

Original Research

Phenotypic Diversity and Variation of *Lonicera caerulea* Populations in the Changbai Mountain Alongside the Elevation Gradient

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Abstract

The shrub, leaf, and fruit traits of natural blue honeysuckle populations at different elevations were measured to explore the key factors influencing phenotypic variation as well as the intra- and inter-population differences in phenotype. Thirteen phenotypic traits were measured at seven elevations (600-1800 m) on Changbai Mountain, China. Analysis of variance indicated that the inter-population variance accounted for 16.2261% of the total variation, whereas the intra-population variance accounted for 14.1178%. Our findings indicated that the leaf and fruit characters varied between and within populations, with the diversity between populations being greater than that within populations. The range in the coefficient of variation (11.9419%-21.7084%) suggested that the phenotypic traits varied to some extent at the species and population level and that these traits demonstrated adaptive potential. The Shannon-Wiener index indicated that the phenotypic diversity of the populations at the individual level was high. The inter-population correlation between phenotypic traits and environmental factors showed that tree height, crown length diameter, crown length diameter/crown short diameter, leaf thickness, leaf shape index, and fruit longitudinal diameter were plastic in response to environmental change. We found that inter-population variation was the main source of phenotypic variation, and the temperature changes associated with altitude primarily influenced the phenotypic changes.

Keywords: *Lonicera caerulea*, Changbai Mountain, elevation gradient, phenotypic diversity, variability

Introduction

Plant phenotypic diversity is the product of the genetic diversity and ecological environment

of a population and is mainly expressed in the morphological features of a population under different habitat conditions within its distribution area [1-3]. Phenotypic and functional traits are plastic in response to the environment, allowing plants to adapt to new habitats [4, 5]. Changes in elevation gradients are associated with significant changes in ecology [6]. For instance, increased elevation is associated with elevated

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precipitation and solar radiation as well as decreased temperature and soil nutrient availability, in addition to vertical changes in vegetation [7, 8]. Environmental heterogeneity can also affect the phenotypic characteristics and reproductive strategies of species [9] and further affect their genetic variation [10]. As the foundation of genetic diversity research, phenotypic changes can reflect genetic and environmental diversity, and the phenotype is the most intuitive embodiment of genetic variation [11, 12]. Phenotypic evaluation is the simplest and most feasible method for detecting the genetic diversity of a population based on the differences in phenotypic traits [13, 14]. Estimating genetic parameters using statistical procedures can be used to effectively evaluate intra- and interpopulation genetic diversity of plants under different habitat conditions [15, 16].

Changes and gradients in habitat conditions force plant populations to either adapt or move [17]. In mountainous areas, rapid changes in abiotic factors over short distances can produce significant changes in the selection pressure for life cycle strategies [18, 19]. For instance, the size of *Rosmarinus officinalis* flowers increases with increases in elevation [20]. In some plants, leaf length, width, and area decrease with increased elevation, whereas leaf thickness and root length increase [21, 22]. Phenotypic plasticity is a fundamental feature of phenotypic evolution [23], and morphological plasticity is an irreversible, long-term change [24]. An elevation gradient provides an experimental opportunity to study plant morphological and physiological responses to the environment [25, 26]. Studying plant phenotypic diversity along an altitude gradient allows us to determine the genetic variation of a species as well as evaluate the potential roles of environmental factors in the ecological adaptation and genetic differentiation of a species.

Blue honeysuckle (*Lonicera caerulea* L., Caprifoliaceae) is native to Europe, Asia, and the United States [27] and is also distributed in northeast and north China [28]. It is an important small-berry species, the fruit of which contains a variety of nutrients and antioxidant substances that are used in medicine,

food, and condiments [29, 30]. Therefore, it having greater agronomic development potential [31]. However, the changes in the phenotypic traits of this species to elevation gradients have not been explored despite such information being critical for the conservation and sustainable use of wild *Lonicera caerulea* resources. Blue honeysuckle provides an excellent study system for assessing the contribution of altitude and genetic factors to inter- and intra- population diversity. We used scientific methods to sample and measure for it without damaging its growth and development through field investigation and demonstration. In this study, blue honeysuckle populations growing at different altitudes in the Changbai Mountain Nature Reserve were sampled for morphological analysis in order to characterize the response of this species along an elevation gradient. The principal factors influencing the phenotypic characteristics and phenotypic diversity of this species were also evaluated. Our findings provide a reference for the germplasm protection, wild resource evaluation, and sustainable exploitation and utilization of this species.

