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VARIATION OF LEAF TRAITS WITH ALTITUDE IN *LONICERA CAERULEA* VAR. *EDULIS* (CAPRIFOLIACEAE) FROM NORTHEASTERN CHINA

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Abstract

Leaf structure, orientation and other properties along elevation gradients in seven populations of *Lonicera caerulea* var. *edulis* in the Changbai Mountains of China were studied to evaluate the relationship between the properties of adaptability to alpine environment. Leaf architecture was isolateral at low altitude, but dorsiventral at high altitudes, whereas the leaf orientation in space changed from upright to horizontal. Raised stomata were present in *Lonicera caerulea* var. *edulis* and their density increased with increasing elevation. Leaf thickness initially increased with altitude, but then decreased. Between 600 m and 1400 m, palisade parenchyma thickness increased but then decreased above 1600 m, reaching a minimum at 1800 m, a pattern also reflected by spongy parenchyma. The palisade parenchyma thickness/spongy parenchyma thickness ratio increased with increasing altitude. By contrast, the thickness of the upper epidermis decreased with increasing altitude, whereas there was no change in the lower epidermis. There were significant differences in the ecological indicators of *L. caerulea* var. *edulis* at seven altitudes. Leaf area (LA) and leaf biomass (LB) were highest at 1800 m, whereas the specific leaf area (SLA) was the highest at 1600 m. The leaf saturated weight (SW), LB and the specific leaf weight (LMA) were lowest at 1200 m. At 600 m, LA, SLA and SW/LB were the lowest, whereas LMA was highest at 800 m. The leaf saturated water content (SWC) and SW/LB were also highest at 600 m. Therefore, there was a significant correlation between leaf and environmental factors at different altitudes, indicating that *Lonicera caerulea* var. *edulis* modifies its structure and morphology to adapt to alpine environments with a thin atmosphere, lower oxygen levels, low temperatures, heavy rainfall and strong solar radiation.

Keywords: Leaf structure; Adaptability properties; Different altitudes; *Lonicera caerulea* var. *edulis*

Introduction

Leaves are the plant organs that are most sensitive to environmental changes and, thus, exhibit traits that are adaptive to different environments (Castro-Diez *et al.*, 2000). In addition, leaves represent the largest surface area of the plant in close contact with the atmosphere (Cai & Song, 2001; Wang & Wang, 1989). Leaves are sensitive and plastic to environment factors, such as sunlight, temperature and water, which can easily influence leaf morphology and structure (Walter, 1973; Köike, 1988; Körner *et al.*, 1989; Desoyla & Kincaid, 1991; Terasjhima & Hikosakfi, 1995; Smith *et al.*, 1998; David *et al.*, 2000). Previous studies of the leaf morphology and mesophyll structure of alpine species—conducted in New Zealand (Körner *et al*., 1986), the Alps (Körner & Diemer, 1994; Körner *et al.*, 1989), the Caucasus (Goryshyna & Hetsuriany, 1980) and Tien-Shan (Miroslavov & Kravkina, 1990a,b, 1991)—documented the most common features of adaptation of plants to high elevations: small thick leaves, an increased number of layers of palisade cells and larger mesophyll cells.

Studying leaf traits along ecological gradients commonly focuses on two points: trait variation and its drivers; and trait spectra and their drivers. Addressing these patterns and their drivers has important ecological and/or evolutionary significance (Dong & He, 2019). For example, trait variation can reflect selective pressures and evolutionary trajectories (Chapin *et al.*, 1993; Dudley, 2004) and trait spectra can be incorporated into the ecological strategies of plants to adapt to changing environments (Wright *et al.*, 2004; Donovan *et al.*, 2011). However, several aspects remain poorly understood. First, previous studies primarily addressed the role of abiotic factors (e.g., climatic, edaphic and topographic gradients) in shaping plant traits (Wright *et al.*, 2004; Craine *et al.*, 2005; Liu *et al.*, 2010; Geng *et al.*, 2014). However, few studies have addressed the role of biotic factors, in particular the relative importance of biotic and abiotic factors in shaping trait variation and spectra. Second, most of the related studies have usually involved different species. Such cross-species studies encompass phylogenetic effects and, thus, identifying ecological effects is difficult (Geng *et al.*, 2014); therefore, examining trait variation and spectra among populations of same species might help to better understand these features mechanistically (Hajek *et al.*, 2014).

