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Effects of Latitude-Related Factors and Geographical Origin on Anthocyanidin Concentrations in Fruits of *Vaccinium myrtillus* L. (Bilberries)

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Two data sets are presented to identify the effect of growth location and origin of parental plant on anthocyanidin concentrations in *Vaccinium myrtillus* fruits. Bilberries were collected from wild populations growing at different latitudes and from cultivated plants originating from different geographical locations but grown in the same location for over 10 years. High-performance liquid chromatography analysis showed that anthocyanidin concentrations varied significantly with latitude and with geographical origin, with higher values form northern latitudes or from a more northerly origin of parent plants. The results show that anthocyanidin concentrations in bilberries are under strong genetic control but are also influenced by climatic factors. Furthermore, the proportions of specific anthocyanidins differed between latitudes and between plants with different parental origins. The diversity in anthocyanidin concentration and composition has important implications for plant breeders and for future development of varieties with high antioxidant capacity.

KEYWORDS: Anthocyanidin composition; breeding; genome; climate; cyanidin; delphinidin

INTRODUCTION

Because of their health-promoting effects, the anthocyanin content in fruits and berries is becoming an important quality factor for the industry and the general public. However, the variation of anthocyanin content within and between populations on both spatial and temporal scales can be very high. The concentration and composition of anthocyanins in small berries are influenced by both genetic factors, such as genotype (*1*), and environmental factors, such as temperature (*2*−*4*), light quality (*3*, *5*−*8*), nutrient availability (*9*, *10*), and the timing of harvest (*4*).

Climate can have a great effect on berry-producing plants. Krebs et al. (*11*) showed that in *Vaccinium vitis-idea* and other species, the following year's yield could be predicted from climate factors and previous yields with a certainty of 80−96%. They found no periodicity in berry yields in their study as previously reported by Sedlås (*12*) when looking at a 50 year time line of bilberry yields in Norway. In a 3 year study in northern Sweden, a periodicity was also suggested to explain observed variations of anthocyanidin concentrations in bilberries that could not otherwise be explained by climate parameters (*4*).

Differences in latitude were interpreted by Strong and Redburn (*13*) as a factor causing understory vegetation to respond to cooler and slightly drier conditions and PAR (photosynthetically active radiation) availability to understory species being reduced by 20%. Temperature effects on anthocyanidin concentrations in berries are apparently inconsistent. Low temperatures (10−15 °C) have been reported to increase the amounts of anthocyanidin in berries of various species (*2*, *3*, *14*), and high temperatures (> 30 °C) have been reported to limit their production (*15*, *16*). However, in strawberries, high temperatures (30 °C day, 22 °C night) have been found to be more favorable than low temperatures (18 °C day, 12 °C night) on anthocyanidin concentrations (*17*). In a 2 year study by Rieger et al. (*18*), the anthocyanin contents of *Vaccinium myrtillus* fruits were found to decrease with altitudes increasing from 800 to 1200 m and 1500 m above sea level. Large differences in day and night temperatures have been suggested to promote anthocyanin formation, and this is supported by the results of Mori et al. (*19*) who found that accumulation of red grape skin anthocyanins decreased if night temperatures were elevated from 15 to 30 °C for grapevines grown under 30 °C day temperature. However, in strawberry, the antioxidant capacity increased when night temperatures were increased from 15 to 22 °C with a constant day temperature of 25 °C (*17*).

Anthocyanin production is photoinduced by light in the UV, visible, and far-red wavelengths (*16*) and inhibited by darkness in, for example, apple (*20*). However, UV-B (280−315 nm) light has been proven to inhibit anthocyanidin synthesis in juvenile *Syzygium* leaves due to DNA damage (*21*). Elevated amounts of red light in the light spectra promote anthocyanidin biosynthesis (*22*). Long days and increased amounts of red light in the light spectrum are two factors that typify growing conditions in northern Scandinavia. Moreover, *V. myrtillus* plants from the same clone produced a better fruit set when grown in climate chambers under long day (24 h) than under short day (12 h) conditions (*3*).

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Various studies have been performed to evaluate the natural variation of anthocyanins in bilberries and blueberries (*6*, *23*−*25*) and variation between populations (*26*) including comparisons between populations from different latitudes, which have reported lower total anthocyanin contents in southern (61° N) as compared to in northern (63° N) Finnish bilberries (*27*). However, identifying the cause of anthocyanidin variation in bilberries remains problematic due to the number of different factors that influence the plant and its fruit.