Material and Methods

Study Site

Changbai Mountain is the highest mountain in northeast China (124°47'-131°19'E 40°52'-44°31'N). It is influenced by horizontal zonal factors (terrain, climate, soil, etc.) and geological historical conditions, particularly non-zonal terrain factors. The climate changes with increases in elevation, resulting in a clear vertical plant distribution. From the bottom to the top, five vertical vegetation zones can be clearly distinguished, including broadleaved forest (<500 m), coniferous and broadleaved mixed forest (500-1100 m), coniferous forest (1100-1700 m), *Betula ermanii* Cham. forest (1700-2100 m), and alpine tundra (>2100 m). Based on a comprehensive survey of the distribution of blue honeysuckle, we selected an area with a continuous honeysuckle distribution and obvious elevation gradient

Table 1. General information of the seven altitudinal populations of wild blue honeysuckle in Changbai Mountain Nature Reserve, Jilin Province.

Population	Altitude/m	Longitude	Latitude	Average annual temperature (°C)	Mean annual precipitation (mm)	June to September precipitation (mm)
A1	600	127°54.571'	42°41.468'	3.35	640.68	455.01
A2	800	128°05.996'	42°20.557'	2.32	703.62	500.40
A3	1000	128°10.497'	42°14.530'	1.29	755.19	537.07
A4	1200	128°07.916'	42°08.688'	0.27	810.53	576.43
A5	1400	128°04.703'	42°05.400'	-0.75	869.92	618.67
A6	1600	128°03.876'	42°04.760'	-1.78	933.67	664.01
A7	1800	128°03.925'	42°03.853'	-2.80	1002.09	712.67

Table 1. Continued.

Population	>5°C accumulated temperature (°C)	January average temperature (°C)	July average temperature (°C)	Annual frost-free period	Frost accumulation days	Drying index	Moisture index
A1	2649.06	-17.00	20.16	123.37	124.02	0.69	1.58
A2	2285.25	-17.64	19.07	116.50	137.58	0.59	2.21
A3	1972.49	-18.27	17.95	108.12	151.16	0.53	2.82
A4	1702.53	-18.89	16.84	100.31	164.73	0.47	3.43
A5	1469.52	-19.52	15.73	93.06	178.31	0.42	4.04
A6	1268.40	-20.15	14.61	86.34	191.88	0.37	4.65
A7	1094.81	-20.77	13.50	80.10	205.46	0.33	5.25

A1: population at altitude of 600 m; A2: altitude of 800 m; A3: altitude of 1000 m; A4: altitude of 1200 m; A5: altitude of 1400 m; A6: altitude of 1600 m; A7: altitude of 1800 m. The same applies below.

as the test area. Standard plots were set using the gradient pattern method [32], and a sample line was set within a 600 m to 1800 m elevation range. Sample plots were set at every 200-m-increase in elevation, resulting in a total of seven populations. The population numbers of each altitude segment and the environmental characteristics of the collection sites are shown in Table 1.

Measurement of Phenotypic Traits

We conducted field surveys from mid-June to late July of 2018. Samples were collected on the north slope of Changbai Mountain, and horizontal sampling was conducted from east to west for each altitude population. For each altitude population, 30 10-year-old blue honeysuckle plants exhibiting good growth and that were free from pests were randomly selected as the sample plants, with the distance between the sample plants being greater than 10 m. Thirteen traits were measured, including shrub traits: height of the tree (TH), crown length ratio (CL), crown short diameter (CS), and crown ratio of length diameter to short diameter (LS); leaf traits: leaf length (LL), leaf width (LW), leaf thickness (LT), leaf area (LA), leaf shape index (LI); and fruit traits: fruit longitudinal diameter (FL), fruit transverse diameter (FT), fruit shape index (FI), and fruit weight (FW). A standard branch consisting of four directions (east, south, west, and north) was collected from the top of each shrub, and the leaves (10 in each direction) were collected from each branch to study the leaf characteristics. A Vernier caliper was used to measure the blade length from the base of the petiole to the tip, as well as to measure the blade width at the maximum width of the blade. Vernier calipers were also used to measure the leaf thickness (avoiding the veins), using the center of the blade as far as possible. LA was measured with a portable leaf area meter (CI-202, ICT International). Twenty fruits were collected from each plant to assess the morphological characteristics. FL and FT were measured with a Vernier caliper. The fruit

shape index was measured as the ratio of FL to FT. The tree characters were measured with a tape measure.

Statistical Analysis

Nested and multiple comparisons of the 13 phenotypic traits in the seven populations were analyzed with SAS 9.0 (SAS INS. INC, NCSU, USA) using the linear model $Y_{ijk} = \mu + \alpha_i + \beta_{j(i)} + \epsilon_{ijk}$, where Y_{ijk} is K^{th} observation of the J^{th} of the i^{th} population; μ represents the population mean; α_i is the effect value of the i^{th} population; $\beta_{j(i)}$ is the effect value of the J^{th} individual in the i^{th} population; and ϵ_{ijk} is the experimental error of the ijk^{th} observed value. SPSS 19.0 software (IBM Corp., Armonk, NY, USA) was used for correlation analysis of the phenotypic traits of each population, as well as for the correlation analysis of phenotypic traits and environmental factors. Pearson's correlation coefficients and a double-tailed tests were used for analysis. The mean value, extreme value, range, standard deviation, coefficient of variation (CV), coefficient of phenotypic differentiation (Vst), and Shannon-Wiener (H') diversity index were calculated. CV indicates the dispersion degree of trait variation, which is calculated by the formula: $CV = \text{standard deviation} \times 100\% / \text{mean}$. Vst is used to describe the contribution of phenotypic variation in a population to diversity. The formula $V_{st} = \sigma_{t/s}^2 / (\sigma_{t/s}^2 + \sigma_s^2)$ was used, where $\sigma_{t/s}^2$ and σ_s^2 are the variances between and within populations. The Shannon-Wiener index was used to describe the degree of variation in trait diversity using the formula:

$$H' = \sum_{i=1}^n (P_i \ln P_i)$$

In the formula, P_i is the effective percentage of the distribution frequency in the material of grade i for a certain character. As for character classification, the measured traits were divided into 10 levels, with $<X-2\sigma$ as the first level and $\geq X+2\sigma$ as the 10th level.

Table 2. Phenotypic variation of wild blue honeysuckle at different altitudes.

Character	Code	Min	Max	Average	Extreme difference value	Standard deviation	Coefficient of variation (%)	Shannon-Wiener index
Tree height/m	TH	0.100	1.900	1.138	1.800	0.294	25.8348	1.916
Crown length diameter/m	CL	0.400	2.000	1.033	1.600	0.291	28.1704	2.038
Crown short diameter/m	CS	0.400	1.800	0.824	1.400	0.259	31.4320	1.741
Crown ratio of length diameter to short diameter	LS	1.000	1.800	1.271	0.800	0.179	14.0834	1.922
Leaf length/mm	LL	29.000	87.000	55.444	58.00	10.308	18.5917	2.048
Leaf width/mm	LW	13.000	37.000	23.304	24.00	4.317	18.5247	2.040
Leaf thickness/mm	LT	0.115	0.255	0.172	0.140	0.033	19.1860	2.051
Leaf area/mm ²	LA	1.125	17.724	8.155	16.599	2.851	34.9601	2.051
Leaf shape index	LI	1.611	3.346	2.400	1.735	0.330	13.7500	2.045
Fruit transverse diameter/mm	FT	4.560	10.690	7.580	6.130	0.983	12.9683	2.082
Fruit longitudinal diameter/mm	FL	7.980	26.140	15.417	18.160	2.761	17.9088	2.068
Single fruit weight/g	FW	0.102	1.056	0.487	0.954	0.151	31.0062	2.035
Fruit shape index	FI	1.147	3.961	2.052	2.814	0.377	18.3723	2.034

σ is the standard deviation of the measured value of the character, with a difference of 0.5σ in each step.

Results and Discussion

Phenotypic Variation of Different Blue Honeysuckle Populations

The phenotypic variation of blue honeysuckle populations is shown in Table 2. The CV values of the 13 phenotypic traits ranged from 12.9683% to 34.9601%, with an average of 21.9068%. The largest CV was LA (34.9601%), which ranged from

1.611 mm²-17.724 mm². Fruit transverse diameter (12.9683%) showed the smallest range of variation, ranging from 4.560 mm to 10.690 mm. The characters with a variation range greater than 30% included crown diameter (31.4320%), leaf area (34.9601%), and single fruit weight (31.0062%). The CV range from large to small was as follows: LA>CS>FW>CL>TH>LT>LW>FI>LL>FT>LS>LI>FL. Based on the CV values, it is evident that the phenotypic traits are relatively stable. The phenotypic traits do not vary greatly at the population level, and there is little geographic population differentiation.

The H' index of the 13 phenotypic traits ranged from 1.741-2.082, with an average of 2.005. The H'

Table 3. Variance analysis of eight phenotypic traits in the seven populations.

Traits	Mean squared			F	
	Among population	Within populations	Random errors	Among population	Within population
LL	1255.4475	199.9585	71.8165	17.48**	2.78**
LT	0.0192	0.0048	0.0003	55.12**	13.64**
LA	118.8355	12.5703	5.1927	22.88**	2.42**
LI	0.3466	0.306	0.0871	3.98**	3.51**
FW	0.0614	0.0401	0.0187	3.28**	2.14**
FL	3.8446	1.6238	0.1914	4.21**	1.78*
FT	26.0569	12.3626	6.7407	3.87**	1.83*
FI	0.5077	0.1902	0.1359	3.73**	1.40

*: $P<0.05$; **: $P<0.01$, the same applies below.

Table 4. Variance components and differentiation coefficients of eight phenotypic traits among and within the populations.