Altitude is an important ecological factor affecting plant growth and development, metabolism, structure and function (Wu *et al.*, 2009). When examining the impact of altitude on plants, most studies have mainly focused on ecological factors of the alpine environment, characterized by lower temperature, lower atmospheric pressure and partial CO_2 pressure, high light intensity, among others (Friend & Woodward, 1990). These pressures have important effects on leaf morphology and physiology (Hovenden & Brodribb, 2000). Research has also considered the adaptability of plants at different altitudes in terms of their physiological functions, such as short-term response and long-term adaption of gas exchange or photosynthesis along an elevation gradient (Lu *et al.*, 2000).However, the adaptability of the morphology and structure of plants to alpine environments is not as fully understood as that of their physiology and molecular biology (Lu *et al.*, 1995; Zhou, 2001).

The function is based on the structure, differences and variations that will influence the physiological functions of a plant (Cai & Da, 2002). Studying the ecological anatomy of leaves not only reveals the influence of the variety of environment factors on plants and adaptability of plants, but also builds the basis for research on the physiological and ecological function of leaves (Li *et al.*, 2006). Therefore, comparative analyses of the leaf structure at different altitudes appear particularly relevant.

Located in northeast China and extending into North Korea, the Changbai Mountains are the highest massif along the coast of Eurasia and the most complex, formed of huge inactive and active volcanoes. They are home to endangered Siberian tigers and the last stands of Virgin Korean pine-mixed hardwood in the world. In addition, there are large areas of undamaged primitive forests comprising abundant plant species and a clear vertical vegetation distribution pattern, from deciduous broad-leaved forests, to coniferous forests and then tundra). The species composition in the Changbai Mountains reflects the impact of factors such as altitude, climate and variation in the ecological environment.

Plants and plant communities in the Changbai Mountains show marked vertical changes. A case in point is *Lonicera caerulea* L. var. *edulis* Turcz. ex Herd. (Caprifoliaceae), which is widely distributed at altitudes from 600 m to 1800 m. This species is well known for its soft, succulent fruits, which are eaten whole and used to make juice and wine. Leaf surface traits of *L. caerulea* var. *edulis* are closely related to environmental factors along elevational gradients. Previous work focused on the ecological anatomy of the shrub along elevation gradients (Li *et al.*, 2006). However, less information is available in terms of the relation between leaf form and altitude.

L. caerulea var. *edulis* inhabits wetlands with sufficient light levels in forest zones, and along hillsides and forest edges. This large habitat range across different elevations implies a strong ability to adapt to different environments. It is one of main species in the Changbai Mountain ecosystem and has an important role in preventing soil erosion, soil water loss and regulating climate, as well as in maintaining ecological stability.

Therefore, the objectives of the current study were to evaluate the changes in leaf traits *L. caerulea* var. *edulis* with increasing elevation and to determine the mechanisms of adaptation of this species to different habitats.

Materials and Methods

Environment in study sites: The study sites were located in the Changbai Mountains (127°33′30″～128°16′48″E, 41°42′45″ ～ 42°45′18″N) with an annual mean temperature of 2～6°C, and an annual mean precipitation of 600～900 mm. This area has temperate continental mountain climate affected by the monsoon. The lowest temperature can reach –39.4°C in January, with the highest in July. Rainfall increases with increasing altitude (Yu *et al*., 2005; Li *et al*., 2021). Table 1 details the climatic data of the survey sites.

Plant materials: Mature leaves of *L. caerulea* var. *edulis* were collected at elevations 600 m, 800 m, 1000 m, 1200 m, 1400 m, 1600 m and 1800 m in August 2019, from eight populations. To smooth any variability in the material, we selected areas containing most of the species in the community to establish fixed plots (every 20 × 20 m) of *L. caerulea* var. *edulis* at each altitude. Each plot was divided into 16 small subsample plots, and we randomly selected 15–20 plants belonging to the same ecotypes at different altitudes; the plants were approximately 10 years old. Leaf orientation was measured based on mature leaves collected from the upper canopy of the plants. The leaves collected were placed in a portable fridge and taken back to the laboratory for sectioning.