In this paper, we present two studies on environmental and genetic factors that may have a potential influence on the variation of anthocyanidin concentrations in *V. myrtillus* fruits. First, we analyze anthocyanidin concentrations in bilberries from wild populations growing at different latitudes. Second, we analyze bilberry anthocyanidin concentrations in cloned plants grown at a single experimental site and so remove any variation due to differences in the latitude and altitude of the location where the plants are growing. However, these plants were all originally sampled from a variety of known geographical locations, each of which is potentially associated with a characteristic genome. That enabled us to examine the effects of plants' genomes on anthocyanidin concentrations in bilberries in the absence of any differences due to latitude or altitude. This type of information is very scarce, and to our knowledge, this is the first time such information has been presented for anthocyanidins in *V. myrtillus*.

In the present study, we test the following hypotheses: (1) that anthocyanidin concentrations are higher in bilberries sampled in northern than southern latitudes and (2) that anthocyanidin concentrations do not differ in berries obtained from plants acclimatized to and growing at one latitude, independent of them originating from different geographical locations.

MATERIALS AND METHODS

Sampling in Wild Populations at Different Latitudes. During 2007 and 2008, we sampled four wild populations of *V. myrtillus* located at different latitudes: Tjuorre (2), Tönnebro (3), Kungsör (4), and Gammel Rye (5). The most southern population was located in Gammel Rye, Denmark (56°50′ N), and the three other populations were located in southern, central, and northern Sweden at Kungsör (59°25′ N), Tönnebro (61°40′ N), and Tjuorre (66°01′ N), respectively (**Figure 1**). In 2008, bilberries were also collected from Kvikkjokk (1) (66°37′ N), but data from that sample location were only included in the regression analysis since it is only represented by 1 year (**Figure 1**). All sampled populations were healthy stands of *V. myrtillus*, as judged by visible inspection, with no infections of fungus or parasites, growing in open or semishaded conditions under either mature spruce or pine. In each of the 2 years of the study, 10 samples, each comprising a minimum of 10 bilberries, were collected from each of the five locations, making a total of 90 samples. Each sample was collected from a single stem, or if necessary, a few stems close together. The precise sample locations were recorded by GPS in the first year. Except in 2007, when Kungsör was sampled before Gammel Rye, samples were collected progressively from the south to the north as more southerly populations tend to mature earlier in the season (**Table 1**). The sampled bilberries were sealed in plastic tubes (Sarstedt 50 mL) and kept on ice while being transported to the laboratory.

Sampling from Cultivated Plants with Different Geographical Origin. For the study on the potential effects of diverse genomes associated with plants of different "geographical origin", bilberries were collected on July 31, 2007, and August 12, 2008, from plants in an experimental field at the University of Oulu. The *V. myrtillus* plants had been growing in the experimental field since 1997, tissue cultured from seeds collected from plants along a transect ranging from northern Germany to northern Finland. The test field was located at 65°01′ N 25°28′ E (**Figure 1**) and contained 123 different *V. myrtillus* genotypes. The six origins were chosen from the following different geographical locations and latitudes: Utsjoki (1) (69°45′ N), Pallasjärvi (2) (68°01′ N), Muhos (3) (64°48′ N), Parkano (4) (62°02′ N), Lapinjärvi (5) (60°37′ N), and Kiel (6) (54°20′ N) (**Figure 1**). For the present study, between 5 and 10 replicate samples were collected from each origin of the parental plants depending on the amount of fully mature bilberries available. Each sample comprised a minimum of 10 bilberries, and in total, more than 100 samples were collected.

Figure 1. Sampling sites of wild populations (●, 1−5), geographical origin of plants growing on the test field (♦, 1−6), and the location of the test field at Oulu University (★).

Table 1. Sample Collecting Dates in the Study of Wild Populations from Different Latitudes

location	2007	2008
Kvikkjokk (66°57′)		August 21
Tjuorre (66°10′)	August 21	August 28
Tönnebro (61°40′)	July 28	July 23
Kungsör (59°25′)	July 11	July 17
Gammel Rye (56°50′)	July 16	July 9

Sample Treatment and Analysis. In both studies, only bilberries that had developed a uniform dark blue skin color and could be considered fully matured were sampled. All bilberries were collected by hand and were immediately placed on ice for transport to the laboratory where they were stored in darkness at −20 °C until required for analyses.