Traits	Variance component			Percentage of variance component (%)			Differentiation coefficients of phenotypic traits (Vst) (%)
	Among population	Within population	Random errors	Among population	Within population	Random errors	
LL	25.8572	13.0113	71.8165	23.3611	11.7553	64.8836	66.5248
LW	6.3500	1.7316	11.5732	32.3065	8.8103	58.8831	78.5725
LT	0.0004	0.0004	0.0003	30.7703	38.9145	30.3152	44.1564
LA	2.6040	0.7491	5.1927	30.4711	8.7657	60.7633	77.6595
LI	0.0010	0.0222	0.0876	0.8767	20.1625	78.9608	4.1670
FW	0.0005	0.0022	0.0187	2.4189	10.1397	87.4414	19.2609
FL	0.0543	0.0721	0.9139	5.2241	6.9288	87.8471	42.9864
FT	0.3349	0.5708	6.7407	4.3804	7.4653	88.1543	36.9788
FI	0.0078	0.0055	0.1359	5.2096	3.655	91.0949	58.7686
Mean (SD)	-	-	-	16.2261 (14.2003)	14.1178 (10.8600)	69.6561 (20.1481)	47.6767 (23.99634)

of fruit transverse diameter (2.082) was the highest, whereas tree crown short diameter (1.741) was the lowest. The H' of the 13 phenotypic traits ranging from high to low was as follows: FT>FL>LA>LT>LL>LI>

LW>CL>FW>FI>LS>TH>CS. The results suggested that there was abundant phenotypic variation at the individual level, thus providing some direction for the development of conservation strategies. The extreme

Table 5. Coefficient of variation of the phenotypic traits in seven populations of wild blue honeysuckle.

Traits	Populations (%)							Mean	SD
	A1	A2	A3	A4	A5	A6	A7		
TH	8.1312	10.2161	25.1037	34.5954	36.8546	22.6117	18.0805	22.2276	10.2505
CL	9.9979	14.4088	24.3888	37.0810	25.8199	40.2766	17.0529	24.1466	10.5266
CS	12.1405	14.9512	28.0818	42.1648	22.4382	49.0998	15.9749	26.4073	13.2310
LS	5.6952	3.9827	15.6696	11.5767	14.0490	17.8101	13.7580	11.7916	4.7572
Mean (SD)	8.9912 (2.3735)	10.8897 (4.3886)	23.3110 (4.6240)	31.3545 (11.7401)	24.7904 (8.1779)	32.4496 (12.7435)	16.2166 (1.6029)	21.1433 (8.6782)	-
LL	9.9492	18.3241	12.5736	18.9946	18.1677	17.9443	17.1895	16.1633	3.2167
LW	10.6352	15.3919	15.4268	15.0177	20.5463	16.2739	15.1666	15.4941	2.6750
LT	11.8071	13.3491	9.8795	19.0271	19.8519	11.2063	15.5124	14.3762	3.6022
LA	19.9285	32.0765	24.1168	31.0472	34.3840	34.0531	28.4907	29.1567	4.9915
LI	6.0701	13.1823	13.9243	13.8256	14.0084	14.3095	15.6653	12.9979	2.9137
Mean (SD)	11.6780 (4.5522)	18.4648 (7.0543)	15.1842 (4.8248)	19.5824 (6.1004)	21.3917 (6.8818)	18.7574 (7.9698)	18.4049 (5.0904)	17.6376 (5.8587)	-
FT	12.0002	13.3058	13.3547	12.2577	12.9047	11.6185	14.1148	12.7938	0.8135
FL	17.9342	16.7237	18.3278	15.3064	17.6961	16.2563	18.0451	17.1842	1.0332
FW	28.8087	34.8176	32.7158	29.2787	27.3060	27.5178	30.2464	30.0987	2.5617
FI	2.1470	2.1428	2.1425	2.0365	2.0715	1.9410	1.8833	2.0521	0.0977
Mean (SD)	15.2225 (9.6604)	16.7475 (11.7438)	16.6352 (10.9801)	14.7198 (9.7370)	14.9946 (9.0863)	14.3334 (9.1988)	16.0724 (10.1237)	15.5322 (10.0514)	-
Total mean (SD)	11.9419 (6.6660)	15.6056 (8.8256)	18.1312 (8.0568)	21.7084 (11.4582)	20.4691 (8.9158)	21.6091 (12.4903)	17.0139 (6.5958)	18.0685 (7.6740)	-

Table 6. The Shannon-Wiener index of the phenotypic traits in seven populations of wild blue honeysuckle.