Measurements of leaf traits: The thickness of each of ten leaves (as a sample) was measured using a vernier caliper, and the thickness of each leaf (LTh) was calculated, with a measurement accuracy of 0.01 cm. Leaf length (LL), leaf width (LW) and leaf area (LA) are characteristic parameters of leaf shape. Each leaf was taken out of the water and the petiole was removed; any surface water was wiped off and the profile of the leaf was scanned with a laser scanner; the LA was then calculated with ArcView 3.2 image processing software. The LL was measured with an electronic digital caliper using the longest segment parallel to the main vein. The widest part of the vertical main vein was used to measure the leaf width and calculate the leaf length/leaf width of the leaf shape index (Li *et al*., 2005).

To determine the ecological characteristic indexes, the leaves were first soaked in distilled water for ~24 h. They were then removed having become fully saturated. The petioles were removed and any surface water wiped off. The saturated weight (SW) of the leaves was measured immediately with a balance. The leaves were then placed in a drying envelope and left to air dry for 24 h. The leaves were heated in an oven at 105°C for 30 min, and dried to constant weight at 70°C. The leaf biomass (LB) was measured and the leaf mass per area (LMA = LB/LA), specific leaf area (SLA = LA/LB) and leaf water content [LWC = (SW–LB)/SW) were calculated. Saturated water content (SWC = (SW–LB)/LB), leaf density (LD = LMA/LTh) and the ratio of saturated to dry weight (SWC/LB) of the whole leaf were also determined.

Table 1. Climate data along the study altitude gradient on the northern slopes of the Changbai Mountains.

Altitude (m)	Annual average temperature (°C)	Annual precipitation (mm)	> 5°C Accumulated temperature (°C)	Precipitation from June to September (mm)	Average January temperature (°C)	Average July temperature (°C)	Frost free period (d)	No. of frost days	Dryness index	Humidity index
600	3.35	640.68	2649.06	455.01	-16.99	20.16	123.37	124.02	0.69	1.58
800	2.32	703.62	2285.25	500.4	-17.64	19.07	116.5	137.58	0.59	2.21
1000	1.29	755.19	1972.49	537.07	-18.27	17.95	108.12	151.16	0.53	2.82
1200	0.27	810.53	1702.53	576.43	-18.89	16.84	100.31	164.73	0.47	3.43
1400	-0.75	869.92	1469.52	618.67	-19.52	15.73	93.06	178.31	0.42	4.04
1600	-1.78	933.67	1268.4	664.01	-20.15	14.61	86.34	191.88	0.37	4.65
1800	-2.8	1002.09	1094.81	712.67	-20.77	13.5	80.1	205.46	0.33	5.25

Leaf orientation: Leaf orientation was determined by the angle between the leaf base and the trunk in an individual leaf with a protractor. Twenty individual angles per field population were used as replicates.

Leaf surface scanning: Fresh mature leaf segments were fixed for 48 h by vacuum infiltration with 2.5% glutaraldehyde in 0.1 M phosphate-buffered solution and dehydrated by an alcohol series. The dehydrated tissue samples were critical-point dried, mounted with CO_2, coated with a gold ion coater and observed with a Hitachi S-3000N scanning electron microscope at an accelerating voltage of 15.0 kv (Luan *et al.*, 2017).

The stomatal type and distribution characteristics were observed. The stomatal density (SD) was defined as the number of stomata per square millimeter of leaf surface, which was calculated using Equation 1 (Meng *et al.*, 1997):

$$SD = N/S \qquad [1],$$

where SD was the stomatal density in a visual field, N was the number of stomata in a visual field, and S was the area of a visual field. Ten visual fields were randomly selected as replicates. The length (SL) and width (SW) of 20 stomata were measured by a vernier caliper in the SEM photographs of samples and then divided by the magnification of the photographs. The stomatal area (SA, mm^2) was calculated using Equation 2 (Wise *et al.*, 2000):

$$SA = 3.14 \times 1/4\ SL \times SW \qquad [2]$$

The types of stomata covering the epidermis were divided into raised stomata and sunken stomata (Li & Zhang, 1983) and 50 stomata were observed randomly of each. The stomata in 20 randomly selected visual fields of photographs were also observed to determine whether it was a random or ordered distribution (Zheng & Gong, 2001).