All extractions and analyses of anthocyanidin concentration were performed at the research facility of Balsgård, Department of Plant Breeding and Biotechnology, Swedish University of Agricultural Sciences (SLU). Material was freeze-dried in a semi-industrial freeze drier (Ehrist, LC3) for 7 days and then finely milled using an electric grinder (IKA A10, Yellow line). From each sample, 50 mg of the milled powder was transferred to sampling tubes (Sarstedt, 13 mL) and then mixed with 2 mL of 2 M HCl to extract the five anthocyanidin aglycones known to occur in bilberries: delphinidin, cyanidin, petunidin, peonidin, and malvidin. For each sample, two analytical replicates were made to allow cross-validation.

The anthocyanidin extraction method followed Åkerström et al. (*4*) and was a modified version of that described by Nyman and Kumpulainen (*28*). In brief, hydrolyzed samples were filtered (Micron, Nylon 0.45), and 0.8 mL of each filtered sample was transferred to 1.5 mL amber glass vials (VWR). Amounts of delphinidin, cyanidin, petunidin, peonidin, and malvidin in each sample were then determined by high-performance liquid chromatography (HPLC) (Shimatzu 460 system with a SPD-M10Avp diode array detector and a Phenomenex Synergi 4 μm Hydro-RP 80A, 250 mm × 4.60 mm column). Gradient elution was performed using 10% formic acid (solution A) and 100% acetonitrite (solution B) in ratios

Table 2. Anthocyanidin Concentrations (Mean ± SE mg g^{-1}) in Fruits of Wild Populations of *V. myrtillus* Originating from Latitudes in 2007 and 2008[a]

			delphinidin		cyanidin		petunidin		peonidin		malvidin		
	total anthocyanidin (mg g^{-1})	SE	mg g^{-1}	%	mg g^{-1}	%	mg g^{-1}	%	mg g^{-1}	%	mg g^{-1}	%	*n*
66°10′	39.71 bA	2.35	15.64b	39	11.77b	30	6.00b	15	1.87b	5	4.43b	11	10
61°40′	24.79 aA	4.08	10.27 a	41	6.58 a	27	4.17 ab	17	0.98 a	4	2.8a b	11	10
59°25′	21.78 aA	0.94	8.53 a	39	7.34 a	34	3.19 a	15	0.9 a	4	1.83 a	8	10
56°50′	20.95 aA	1.5	6.8 a	33	8.37 ab	40	2.79 a	13	1.29 ab	6	1.7 a	8	10
66°57′	22.1	1.2	9.33	42	6.82	31	3.1	14	0.77	4	2.07	9	10
66°10′	25.41 bB	0.92	11.57 b	46	7.63 b	30	3.44 b	14	0.76 a	3	2.01 b	8	10
61°40′	21.69 abB	2.41	9.08 ab	42	6.14 ab	28	3.53 ab	16	0.83 a	4	2.13 ab	10	10
59°25′	15.77 aA	0.83	5.93 a	38	5.04 a	32	2.48 a	16	0.73 a	5	1.59 a	10	10
56°50′	17.39 aB	1.05	6.33 a	36	6.08 ab	35	2.56 a	15	0.92 a	5	1.51 a	9	10
	<0.001		<0.001		0.001		<0.001		0.003		<0.001		
	<0.001		<0.001		0.015		0.002		0.307		0.005		

[a] Lowercase letters indicate significant differences between populations at different latitudes and uppercase letters indicate differences between the studied years at a 95% confidence interval (Tukey's posthoc). Sampled populations are marked with latitude North, and the relative abundance of specific anthocyanidins is expressed as parts of total anthocyanidin (%).

ranging from 96% A:4% B to 20% A:80% B and time intervals of 0, 8, 23, 24, 33, and 37 min, with a flow rate of 0.8 mL min^{-1} and an injection volume 10 μL. The wavelengths used for quantification were 530 nm for delphinidin and 510 nm for cyanidin, peonidin, petunidin, and malvidin. Standard curves were prepared using pure anthocyanidins supplied by Extrasynthese (Lyon, France), dissolved in acidic MeOH (0.1% HCl) in four serial dilutions, with concentrations ranging from 9.0 to 291.0 μg mL^{-1}, depending on the specific anthocyanidin. Total anthocyanidins were calculated as the sum of all five anthocyanidins analyzed. Anthocyanidin concentrations are reported as the mean of the two analytical replicates (mg g^{-1} dry weight). During the extraction of anthocyanidins, some samples from the geographical data set were found to be either too small, not mature enough, or fell out during the extraction for other reasons.