Traits	Populations						
	A1	A2	A3	A4	A5	A6	A7
TH	1.0549	1.3321	1.8344	1.8867	1.4708	1.5571	1.4979
CL	0.9503	1.3322	1.7481	1.6434	1.6957	1.6957	1.6094
CS	1.3322	1.3322	1.7481	1.4708	1.6434	1.6434	1.5048
LS	1.3322	1.3322	1.6957	1.5048	1.6094	1.4979	1.7481
Mean (SD)	1.1674 (0.1689)	1.3322 (0.0000)	1.7566 (0.0498)	1.6264 (0.1636)	1.6048 (0.0833)	1.5685 (0.0763)	1.5901 (0.1014)
LL	2.0306	1.7704	1.9859	1.9377	2.0357	1.9926	1.9804
LW	1.9431	1.9413	1.9724	1.8076	1.9865	1.9331	1.9664
LT	1.3322	1.6094	1.5475	1.6434	1.5571	1.6957	1.4271
LA	1.9769	1.9306	2.0246	2.0505	2.0163	2.0771	1.9836
LI	2.0549	2.0091	1.9678	2.0728	2.0495	1.9505	2.0441
Mean (SD)	1.8675 (0.2705)	1.8522 (0.1445)	1.8996 (0.1772)	1.9024 (0.1602)	1.9290 (0.1872)	1.9298 (0.1272)	1.8803 (0.2282)
FT	2.0278	2.0363	2.0569	2.0127	1.9798	1.9882	2.0479
FL	2.0470	2.0571	2.0596	2.0059	2.0132	2.0335	2.0397
FW	2.0186	1.1925	1.9181	2.0316	1.9341	1.9935	2.0400
FI	2.0377	1.9849	2.0429	2.0406	1.7863	2.0097	1.9450
Mean (SD)	2.0328 (0.0106)	1.8177 (0.3619)	2.0194 (0.0588)	2.0227 (0.0140)	1.9284 (0.0867)	2.0062 (0.0176)	2.0182 (0.0424)
Total mean (SD)	1.7030 (0.4112)	1.6816 (0.3206)	1.8925 (0.1567)	1.8545 (0.2092)	1.8291 (0.2007)	1.8514 (0.1937)	1.8334 (0.2309)

value ratio of plant phenotypic traits typically reflects the evolutionary and adaptative potential of traits in different environments [33]. The maximum value of the 13 phenotypic traits was 1.80 (fruit longitudinal diameter), which is approximately 19.00 (tree height) times as much as minimum value. The evolutionary and adaptative potential in descending order was as follows: TH>LA>FW>CL>CS>FI>FL>LL>LW>FT>LT>LI>LS. The results showed that TH, LA, and FW had high evolutionary or adaptive potential, whereas the ratio of LS had the least evolutionary and adaptive potential.

Source and Differentiation of the Phenotypic Variation

Table 3 shows the ANOVA results of the eight phenotypic traits of the blue honeysuckle leaves and fruits across and within populations. FL and FT differed significantly within populations and were highly significantly different across populations, while the other traits were all highly significantly different both within and across populations, indicating variation in the morphological characters. The phenotypic

Table 7. Phenotypic traits and multiple comparisons of the seven wild blue honeysuckle populations (mean±SD).

Population	TH	CL	CS	LS	LL	LW	LT
A1	1.1±0.05 ^a	0.98±0.05 ^a	0.84±0.05 ^a	1.17±0.03 ^a	61.44±0.87 ^b	25.58±0.42 ^c	0.15±0.003 ^a
A2	1.09±0.04 ^a	1.02±0.05 ^a	0.78±0.04 ^a	1.31±0.02 ^a	50.7±1.49 ^a	21.05±0.52 ^a	0.15±0.003 ^a
A3	1.14±0.09 ^a	1.07±0.09 ^a	0.84±0.08 ^a	1.3±0.07 ^a	51.63±1.04 ^a	21.8±0.54 ^a	0.17±0.003 ^b
A4	1.08±0.12 ^a	1±0.12 ^a	0.87±0.12 ^a	1.17±0.04 ^a	51.8±1.58 ^a	21.53±0.52 ^a	0.17±0.005 ^b
A5	1.03±0.13 ^a	0.9±0.08 ^a	0.72±0.05 ^a	1.26±0.06 ^a	49.93±1.45 ^a	20.9±0.59 ^a	0.19±0.006 ^c
A6	1.22±0.09 ^a	1.03±0.14 ^a	0.81±0.13 ^a	1.33±0.08 ^a	58.95±1.69 ^b	24.2±0.63 ^b	0.18±0.003 ^b
A7	1.29±0.08 ^a	1.21±0.07 ^a	0.92±0.05 ^a	1.32±0.06 ^a	62.92±1.83 ^b	25.19±0.65 ^b	0.21±0.005 ^d

Table 7. Continued.