Anatomical observations: Fifteen sampled leaves ranging from 45° to 90° at every altitude were all cut at the central part of their basal midrib (0.25 cm^2). The segments were fixed in FAA and then dehydrated through an alcohol series and embedded in paraffin. Cross-sections (8–20 μm) were cut with a rotary microtome and stained with 1% safranin and 0.5% fast green. The thicknesses of the upper and lower epidermis, and sponge and palisade parenchymas were measured. In addition, the ratio of palisade and sponge parenchyma thickness (P/S) was calculated at each altitude. Leaf architecture was observed and divided into either dorsiventral or isobilateral.

Statistical analysis: Data were analyzed using SAS8.2. Differences in leaf traits at different altitudes were examined using one-way ANOVA analysis and LSD test multiple comparisons.

Results

Leaf morphological characteristics: LL, LW and LA varied significantly with altitude ($p<0.01$) in response to the environmental changes at different altitudes (Table 2).

LL was highest at 1800 m and lowest at 1400 m (Table 3). There was no significant difference among LL at 1800, 1600, or 600 m; however there were significant differences among LL at the other four altitudes ($p<0.01$). LW was highest at 600 m and lowest at 1400 m. The difference between LL at 600 m and other altitudes was significant, but not between 1800 and 1600 m or 800 and 1400 m. LA at 1800 m was not significantly different from LA at 600 m, but was significantly different from LA at 1600 m ($p<0.05$); LA was more significantly different at the remaining altitudes ($p<0.01$). LA at 800 m was not significantly different from 1000 m, 1200 m or 1400 m, but was significantly different from LA at the remaining altitudes.

Table 4 shows the correlation analysis between leaf morphological characteristics and environmental meteorological factors. Environmental factors showed specific correlations with leaf morphological characteristics. LL, LW and LA were positively correlated with meteorological factors, such as annual precipitation, precipitation from June to September, frost days and humidity index.

Leaf orientation: The angle between the leaf base and the stem ranged from 30° and 90° and increased significantly with altitude, except between 1000 m and 1200 m and between 1400 m and 1600 m (Fig. 1). The degree of inclination of leaves gradually became horizontal and, thus, the leaf orientation significantly changed with altitude.

Leaf anatomy structure: The stomata were liplike, wide and deep, with a smooth edge. The raised stomata (Fig. 2A) did not change with altitude. The stomatal area at different altitudes also showed no significant difference (Table 5). However, the SD significantly increased with altitude ($p<0.01$). The leaf SD at 1800 m was significantly higher than that of other elevations except 1600 m and was more than four times higher than that at 600 m (Table 6). At lower altitudes (600 m, 800 m and 1000 m), there were no significant differences in SD.

The leaf epidermis of *L. caerulea* var. *edulis* comprised a regular pinacocyte layer, mesophyll and vein (Fig. 2B). However, the leaf architecture differed between 600 m and the other altitudes. At 600 m, palisade parenchyma was distributed under the upper epidermis and above the lower epidermis, forming an isolateral leaf architecture was (Fig. 2C). From 800 m to 1800 m, however, palisade parenchyma was only distributed under upper epidermis, below which appeared spongy parenchyma. Thus, the leaf architecture became dorsiventral (Fig. 2D–I).

The upper epidermis thickness (EuT) decreased with increasing altitude and, at 1800 m, was significantly thinner that at 600, 800, 1000 or 1200 m ($p<0.05$). There were no significant differences in EuT at 600 m to 1600 m except at 800 m, nor in the lower epidermis thickness at different altitudes (Table 6).

The palisade parenchyma thickness (PpT) was significantly influenced by altitude ($p<0.05$). PpT was thickest at 1400 m and then decreased above or below this altitude. By contrast, the sponge parenchyma (PsT) at 1000 m was significantly thicker than at the higher or lower altitudes ($p<0.05$). The value of P/S was in the order: 1600 m > 1800 m > 1400 m > 1200 m > 800 m > 600 m > 1000 m (Table 6).

Table 5. ANOVA of leaf structure of *L. caerulea* var. *edulis* at different altitudes.

Source	DF	F	P
Stomatal density (SD)	6	21.645***	0.000
Stomatal area (SA)	6	0.088^{NS}	0.997
Upper epidermis thickness	6	5.065**	0.006
Lower epidermis thickness	6	0.267^{NS}	0.944
Palisade parenchyma thickness	6	4.939**	0.007
Spongy parenchyma thickness	6	22.657***	0.000

Note: ** indicates $p<0.01$; *** indicates $p<0.001$; NS indicates $p>0.05$

Table 2. Analysis of variance of leaf morphological characteristics at different altitudes.