The effects of year and latitude or year and geographical origin on the anthocyanidin levels obtained were tested using statistical procedures in SPSS 16.0. The effects of latitude and geographical origin on concentrations of anthocyanidins, delphinidin, cyanidin, petunidin, peonidin, and malvidin, and total anthocyanidin levels were tested statistically using GLM procedure, with year and latitude as fixed factors for the first data set using data from sites 2−5 [balanced analysis of variance (ANOVA)] or using the GLM procedure, with year and geographical origin as fixed factors for the second data set derived from plants sampled from the Oulu test field (unbalanced ANOVA). In addition, a robust one-way ANOVA was used as a complement for all samples that did not obtain equal variances, but the output did not differ from the results obtained with the GLM procedure; thus, the GLM procedure was used to evaluate differences between treatments. Tukey's posthoc test was enabled to test differences between sample categories. In those cases, equal variance was not obtained; Tamhane's T2 test was enabled as a complementary posthoc (equal variances not assumed). Regression analysis using curve estimation was performed for both data sets and for each year separately. For the latitude data set, site 1 (Kvikkjokk) was only included in the regression analysis for 2008.

RESULTS

Wild Populations. Total anthocyanidin concentrations were significantly affected by both year ($F = 21.260$, $P < 0.001$) and latitude ($F = 18.996$, $P < 0.001$) with higher values in 2007 than 2008 and the highest anthocyanidin concentrations occurring in samples from the most northern Swedish location (Tjuorre, 66°10′ N) (**Table 2**). However, significant interactions between year and latitude occurred both for total and several individual anthocyanidins. Results for each year are therefore presented separately.

The levels of all individual anthocyanidins except peonidin ($P = 0.307$) in 2008 were significantly affected by latitude (Tukey posthoc, $P < 0.05$) (**Table 2**). In 2007, all concentrations of individual anthocyanidins were significantly affected by latitude (Tukey posthoc, $P < 0.05$) (**Table 2**) with higher concentrations at the north Swedish site (Tjuorre) as compared with the south Swedish site (Kungsör).

The relative occurrence of the specific anthocyanidins, irrespective of location, did not differ between years, with delphinidin and cyanidin being the most abundant accounting for 39 and 31% of total anthocyanidins, respectively. Petunidin, peonidin, and malvidin accounted for 15, 4, and 10%, respectively. However, there was a shift in the ratio of relative occurrence between delphinidin and cyanidin depending on location. In the Gammel Rye samples (Denmark, 56°50′ N), cyanidin was more abundant than delphinidin, at 38 and 34%, respectively, while delphinidin was relatively more abundant than cyanidin in samples from the central Swedish location (Tönnebro) at 42 and 27%, respectively, and the northern location (Tjuorre) with 42 and 30%, respectively (**Figure 2**).

Linear regression analyses of total anthocyanidin concentrations against latitude were significant ($P < 0.001$) when both years were treated separately and when they were combined (**Figure 3**), even though variation among samples was rather high (**Table 2** and **Figure 3**). The R^2 values for 2008 and 2007 were 0.388 ($y = 0.9876x - 39.914$) and 0.444 ($y = 2.029x - 96.605$), respectively (**Figure 3**). Linear regression analysis of relative amounts of delphinidin and cyanidin was significant ($P < 0.023$) for both anthocyanidins and for both studied years with R^2 values of 0.426 and 0.125 in 2008 for delphinidin and cyanidin, respectively, and 0.137 and 0.275 in 2007 for delphinidin and cyanidin, respectively (**Figure 2**).

Cultivated Plants. In the data set derived from plants of diverse geographical origin grown at a single site, both year ($F = 112.751$, $P = 0.003$) and origin ($F = 464.333$; $P < 0.001$) were found to be significant when tested against total anthocyanidin concentrations, with higher anthocyanidin concentrations in 2008 than in 2007, and increasing concentrations with increasing latitude of origin (Tukey posthoc) (**Table 3**). Viewing all data together, the most northern Finnish clone (Utsjoki, 69°45′ N) had higher anthocyanidn levels than all other clones, and anthocyanidin levels in the clones from Germany (Kiel, 54°20′ N) and southern Finland (Lapinjärvi, 60°37′ N) were lower than all others. Mean anthocyanidin values in samples from south central Finland (Parkano, 62°02′ N) were higher than in those originating from central (Muhos, 64°48′ N) and north central (Pallasjärvi, 68°02′ N) Finland (**Table 3**).