Population	LA	LI	FL	FT	FW	FI
A1	9.8±0.28 ^{cd}	2.23±0.02 ^a	7.82±0.09 ^c	16.62±0.3 ^a	0.56±0.016 ^a	2.15±0.042 ^c
A2	5.96±0.31 ^a	2.42±0.05 ^b	7.46±0.1 ^{ab}	15.84±0.27 ^b	0.49±0.017 ^b	2.14±0.038 ^c
A3	6.95±0.27 ^{ab}	2.4±0.05 ^b	7.34±0.1 ^a	15.69±0.29 ^{bc}	0.49±0.016 ^b	2.14±0.033 ^{cd}
A4	7.54±0.37 ^b	2.41±0.05 ^b	7.45±0.09 ^a	15.06±0.23 ^{bc}	0.46±0.014 ^{bc}	2.04±0.031 ^c
A5	6.94±0.38 ^{ab}	2.42±0.05 ^b	7.62±0.1 ^{abc}	15.65±0.28 ^{bc}	0.48±0.013 ^{bc}	2.07±0.039 ^c
A6	9.16±0.5 ^c	2.45±0.06 ^b	7.76±0.09 ^{bc}	14.94±0.24 ^b	0.48±0.013 ^{bc}	1.94±0.032 ^{ab}
A7	10.58±0.51 ^d	2.52±0.07 ^b	7.61±0.11 ^{abc}	14.11±0.26 ^a	0.44±0.013 ^a	1.88±0.040 ^a

differentiation coefficient V_{st} reflects the variation among populations and can estimate the phenotypic differentiation in each population. As indicated in Table 4, the differentiation coefficients of the eight phenotypic traits ranged from 4.1670% to 78.5725%. The inter-population variance component accounted for 16.2261% of the total variation, while the intra-population variance component accounted for 14.1178%. The results demonstrated that there was a certain degree of variation in the eight traits between and within populations, with the inter-population variance being the main source of phenotypic variation.

Variation in Phenotypic Traits at Different Altitudes

According to Table 5, the CV values of the seven populations ranged from 11.9419% (A1) to 21.7084 (A4), with an average of 18.0685%. All were above 15%, except for the A1 population, indicating that the phenotypic trait variation in the populations was

high. The CV values of the tree phenotypic traits were greater than that of the fruit and leaf traits; for instance, the variation coefficient of the crown length and crown width of the A6 population exceeded 40%. The phenotypic variation of the A4, A5, and A6 populations was relatively high, while the A1 population was the lowest. The variation coefficient of the phenotypic traits of A1 and A2 was lower than that of the other populations, which may be attributed to the similar environment at low altitudes. The variation in FW was greatest, while LS was the lowest. The average CV values of the phenotypic traits at the species level, with the exception of CL, LT, LI and FL, were all greater than 15%, indicating that these four traits were relatively stable. In summary, the phenotypic traits at different elevations varied at both the species and population levels, exhibiting a degree of evolutionary potential.

The H^2 of the various groups ranged between 1.6816 (A2) and 1.8925 (A3) (Table 6). For tree phenotypic traits, A3 was the largest and A1 was the smallest; for leaf phenotypic traits, A6 was the largest and A2

Table 8. Correlation analysis of wild blue honeysuckle population phenotypic characters.

Phenotypic	TH	CL	CS	LS	LL	LW	LT
TH	1						
CL	0.682**	1					
CS	0.652**	0.900**	1				
LS	0.045	0.115	-0.305*	1			
LL	-0.228	-0.195	-0.197	0.104	1		
LW	-0.295*	-0.239	-0.205	0.007	0.739**	1	
LT	-0.059	-0.087	-0.180	0.253*	0.234**	0.024	1
LA	-0.263	-0.208	-0.168	0.007	0.838**	0.784**	0.216**
LI	0.153	0.091	0.017	0.189	0.368**	-0.343**	0.299**
FL	0.055	0.093	0.042	0.042	-0.019	0.011	-0.170**
FT	0.214	0.139	0.138	-0.022	0.045	0.036	-0.131*
FW	0.090	0.000	-0.022	-0.006	0.046	0.063	-0.179**
FI	0.196	0.084	0.109	-0.029	0.051	0.015	-0.004

Table 8. Continued.

Phenotypic	LA	LI	FL	FT	FW	FI
LA	1					
LI	0.074	1				
FL	-0.027	-0.024	1			
FT	0.025	0.032	0.347**	1		
FW	0.017	-0.007	0.73**	0.719**	1	
FI	0.028	0.057	-0.369**	0.734**	0.176**	1

* Indicates significant correlation at the 0.05 level; ** indicates significant correlation at the 0.01 level.

was the smallest; and for fruit phenotypic traits, A1 was the largest and A2 was the smallest. The H' of each group was ranked from high to low as follows: A3>A4>A6>A7>A5>A1>A2. The phenotypic diversity of the A1 and A2 populations was low, while that of the other populations was comparatively high, suggested that the higher altitudes constitute key areas for resource collection.

Variation in Phenotypic Traits in Different Populations

The variations in the 13 traits in each population are shown in Table 7. There were no significant differences in the four characters of SH, CL, CS, and LS among the different populations, while the other nine characters differed significantly among the different populations ($P<0.05$). The LW, FT, FL, FI and FW of the A1 population were the largest, while LT and LI were the smallest, indicating that this population had the characteristics of large fruits and small, thin leaves. The A5 population had the lowest TH, CL, CS, LL, and LW, indicating that this population exhibits a stunted tree body, a small leaf blade, and a short petiole. The LS of the A6 population was the largest, indicating that the tree crown is almost oval in shape. The TH, CL, CS, LL, LI, LT, and LA of the A7 population were the largest, whereas FT, FI, and FW were the smallest, indicating that this population is characterized by a tall body, large and thick leaves, and small fruit. The phenotypic indexes of the A2, A3, and A4 populations were all moderate, with no special phenotypic characteristics observed.

