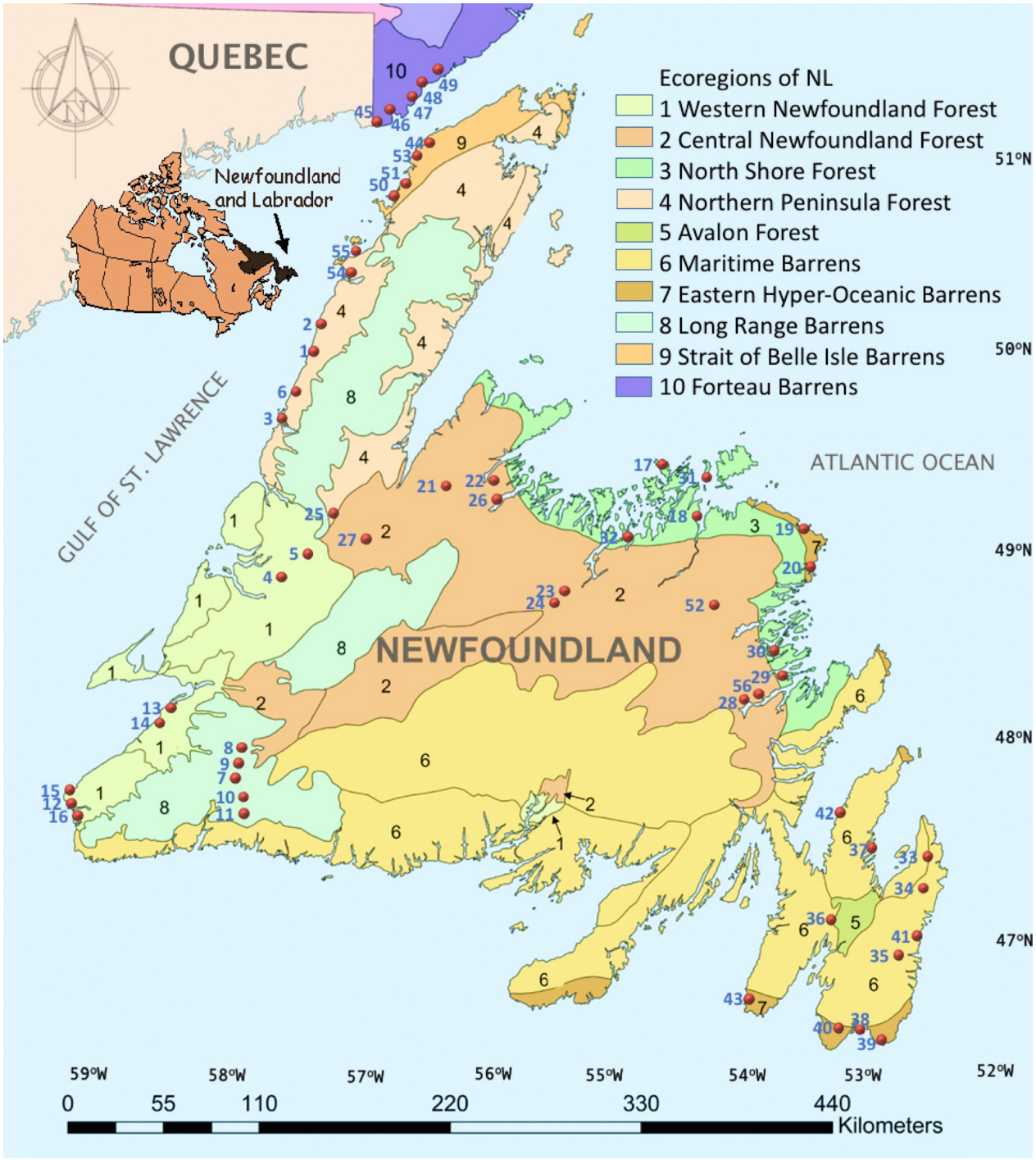
| | N | TPC | AC |
|---|---|---|---|
| **COASTAL PROXIMITY** | | | |
| *COASTAL* | 28 | 333.30±12.25 a | 272.96±7.43 a |
| *INLAND* | 28 | 319.38±14.39 a | 280.14±7.37 a |
| *T-TEST (P-VALUE)* | | 0.74 (0.47) | -0.75 (0.46) |

**Table 2.6** Pearson correlation coefficient matrix for total phenolic content (TPC) and antioxidant capacity (AC) for leaves in *V. vitis-idaea* from Newfoundland and Labrador and geographical coordinates. Significant correlations at $p<0.05$ are shown in bold font.

| | TPC LEAVES | AC LEAVES |
|---|---|---|
| **TPC LEAVES** | - | |
| **AC LEAVES** | 0.154 | - |
| **LATITUDE** | -0.245 | -0.127 |
| **LONGITUDE** | 0.089 | **0.478** |

**Figures**

**Fig. 2.1 Geographical distribution of sampled *Vaccinium vitis-idaea* sites in Newfoundland and Labrador.** The map indicates the distribution of samples (red dots) by ecoregions as defined in Meades (1990). Samples are numbered 1-56, and the environmental categories associated with each appear in Table S2.1. The inset is a map of Canada showing the location of Newfoundland and Labrador. Ecoregions map taken from the Government of Newfoundland and Labrador (1992).



**Supplementary Tables**

**Table S2.1** Sampled *V. vitis-idaea* sites in Newfoundland and Labrador, Canada. Each site is coded by ecoregion, mean annual temperature, mean summer temperature, mean annual precipitation, mean annual runoff, surface water pH, surface water sensitivity to acid rain, and proximity to the coast (Government of Newfoundland and Labrador 1992).

| SITE | LATITUDE | LONGITUDE | ECORE-GION | MEAN ANNUAL TEMPERA-TURE | MEAN SUMMER TEMPERA-TURE | MEAN ANNUAL PRECIPITA-TION | MEAN ANNUAL RUN OFF | SUR-FACE WA-TER PH | SURFACE WATER SENSITI-VITY | COAS-TAL PROXI-MITY |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 50.258204 | -57.570914 | 4 | 2 | 2 | 1 | 3 | 3 | 4 | 1 |
| 2 | 50.29574 | -57.554394 | 4 | 2 | 2 | 1 | 3 | 3 | 4 | 1 |
| 3 | 49.921719 | -57.807381 | 4 | 2 | 2 | 2 | 3 | 3 | 3 | 1 |
| 4 | 49.074177 | -57.570234 | 1 | 3 | 3 | 2 | 3 | 3 | 4 | 2 |
| 5 | 49.230142 | -57.297187 | 1 | 3 | 3 | 1 | 3 | 3 | 4 | 2 |
| 6 | 50.045723 | -57.704007 | 4 | 2 | 2 | 1 | 3 | 3 | 3 | 1 |
| 7 | 47.977643 | -57.628318 | 8 | 2 | 2 | 3 | 4 | 2 | 2 | 2 |
| 8 | 48.182911 | -57.739559 | 8 | 2 | 2 | 3 | 4 | 3 | 3 | 2 |
| 9 | 48.022422 | -57.673641 | 8 | 2 | 2 | 3 | 4 | 2 | 3 | 2 |
| 10 | 47.918525 | -57.646175 | 8 | 2 | 2 | 3 | 5 | 1 | 1 | 2 |
| 11 | 47.829422 | -57.675010 | 8 | 2 | 2 | 3 | 5 | 1 | 1 | 2 |
| 12 | 47.882011 | -59.393378 | 1 | 3 | 2 | 2 | 5 | 4 | 4 | 1 |
| 13 | 48.439436 | -58.453665 | 1 | 3 | 3 | 2 | 4 | 4 | 4 | 1 |
| 14 | 48.379947 | -58.588072 | 1 | 3 | 3 | 2 | 4 | 3 | 4 | 1 |
| 15 | 47.887137 | -59.397337 | 1 | 3 | 2 | 2 | 5 | 4 | 4 | 1 |
| 16 | 47.852131 | -59.343778 | 1 | 3 | 2 | 2 | 5 | 4 | 4 | 1 |
| 17 | 49.652608 | -54.762294 | 3 | 3 | 3 | 1 | 1 | 3 | 2 | 1 |
| 18 | 49.364175 | -54.499142 | 3 | 3 | 3 | 1 | 1 | 3 | 2 | 1 |
| 19 | 49.305873 | -53.646194 | 7 | 3 | 3 | 1 | 1 | 1 | 1 | 1 |
| 20 | 49.159781 | -53.573217 | 7 | 3 | 3 | 1 | 1 | 1 | 1 | 1 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 21 | 49.55919 | -56.462041 | 2 | 2 | 3 | 1 | 1 | 3 | 3 | 2 |
| 22 | 49.573004 | -55.993633 | 2 | 2 | 3 | 1 | 1 | 3 | 4 | 2 |
| 23 | 49.019023 | -55.490362 | 2 | 3 | 3 | 1 | 1 | 2 | 3 | 2 |
| 24 | 48.975324 | -55.568296 | 2 | 3 | 3 | 1 | 1 | 2 | 2 | 2 |
| 25 | 49.474919 | -57.142606 | 4 | 3 | 3 | 2 | 3 | 4 | 4 | 2 |
| 26 | 49.522484 | -56.003895 | 2 | 2 | 3 | 1 | 1 | 3 | 4 | 1 |
| 27 | 49.22833 | -57.076607 | 2 | 3 | 3 | 1 | 2 | 3 | 3 | 2 |
| 28 | 48.41931 | -54.134101 | 2 | 3 | 3 | 2 | 2 | 1 | 3 | 1 |
| 29 | 48.658204 | -53.846070 | 3 | 3 | 3 | 2 | 2 | 2 | 2 | 1 |
| 30 | 48.80791 | -54.182040 | 3 | 3 | 3 | 2 | 2 | 1 | 1 | 1 |
| 31 | 49.592478 | -54.412050 | 3 | 3 | 3 | 1 | 1 | 2 | 2 | 1 |
| 32 | 49.259259 | -55.033839 | 3 | 3 | 3 | 2 | 1 | 2 | 3 | 1 |
| 33 | 47.583102 | -52.758423 | 6 | 3 | 2 | 3 | 3 | 4 | 3 | 2 |
| 34 | 47.427251 | -52.766663 | 6 | 3 | 2 | 3 | 4 | 1 | 1 | 2 |
| 35 | 47.095463 | -52.958923 | 6 | 3 | 2 | 3 | 4 | 1 | 2 | 1 |
| 36 | 47.194471 | -53.381897 | 6 | 3 | 2 | 3 | 4 | 2 | 2 | 2 |
| 37 | 47.668254 | -53.272034 | 6 | 3 | 2 | 2 | 4 | 2 | 2 | 1 |
| 38 | 46.739389 | -53.341040 | 7 | 3 | 1 | 3 | 5 | 1 | 1 | 2 |
| 39 | 46.650565 | -53.185740 | 7 | 3 | 1 | 3 | 5 | 1 | 1 | 2 |
| 40 | 46.709071 | -53.486851 | 7 | 3 | 1 | 3 | 5 | 1 | 1 | 2 |
| 41 | 47.167128 | -52.908556 | 6 | 3 | 2 | 3 | 4 | 1 | 1 | 2 |
| 42 | 47.813517 | -53.457461 | 6 | 3 | 3 | 2 | 4 | 1 | 1 | 1 |
| 43 | 46.922322 | -54.171964 | 7 | 3 | 2 | 2 | 4 | 1 | 1 | 1 |
| 44 | 51.311631 | -56.726437 | 9 | 1 | 1 | 1 | 2 | 3 | 4 | 1 |
| 45 | 51.420523 | -57.106342 | 10 | 1 | 1 | 1 | 1 | 3 | 3 | 1 |
| 46 | 51.447211 | -57.015381 | 10 | 1 | 1 | 1 | 1 | 3 | 3 | 2 |
| 47 | 51.486962 | -56.890261 | 10 | 1 | 1 | 1 | 1 | 3 | 3 | 2 |
| 48 | 51.537334 | -56.812801 | 10 | 1 | 1 | 1 | 1 | 3 | 3 | 2 |
| 49 | 51.565912 | -56.744939 | 10 | 1 | 1 | 1 | 1 | 3 | 3 | 1 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 50 | 50.814569 | -57.060627 | 9 | 1 | 2 | 1 | 2 | 3 | 4 | 2 |
| 51 | 51.056136 | -56.920986 | 9 | 1 | 2 | 1 | 2 | 3 | 4 | 1 |
| 52 | 48.949858 | -54.599919 | 2 | 3 | 3 | 2 | 1 | 1 | 1 | 2 |
| 53 | 51.221539 | -56.754309 | 9 | 1 | 1 | 1 | 2 | 3 | 4 | 1 |
| 54 | 50.639775 | -57.206044 | 4 | 1 | 2 | 1 | 2 | 3 | 4 | 2 |
| 55 | 50.727439 | -57.185445 | 9 | 1 | 2 | 1 | 2 | 3 | 4 | 2 |
| 56 | 48.443822 | -54.013736 | 2 | 3 | 3 | 2 | 2 | 2 | 2 | 2 |

**Ecoregions:** 1=Western Nfld Forest, 2=Central Nfld Forest, 3=North Shore Forest, 4=Northern Peninsula Forest, 6=Maritime Barrens, 7=Eastern Hyper Oceanic Barrens, 8=Long Range Barrens, 9=Strait of Belle Isle Barrens, 10=Forteau Barrens. **Mean annual temperature**: 1=1.0-2.9°C, 2=3.0-3.9°C, 3=4.0-7.9°C. **Mean summer temperature**: 1=6-11.9°C, 2=12-13.9°C, 3=14-19.9°C. **Mean annual precipitation**: 1=700-1100mm, 2=1100-1300 mm, 3=1300-1500 mm. **Mean annual runoff**: 1=500-800 mm, 2=800-1000 mm, 3=1000-1300 mm, 4=1300-1800 mm, 5=1800-2200 mm. **Surface water pH**: 1=<6.1, 2=6.2-6.5, 3=6.6-7.0, 4=>7.0. **Surface water sensitivity to acid rain**: 1=extremely sensitive (<60 µeq/l), 2=highly sensitive (60-100 µeq/l), 3=moderate (100-200 µeq/l), 4=low (>200 µeq/l). **Coastal proximity**: 1=coastal, 2=inland.