However, when the 2008 data were analyzed separately, samples of the two genotypes from the north central (Pallasjärvi) and northern (Utsjoki) did not differ from each other significantly ($P = 0.119$), and the genotype from south central Finland (Parkano) did not differ significantly from the genotype originating from central Finland (Muhos) ($P = 0.826$), north central Finland (Pallasjärvi)

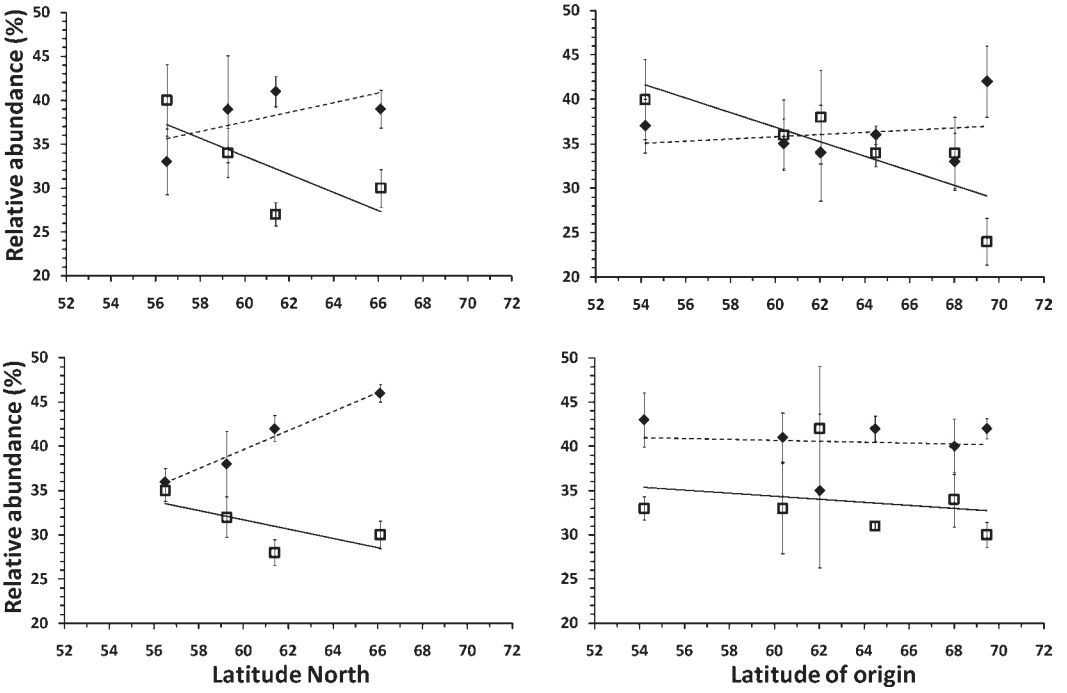

Figure 2. Relative abundance of delphinidin (♦) and cyanidin (□) as a percentage of total anthocyanidin for the latitude study in 2007 (upper left) and 2008 (bottom left) and for the geographical origin study in 2007 (upper right) and 2008 (bottom right).

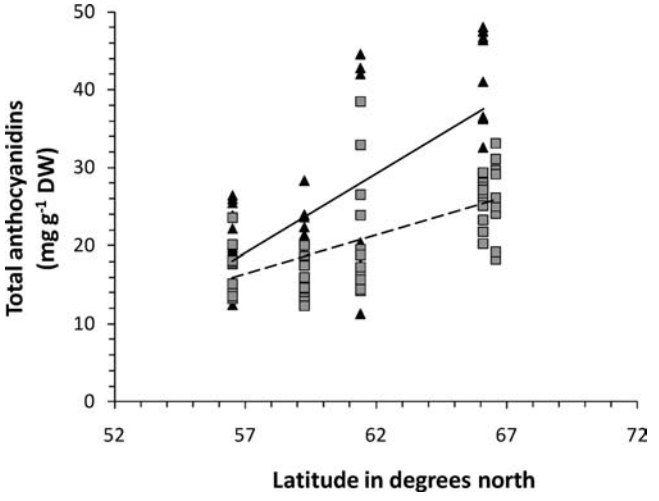

Figure 3. Regression lines for total anthocyanidins in fruits of *V. myrtillus* vs latitude for samples collected in Sweden and Denmark during 2007 (▲; $P < 0.001$, $R^2 = 0.444$, and $y = 2.0294x - 96.605$) and 2008 (■; $P < 0.001$, $R^2 = 0.388$, and $y = 0.9876x - 39.914$).