Correlations between Phenotypic Traits

According to Table 8, TH was significantly positively correlated with CL and CS and negatively correlated with LW. There was a significant positive correlation between CL and CS, and a significant negative correlation between CS and LS. Taller individuals tended to have longer crown diameters and shorter diameters, but smaller blade widths. LL

was positively correlated with LW, LT, LA, and LI. LW was significantly positively correlated with LA, but significantly negatively correlated with LI. LT was significantly positively correlated with LA and LI, and significantly negatively correlated with FT, FW, and FL. The results suggested that an increase in LL results in an increase in other blade index values. Moreover, the blade correlation index was more greatly affected by the change in blade thickness. FT was positively correlated with FL and FW, and negatively correlated with FI, while FL was positively correlated with FW and FI. There was a significant positive correlation between FW and FI. The results showed that FW and FI were mainly influenced by FT and FL.

Correlations of the Phenotypic Traits with Geographical Factors

Table 9 shows that numerous traits were strongly correlated with geographical factors. However, the three traits of CS, LL, and FT were not correlated with any of the geographical factors, indicating that these three traits are relatively stable and not easily affected by habitat conditions. There was no correlation between TH and annual average temperature; CL was negatively correlated with accumulated temperature greater than 5°C; and there was no correlation between LS and elevation. However, these three traits were significantly positively or negatively correlated with other environmental factors. LW was correlated with accumulated temperature greater than 5°C and positively correlated with drying index, but not with other environmental factors. LT and LI were positively correlated with all environmental factors. LA was not correlated with accumulated temperature or drying index greater than 5°C, but extreme significant positive and negative correlations between LA and other environmental factors were observed. FL and FI were significantly positively or negatively correlated with all environmental factors, while FW was significantly positively correlated with all environmental factors. As indicated above, as the elevation gradient increased, temperature indexes such as the annual average temperature decreased, and temperature-sensitive

morphological indexes changed rapidly to reduce the influence of extreme weather on growth.

Discussion

Variation and stability in plant phenotypic traits are closely related to the genetic characteristics of the species and its environment [34, 35]. For instance, the temperature, water, light, and nutrients of different environment can response plant traits change [36]. Across an elevation gradient, plant phenotypic traits exhibit diversity in order to tolerate the abiotic stress caused by the environment [37, 38]. An earlier study found that phenotypic plasticity allows for rapid responses to environmental changes, but has little evolutionary potential [38]. In this study, leaf and fruit traits were found to differ significantly both between and within populations (Table 3, 4). The phenotypic differentiation coefficient V_{st} showed that inter-population variation was the main source of the phenotypic variation. These findings also demonstrated that the blue honeysuckle populations at different elevation gradients responded to environmental changes by altering their phenotypic traits.

The CV reflects the degree of phenotypic dispersion of different populations of the same species, while H' represents the diversity of plant germplasm resources [39, 40]. In this study, the CV values of the phenotypic traits in the different populations indicated that the populations at low elevations were less diverse, whereas the populations at middle and higher elevations were comparatively more diverse. The CV values of each population at altitude were similar to the normal distribution, and the distribution trend of Shannon-

Winner was similar in the phenotypic traits of each population at altitude. The temperature and precipitation of middle elevation environments are more complex in Changbai Mountain [41], thus, environmental variability leads to greater variation in phenotypic traits. This might explain why the phenotypic of middle elevation was high. The phenotypic traits of blue honeysuckle varied greatly, but the distribution of the phenotypic traits was unbalanced. In other words, the distribution of the phenotypic traits of each population was relatively concentrated, and the proportion of variable traits was small. This suggests that individuals with unique phenotypic traits should be paid greater attention and collected and preserved for breeding applications.

As a result of increased solar radiation intensity and decreased nutrient availability in the soil with elevation, leave traits will change in order to tolerate the more extreme conditions [42]. The LT increased with elevation, which is consistent with the trend observed in other plants [43, 44]. Increased blade thickness is an adaptive mechanism in response to the environmental changes at high altitude [21]. For instance, changing leaf traits allows plants to absorb nutrients from the environment faster, and to improve their stress resistance and life span [45]. In addition, LT plays an important role in carbon assimilation [46]. The carbon assimilation rate per unit leaf area is higher in nutrient-deficient high-altitude regions [47]. This mechanism results in an increase in leaf longevity with the increase in leaf thickness, which prolongs the period for nutrients to remain in the leaf [48] and thus supports plant growth. We also found that the LW of the various populations increased with elevation, and the leaf thickness was positively correlated with elevation.