**Table S2.2.** Sampling site, name, total phenolic content (TPC) and antioxidant capacity (AC) measured for each *V. vitis-idaea* composite sample (plant genotype) of leaves in Newfoundland and Labrador.

| SAMPLING SITE | NAME | TPC LEAVES (MG GAE/100 G FW) | AC LEAVES (MG TE/100G FW) |
|---|---|---|---|
| **1** | DHB_01 | 240.55 | 233.90 |
| **2** | DHB2_02 | 258.65 | 228.34 |
| **3** | CWH_03 | 313.92 | 280.73 |
| **4** | CRP_04 | 295.94 | 235.32 |
| **5** | DLK_05 | 245.41 | 253.16 |
| **6** | PRP_06 | 334.24 | 256.13 |
| **7** | TPD_07 | 284.71 | 277.24 |
| **8** | KGP_08 | 313.83 | 230.53 |
| **9** | KPG2_09 | 303.93 | 273.41 |
| **10** | KGP3_10 | 389.58 | 276.15 |
| **11** | R480_11 | 240.85 | 254.69 |
| **12** | COD_12 | 383.45 | 279.61 |
| **13** | STG_13 | 330.81 | 254.55 |
| **14** | STGP_14 | 334.58 | 218.99 |
| **15** | COD2_15 | 389.08 | 230.49 |
| **16** | COD3_16 | 406.02 | 231.51 |
| **17** | TWL_17 | 246.03 | 220.16 |
| **18** | GBY_18 | 318.21 | 295.68 |
| **19** | LMS_19 | 228.72 | 267.68 |
| **20** | NWV1_20 | 387.90 | 280.94 |
| **21** | BLK_21 | 287.52 | 279.05 |
| **22** | SPG360_22 | 396.89 | 329.72 |
| **23** | BF1_23 | 444.29 | 357.93 |
| **24** | BF2_24 | 317.42 | 353.33 |
| **25** | RC1_25 | 479.47 | 189.33 |
| **26** | SPG_26 | 349.42 | 205.09 |
| **27** | SLK_27 | 351.13 | 282.83 |
| **28** | TNN1_28 | 354.54 | 359.24 |
| **29** | SBC1_29 | 357.94 | 318.01 |
| **30** | GMB1_30 | 336.79 | 326.50 |
| **31** | DVT1_31 | 374.04 | 342.37 |
| **32** | LWP_32 | 297.65 | 282.13 |
| **33** | AVA1_33 | 433.83 | 332.42 |
| **34** | AVA2_34 | 218.81 | 316.94 |
| **35** | AVA3_35 | 454.21 | 321.26 |
| **36** | AVA4_36 | 265.68 | 309.08 |
| **37** | AVA5_37 | 319.78 | 304.15 |
| **38** | AVA6_38 | 223.30 | 291.17 |

| | | | |
|---|---|---|---|
| **39** | AVA7_39 | 234.51 | 308.70 |
| **40** | AVA8_40 | 395.34 | 253.14 |
| **41** | AVA9_41 | 398.74 | 257.89 |
| **42** | AVA10_42 | 448.81 | 273.66 |
| **43** | AVA11_43 | 408.98 | 281.61 |
| **44** | FCV_44 | 371.17 | 287.30 |
| **45** | LAB1_45 | 239.96 | 279.99 |
| **46** | LAB2_46 | 319.90 | 282.15 |
| **47** | LAB3_47 | 260.55 | 247.73 |
| **48** | LAB4_48 | 368.53 | 296.56 |
| **49** | LAB5_49 | 252.31 | 294.72 |
| **50** | BHB_50 | 341.11 | 331.94 |
| **51** | BDC_51 | 230.59 | 237.94 |
| **52** | GND_52 | 241.02 | 250.74 |
| **53** | STBS_53 | 364.06 | 250.44 |
| **54** | PSD_54 | 299.03 | 276.86 |
| **55** | PSD2_55 | 195.44 | 251.96 |
| **56** | CHT_56 | 396.01 | 262.97 |

**References**


- Aiken, S., Dallwitz, M., Consaul, L., McJannet, C., Boles, R., Argus, G., & Harris, J. (2011). *Flora of the Canadian Arctic Archipelago: Descriptions, Illustrations, Identification, and Information Retrieval*. Retrieved January 16, 2016, from National Research Council of Canada, Ottawa: http://nature.ca/aaflora/data

- Åkerström, A., Jaakola, L., Bång, U., & Jäderlund, A. (2010). Effects of latitude-related factors and geographical origin on anthocyanidin concentrations in fruits of *Vaccinium myrtillus* L. (bilberries). *Journal of Agricultural and Food Chemistry*, *58*(22), 11939-11945.

- Ahmad, P., Ozturk, M., Sharma, S., & Gucel, S. (2014). Effect of sodium carbonate-induced salinity–alkalinity on some key osmoprotectants, protein profile, antioxidant enzymes, and lipid peroxidation in two mulberry (*Morus alba* L.) cultivars. *Journal of Plant Interactions*, *9*(1), 460-467.

- Antolin, M. C., Santesteban, H., Santa María, E., Aguirreolea, J., & Sanchez-Diaz, M. (2008). Involvement of abscisic acid and polyamines in berry ripening of Vitis vinifera (L.) subjected to water deficit irrigation. *Australian Journal of Grape and Wine Research*, *14*(2), 123-133.

- Arslan, D., & Özcan, M. M. (2011). Phenolic profile and antioxidant activity of olive fruits of the Turkish variety "Sarıulak" from different locations. *International Journal of Fats and Oils*, *62*(4), 453-461.

- Bakowska-Barczak, A. M., Marianchuk, M., & Kolodziejczyk, P. (2007). Survey of bioactive components in Western Canadian berries This article is one of a selection of papers published in this special issue (part 2 of 2) on the Safety and Efficacy of Natural Health Products. *Canadian Journal of Physiology and Pharmacology*, *85*(11), 1139-1152.

- Balsdon, J. L., Smith, T. W., & Lundholm, J. T. (2011). Phenotypic and genotypic differentiation of *Vaccinium vitis-idaea* between coastal barrens and forests in Nova Scotia, Canada. *Botany*, *89*(3), 147-155.

- Boege, K. (2004). Induced responses in three tropical dry forest plant species–direct and indirect effects on herbivory. *Oikos*, *107*(3), 541-548.

- Bomser, J., Madhavi, D. L., Singletary, K., & Smith, M. A. L. (1996). In vitro anticancer activity of fruit extracts from *Vaccinium* species. *Planta Medica*, *62*(03), 212-216.

- Camire, M. E. (2002). Phytochemicals in the Vaccinium family: bilberries, blueberries, and cranberries. *Phytochemicals in nutrition and health*, 19.

- Cao, G., Sofic, E., & Prior, R. L. (1997). Antioxidant and prooxidant behavior of flavonoids: structure-activity relationships. *Free Radical Biology and Medicine*, *22*(5), 749-760.

- Castrejon, A. D. R., Eichholz, I., Rohn, S., Kroh, L. W., & Huyskens-Keil, S. (2008). Phenolic profile and antioxidant activity of highbush blueberry (*Vaccinium corymbosum* L.) during fruit maturation and ripening. *Food Chemistry*, *109*(3), 564-572.

- Curran, N. (2012). *"Antioxidant Properties of Lingonberry (Vaccinium vitis-idaea L.) as Affected by Different Geographical Locations"*. Memorial University of Newfoundland, Biology. St. John's, NL: Memorial University of Newfoundland.

- Dannehl, D., Huber, C., Rocksch, T., Huyskens-Keil, S., & Schmidt, U. (2012). Interactions between changing climate conditions in a semi-closed greenhouse and plant development, fruit yield, and health-promoting plant compounds of tomatoes. *Scientia Horticulturae*, *138*, 235-243.

- Debnath, S. C. (2007). Inter simple sequence repeat (ISSR) to assess genetic diversity within a collection of wild lingonberry (*Vaccinium vitis-idaea* L.) clones. *Canadian Journal of Plant Science*, *87*(2), 337-344.

- Debnath, S. C., & Sion, M. (2009). Genetic diversity, antioxidant activities, and anthocyanin contents in lingonberry. *International Journal of Fruit Science*, *9*(2), 185-199.

- Debnath, S. C., & Bishop, G. A. (2010). Lingonberry Germplasm Characterization for Berry Characters, Anthocyanin Content and Antioxidant Activity. In *XXVIII International Horticultural Congress on Science and Horticulture for People*. (pp. 647-654).

- Debnath, S. C., Vyas, P., Goyali, J. C., & Igamberdiev, A. U. (2012). Morphological and molecular analyses in micropropagated berry plants acclimatized under *ex vitro* condition. *Canadian Journal of Plant Science*, *92*(6), 1065-1073.

- Deluc, L. G., Quilici, D. R., Decendit, A., Grimplet, J., Wheatley, M. D., Schlauch, K. A., & Cramer, G. R. (2009). Water deficit alters differentially metabolic pathways affecting important flavor and quality traits in grape berries of Cabernet Sauvignon and Chardonnay. *BMC Genomics*, *10*(1), 212.

- Faria, A., Oliveira, J., Neves, P., Gameiro, P., Santos-Buelga, C., de Freitas, V., & Mateus, N. (2005). Antioxidant properties of prepared blueberry (*Vaccinium myrtillus*) extracts. *Journal of Agricultural and Food Chemistry*, *53*(17), 6896-6902.

- Ehlenfeldt, M. K., & Prior, R. L. (2001). Oxygen radical absorbance capacity (ORAC) and phenolic and anthocyanin concentrations in fruit and leaf tissues of highbush blueberry. *Journal of Agricultural and Food Chemistry*, *49*(5), 2222-2227.

- Ferrier, J., Djeffal, S., Morgan, H. P., Vander Kloet, S. P., Redžić, S., Cuerrier, A., Ballick, M.J. & Arnason, J. T. (2012). Antiglycation activity of *Vaccinium* spp. (*Ericaceae*) from the Sam Vander Kloet collection for the treatment of type II diabetes 1 1 This article is part of a Special Issue entitled "A tribute to Sam Vander Kloet FLS: Pure and applied research from blueberries to heathland ecology". *Botany*, *90*(5), 401-406.

- Fu, J., & Huang, B. (2001). Involvement of antioxidants and lipid peroxidation in the adaptation of two cool-season grasses to localized drought stress. *Environmental and Experimental Botany*, *45*(2), 105-114.

- George, M. J., Lets'aba, R., Khotso, M. J., Bese, L. E., Taole, K., & Maroba, M. (2015). Determination of antioxidants and total phenolic content in some wild vegetables used widely in Lesotho. *British Journal of Applied Science and Technology, 11*(1), 1-8.

- Government of Newfoundland and Labrador. (1992). Water resources atlas of Newfoundland. Available from http://www.canal.gov.nl.ca/reports/Water_Resources_Atlas/Water_Resources_Atlas_of_Newfoundland_1992.pdf. Water Resources Division, St. John's.

- Haborne, J.B. 1997. Role of phenolic secondary metabolites in plants and their degradation in nature. *In* Driven by nature: plant litter quality and decomposition. *Edited by* G. Cadish and K.E. Giller. CAB International, Wallingford, Oxfordshire, UK. pp. 67–74.

- Hansen, A. H., Jonasson, S., Michelsen, A., & Julkunen-Tiitto, R. (2006). Long-term experimental warming, shading and nutrient addition affect the concentration of phenolic compounds in arctic-alpine deciduous and evergreen dwarf shrubs. *Oecologia*, *147*(1), 1-11.

- Haselgrove, L., Botting, D., Heeswijck, R. V., Høj, P. B., Dry, P. R., Ford, C., & Land, P. G. I. (2000). Canopy microclimate and berry composition: The effect of bunch exposure on the phenolic composition of *Vitis vinifera* L cv. Shiraz grape berries. *Australian Journal of Grape and Wine Research*, *6*(2), 141-149.

- Jaakola, L., & Hohtola, A. (2010). Effect of latitude on flavonoid biosynthesis in plants. *Plant, cell & environment*, *33*(8), 1239-1247.

- Jakopic, J., Stampar, F., & Veberic, R. (2009). The influence of exposure to light on the phenolic content of 'Fuji'apple. *Scientia Horticulturae*, *123*(2), 234-239.

- Jonasson, S., Bryant, J. P., Chapin III, F. S., & Andersson, M. (1986). Plant phenols and nutrients in relation to variations in climate and rodent grazing. *The American Naturalist*, *128*(3), 394-408.

- Kalt, W., Ryan, D. A., Duy, J. C., Prior, R. L., Ehlenfeldt, M. K., & Vander Kloet, S. P. (2001). Interspecific variation in anthocyanins, phenolics, and antioxidant capacity among genotypes of highbush and lowbush blueberries (*Vaccinium* section *cyanococcus* spp.). *Journal of Agricultural and Food Chemistry*, *49*(10), 4761-4767.

- Kalt, W., Lawand, C., Ryan, D. A., McDonald, J. E., Donner, H., & Forney, C. F. (2003). Oxygen radical absorbing capacity, anthocyanin and phenolic content of highbush blueberries (*Vaccinium corymbosum* L.) during ripening and storage. *Journal of the American Society for Horticultural Science*, *128*(6), 917-923.

- Kaplan, I., Halitschke, R., Kessler, A., Sardanelli, S., & Denno, R. F. (2008). Constitutive and induced defenses to herbivory in above and below ground plant tissues. *Ecology*, *89*(2), 392-406.

- Karlsson, P. S. (1985). Photosynthetic characteristics and leaf carbon economy of a deciduous and an evergreen dwarf shrub: *Vaccinium uliginosum* L. and *V. vitis-idaea* L. *Ecography*, *8*(1), 9-17.