Leaf morphological characteristics	df	Variance	Mean square	F value	*P* value
LL	6	7532.68490	1255.44748	15.33	<0.0001
LW	6	1726.44108	287.74018	22.27	<0.0001
LA	6	713.01301	118.8355	20.58	<0.0001

Table 3. Multiple comparison analysis of leaf morphological characteristics at different altitudes[a].

Altitude (m)	LL (cm)	LW (cm)	LA (cm^2)
600	61.44 ± 0.87aA	27.58 ± 0.42aA	9.80 ± 0.28abA
800	50.70 ± 1.49bB	21.05 ± 0.52cC	5.95 ± 0.31dC
1000	51.63 ± 1.04 bB	21.80 ± 0.54 cC	6.95 ± 0.27cdB
1200	51.80 ± 1.58 bB	21.53 ± 0.52 cC	7.54 ± 0.37cB
1400	49.93 ± 1.45 bB	20.90 ± 0.69 cC	6.94 ± 0.38cdBC
1600	58.95 ± 1.69aA	24.20 ± 0.63 bB	9.16 ± 0.50bA
1800	62.92 ± 1.83aA	25.19 ± 0.65bB	10.58 ± 0.51aA

[a] Lower-case letters and upper-case letters indicate significant differences between treatments at $p<0.05$ and $p<0.01$, respectively

Table 4. Correlation coefficients between leaf morphological characteristics and environmental factors[a].

Leaf morphological characteristic	Annual average temperature (°C)	Annual precipitation (mm)	>5°C Accumulated temperature (°C)	Precipitation from June to September (mm)	Average January temperature (°C)	Average July temperature (°C)	No. of frost-free days	No. of frost day	Dryness index	Humidity index
LL	-0.36**	0.38**	-0.34**	0.38**	-0.36**	-0.36**	-0.35**	0.36**	-0.35**	0.36**
LW	-0.32**	0.33**	-0.30**	0.33**	-0.32**	-0.32**	-0.31**	0.32**	-0.31**	0.32**
LA	-0.48**	0.49**	-0.47**	0.49**	-0.48**	-0.48**	-0.47**	0.48**	-0.47**	0.48**

**Indicates $p<0.01$

Table 7. Correlation coefficients between leaf anatomical characteristics and environmental factors.

Leaf anatomical characteristic	Annual average temperature (°C)	Annual precipitation (mm)	>5°C accumulated temperature (°C)	Precipitation from June to September (mm)	Average January temperature (°C)	Average July temperature (°C)	No. of frost free days	No. of frost days	Dryness index	Humidity index
SD	-0.92**	0.93**	-0.91**	0.93**	-0.92**	-0.92**	0.92**	-0.91**	0.92**	-0.92**
SA	-0.15	0.15	-0.15	0.15	-0.15	-0.15	0.15	-0.15	0.15	-0.15
EuT	0.88**	-0.88**	0.86**	-0.88**	0.88**	0.87**	-0.88**	0.86**	-0.88**	0.88**
ElT	-0.02	0.00	-0.06	0.00	-0.02	-0.04	0.02	-0.05	0.02	-0.02
PpT	0.23	-0.28	0.14	-0.28	0.23	0.19	-0.23	0.17	-0.23	0.23
PsT	0.83**	-0.85**	0.78**	-0.85**	0.83**	0.80**	-0.83**	0.80**	-0.83**	0.83**

Note: ** Indicates $p<0.01$; *** Indicates $p<0.001$

The stomatal density showed a very significant negative correlation with the annual average temperature, > 5°C accumulated temperature, dryness index, frost-free period and average temperature in July, and a very significant positive correlation with annual precipitation, precipitation from June to September, humidity index and snow days (Table 7). The thickness of the upper epidermis (EuT) and of the sponge tissue (PsT) were negatively correlated with annual precipitation, precipitation from June to September, humidity index and number of frost days, but was positively correlated with the annual average temperature, the > 5°C accumulated temperature, dryness index, frost-free period and average temperature in July. There was no significant correlation among stomatal area (SA) and palisade tissue thickness (PpT) with meteorological factors.

Table 8. Analysis of variance for ecological characteristics of *L. caerulea* var. *edulis* at different altitude.