Table 9. Correlation analysis of phenotypic traits and geographical factors in wild blue honeysuckle populations.

Phenotypic	Altitudes	Year average temperature	Mean annual precipitation	>5°C accumulated temperature	June to September amount of precipitation	January average temperature
TH	0.2093**	-0.2090	0.2177**	-0.1778**	0.2174**	-0.2091**
CL	0.1848**	-0.1844**	0.2079**	-0.1280*	0.2075**	-0.1842**
CS	0.0625	-0.0618	0.0854	0.0006	0.0848	-0.0609
LS	0.1738	-0.1743**	0.1750**	-0.1797**	0.1753**	-0.1753**
YC	0.0839	-0.0833	0.0951	-0.0262	0.0942	-0.0821
LW	-0.0887	0.0894	-0.0786	0.1512*	-0.0797	0.0908
LT	0.5449**	-0.5448**	0.5432**	-0.5408**	0.5431**	-0.5446**
LA	0.1643**	-0.1636**	0.1730**	-0.1052	0.1719**	-0.1622**
LI	0.2205**	-0.2207**	0.2219**	-2.2262**	0.2221**	-0.2210**
FL	-0.0167	0.0171	-0.0093	0.0503	-0.0098	0.0178
FT	-0.2237**	0.2238**	-0.2250**	0.2240**	-0.2251**	0.2238**
FW	-0.1475*	0.1479*	-0.1438*	0.1670**	-0.1441*	0.1481*
FI	-0.1870**	0.1869**	-0.1925**	0.1666**	-0.1923**	-0.2091**

Table 9. Continued.

Phenotypic	July average temperature	Annual frost-free period	Frost accumulation days	Drying index	Moisture index
TH	-0.2096**	-0.1995**	0.2093**	-0.1788**	0.2085**
CL	-0.1849**	-0.1605**	0.1848**	-0.1367*	0.1831**
CS	-0.0628	-0.0392	0.0626	-0.0024	0.0599
LS	-0.1738**	-0.1717**	0.1738**	-0.1872**	0.1749**
YC	-0.0849	-0.0719	0.0839	-0.0186	0.0813
LW	0.0874	0.0993	-0.0886	0.1631**	-0.0917
LT	-0.5449**	-0.5460**	0.5449**	-0.5341**	0.5445**
LA	-0.1655**	-0.1548**	0.1643**	-0.0929	0.1614**
LI	-0.2203**	-0.2189**	0.2205**	-0.2311**	0.2211**
FL	0.0162	0.0245	-0.0166	0.0529	-0.0181
FT	0.2237**	0.2222**	-0.2237**	0.2258**	-0.2238**
FW	0.1473*	0.1516*	-0.1475*	0.1690**	-0.1484*
FI	0.1873**	0.1812**	-0.1871**	0.1664**	-0.1863**

During plant adaptation and evolution, different phenotypic traits often adapt to the environment through mutual adjustments, and changes in one trait shape can directly or indirectly lead to changes in other traits [49]. Most of the correlations among the 13 phenotypic traits of blue honeysuckle reached significant or highly significant levels, which indicated that these traits showed strong mutual regulation in the process of evolution. The differences in the adaptative mechanisms and the degree of sensitivity of different plants to environmental factors can present different geographical variation rules. For example, changes in climate may influence the distribution of rhododendrons, with the effects likely being felt most by species with either a narrow geographical or elevational range [50]. The environmental variables tested in this study were significantly or extremely significantly correlated with most of the observed traits, which indicated that the changes in habitat conditions greatly impacted on the phenotypic traits of the blue honeysuckle populations. These findings also demonstrated that blue honeysuckle could meet its growth requirements via adaptive regulation in alpine regions.

Most berry plants have a wide distribution range, which requires the plants to have excellent regulatory mechanisms to adapt to changes in different habitat conditions. Blue honeysuckle is widely globally distributed and is highly economically and socially valuable. Future research should thus focus on: 1) the protection of wild blue honeysuckle populations in order to realize their ecological and economic benefits; 2) ecological genetics research to explore the interactions between genetic mechanisms and geographical and climatic factors at the molecular level to provide a foundation for breeding protection; 3) increasing the

power of the present study by extending the distribution range; and (4) combining provenance selection with excellent single wild plants and constructing a mother forest or seed orchard to develop ecologically and economically suitable berry tree species.

Conclusions

Blue honeysuckle exhibits high variation at the population level. The phenotypic traits are relatively stable in the population (rare phenotypes are less common and less dispersed), and population differentiation is low. Inter-population variation is the main source of phenotypic variation in blue honeysuckle. The phenotypic traits are sensitive to changes in environmental factors, being negatively or positively regulated by the changes in the environment. Temperature-related factors were found to be most important and could be used to inform cultivation strategies. The fruit and leaves possessed high H' index values, which should be considered when collecting and protecting plant resources.

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Conflict of Interest

The authors declare no conflict of interest.

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