- Kedare, S. B., & Singh, R. P. (2011). Genesis and development of DPPH method of antioxidant assay. *Journal of Food Science and Technology*, *48*(4), 412-422.

- Kraujalytė, V., Venskutonis, P. R., Pukalskas, A., Česonienė, L., & Daubaras, R. (2015). Antioxidant properties, phenolic composition and potentiometric sensor array evaluation of commercial and new blueberry (*Vaccinium corymbosum*) and bog blueberry (*Vaccinium uliginosum*) genotypes. *Food Chemistry*, *188*, 583-590.

- Kujala, T. S., Loponen, J. M., Klika, K. D., & Pihlaja, K. (2000). Phenolics and betacyanins in red beetroot (*Beta vulgaris*) root: Distribution and effect of cold storage on the content of total phenolics and three individual compounds. *Journal of Agricultural and Food Chemistry*, *48*(11), 5338-5342.

- Martineau, L. C., Couture, A., Spoor, D., Benhaddou-Andaloussi, A., Harris, C., Meddah, B. & Prentki, M. (2006). Anti-diabetic properties of the Canadian lowbush blueberry V*accinium angustifolium* Ait. *Phytomedicine*,*13*(9), 612-623.

- Martinussen, I., Rohloff, J., Uleberg, E., Junttila, O., Hohtola, A., Jaakola, L., & Häggman, H. (2009). Climatic effects on the production and quality of bilberries (*Vaccinium myrtillus*). *Latvian Journal of Agronomy/Agronomija Vestis*, (12), 71-74.

- Martz, F., Jaakola, L., Julkunen-Tiitto, R., & Stark, S. (2010). Phenolic composition and antioxidant capacity of bilberry (*Vaccinium myrtillus*) leaves in Northern Europe following foliar development and along environmental gradients. *Journal of Chemical Ecology*, *36*(9), 1017-1028.

- Mayer, A. M. (2004). Resistance to herbivores and fungal pathogens: variations on a common theme? A review comparing the effect of secondary metabolites, induced and constitutive, on herbivores and fungal pathogens. *Israel Journal of Plant Sciences*, *52*(4), 279-292.

- McDonald, S., Prenzler, P. D., Antolovich, M., & Robards, K. (2001). Phenolic content and antioxidant activity of olive extracts. *Food Chemistry*, *73*(1), 73-84.

- McKay, D. L., & Blumberg, J. B. (2007). Cranberries (*Vaccinium macrocarpon*) and cardiovascular disease risk factors. *Nutrition Reviews*, *65*(11), 490-502.

- Meades, L. (1990). Ecoregions of Newfoundland and Labrador. Retrieved January 20, 2017, from http://www.env.gov.nl.ca/env/publications/parks/index.html#brochures

- Moyer, R. A., Hummer, K. E., Finn, C. E., Frei, B., & Wrolstad, R. E. (2002). Anthocyanins, phenolics, and antioxidant capacity in diverse small fruits: *Vaccinium, Rubus,* and *Ribes*. *Journal of Agricultural and Food Chemistry*, *50*(3), 519-525.

- Naczk, M., Williams, J., Brennan, K., Liyanapathirana, C., & Shahidi, F. (2004). Compositional characteristics of green crab (*Carcinus maenas*). *Food Chemistry*, *88*(3), 429-434.

- Nagle, A. M., McPherson, B. A., Wood, D. L., Garbelotto, M., & Bonello, P. (2011). Relationship between field resistance to *Phytophthora ramorum* and constitutive phenolic chemistry of coast live oak. *Forest Pathology*, *41*(6), 464-469.

- Neto, C. C. (2007). Cranberry and blueberry: evidence for protective effects against cancer and vascular diseases. *Molecular Nutrition & Food Research*, *51*(6), 652-664.

- Nybakken, L., Selås, V., & Ohlson, M. (2013). Increased growth and phenolic compounds in bilberry (*Vaccinium myrtillus* L.) following forest clear-cutting. *Scandinavian Journal of Forest Research*, *28*(4), 319-330.

- Niculcea, M., Martinez-Lapuente, L., Guadalupe, Z., Sánchez-Díaz, M., Ayestarán, B., & Antolín, M. C. (2015). Characterization of phenolic composition of Vitis vinifera L.'Tempranillo'and 'Graciano'subjected to deficit irrigation during berry development. *VITIS-Journal of Grapevine Research*, *54*(1), 9-16.

- Nowack, R., & Schmitt, W. (2008). Cranberry juice for prophylaxis of urinary tract infections–Conclusions from clinical experience and research. *Phytomedicine*, *15*(9), 653-667.

- Pascual-Alvarado, E., Cuevas-Reyes, P., Quesada, M., & Oyama, K. (2008). Interactions between galling insects and leaf-feeding insects: the role of plant phenolic compounds and their possible interference with herbivores. *Journal of Tropical Ecology*, *24*(03), 329-336.

- Penhallegon, R. (2006). Lingonberry Production Guide for the Pacific Northwest. Retrieved March 14, 2017, from OSU Extension Service OSU Extension Catalog: https://catalog.extension.oregonstate.edu/sites/catalog/files/project/pdf/pnw583.pdf

- Penney, B. G., Gallagher, C. A., Hendrickson, P. A., Churchill, R. A., & Butt, E. (1996). The wild partridgeberry (*Vaccinium vitis-idaea* L. var. *minus* Lodd) industry in Newfoundland and Labrador and the potential for expansion utilizing European cultivars. In *VI International Symposium on Vaccinium Culture* (pp. 139-142).

- Perez-Lopez, F. R., Haya, J., & Chedraui, P. (2009). *Vaccinium macrocarpon*: an interesting option for women with recurrent urinary tract infections and other health benefits. *Journal of Obstetrics and Gynaecology Research*, *35*(4), 630-639.

- Prior, R. L., Cao, G., Martin, A., Sofic, E., McEwen, J., O'Brien, C., & Mainland, C. M. (1998). Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity, and variety of *Vaccinium* species. *Journal of Agricultural and Food Chemistry*, *46*(7), 2686-2693.

- Rahmati, M., Vercambre, G., Davarynejad, G., Bannayan, M., Azizi, M., & Génard, M. (2015). Water scarcity conditions affect peach fruit size and polyphenol contents more severely than other fruit quality traits. *Journal of the Science of Food and Agriculture*, *95*(5), 1055-1065.

- Reddy, A. R., Chaitanya, K. V., & Vivekanandan, M. (2004). Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. *Journal of Plant Physiology*, *161*(11), 1189-1202.

- Reyes, L. F., & Cisneros-Zevallos, L. (2003). Wounding stress increases the phenolic content and antioxidant capacity of purple-flesh potatoes (*Solanum tuberosum* L.). *Journal of Agricultural and Food Chemistry*, *51*(18), 5296-5300.

- Rice-Evans, C. A., Miller, N. J., & Paganga, G. (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free radical biology and medicine*, *20*(7), 933-956.

- Rimando, A. M., Kalt, W., Magee, J. B., Dewey, J., & Ballington, J. R. (2004). Resveratrol, pterostilbene, and piceatannol in *Vaccinium* berries. *Journal of Agricultural and Food Chemistry*, *52*(15), 4713-4719.

- Rosen, C. J., Allan, D. L., & Luby, J. J. (1990). Nitrogen form and solution pH influence growth and nutrition of two Vaccinium clones. *Journal of the American Society for Horticultural Science*, *115*(1), 83-89.

- Roy, B. A., & Mulder, C. P. (2014). Pathogens, herbivores, and phenotypic plasticity of boreal *Vaccinium vitis-idaea* experiencing climate change. *Ecosphere*, *5*(3), 1-19.

- Scalzo, J., Politi, A., Pellegrini, N., Mezzetti, B., & Battino, M. (2005). Plant genotype affects total antioxidant capacity and phenolic contents in fruit. *Nutrition*, *21*(2), 207-213.

- Sellappan, S., Akoh, C. C., & Krewer, G. (2002). Phenolic compounds and antioxidant capacity of Georgia-grown blueberries and blackberries. *Journal of Agricultural and Food Chemistry*, *50*(8), 2432-2438.

- Semerdjieva, S. I., Sheffield, E., Phoenix, G. K., Gwynn-Jones, D., Callaghan, T. V., & Johnson, G. N. (2003a). Contrasting strategies for UV-B screening in sub-Arctic dwarf shrubs. *Plant, Cell & Environment*, *26*(6), 957-964.

- Semerdjieva, S. I., Phoenix, G. K., Hares, D., Gwynn-Jones, D., Callaghan, T. V., & Sheffield, E. (2003b). Surface morphology, leaf and cuticle thickness of four dwarf shrubs from a sub-Arctic heath following long-term exposure to enhanced levels of UV-B. *Physiologia Plantarum*, *117*(2), 289-294.

- Skupien, K., Oszmiański, J., Kostrzewa-Nowak, D., & Tarasiuk, J. (2006). *In vitro* antileukaemic activity of extracts from berry plant leaves against sensitive and multidrug resistant HL60 cells. *Cancer Letters*, *236*(2), 282-291.

- Stevens, M. T., & Lindroth, R. L. (2005). Induced resistance in the indeterminate growth of aspen (Populus tremuloides). *Oecologia*, *145*(2), 297-305.

- Takeshita, M., Ishida, Y. I., Akamatsu, E., Ohmori, Y., Sudoh, M., Uto, H., & Kataoka, H. (2009). Proanthocyanidin from blueberry leaves suppresses expression of subgenomic hepatitis C virus RNA. *Journal of Biological Chemistry*, *284*(32), 21165-21176.

- Taruscio, T. G., Barney, D. L., & Exon, J. (2004). Content and profile of flavonoid and phenolic acid compounds in conjunction with the antioxidant capacity for a variety of northwest *Vaccinium* berries. *Journal of Agricultural and Food Chemistry*, *52*(10), 3169-3176.

- Thirunavukkarasu, P., Ramanathan, T., Ramkumar, L., Shanmugapriya, R., & Renugadevi, G. (2011). The antioxidant and free radical scavenging effect of *Avicennia officinalis*. *Journal of Medicinal Plants Research*, *5*(19), 4754-4758.

- Vega-Galvez, A. A., Lemus-Mondaca, R. R., Tello-Ireland, C. C., Miranda, M. M., & Yagnam, F. F. (2009). Kinetic study of convective drying of blueberry variety O'Neil (*Vaccinium corymbosum* L.) *Chilean Journal of Agricultural Research*, 69(2), 171-178.

- Vyas, P., Curran, N. H., Igamberdiev, A. U., & Debnath, S. C. (2015). Antioxidant properties of lingonberry (*Vaccinium vitis-idaea* L.) leaves within a set of wild clones and cultivars. *Canadian Journal of Plant Science*, *95*(4), 663-669.

- Wang, M., Li, J., Rangarajan, M., Shao, Y., LaVoie, E. J., Huang, T. C., & Ho, C. T. (1998). Antioxidative phenolic compounds from sage (Salvia officinalis). *Journal of Agricultural and Food Chemistry*, *46*(12), 4869-4873.

- Wang, L., Zhang, X. T., Zhang, H. Y., Yao, H. Y., & Zhang, H. (2010). Effect of *Vaccinium bracteatum Thunb*. leaves extract on blood glucose and plasma lipid levels in streptozotocin-induced diabetic mice. *Journal of Ethnopharmacology*, *130*(3), 465-469.

- Yang, B., Zheng, J., Laaksonen, O., Tahvonen, R., & Kallio, H. (2013). Effects of latitude and weather conditions on phenolic compounds in currant (Ribes spp.) cultivars. *Journal of Agricultural and Food Chemistry*, *61*(14), 3517-3532.

- Yuan W, Zhou L, Deng G, Wang P, Creech D, Li S. (2011). Anthocyanins, phenolics, and antioxidant capacity of *Vaccinium* L. in Texas, USA. *Pharmaceutical Crops,* 2(1) 11-23.

- Zadernowski, R., Naczk, M., & Nesterowicz, J. (2005). Phenolic acid profiles in some small berries. *Journal of Agricultural and Food Chemistry*, *53*(6), 2118-2124.

- Zheng, W., & Wang, S. Y. (2003). Oxygen radical absorbing capacity of phenolics in blueberries, cranberries, chokeberries, and lingonberries. *Journal of Agricultural and Food Chemistry*, *51*(2), 502-509.

- Zoratti, L., Karppinen, K., Luengo Escobar, A., Häggman, H., & Jaakola, L. (2014). Light-controlled flavonoid biosynthesis in fruits. *Frontiers in Plant Science*, *5*, 534.

# CHAPTER 3: LINKING GENETIC STRUCTURE, BIOCHEMICAL PROPERTIES, AND ENVIRONMENTAL CONDITIONS OF *VACCINIUM VITIS-IDAEA* IN NEWFOUNDLAND AND SOUTHERN LABRADOR


## Abstract

Partridgeberry (*Vaccinium vitis-idaea* L.) is native to Eurasia, Iceland, Greenland and North America. Partridgeberries are well known for their nutritional and health benefits, making them an increasingly important fruit for cultivation. Knowledge of the genetic diversity and chemical composition of partridgeberry is necessary for the selection of superior genotypes for cultivation. The genetic diversity among partridgeberry populations and its relationship with environmental conditions and biochemical compound variation is of great importance, but this knowledge is incomplete. Therefore, the genetic diversity of 56 partridgeberry individuals distributed across nine ecoregions of Newfoundland and Southern Labrador were investigated in the present study. This study also addresses whether environmental factors or genetic proximity plays a role on the biochemical variation of partridgeberry leaves. Individuals from three genetic clusters that show geographic structure according to ecoregion and temperature. The first group includes individuals from the Maritime Barrens, Eastern Hyper-Oceanic Barrens, Strait of Belle Isle Barrens, and the Forteau Barrens; the second genetic group includes individuals from the Western and Central Newfoundland Forest, and the North Shore Forest; and the third genetic group includes individuals from the Northern Peninsula Forest and the Long Range Barrens. Individuals collected in areas with the coldest annual and summer temperatures are clustered within the first genetic group. In addition, geographically close individuals were also genetically close and contained similar quantities of total phenolics. However, I did not find any correlation between genetic distance and total phenolics or antioxidant capacity.