($P = 1.0$), or northern Finland (Utsjoki) ($P = 0.129$). The German genotype from Kiel differed only from the south central and northern Finnish genotypes, while the south Finnish genotype (Lapinjärvi) differed from all except the German. In 2007, all genotypes differed significantly from each other except the south central Finnish genotype (Parkano) when compared with the north central Finnish genotype (Pallasjärvi) ($P = 0.768$) and the south Finnish genotype (Lapinjärvi) when compared with the central Finnish genotype (Muhos) ($P = 0.950$) (**Table 3**).

Similar to the results for total anthocyanidin, delphinidin and cyanidin concentrations were significantly higher in 2008 than in 2007 ($P < 0.05$). The concentrations of all individual anthocyanidins were strongly affected by the geographical origin of the parental plant ($P < 0.005$) (**Table 3**). Anthocyanidins delphinidin, petunidin, and malvidin showed significant year × origin interactions ($P < 0.05$).

There were fewer significant differences between the different parental origins with respect to cyanidin and peonidin than the other anthocyanidins. Malvidin varied most among the different parental origins but with no significant difference between the Parkano and Muhos clones. Both anthocyanidins cyanidin and peonidin differed significantly between these two origins in 2007, but delphinidin and petunidin did not (**Table 3**). However, in 2008, only cyanidin differed significantly.

The relative abundance of specific anthocyanidins, irrespective of geographical origin, did not differ between years. Anthocyanidins delphinidin and cyanidin were the most abundant and in both years combined accounted for 38 and 35% of total anthocyanidins, respectively. Anthocyanidins petunidin, peonidin, and malvidin accounted for 14, 4, and 9%, respectively, irrespective of parental origin. However, the south central Finnish genotypes (Parkano) contained relatively higher levels of cyanidin than of delphinidin, at 39 and 34%, respectively. Furthermore, the northern Finnish genotypes (Utsjoki) expressed very high relative amounts of delphinidin as compared to cyanidin, at 42 and 27%, respectively (**Figure 2**).

Linear regressions of total anthocyanidin concentrations against geographical origin were significant ($P < 0.001$) when data for years were regressed separately or combined in a single regression (**Figure 4**), even though variation between replicates was rather high (**Table 3** and **Figure 4**). The R^2 values for 2008 and 2007 were 0.295 ($y = 0.7667x - 26.152$) and 0.490 ($y = 1.0x - 42.452$), respectively (**Figure 4**). Linear regression analysis of relative amounts of delphinidin and cyanidin was only significant for cyanidin in 2007 ($P < 0.001$) with a R^2 value of 0.027 and for delphinidin and cyanidin in 2008 ($P < 0.001$), with R^2 values of 0.002 and 0.270, respectively (**Figure 2**).

DISCUSSION

Our hypothesis that anthocyanidin concentrations are higher in bilberries sampled from northern latitudes than southern locations is supported by our results and in agreement with data

Table 3. Anthocyanidin Concentrations (Mean ± SE mg g^{-1}) in Fruits of *V. myrtillus* Originating from Sites at Various Latitudes and Cultivated at Oulu in 2007 and 2008[a]

			delphinidin		cyanidin		petunidin		peonidin		malvidin		
	total anthocyanidin (mg g^{-1})	SE	mg g^{-1}	%	mg g^{-1}	%	mg g^{-1}	%	mg g^{-1}	%	mg g^{-1}	%	*n*
69°45′	32.78 dA	2.33	13.91 e	42	7.95 bc	24	5.74 d	18	1.08 bc	3	4.1 e	13	3
68°01′	23.72 cA	0.42	7.9 cd	33	8.13 c	34	3.43 c	15	1.34 c	7	2.93 d	12	10
64°48′	18 bA	1.39	6.61 bc	36	6.16 ab	34	2.58 b	14	0.8 ab	4	1.89 bc	11	6
62°02′	25.62 cA	1.13	8.63 d	34	9.73 c	38	3.51 c	14	1.32 c	5	2.43 cd	10	10
60°37′	16.75 bA	0.64	5.9 b	35	5.96 ab	36	2.39 b	14	0.91 b	5	1.59 b	10	16
54°20′	12 aA	0.47	4.42 a	37	4.85 a	40	1.5 a	13	0.52 a	4	0.72 a	6	10
69°45′	35.06 cA	1.99	14.77 c	42	10.66 bc	30	5.01 b	14	1.07 a	3	3.55 c	10	2
68°01′	25.31 acA	2.1	10.11 ab	40	8.59 ac	34	3.4 a	13	0.9 a	4	2.32 bc	9	3
64°48′	22.89 abB	1.31	9.5 b	42	7.17 ab	31	3.28 a	14	0.8 a	4	2.14 b	9	8
62°02′	25.95 bcA	1.7	9.14 ab	35	10.86 c	42	3.07 a	12	1.08 a	4	1.8 ab	7	4
60°37′	18.03 aA	0.97	7.36 a	41	5.99 a	33	2.53 a	14	0.69 a	4	1.45 a	8	15
54°20′	18.03 aB	0.61	7.82 ab	43	5.86 a	33	2.5 a	14	0.59 a	3	1.26 a	7	9
	<0.001		<0.001		<0.001		<0.001		0.003		<0.001		
	<0.001		<0.001		<0.001		<0.001		0.011		<0.001		