Trait	df	Variance	Mean square	F value	*P* value
SW	6	0.3003	0.0500	29.70	<0.0001
LB	6	0.0328	0.0055	5.80	<0.0001
SLA	6	1011662.96	168610.49	43.65	<0.0001
LMA	6	0.0002	0.0000	3.41	0.0029
SWC	6	25.6722	4.2787	12.89	<0.0001
SW/LB	6	25.6722	4.2787	12.89	<0.0001

Leaf ecological characteristics: The *P* values of the analysis of variance for each ecological index of leaves were <0.01, indicating that there were extremely significant differences among the ecological index for each of the seven altitudes (Table 8).

SW was highest (0.1787 ± 0.0021 g) at 600 m, which was very significant compared with other altitudes (Table 9). It was lowest (0.0825 ± 0.0014 g) at 1200 mm but this was not significant compared with at 1400 m but was significantly different at other altitudes. LB was highest (0.0566 ± 0.0010 g) at 1800 m, which was not significantly different from that at 600 m and other altitudes. LB was lowest (0.0236 ± 0.0001 g) at 1200 m and was not significantly different from that at 1400 m and 1600 m. SLA was highest (339.91 ± 0.0189 cm^2/g) at 1600 m, which was significantly different from other altitudes except 1200 m, and lowest (174.42 ± 0.0162 cm^2/g) at 800 m, which was significantly different from at 600 m and 1000 m. LMA was highest (0.0059 ± 0.0002 g/cm^2) at 800 m, which was not significantly different from 1000 m, but significantly different from 600 m ($p<0.05$) and extremely significant from other altitudes ($p<0.01$). LMA was lowest (0.0032 ± 0.0001 g/cm^2) at 1200 m, which was significantly different from 600, 800 and 1000 m. SWC was highest at 600 m (3.0339 ± 0.1300), which was significantly different from other altitudes. SWC was lowest at 800 m (2.1737 ± 0.0564), which was not significantly different from 1000 m and 1800 m, but significantly different from other altitudes. SW/LB was highest at 600 m (4.0339 ± 0.1300), which was significantly different from other altitudes. It was lowest was at 800 m (3.1737 ± 0.0564), which was also not significantly different from 1000 m and 1800 m, but significantly different from other altitudes.

Table 6. Upper epidermis thickness (EuT), lower epidermis thickness (ElT), palisade parenchyma thickness (PpT) and spongy parenchyma thickness (PsT) of *L. caerulea* var. *edulis* at different altitudes.

A (m)	600	800	1000	1200	1400	1600	1800
EuT (μm)	(2.33±0.22)ab	(2.42±0.44)a	(2.00±0.14)ab	(2.00±0.14)ab	(1.67±0.08)bc	(1.67±0.08)bc	(1.00±0.14)c
ElT (μm)	(0.92±0.08)a	(1.00±0.14) a	(1.08±0.17) a	(1.17±0.22) a	(1.17±0.55) a	(1.25±0.14) a	(0.92±0.17)a
PpT (μm)	(2.00±0.14)cd	(2.50±0.25)bcd	(2.58±0.08)abcd	(3.58±0.08)ab	(3.83±0.96)a	(3.17±0.08)abc	(1.42±0.17)d
PsT (μm)	(3.33±0.22)bc	(4.00±0.25)b	(5.33±0.33)a	(4.00±0.38)b	(3.75±0.29)b	(2.75±0.14)c	(1.25±0.14)d
P/S	0.60	0.63	0.48	0.90	1.02	1.52	1.13

Note: Different superscript letters in the same species indicate significant differences ($p<0.05$), No superscript letters indicate $p>0.05$

Table 9. Duncan's Multiple Range Test for ecological characteristics of *L. caerulea* var. *edulis* at different altitudes.