***Keywords: genotyping-by-sequencing, Vaccinium vitis-idaea, phylogenetic tree, ecoregions, environmental factors, genetic distance, geographic distance, total phenolic content, antioxidant capacity***

## Introduction

Among the research goals of horticultural scientists are to connect phenotype to genotype because desired traits such as a high phenolic content and antioxidant capacity could be associated with particular genetic factors or a genotype, and the conditions where plants grow (Poland and Rife, 2012). In plant breeding, the genotype can then be used to predict phenotypes and select improved cultivars. But further understanding of the connection between heritable genetic factors and the resulting phenotypes can only enable marker-assisted breeding to help on the scale needed to increase healthier food production (Poland and Rife, 2012).

Species in the genus *Vaccinium* are recognized as stress-adapted, long-lived perennials, and have high antioxidant capacity and phenolic content (Debnath, 2009). In the Canadian province of Newfoundland and Labrador, *V. vitis-idaea* exhibits a high phenolic profile and high antioxidant capacity (Prior, 1998, 2001; Debnath, 2009, 2012a; Vyas *et al*., 2015; Alam *et al*., 2016), but no study has connected those desired agronomic characteristics with population genetic structure, the environmental conditions where they grow, and interactions between genetic structure and type of soil, and genetic structure and latitude and longitude. While genetic structure (which might result from breeding programs), and environmental regimes can lead to the development of different phytochemical constituents, the bioactive value of partridgeberries leaves could be improved by careful selection of genotypes with high phenolic content. Studies that take into account the genetic variation and structure of populations, and environmental variation can inform future selection of clones with desired bioactive traits.

Overall, few studies have characterized the genetic diversity of wild partridgeberry populations in North America (Debnath, 2007a; Balsdon *et al.*, 2011). In addition, few studies have evaluated the effects of environmental variables on the total phenolic and antioxidant capacity of *Vaccinium vitis-idaea* (Hansen *et al*., 2006; Semerdjieva *et al*., 2003; Roy and Mulder, 2014; Alam *et al*. 2016). In this study, I evaluated the genetic relatedness among partridgeberries individuals sampled in Newfoundland and Southern Labrador, and their links to environmental conditions that promote differential synthesis of phenolic compounds and antioxidant capacity.

**Research questions**

I want to distinguish, *what are the genetic relationships among partridgeberry individuals collected in Newfoundland and Southern Labrador*. As well, *what environmental factors, such as ecoregion, temperature, precipitation, and water quality are associated with genetic relatedness among individuals*. From a geographically perspective, I *want to recognize if geographically close individuals also genetically close or geographically and genetically close individuals have similar phenolic contents and antioxidant capacities*. Finally, I want to identify which factor (i.e. genetics or environment) seems to influence the most the quantity of phenolic compounds and antioxidant capacity in partridgeberry.

This study augments the existing genetic knowledge of partridgeberries in North America. Furthermore, it provides useful information for future berry breeding programs in this continent.

## Methods

### Sampling and DNA extraction

The island of Newfoundland and Southern Labrador was visited from mid-August to mid-September in 2014, and leaves from 56 wild adult individuals of *V. vitis-idaea* were collected from 56 sites using the sampling design described in Fig. 2.1. Leaves were dried in silica gel for DNA extraction. Up to 30 µg/µl of total genomic DNA from partridgeberry leaves were extracted using the DNeasy Plant Mini Kit from QIAGEN (Venlo, Netherlands). Typical yields were 30 µg/µl of high-quality DNA. A final standardization of the DNA concentration at 10 µg/µl was achieved using a Qubit Fluorometer (ThermoFischer Scientific) for subsequent library preparation and DNA sequencing.

### Library construction and genotyping-by-sequencing (GBS)

The 56 DNA samples of leaf material were sent to Laval University in Quebec for library construction where highly-multiplexed GBS libraries were constructed using a protocol modified from Elshire *et al.,* (2011). The GBS method provides an inexpensive approach to reduce genome complexity using two restriction enzymes that cut in the low-copy fraction of the genome. In this technique, there is no random trimming or size selection as in other next generation genotyping techniques (e.g RADseq). This technique uses a simple barcoding classification that adds a series of short stretch DNA sequences to the sequencing adaptors that are ligated to the cut DNA fragments. DNA samples, barcodes, and collective adapter pairs were laid on a plate and dried. Samples were then digested with the two restriction enzymes, *Pst*I and *Msp*I, and adapters were ligated to the ends of the whole genome DNA fragments. Heating deactivated a T4 ligase, and an aliquot of each sample was pooled and size selected to remove additional sequencing adapters. Suitable primers

with the necessary sites on the ligated adapters were added to the samples, and a series of polymerase chain reactions (PCR) were performed to increase the fragment pool. PCR products were cleaned, and the fragment sizes of the resulting library were assessed on a DNA bioanalyzer. Single-end sequencing of 100 base pairs (bp) reads were obtained in a single lane of Illumina Hiseq2500 DNA sequencing, producing a FASTQ file containing the raw information of the 56 samples. DNA sequencing was conducted at McGill University-Génome Québec Innovation Center in Montreal, Canada.

**Filtering Raw Sequence Data**

To process the raw reads, I used The FASTX-Toolkit with default parameters (Gordon and Hannon, 2010) to trim adaptors and quality-filter all reads (90-95%). Sequences were subjected to the FASTQ-to-FASTA converter. The FASTA Collapser was used to collapse identical sequences in the FASTA file into a single sequence. Subsequently, the FASTA Trimmer was used to shorten reads to remove the barcodes of the Illumina Sequencing platform. The FASTA Renamer retitled the sequence identifiers in a FASTA file, and the FASTA Clipper was used to remove sequencing adapters. The FASTA Barcode splitter was used to split the FASTA files containing multiple samples, and the FASTA Quality Filter was applied to filter sequences based on a 95% Phred score quality. Finally, the FASTA Quality Trimmer was used to trim reads based on Phred score, as a measure for the quality of identification for nucleotides. The results after performing all these steps were 56 FASTA files containing quality DNA sequences that belonged to each sample that were ready to be used for further analysis.

**Selecting stacked sequences using UStacks**

Stacks (v. 1.0.0.1, Catchen *et al.,* 2013) is a software pipeline for building and binding genes or loci from short-read sequences, such as those generated on the Illumina platform. Stacks was developed to work with restriction enzyme-based data, such as GBS, for the purpose of building genomic maps or working with populations that lack a reference genome, such as *V. vitis-idaea* (Catchen *et al.,* 2013). In order to eliminate redundant genomic information (paralogs) from my 56 samples (redundancy near 80%), I used functions in UStacks. This option from the command line Stacks program takes a set of short-read sequences as the input and aligns them into matching within a threshold stacks that can then be extracted to align one population with the rest (Byrne *et al.,* 2013). From this software I obtained the consensus sequences for each position of the contig, which is the calculated order of most frequent nucleotide residues. I concatenated all processed reads from each sample and ran them through UStacks with the deleveraging algorithm turned off in order to report all loci, no matter how many stacks were merged together to create that locus. A minimum read depth for stack formation was set to 3. The maximum nucleotide distance allowed between stacks was set to 2. The maximum nucleotide distance allowed to align was set to 4, as suggested by Byrne *et al.,* (2013).

**Multiple sequence alignment using MAFFT 7.0**

The accuracy and scalability of multiple DNA sequence alignment (MSA) of DNA information is an essential step in robust phylogenetic inference (Katoh and Standley, 2013). I used MAFFT 7.0, suggested to be one of the best tools for large alignments (Katoh and Standley, 2013). To achieve a reasonable balance between speed and accuracy, I used MAFFT with its default conditions using a two-cycle progressive method and the

BLOSUM62 (Katoh and Standley, 2013). The ratio of transitions to transversions was set to 2 by default (Katoh and Standley, 2013). After these conditions were set up, a multiple sequence alignment was obtained.

**Genetic tree reconstruction**

For the genetic tree reconstruction, I used a Bayesian analysis (BA). The software implemented for this purpose was Mr. Bayes v 3.2.6 (Ronquist *et al.,* 2012). The settings for the number of generations, number of runs, sample frequency, substitution model, and tree prior on the MCMC analysis are shown in Table 3.1. This analysis allowed us to obtain the best posterior probability genetic trees (Suchard and Rambaut, 2009; Ronquist *et al*., 2012).

**Table 3.1.** Parameters used for the Bayesian genetic tree reconstruction in Mr. Bayes v 3.2.6

| PARAMETER | DESCRIPTION |
|---|---|
| NUMBER OF GENERATIONS AND RUNS | 5,000,000 generations, 2 runs |
| HEAT CHAINS | 4 |
| SAMPLE FREQUENCY | Every 1000 generations |
| MCMC PARAMETERS | MCMC stamp = 420563589<br>Seed = 1129787293<br>Swapseed = 1453354370 |
| DATA TYPE | DNA<br>Nucmodel = 4by4<br>Nst = 1, GTR + GAMMA<br>Covarion = No<br># States = 4<br>State frequencies have a Dirichlet prior (0.25,0.25,0.25,0.25)<br>Nucleotide Rate Variation = Equal |
| AVERAGE STANDARD DEVIATION OF SPLIT FREQUENCIES | 0.009984 |
| PSRF = POTENTIAL SCALE REDUCTION FACTOR | Set to 1 (Real value was 1.001) |

After the resulting 50,001 trees (looked at an overview in Tracer, Edinburgh, Scotland), I then used Log-Combiner to discard 10, 000 burn-in to preserve those that reached the

convergence of the run. The consensus tree topology and posterior probability values resulting from this Bayesian analysis was used to assess the genetic relationships among the 56 partridgeberry individuals in Newfoundland and Southern Labrador (Rambaut *et al*., 2014).

**Association of environmental variables with genetic groups**

To examine the existence of an association between the genetic relationships and environmental variables, I used the nine variables: ecoregion, mean annual precipitation, mean summer temperature, mean annual temperature, coastal proximity, mean annual runoff, surface water pH, and surface water sensitivity to acid rain for Newfoundland and Labrador. I identified clades based on the phylogenetic analysis. Associations between clade membership and the variables of interest were explored using pivot tables in Microsoft Excel. A pivot table allowed us to associate each environmental variable and category with a clade(s) in the phylogeny (Dierenfeld and Merceron, 2012; Ojanen *et al*., 2013; Alcaide *et al*., 2014). I also examined this association visually by plotting the environmental variable and category on each of the 56 samples on the phyogeny.

**Genetic, geographic, AC and TPC distance matrices**

In order to elucidate the associations between genetics, geographic location, and biochemistry, I estimated distance matrices among all sampled individuals for genetics, geography, AC, and TPC. To measure the pairwise genetic distances among individuals, I utilized TASSEL 5.0 (Bradbury *et al.,* 2007) and my Multiple Sequence Alignment. TASSEL calculates genetic distance as 1 minus the IBS (identity-by-state) similarity, with IBS defined as the probability that alleles drawn at random from two individuals at the same locus are

the same (Endelman and Jannink, 2012). The result was a 56x56 genetic distance matrix that was used for further analysis.

I used the Geographic Distance Matrix Generator by Ersts (2011), an application that implements a Perpendicular Distance Calculator to compute all pairwise distances from a list of geographic coordinates. A 56x56 geographic distance matrix that was used for further analysis. The AC and TPC matrices were constructed using SPSS 23.0 for Mac (SPSS, Chicago, Illinois, USA), which uses Euclidean distance as a parameter with which to estimate ordinary distance between two samples.

**Mantel test**

A Mantel test measures the correlation between two matrices and is a non-parametric assessment of the correlation amongst the distance matrices (geographic, genetic, AC, and TPC). The test statistic is the Pearson product-moment correlation coefficient 'R'. 'R' has values close to -1 indicating a strong negative correlation, and values close to +1 indicate a strong positive correlation. An R-value of 0 indicates no correlation. Using functions in the ade4 library (Chessel *et al*., 2004), I performed a Mantel test in R (R development Core Team, 2008). Based on these results, I can reject or accept the null hypothesis that these four matrices: genetic, geographic, AC, and TPC, are related at an alpha value of = 0.05. If TPC and AC matrices were correlated with the genetic distance matrix but not with the geographic matrix, then this would suggest that the genetic diversity of individuals can explain better TPC and AC than the geographic proximity of individuals.

## Results

### Genetic relationships among *V. vitis-idaea* individuals

The Bayesian inference tree (Fig. 3.1) showed posterior probability values ranging from 0.51 to 1. Three genetic groups were recovered with posterior probabilities of 0.63, 0.82, and 0.86, respectively. The Bayesian analysis recovered relationships with high statistical support. The environment variables tested were plotted in this tree.

### Association of environmental variables with genetic groups

I plotted the environmental variables on the Bayesian inference tree, and out of the nine analyzed, phylogenetic structure was found in: ecoregion, mean summer temperature, mean annual temperature, and mean annual runoff (Figs. 3.1, 3.3, 3.4 and 3.6). Water pH and sensitivity to acid rain showed no structure at all, and therefore are not shown. Table 3.2 summarizes selected environmental variables and factors that appeared to be linked with each of the three genetic groups. The parameter I used to identify links between environmental factors and the phylogeny was a 50% rule, where if 50% or more of individuals in a particular environmental category appear in one genetic group, I interpreted this as a link (a positive association between a genetic group and an environmental factor).