[a] Lowercase letters indicate significant differences between clones of different geographical origin, and uppercase letters indicate differences between the studied years at a 95% confidence interval (Tukey's posthoc). The origins of plants are marked with latitude North, and the relative abundance of specific anthocyanidins is expressed as parts of total anthocyanidin (%).

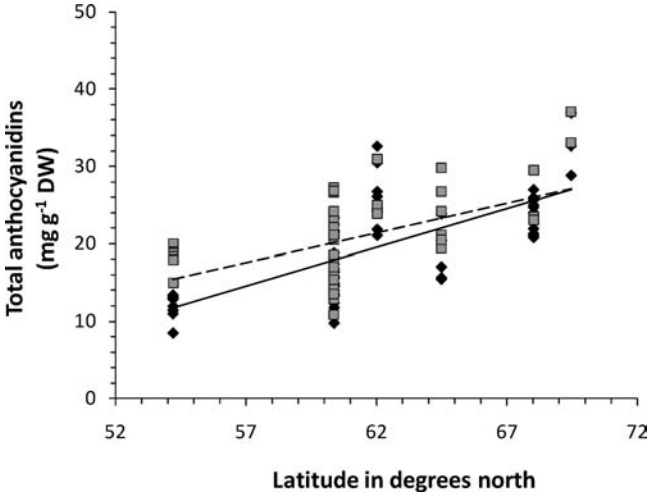

Figure 4. Regression lines for total anthocyanidins in fruits of *V. myrtillus* vs geographical origin of parental plants for samples collected during 2007 (♦; $P < 0.001$, $R^2 = 0.490$, and $Y = 1.0x - 42.452$) and 2008 (■; $P < 0.001$, $R^2 = 0.295$, and $Y = 0.7667x - 26.152$) from plants of different geographical origin but grown at the same location in Finland.

presented by Kähkönen (*26*) and Lätti et al. (*27*), both of whom found lower anthocyanidin levels in populations of bilberry growing in southern as compared to northern Finland. However, it is debatable whether the results obtained are directly caused by latitude specific factors such as day length and spectral composition or whether other climatic conditions, genotype or a combination of these, are also involved. Nonetheless, even without being able to identify the mechanism underlying the observed variation in antocyanidin concentrations, it is clear that bilberries in northern latitudes have higher concentrations than bilberries from more southern locations. The significant interaction term between year and latitude is most probably due to the high level of variation within the data and the lack of any significant difference between years for the southern Swedish location (Kungsör).

Our second hypothesis can be rejected since anthocyanidin concentrations in the bilberries obtained from plants of different geographical origin differed significantly, even though these plants had been grown from seed at the same location and latitude for over 10 years. The gradient of increasing anthocyanidin concentrations from genotypes with southern origins to northern origins indicates a strong contribution from inherent genetic factors in the quantitative and qualitative composition of anthocyanidins in bilberries. Thus, although the different genotypes had been cultivated at the same latitude, they nevertheless inherited the anthocyanidin production potential associated with their parental environments.