Ecological character	600 m	800 m	1000 m	1200 m	1400 m	1600 m	1800 m
SW (g)	0.18±0.00aA	0.11±0.00cC	0.11±0.00cC	0.08±0.00eE	0.09±0.00deCD	0.11±0.00cdCD	0.14±0.01bB
LB (g)	0.05±0.00aA	0.04±0.00bB	0.03±0.00bB	0.02±0.00cC	0.03±0.00cC	0.03±0.00cC	0.06±0.00aA
SLA (cm^2/g)	224.83±0.01dCD	174.42±0.02eE	207.02±0.01dDE	336.84±0.01 aA	293.53±0.01 bB	339.91±0.02 aA	244.80±0.08 cC
LMA (g/cm^2)	0.005±0.00bcABC	0.006±0.00aA	0.005±0.00abAB	0.003±0.00dC	0.004±0.00cdBC	0.004±0.00 cdBC	0.005±0.00bcdBC
SWC	3.03±0.13aA	2.17±0.06cD	2.33±0.09cDC	2.59±0.10bBC	2.60±0.06 bBC	2.86±0.05 aAB	2.28±0.08 cCD
SW/LB	4.03±0.13 bB	3.17±0.06 aA	3.33±0.09 cCD	3.59±0.10 bBC	3.60±0.06 bBC	3.86±0.05 aAB	3.28±0.08 cCD

Note: Different superscript letters in the same species indicate significant differences ($p<0.05$), No superscript letters indicate $p>0.05$

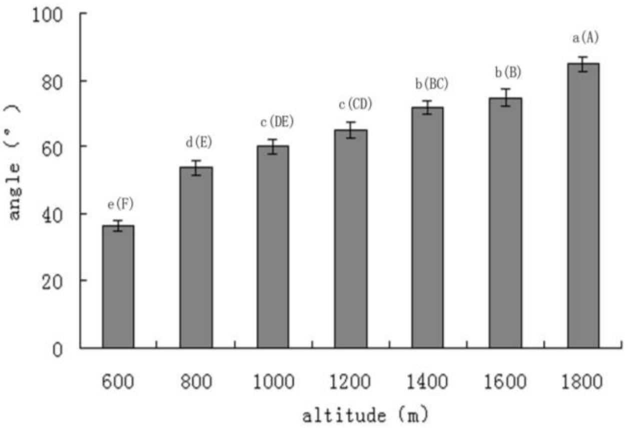

Fig. 1. The relationship between leaf orientation (angle) and altitude. Lower case letters indicate $p<0.05$ level; upper case letters indicate $p<0.01$ level ($N = 20$). Two means marked with the same letter indicates that the difference was not significant; two means marked with different letters indicates that the difference was significant (two means marked with different capital letters indicates that the difference was very significant).

Table 10 shows that SLA was influenced significantly by precipitation from June to September and the frost-free period, and that the annual average temperature and precipitation affected SWC.

Discussion

SD, SA and EuT were more significantly correlated with environmental factors than were the other traits measured in this study. To survive at high altitudes, *L. caerulea*var. *edulis* has evolved special morphologies that can resist the impacts of high elevation, low temperatures and freezing stress. The leaf architecture of *L. caerulea* var. *edulis* was isolateral at 200 m, becoming dorsiventral> 740 m. Some plants on the Qinghai-Tibet Plateau (Yang *et al.*, 2019), such as *Polygonum aviculare* L. and *Chenopodium album* L., are also able to change their leaf structure from isolateral into dorsiventral with increasing elevation. Given that solar radiation has important effects on the growth and development of plants, they can respond to different levels of sunlight exposure by either orienting leaves so that the incident light is altered or by altering the leaf structure (Smith, 1998). From low to high altitudes, the leaf orientation changed from upright to horizontal, which would enable leaves of *L. caerulea* var. *edulis* to develop mesophyll parenchyma in the upper epidermis and to become dorsiventral. Therefore, the dorsiventral leaf architecture is considered to be more advantageous at higher elevations with more intense solar irradiance.

The anatomy and micromorphological structures of leaves of *L. caerulea* var. *edulis* showed that they had a single epidermis cell layer with raised stomata. Thus, *L. caerulea* var. *edulis* is a mesophyte (Li, 1983).

High altitudes are characterized by decreasing air density and intense solar radiation, causing plants to expand their SD to increase the respiration area to compensate for respiration inefficiency and for have an increased amount of aeration (Qiu *et al.*, 1998). The SD of *L. caerulea* var. *edulis* increased with increasing altitude and was correlated with all environmental factors (Table 4); this could enable the plants to regulate their transpiration rate effectively under conditions of high altitude, higher precipitation and intense solar radiation.

In general, leaf thickness increases with increasing altitude (Hultine & Marshall, 2000; Cordell *et al.*, 1999; Körner, 2003). In *L. caerulea*var. *edulis*, leaf thickness increased initially but then became thinner at higher elevations. This was because EuT correlated positively with MT and D and correlated negatively with MR and W and ElT correlated positively with MR, W and S (Table 4). Thus, high precipitation, low temperatures and intense solar radiation at high altitudes result in increased transpiration rates and thickening of the upper epidermis, but restrain the growth of mesophyll cells.