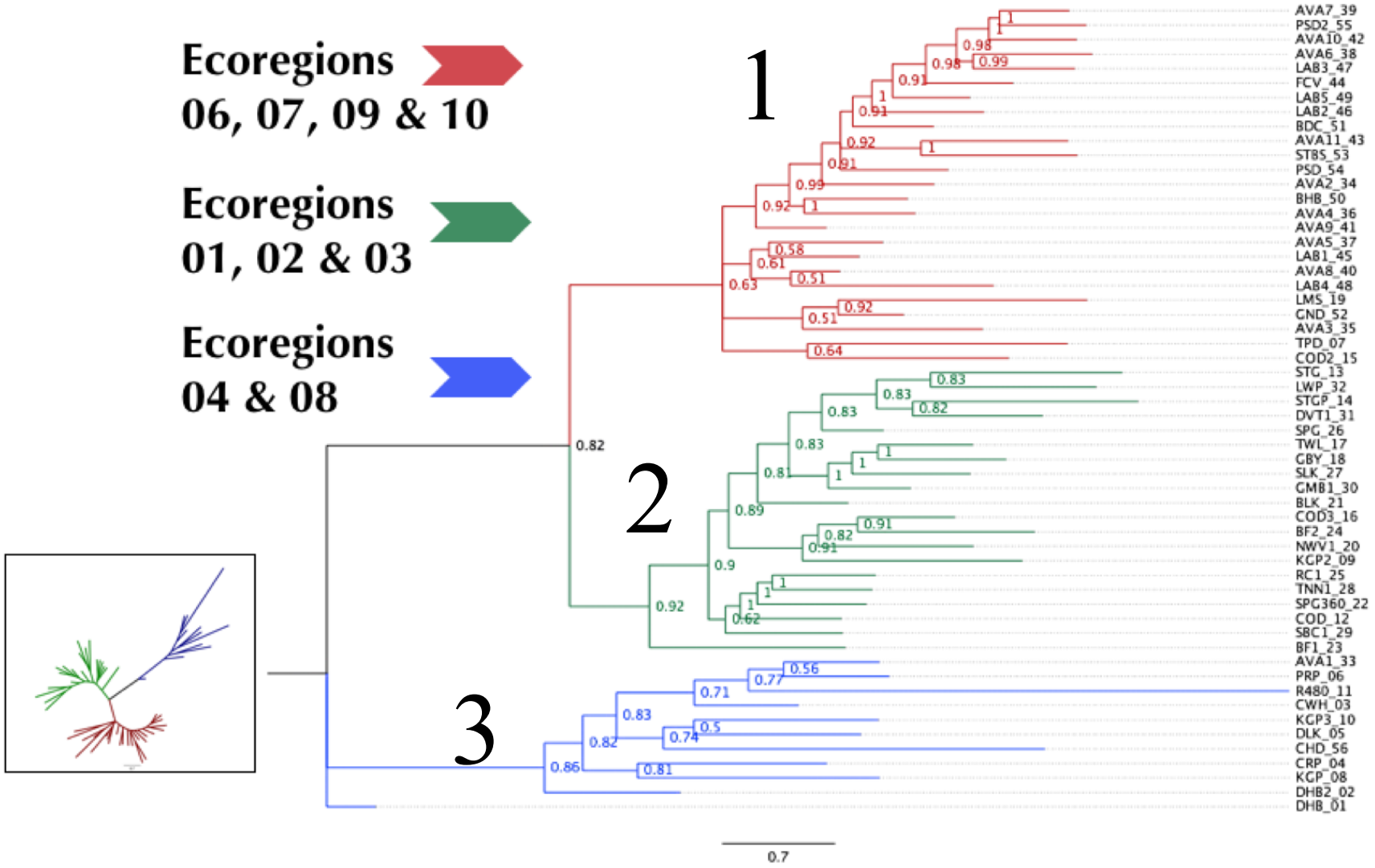



**Fig. 3.1 Bayesian inference tree using Mr. Bayes v 3.2.6.** Three major genetic groups were identified in red (1), green (2) and blue (3), showing an ecological structuring on the genetic tree. Taxa are represented by a unique code where the first three or four letters represent the sampling site, and the number represent the sample number from 1 to 56 as in Table S2.2. Numbers at nodes represent posterior probability values from 0 to 1. Inset represents an unrooted tree showing the three major genetic groups.

The most evident phylogenetic structure was in coldest mean summer temperature (6-11.9 C) and coldest mean annual temperatures (1-2.9 C), which both clustered within genetic group 1. Individuals with mean annual runoff of 1000-1300 mm were clustered within genetic group 3. I observed phylogenetic structure of individuals by ecoregions. Genetic group 1 corresponded to individuals collected from ecoregions 6 (Maritime Barrens), 7 (The Eastern Hyper-Oceanic Barrens), 9 (Strait of Belle Isle Barrens), and 10 (Forteau Barrens). Genetic group 2 corresponded to individuals collected from ecoregions 1 (Western Newfoundland Forest), 2 (Central Newfoundland Forest), and 3 (North Shore Forest). Genetic group 3 matched individuals collected from ecoregions 4 (Northern Peninsula Forest) and 8 (Long Range Barrens).

**Mantel test results**

I found a significant correlation on three out of the six Mantel tests performed (Table 3.3). The correlation between genetics and geography was the strongest (R=0.2239), suggesting an isolation-by-distance effect. Geographically closer individuals were not only genetically closer, but also shared similar levels of total phenolic content but not antioxidant capacity (Table 3.3). Genetically close individuals did not have similar total phenolic content nor antioxidant capacity (Table 3.3). Contrary to my expectation, total phenolics was not correlated with antioxidant capacity in *V. vitis-idaea* samples.

**Table 3.2.** Summary of relevant environmental variables linked to the identified three genetic groups as evaluated by the 50% majority rule in pivot tables. The following categories were considered relevant while harmonizing them with the individual genetic groups presented in Fig. 3.1. Environmental variables and their categories can be found in chapter 2 of this thesis, while genetic groups can be found in Fig. 3.1 of this chapter.

| ENVIRONMENTAL CONDITIONS THAT MATCH WITH GENETIC GROUP 1(RED) | ENVIRONMENTAL CONDITIONS THAT MATCH WITH GENETIC GROUP 2 (GREEN) | ENVIRONMENTAL CONDITIONS THAT MATCH WITH GENETIC GROUP 3 (BLUE) |
|---|---|---|
| • **MEAN ANNUAL PRECIPITATION OF 1300 – 1500 MM PER YEAR** | • MEAN ANNUAL PRECIPITATION OF 1100 – 1300 MM PER YEAR | • MEAN ANNUAL TEMPERATURE FROM 4.0-7.9$^{O}$C |
| • **MEAN SUMMER TEMPERATURE 6-11.9$^{O}$C AND 12-13.9$^{O}$C** | • MEAN SUMMER TEMPERATURE OF 14-19.9 $^{O}$C | • MEAN ANNUAL RUNOFF OF 1000-1300 MM |
| • **MEAN ANNUAL TEMPERATURE FROM 1.0-2.9 $^{O}$C** | • MEAN ANNUAL RUNOFF OF 500-800 MM | |
| • **INLAND POPULATIONS** | | |
| • **MEAN ANNUAL RUNOFF OF 800-1000 MM, 1300-1800 MM AND 1800-2200** | | |

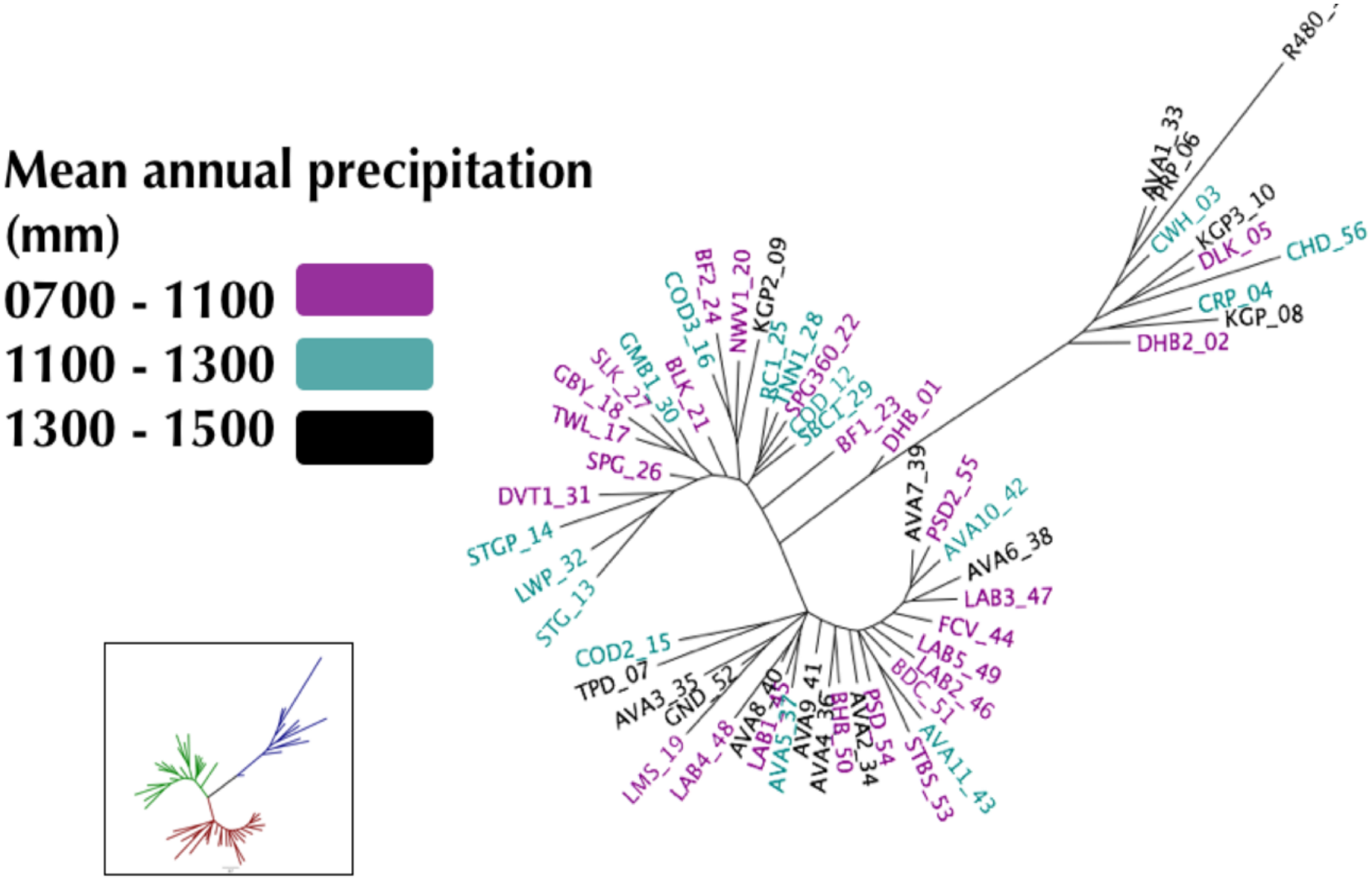



**Fig. 3.2 Mean annual precipitation of samples plotted on the Bayesian inference tree.** Taxa are represented by a unique code in which the first three or four letters denote the sampling site name followed by the sample number from 1 to 56. The inset represents an unrooted tree showing the three major genetic groups in red (1), green (2) and blue (3).

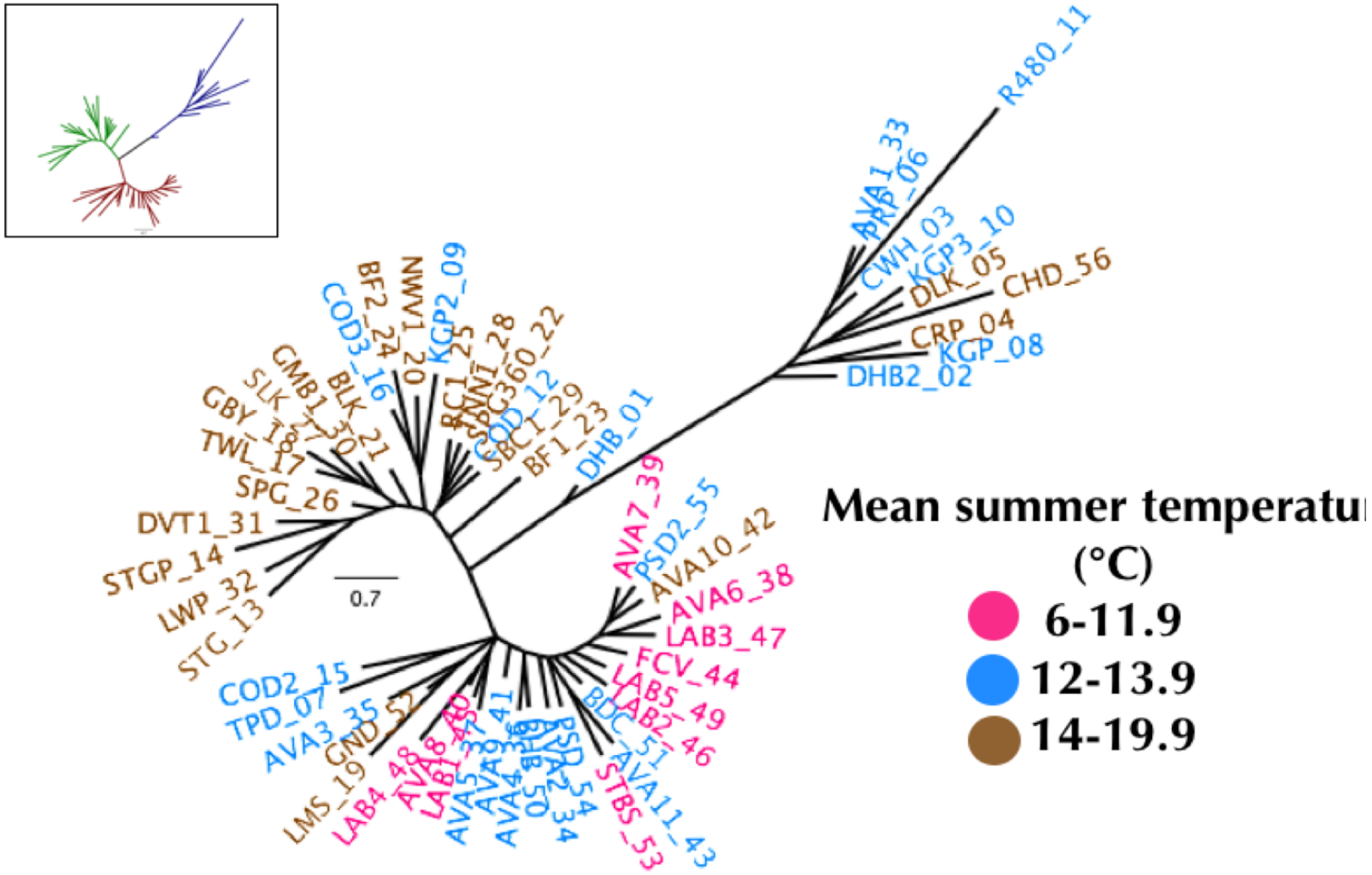



**Fig. 3.3 Mean summer temperature of samples plotted on the Bayesian inference tree.** Taxa are represented by a unique code in which the first three or four letters denote the sampling site name followed by the sample number from 1 to 56. The inset represents an unrooted tree showing the three major genetic groups in red (1), green (2) and blue (3).