The different clones from the geographical origin study can be divided into three major groups based on their anthocyanidin concentrations: a southern group, Kiel (54°20′ N) and Lapinjärvi (60°37′ N); a central group, Parkano (62°02′ N), Muhos (64°48′ N), and Pallasjärvi (68°01′ N); and a northern group, Utsjoki (69°45′ N). The latitudinal range of members of the central group extends 3° north and south of the latitude of the test field at Oulu University (65°01′ N). The northern group lies a further 1°44′ north of the upper bound of the central group, and the southern group lies 1°65′ south of the lower bound of the central group. This grouping may indicate an effect of relocalization since the genotypes that characterize the parent populations are probably optimally adapted to their original environment, climate, and growth conditions. The time spent growing in the test field at Oulu has probably not been sufficient to change the levels of expression of secondary metabolites such as anthocyanidins. As temperature is one of the main factors that varies with latitude, the German and southern Finnish populations may have responded negatively, in terms of their anthocyanidin production, when relocated to the colder more northerly climate between 4°64′ N and 10°81′ N, while the genotype from Utsjoki in northern most Finland may have reacted positively to being relocalized to a warmer environment 4°44′ further south. Such negative effects of cooler growth climate on anthocyanidin concentrations have been observed in strawberries grown in either 18 or 30 °C (*17*).

Differences in day length and the wavelengths of ambient light are much more closely associated with latitude and far less variable than other parameters, for example, temperature. It has been shown that light conditions with long days (24 h) produce more bilberries as compared to light conditions with short days (12 h light) under the same temperature conditions (*3*). Furthermore, long day conditions have been shown to increase anthocyanidin concentrations in cloudberry, *Rubus chamaemorus* (*14*). The significant effect on anthocyanidin concentrations between the genotypes sampled at the Oulu University test

field indicates a strong genetic component in the regulation of anthocyanidin formation. However, the high level of variation between replicates observed in both data sets suggests that anthocyanidin concentrations in bilberries are also strongly influenced by their site of growth. Jaakola et al. (*8*) found that anthocyanidin concentrations in bilberry leaves sampled from a single bush varied depending on whether they were from upper or lower branches of the bush. This difference was attributed to a positive effect of the higher light levels available to leaves in the upper parts of the bush.

Both data sets analyzed in the present study revealed, for most latitudes and geographical origins, variation in anthocyanidin concentrations between years. Among the samples from different latitudes, this variation could be attributed to differences in large-scale climatic patterns. However, no such climate differences could be associated with the data set derived from the bilberries sampled from the single field at Oulu. A natural periodicity in anthocyanidin concentrations has been observed, which may be driven by the cost of producing secondary metabolites as suggested by Åkerström et al. (*4*). The amplitude of this periodicity in anthocyanidin concentration within bilberries may be either increased or diminished by weather conditions. This may explain why some bilberry clones from both data sets reacted differently in the 2 years, and it may further explain the extreme difference in mean anthocyanidin concentrations between years in the samples from Tjuorre (36%), Kungsör (28%), and Kiel (33%) (**Tables 2** and **3**). It is likely, therefore, that the effect of latitude and year on anthocyanin concentration results from a combination of environmental factors due to temperature and light varying between latitudes and between years and genetic factors due to differences in the genome of plants from different geographical locations. The quantitative effect of each factor is, however, hard to evaluate.

Plants from northern latitudes produced bilberries that contained more delphinidin than cyanidin, while the ratio was reversed for plants originating from southern latitudes. The same phenomena have been detected earlier in other studies (*26*, *29*). It has been suggested that in the northern climate, especially increased irradiation in long day conditions, would favor the production of flavonoids with higher hydroxylation level (*30*), which also have been shown to possess greater antioxidant capacity. However, the higher delphinidin to cyanidin ratio was detected also in the more northern clones growing at the same experimental field, which suggest long-term genetic adaptation to northern growth conditions. Furthermore, both daily UV-B and PAR irradiation levels were approximately the same over the geographical sampling area during the studied years according to the Swedish metrological and hydrological institute (SMHI).

The results presented in this study clearly show that there is strong genetic regulation of anthocyanidin concentrations in bilberries. The bilberries sampled from the same experimental field from plants with parents from diverse locations were not subjected to any differences in weather on a large scale; yet, the results still show differences in anthocyanidin concentrations occurring between years and between genotypes. Furthermore, because this genetic influence has probably not diminished since the plants were first planted, there may be interesting possibilities for future plant breeding. The higher concentrations of anthocyanidins in northern genotypes make those bilberries better for health in terms of their antioxidant capacity, which is further enhanced by the fact that delphinidin, as the dominant product in northern bilberries is a more powerful antioxidant than cyanidin (*31*). We believe that this comparative study between latitude-related effects and genome-related effects on anthocyanidin concentrations in bilberries gives a good overview of the complex relationship of anthocyanidin concentrations in *V. myrtillus* fruits and the factors affecting that concentration. The results indicate that growing suitably selected genotypes in controlled conditions could greatly enhance the natural anthocyanidin concentrations in bilberries.

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