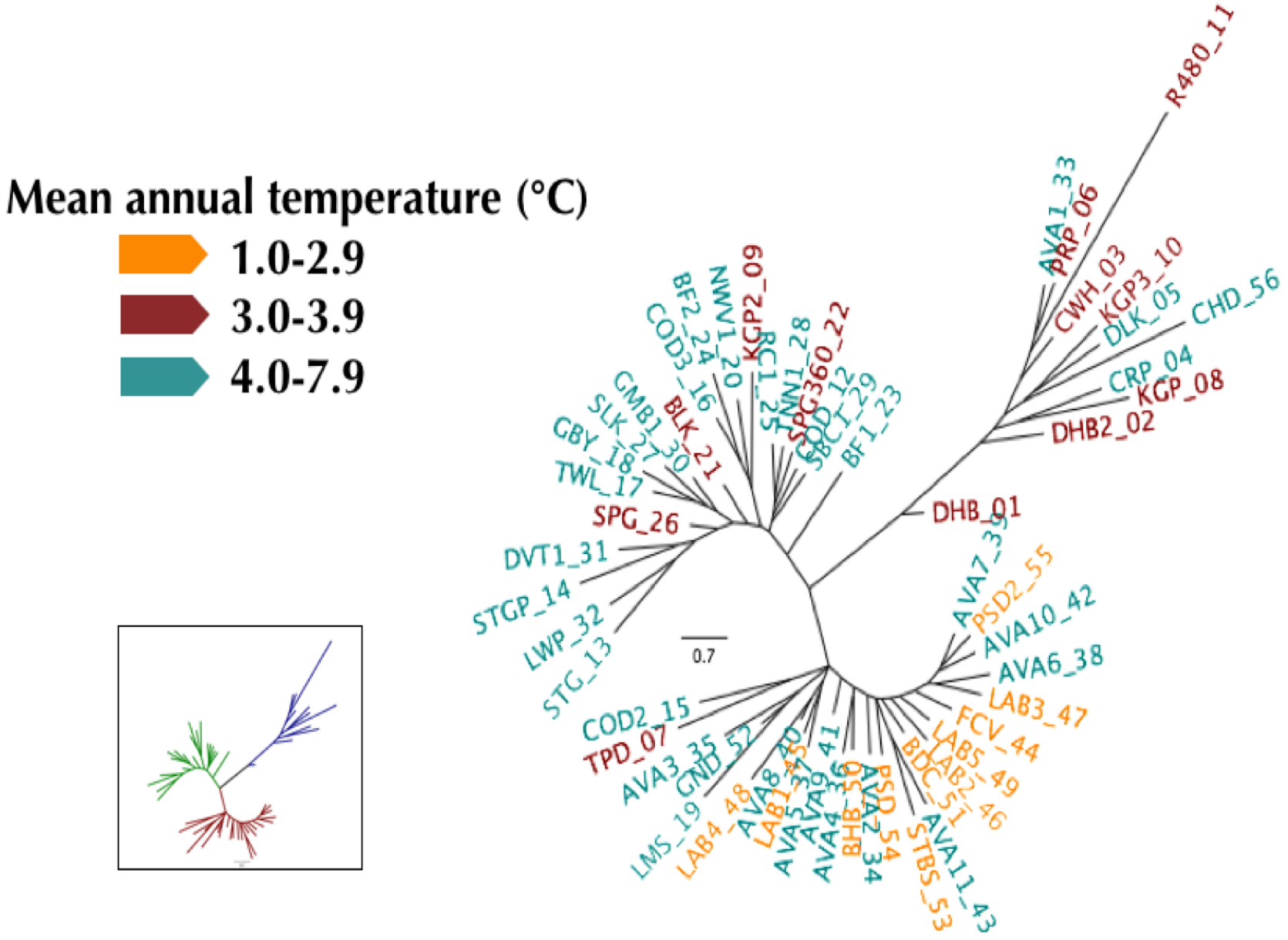



**Fig. 3.4 Mean annual temperature of samples plotted on the Bayesian inference tree.** Taxa are represented by a unique code in which the first three or four letters denote the sampling site name followed by the sample number from 1 to 56. The inset represents an unrooted tree showing the three major genetic groups in red (1), green (2) and blue (3).

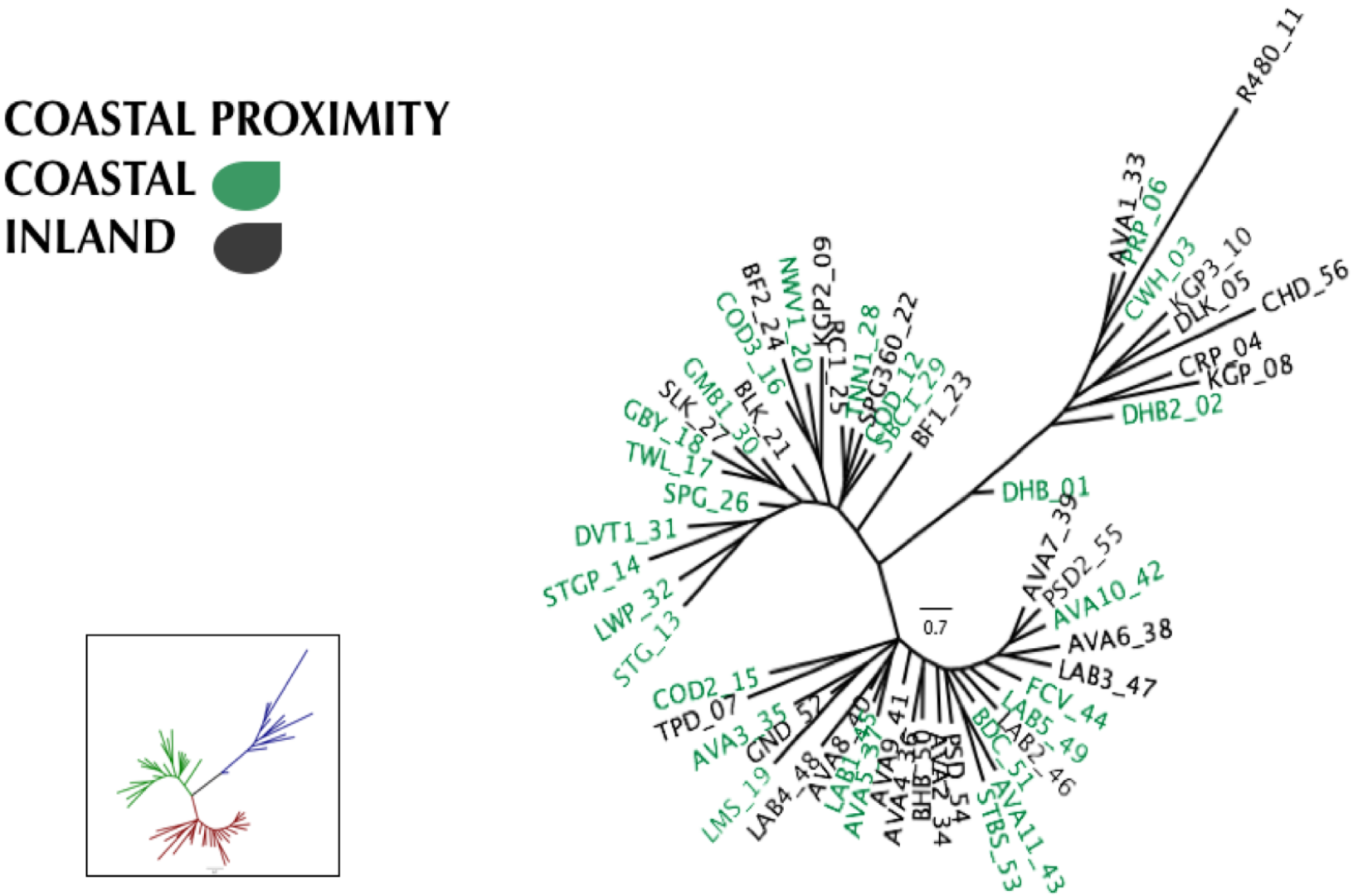



**Fig. 3.5 Coastal proximity of samples plotted on the Bayesian inference tree.** Taxa are represented by a unique code in which the first three or four letters denote the sampling site name followed by the sample number from 1 to 56. The inset represents an unrooted tree showing the three major genetic groups in red (1), green (2) and blue (3).

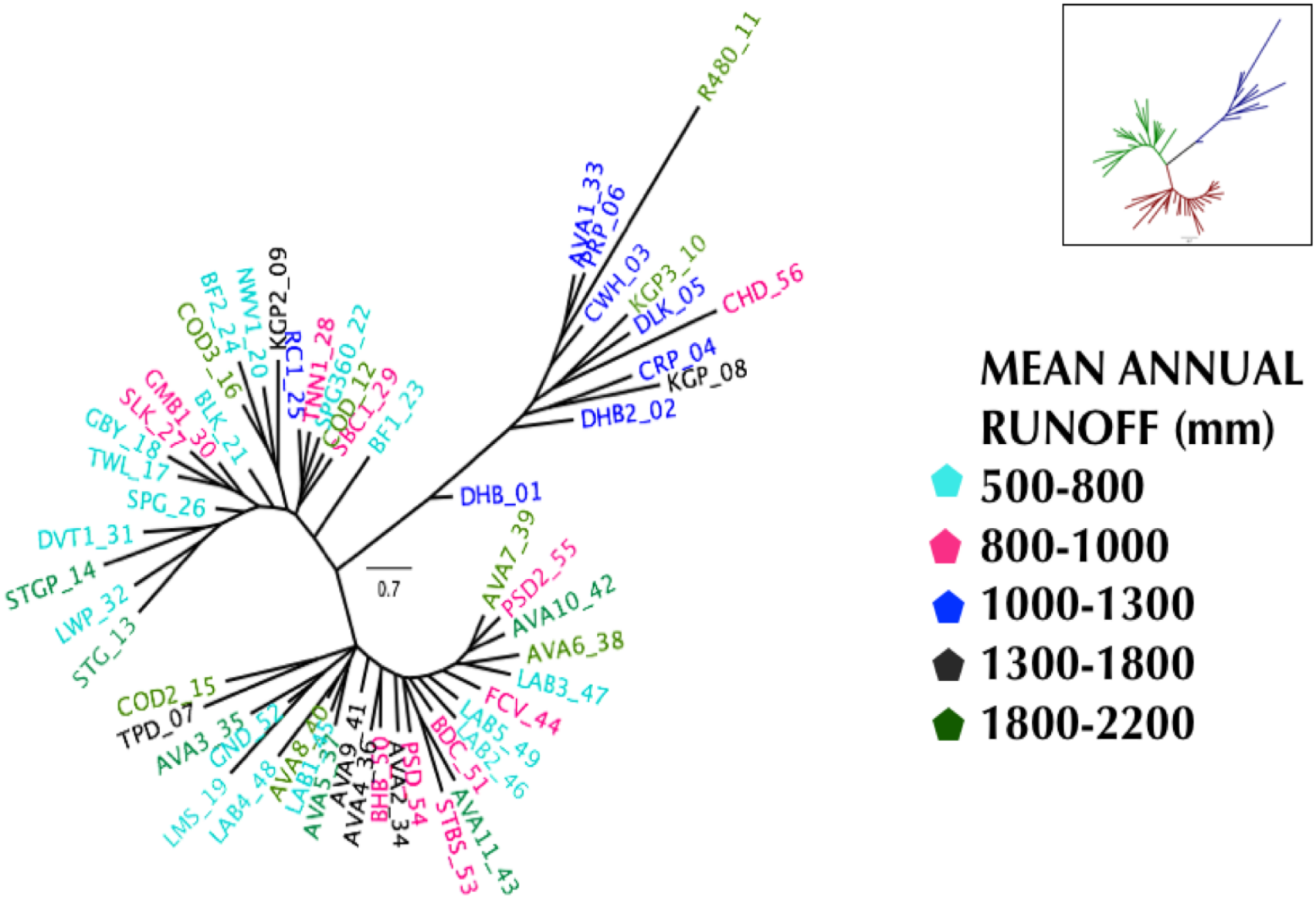



**Fig. 3.6 Mean annual runoff of samples plotted on the Bayesian inference tree.** Taxa are represented by a unique code in which the first three or four letters denote the sampling site name followed by the sample number from 1 to 56. The inset represents an unrooted tree showing the three major genetic groups in red (1), green (2) and blue (3).

**Table 3.3.** Mantel test of matrix correspondence. The following table summarizes the results of pairwise comparisons of four sets of distance matrices: geographic distances (Km), genetic distances (1- Identity-by-State), antioxidant capacity distance (Euclidean), and phenolic compound distance (Euclidean). The alpha for the test is $\alpha$=0.05, based on 9999 replicates. Numbers with a star * represent that the matrix entries are positively associated. **Marginally positively correlated

| | **GEOGRAPHIC DISTANCE** | **GENETIC DISTANCE** | **ANTIOXIDANT CAPACITY DISTANCE** | **TOTAL PHENOLIC CONTENT DISTANCE** |
|---|---|---|---|---|
| **GEOGRAPHIC DISTANCE** | - | | | |
| **GENETIC DISTANCE** | R=0.2239 (p=0.0229)* | - | | |
| **ANTIOXIDANT ACTIVITY DISTANCE** | R=0.0155 (p=0.3221) | R=0.0709 (p=0.9634) | - | |
| **TOTAL PHENOLIC CONTENT DISTANCE** | R=0.1147 (p=0.0034)* | R=0.0046 (p=0.52) | R=0.0752 (p=0.0667)** | - |

**Discussion**

I reconstructed a phylogenetic tree showing the relationships among individuals and evaluated how particular environmental factors were associated with the genetics groups reconstructed in the Island of Newfoundland and Southern Labrador. Individuals collected from specific ecoregions shared a genetic relatedness as seen in Fig. 3.2.

Individuals from group 2, collected in the Western and Central Newfoundland Forest and the North Shore Forest, share similar soil types. These ecoregions have humoferric podzols, generally strongly acid, containing inorganic material that occur in relatively dry sites, with coniferous and mixed forests, and low moisture levels. The warmest summers of any coastal area in Newfoundland occur in the area of the Central Newfoundland Forest

and the North Shore Forest. Particularly the North Shore Forest is the driest ecoregion on the Island, with moisture deficiencies common in summer. It has been reported that acidic soil conditions (pH 5), as the ones in these ecoregions are very positive for growth and maturity in many *Vaccinium* species including partridgeberry (Ingestad, 1973).

Individuals clustered in group 1 were collected in the Maritime Barrens, the Eastern Hyper-Oceanic Barrens, the Strait of Belle Isle Barrens and the Forteau Barrens, sharing a similar soil composed of a mixture of low organic remains, clay, and different oceanic rock particles, volcanic rock, and sandstones (Meades, 1990). The barrens are known to have soils with relatively low potassium and high deposits of calcium due to limestone underneath its surface, and is exposed directly to light (a well-known factor for partridgeberry growth), is a well-drained area, and it has a basic pH, unusual for the growth of partridgeberry (Debnath, 2007a). Nevertheless, species of trees such as birch (*Betula* L.) and some species in the genus *Vaccinium* grow well under these conditions (Meades, 1990). Other shared characteristics of these barrens for optimal growth of partridgeberry include similar nitrogen sources, ammonium, and nitrate, both found at low concentrations, typical of areas exposed directly to the Atlantic Ocean (Meades, 1990).

The sensitivity to high salt concentrations in soil has been reported in *Vaccinium* species, as those conditions would affect their normal growth and reproduction. The Island of Newfoundland, however, has naturally those conditions in the Northern Peninsula Forest, and the Long Range Barrens even more than those individuals in group 1, where individuals from the third genetic group clustered. Arctic-alpine plants, such as diapensia (*Diapensia lapponica*), can be found on both ecoregions (Ingestad, 1973; Meades, 1990).

Partridgeberry individuals collected in the serpentine soils of the Western Newfoundland Forest clustered mainly within the second genetic group. The serpentine rocks result in basic soils from which many common plants on the Island cannot take up nutrients, and thus cannot grow (Meades, 1990). Future research should test whether the genes that confer adaptation to serpentine soils in *Arabidopsis* also play a role in *Vaccinium*.

Specific soil conditions for partridgeberry and other *Vaccinium* species has gained considerable attention in the past few years (Debnath, 2007a). Accordingly, a synthesis of the genetic, physiological, and ecological aspects of specific soil conditions as the ones present in each ecoregion, can bring a better understanding of the relationships between the environment and the genetics of the plant, essential for an efficient breeding program. Genome wide association studies, transcriptomics, quantitative trait loci, and high throughout sequencing studies can provide genetic resources of partridgeberry as they help to identify the degree to which protein-coding polymorphisms and variation in gene expression contribute to controlling natural trait variation due to environment or genetics (e.g. Hoekstra and Coyne, 2007; Giraud *et al.*,2008).

**Association of environmental variables with genetic groups**

Genetic variation in plants could be driven by temperature and precipitation factors. For example, Manel et al. (2012) tested thirteen species of plants from the European Alps and found that their amplified fragment length polymorphisms (AFLPs) varied according to temperature and precipitation. These associations allowed the identification of loci of ecological importance as these two environmental factors were the best predictors for broad-scale adaptive genetic variation (Manel *et al*. 2012). In another study, increased

intervals between precipitation events reduced transcription of genes related to photosynthesis and carbon fixation, and increased transcription of a variety of heat shock proteins and kinases in *Andropogon gerardii* (Travers *et al*., 2007). Greater genomic dissimilarity among individuals was found growing under an experimental treatment of increased precipitation variability, suggesting that the effects of variable precipitation regimes on population-level genetic diversity are complex (Avolio *et al*., 2013).

**Correlations between genetics, geographic location, total phenolic content and antioxidant capacity**

Genetically similar samples were geographically close in the province of Newfoundland and Labrador, and geographically close sampled partridgeberries also have similar levels of TPC (Table 3.3).

The relationships between geographic and genetic distances is a very common association found in multiple studies, regarded as "Isolation by distance" (Pusadee *et al*., 2009; Yakimowsky and Eckert, 2008). This is a term refers to the accumulation of local genetic variation under geographically limited dispersal (Pusadee *et al*., 2009). Isolation by distance can be influenced by a series of factors including, growing conditions, bioactive phenolic compounds, and its genetic pool in *V. vitis idaea* (Yakimowsky and Eckert, 2008; Krüger, 2009; Pusadee *et al*., 2009). Greater spatial isolation of peripheral samples of partridgeberries may reduce gene flow with continental populations, further decreasing genetic diversity and increasing differentiation. Future studies should test if populations in Newfoundland have decreased genetic diversity and increased differentiation, compared to those across its distribution. A study on partridgeberries wild populations collected in

four geographically different regions of Eastern Canada concluded that despite a wide genetic diversity among clones, only 10% of the total genetic variation could be explained by geographical distribution (Debnath, 2007a). This result suggests that sources of genetic and geographic differentiation in partridgeberry are extremely varied. A decrease in genetic similarity among populations was observed as the geographic distance between them increases, supporting the isolation by distance pattern.

Geographically close sampled partridgeberries in Newfoundland and Southern Labrador contained similar TPC as revealed by the Mantel test of matrix correspondence between these two distance matrices. This result suggests a clear role of the environment on the TPC content in partridgeberry. Growing conditions under different geographic locations can influence the amount of the bioactive phenolic compounds in *Vaccinium*.

Contrary to my expectations, genetically closer individuals did not share the same amount of TPC and AC. This might suggest that the environment plays a more important role in determining the TPC and AC, and that similar genotypes might not produce the same levels of TPC and AC if growing in different environmental conditions, thus we can cite some studies corroborating the genetic x environment interaction in *Vaccinium* species (Gilbert et al., 2015; Lazaro et al., 2015; Scalzo et al., 2016). This hypothesis was confirmed by Mpofu *et al*. (2006) where environmental effects were considerably larger than genotype effects in hard spring wheat. Independent of their genotype, diurnal temperature variation increased phenolic and anthocyanin content in strawberries (Wang and Stretch, 2001), and increases in the levels of carbon dioxide have also been linked to higher concentrations of phenolic compounds and antioxidant capacity in strawberry and

blueberries (Wang and Stretch, 2001). Contrasting soil conditions can affect plant phenolic composition, rather than genetic diversity in *Camelia sinensis* (Jeyaramraja *et al.,* 2003). As an increase in soil humidity deficit led to an inferior activity of phenylalanine ammonia lyase (a critical enzyme in several secondary compounds) and therefore to a lower synthesis of phenolics in tea plants (Jeyaramraja *et al.,* 2003). Further, soil fertilization, particularly high levels of nitrogen compounds seems to lower the levels of certain phenolics such as flavonols in *Betula pendula* (Keinänen *et al.,* 1999).

The literature however reports cases in which measurements of genetic diversity could better explain variation in polyphenol's content. For example, in asparagus it has been shown that genetic material was more important than pre-harvest conditions, season, spear portion and spear tip color for the total phenolic content (Papoulias *et al*., 2009). In a study in blueberry, the variation of phenolic composition among genotypes was much greater than that found between the growing seasons and geography (Howard *et al.,* 2003), emphasizing the role of geographical distance between populations as a variable to consider when selecting genetic resources in *Vaccinium vitis idaea*.

**Limitations of the study and further direction**

I conducted a study that evaluates the genetic variation of partridgeberries in different climatic regions across the Island of Newfoundland and Southern Labrador. The genomic resources of the wild plant species in Newfoundland are still largely unexplored. Proper evaluation of the genetic resources of native wild species is especially important during the initial stages of plant domestication (Debnath and McRae, 2001; 2007b). A lack of abundant, genome-wide molecular markers has limited the adoption of modern molecular

assisted selection approaches in partridgeberry breeding programs. To increase the number of available markers in this species, there is a need for studies that identify, test, and validate microsatellite, EST-PCR, and SNPs markers, as a prerequisite for initiating a marker-assisted breeding program, and my study has partially filled this gap of knowledge. Conclusions reached in my study need to be corroborated upon a larger sampling of individuals across North America. Complementary studies can look at the effect of the environment on the expression of genes in the phenyl-propanoid metabolism and flavonoid biosynthesis in plants (i.e., mRNA, proteins, metabolites). Future studies with greater genomics coverage will help to elucidate how partridgeberry of Newfoundland has adapted to a wide variety of environmental conditions.

**References**


- Akula, R., & Ravishankar, G. A. (2011). Influence of abiotic stress signals on secondary metabolites in plants. *Plant Signaling & Behavior*, *6*(11), 1720-1731.
- Alam, Z., Morales, H. R., & Roncal, J. (2016). Environmental conditions affect phenolic content and antioxidant capacity of leaves and fruit in wild partridgeberry (*Vaccinium vitis-idaea*). *Botany*, *94*(7), 509-521.
- Alcaide, M., Scordato, E. S., Price, T. D., & Irwin, D. E. (2014). Genomic divergence in a ring species complex. *Nature*, *511*(7507), 83-85.
- Atkinson, N. J., & Urwin, P. E. (2012). The interaction of plant biotic and abiotic stresses: from genes to the field. *Journal of Experimental Botany*, *63*(10), 3523-3543.
- Avolio, M. L., Beaulieu, J. M., & Smith, M. D. (2013). Genetic diversity of a dominant C4 grass is altered with increased precipitation variability. *Oecologia*, *171*(2), 571-581.

- Balsdon, J. L., Smith, T. W., & Lundholm, J. T. (2011). Phenotypic and genotypic differentiation of *Vaccinium vitis-idaea* between coastal barrens and forests in Nova Scotia, Canada. *Botany*, *89*(3), 147-155.

- Bell, D. J., Rowland, L. J., Polashock, J. J., & Drummond, F. A. (2008). Suitability of EST-PCR markers developed in highbush blueberry for genetic fingerprinting and relationship studies in lowbush blueberry and related species. *Journal of the American Society for Horticultural Science*, *133*(5), 701-707.

- Bradbury, P. J., Zhang, Z., Kroon, D. E., Casstevens, T. M., Ramdoss, Y., & Buckler, E. S. (2007). TASSEL: software for association mapping of complex traits in diverse samples. *Bioinformatics*, *23*(19), 2633-2635.

- Brambilla, A., Lo-Scalzo, R., Bertolo, G., & Torreggiani, D. (2008). Steam-blanched highbush blueberry (*Vaccinium corymbosum* L.) juice: phenolic profile and antioxidant capacity in relation to cultivar selection. *Journal of Agricultural and Food Chemistry*, *56*(8), 2643-2648.

- Buter, B., Orlacchio, C., Soldati, A., & Berger, K. (1998). Significance of genetic and environmental aspects in the field cultivation of *Hypericum perforatum*. *Planta Medica*, *64*(5), 431-437.
- Byrne, S., Czaban, A., Studer, B., Panitz, F., Bendixen, C., & Asp, T. (2013). Genome wide allele frequency fingerprints (GWAFFs) of populations via genotyping by sequencing. *PLoS One*, *8*(3), e57438.

- Cano, A., Medina, A., & Bermejo, A. (2008). Bioactive compounds in different citrus varieties. Discrimination among cultivars. *Journal of Food Composition and Analysis*, *21*(5), 377-381.

- Catchen, J., Hohenlohe, P. A., Bassham, S., Amores, A., & Cresko, W. A. (2013). Stacks: an analysis tool set for population genomics. *Molecular Ecology*, *22*(11), 3124-3140.

- Chessel, D., Dufour, A. B., & Thioulouse, J. (2004). The ade4 package-I-One-table methods. *R news*, *4*(1), 5-10.

- Debnath, S. C., & McRae, K. B. (2001). In vitro culture of lingonberry (*Vaccinium vitis-idaea* L.) the influence of cytokinins and media types on propagation. *Small Fruits Review*, *1*(3), 3-19.

- Debnath, S. C. (2007a). Inter simple sequence repeat (ISSR) to assess genetic diversity within a collection of wild lingonberry (*Vaccinium vitis-idaea* L.) clones. *Canadian Journal of Plant Science*, *87*(2), 337-344.

- Debnath, S. C. (2007b). Strategies to propagate *Vaccinium* nuclear stocks for the Canadian berry industry. *Canadian Journal of Plant Science*, *87*(4), 911-922.

- Debnath, S. C. (2009). Development of ISSR markers for genetic diversity studies in *Vaccinium angustifolium*. *Nordic Journal of Botany*, *27*(2), 141-148.

- Debnath, S. C. (2012). Molecular markers in biodiversity of *Vaccinium* and *Rubus* wild germplasm. In *X International Symposium on Vaccinium and Other Superfruits 1017*(1), 85-92.

- Dierenfeld, H., & Merceron, A. (2012). Learning analytics with excel pivot tables. *1st Moodle Research Conference*, *1*(1), 115-121.

- Dorman, H. D., Koşar, M., Kahlos, K., Holm, Y., & Hiltunen, R. (2003). Antioxidant properties and composition of aqueous extracts from *Mentha* species, hybrids, varieties, and cultivars. *Journal of Agricultural and Food Chemistry*, *51*(16), 4563-4569.

- Elshire, R. J., Glaubitz, J. C., Sun, Q., Poland, J. A., Kawamoto, K., Buckler, E. S., & Mitchell, S. E. (2011). A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PloS one*, *6*(5), e19379.

- Endelman, J. B., & Jannink, J. L. (2012). Shrinkage estimation of the realized relationship matrix. *G3: Genes| Genomes| Genetics*, *2*(11), 1405-1413.

- Ersts, P. J. (2011). Geographic distance matrix generator (version 1.2. 3). American Museum of Natural History. *Center for Biodiversity and Conservation*.

- Gilbert, J. L., Guthart, M. J., Gezan, S. A., de Carvalho, M. P., Schwieterman, M. L., Colquhoun, T. A., ... & Olmstead, J. W. (2015). Identifying breeding priorities for blueberry flavor using biochemical, sensory, and genotype by environment analyses. *PloS one*, *10*(9), e0138494.

- Giraud, E., Ho, L. H., Clifton, R., Carroll, A., Estavillo, G., Tan, Y. F., Howell, K., Ivanova, A., Pogson, A., Millar, B., & Whelan, J. (2008). The absence of Alternative Oxidase 1a in *Arabidopsis* results in acute sensitivity to combined light and drought stress. *Plant Physiology*, *147*(2), 595-610.

- Gordon, A., & Hannon, G. J. (2010). Fastx-toolkit. *FASTQ/A short-reads preprocessing tools (unpublished) http://hannonlab. cshl. edu/fastx_toolkit*.

- Government of Newfoundland and Labrador. (1992). Water resources atlas of Newfoundland. Available from http://www.canal.gov.nl.ca/reports/Water_Resources_Atlas/Water_Resources_Atlas_of_Newfoundland_1992.pdf. Water Resources Division, St. John's.

- Hansen, A. H., Jonasson, S., Michelsen, A., & Julkunen-Tiitto, R. (2006). Long-term experimental warming, shading and nutrient addition affect the concentration of phenolic compounds in arctic-alpine deciduous and evergreen dwarf shrubs. *Oecologia*, *147*(1), 1-11.

- Ho, K. Y., Huang, J. S., Tsai, C. C., Lin, T. C., Hsu, Y. F., & Lin, C. C. (1999). Antioxidant activity of tannin components from *Vaccinium vitis-idaea* L. *Journal of Pharmacy and Pharmacology*, *51*(9), 1075-1078.

- Hoekstra, H. E., & Coyne, J. A. (2007). The locus of evolution: evo devo and the genetics of adaptation. *Evolution*, *61*(5), 995-1016.

- Howard, L. R., Pandjaitan, N., Morelock, T., & Gil, M. I. (2002). Antioxidant capacity and phenolic content of spinach as affected by genetics and growing season. *Journal of Agricultural and Food Chemistry*, *50*(21), 5891-5896.
- Howard, L. R., Clark, J. R., & Brownmiller, C. (2003). Antioxidant capacity and phenolic content in blueberries as affected by genotype and growing season. *Journal of the Science of Food and Agriculture*, *83*(12), 1238-1247.

- Hussain, M. S., Fareed, S., Ansari, S., Rahman, M. A., Ahmad, I. Z., & Saeed, M. (2012). Current approaches toward production of secondary plant metabolites. *Journal of Pharmacy and Bioallied Sciences*, *4*(1), 10.

- Ingestad, T. (1973). Mineral nutrient requirements of *Vaccinium vitis idaea* and *V. myrtillus*. *Physiologia Plantarum*, *29*(2), 239-246.

- Jaakola, L., Määttä-Riihinen, K., Kärenlampi, S., & Hohtola, A. (2004). Activation of flavonoid biosynthesis by solar radiation in bilberry (*Vaccinium myrtillus* L.) leaves. *Planta*, *218*(5), 721-728.

- Jeyaramraja, P. R., Pius, P. K., Raj Kumar, R., & Jayakumar, D. (2003). Soil moisture stress-induced alterations in bioconstituents determining tea quality. *Journal of the Science of Food and Agriculture*, *83*(12), 1187-1191.

- Karppinen, K., Zoratti, L., Nguyenquynh, N., Häggman, H., & Jaakola, L. (2016). On the developmental and environmental regulation of secondary metabolism in *Vaccinium* spp. berries. *Frontiers in Plant Science*, *7*(1), 1-12.

- Katoh, K., & Standley, D. M. (2013). MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution*, *30*(4), 772-780.

- Keinänen, M., Julkunen-Tiitto, R., Mutikainen, P., Walls, M., Ovaska, J., & Vapaavuori, E. (1999). Trade-offs in phenolic metabolism of silver birch: effects of fertilization, defoliation, and genotype. *Ecology*, *80*(6), 1970-1986.

- Lázaro-Nogal, A., Matesanz, S., Godoy, A., Pérez-Trautman, F., Gianoli, E., & Valladares, F. (2015). Environmental heterogeneity leads to higher plasticity in dry-edge populations of a semi-arid Chilean shrub: insights into climate change responses. *Journal of Ecology*, *103*(2), 338-350.

- Lake, J. A., Field, K. J., Davey, M. P., Beerling, D. J., & Lomax, B. H. (2009). Metabolomic and physiological responses reveal multi-phasic acclimation of *Arabidopsis thaliana* to chronic UV radiation. *Plant, cell & environment*, *32*(10), 1377-1389.

- Meades, L. (1990). Ecoregions of Newfoundland and Labrador. Retrieved January 20, 2017, from http://www.env.gov.nl.ca/env/publications/parks/index.html#brochures

- Mpofu, A., Sapirstein, H. D., & Beta, T. (2006). Genotype and environmental variation in phenolic content, phenolic acid composition, and antioxidant activity of hard spring wheat. *Journal of Agricultural and Food Chemistry*, *54*(4), 1265-1270.

- Ojanen, S. P., Nieminen, M., Meyke, E., Pöyry, J., & Hanski, I. (2013). Long-term metapopulation study of the Glanville fritillary butterfly (Melitaea cinxia): survey methods, data management, and long-term population trends. *Ecology and evolution*, *3*(11), 3713-3737.

- Papoulias, E., Siomos, A. S., Koukounaras, A., Gerasopoulos, D., & Kazakis, E. (2009). Effects of genetic, pre-and post-harvest factors on phenolic content and antioxidant capacity of white asparagus spears. *International Journal of Molecular Sciences*, *10*(12), 5370-5380.

- Persson, H. A., & Gustavsson, B. A. (2001). The extent of clonality and genetic diversity in lingonberry (*Vaccinium vitis-idaea* L.) revealed by RAPDs and leaf-shape analysis. *Molecular Ecology*, *10*(6), 1385-1397.Pichersky, E., & Gang, D. R. (2000). Genetics and biochemistry of secondary metabolites in plants: an evolutionary perspective. *Trends in Plant Science*, *5*(10), 439-445.

- Poland, J. A., & Rife, T. W. (2012). Genotyping-by-sequencing for plant breeding and genetics. *The Plant Genome*, *5*(3), 92-102.

- Prior, R. L., Cao, G., Martin, A., Sofic, E., McEwen, J., O'Brien, C., & Mainland, C. M. (1998). Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity, and variety of *Vaccinium* species. *Journal of Agricultural and Food Chemistry*, *46*(7), 2686-2693.

- Pusadee, T., Jamjod, S., Chiang, Y. C., Rerkasem, B., & Schaal, B. A. (2009). Genetic structure and isolation by distance in a landrace of Thai rice. *Proceedings of the National Academy of Sciences*, *106*(33), 13880-13885.

- *R Development Core Team (2008). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria*. Available from *http://www.R-project.org*.

- Rambaut A, Suchard MA, Xie D & Drummond AJ (2014) *Tracer v1.6*, Available from *http://beast.bio.ed.ac.uk/Tracer*
- Ribeiro, S. M. R., Barbosa, L. C. A., Queiroz, J. H., Knödler, M., & Schieber, A. (2008). Phenolic compounds and antioxidant capacity of Brazilian mango (*Mangifera indica* L.) varieties. *Food Chemistry*, *110*(3), 620-626.

- Ronquist, F., Teslenko, M., Van Der Mark, P., Ayres, D. L., Darling, A., Höhna, S., & Huelsenbeck, J. P. (2012). MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, *61*(3), 539-542.

- Roy, B. A., & Mulder, C. P. (2014). Pathogens, herbivores, and phenotypic plasticity of boreal *Vaccinium vitis-idaea* experiencing climate change. *Ecosphere*, *5*(3), 1-19.

- Sakai, A., & Larcher, W. (2012). *Frost survival of plants: responses and adaptation to freezing stress* (Vol. 62). Springer Science & Business Media.

- Scalzo, J., Donno, D., Miller, S., Ghezzi, M., Mellano, M. G., Cerutti, A. K., & Beccaro, G. L. (2016). Effect of genotype, medium and light on in vitro plant proliferation of Vaccinium spp. *New Zealand Journal of Crop and Horticultural Science*, *44*(4), 231-246.

- Semerdjieva, S. I., Sheffield, E., Phoenix, G. K., Gwynn-Jones, D., Callaghan, T. V., & Johnson, G. N. (2003). Contrasting strategies for UV-B screening in sub-Arctic dwarf shrubs. *Plant, Cell & Environment*, *26*(6), 957-964.

- Stamatakis, A. (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, *30*(9), 1312-1313.

- Suchard, M. A., & Rambaut, A. (2009). Many-core algorithms for statistical phylogenetics. *Bioinformatics*, *25*(11), 1370-1376.

- Travers, S. E., Smith, M. D., Bai, J., Hulbert, S. H., Leach, J. E., Schnable, P. S., & Garrett, K. A. (2007). Ecological genomics: making the leap from model systems in the lab to native populations in the field. *Frontiers in Ecology and the Environment*, *5*(1), 19-24.

- Turner, T. L., Bourne, E. C., Von Wettberg, E. J., Hu, T. T., & Nuzhdin, S. V. (2010). Population resequencing reveals local adaptation of *Arabidopsis lyrata* to serpentine soils. *Nature Genetics*, *42*(3), 260-263.

- Tuteja, N. (2007). Chapter Twenty-Four. Mechanisms of high salinity tolerance in plants. *Methods in Enzymology*, *428*, 419-438.
- Vyas, P., Curran, N. H., Igamberdiev, A. U., & Debnath, S. C. (2015). Antioxidant properties of lingonberry (*Vaccinium vitis-idaea* L.) leaves within a set of wild clones and cultivars. *Canadian Journal of Plant Science*, *95*(4), 663-669.

- Wang, S. Y. (2007). Antioxidant capacity and phenolic content of berry fruits as affected by genotype, preharvest conditions, maturity, and postharvest handling. *Berry fruit: value-added products for health promotion/edited by Yanyun Zhao*. *Food Science and Technology*, *168*(1), 1-147.

- Wang, S. Y., & Stretch, A. W. (2001). Antioxidant capacity in cranberry is influenced by cultivar and storage temperature. *Journal of Agricultural and Food Chemistry*, *49*(2), 969-974.

- Wang, S. Y., Chen, H., & Ehlenfeldt, M. K. (2011). Antioxidant capacities vary substantially among cultivars of rabbiteye blueberry (*Vaccinium ashei* Reade). *International Journal of Food Science & Technology*, *46*(12), 2482-2490.

- Wiens, J. J., & Moen, D. S. (2008). Missing data and the accuracy of Bayesian phylogenetics. *Journal of Systematics and Evolution*, *46*(3), 307-314.

- Yakimowski, Sarah B., and Christopher G. Eckert. Populations do not become less genetically diverse or more differentiated towards the northern limit of the geographical range in clonal *Vaccinium stamineum* (*Ericaceae*). *New Phytologist* 180.2 (2008): 534-544.

- Zhu, J. K. (2000). Genetic analysis of plant salt tolerance using *Arabidopsis*. *Plant Physiology*, *124*(3), 941-948.

# CHAPTER 4: CONCLUSIONS

Successful cultivation of partridgeberry depends on selecting desired agricultural and nutritious characteristics on plant clones and varieties adapted to different growing conditions.

Partridgeberry individuals were sampled in Newfoundland and Southern Labrador. I evaluated the genetic structure of wild individuals, and its relationship with environmental conditions, phenolic content (TPC), and antioxidant capacity (AC) to provide necessary information on desirable genotypes. This study augmented the knowledge in this area, but still is incomplete. This thesis examined the genetic structure among 56 wild partridgeberry individuals distributed across nine ecoregions of Newfoundland and Labrador. This revision appraised the effects of environmental factors on the TPC and AC of partridgeberry leaves and revealed that, significantly higher TPC on leaves was found in individuals growing under elevated levels of surface water pH (>7). Significantly higher AC was established in individuals from the Central Newfoundland, North Shore Forest, and Maritime Barrens ecoregions and in individuals with low surface water pH (<6.6). Significantly lower AC was uncovered for individuals with low sensitivity to acid rain (alkalinity of >200 μeq/L). Temperature and precipitation had no effect on TPC or AC and there was no correlation between TPC and AC.

While linking genetic structure, biochemical properties and environmental conditions for partridgeberry I found that, individuals formed three genetic groups, which showed some geographic structure according to ecoregion and temperature. Individuals collected in areas with the coldest mean annual and mean summer temperatures were clustered within

one genetic group. Geographically closer individuals were also genetically closer and contained similar quantities of TPC. However, I did not find any correlation between genetic distance and TPC or AC.

My results suggest that TPC and AC for plant breeding programs might be better determined by the environment than by genetics. Future research should look at the environmental effects I found over a longer period of time, under controlled conditions in the field, nurseries or in greenhouses, and the expression of genes in the phenyl propanoid metabolism in partridgeberry leaves and fruits.