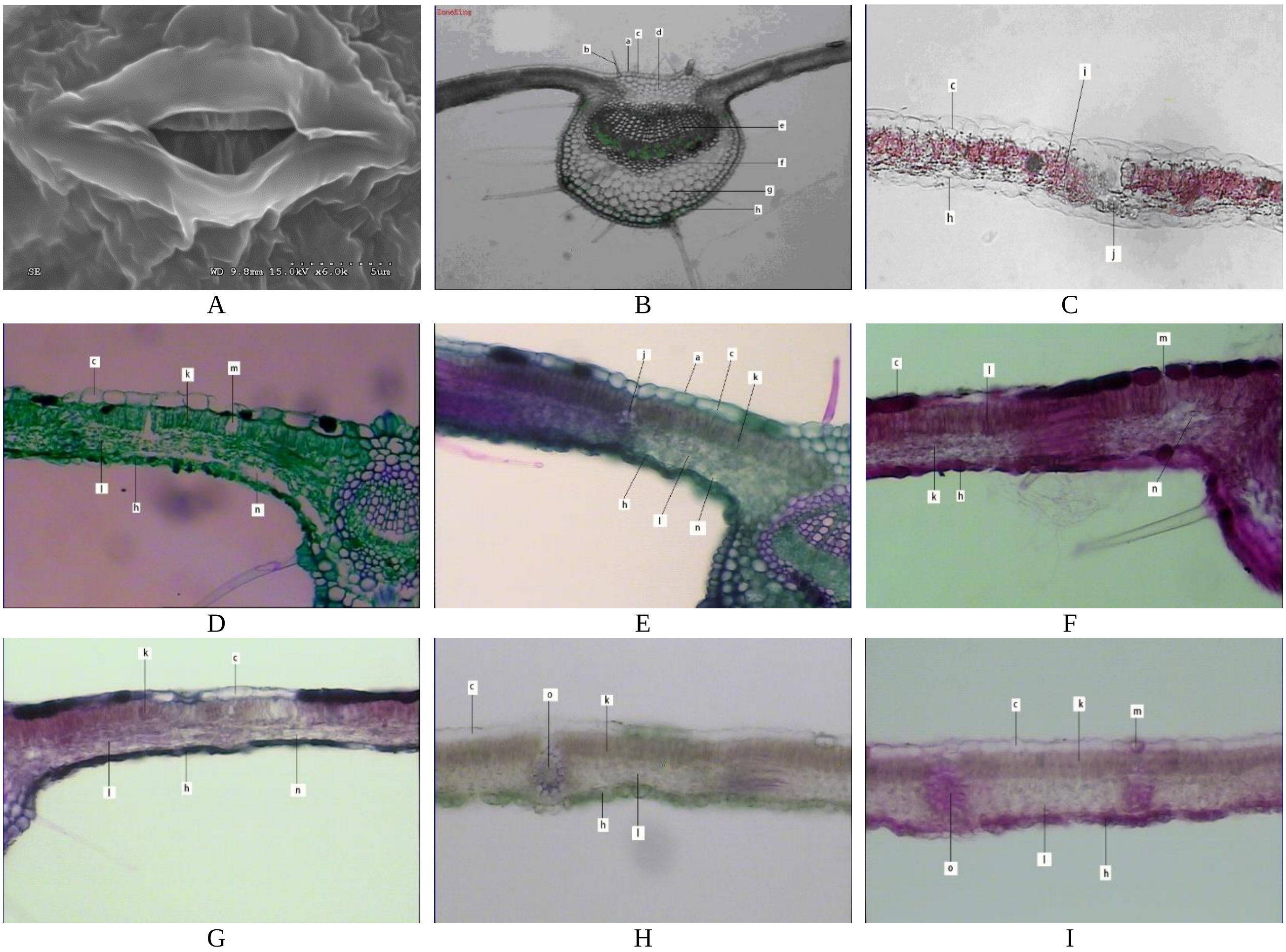

Fig. 2. Leaf anatomy structure of *Lonicera caerulea* var. *edulis*. (A) Stomatal morphology (×6.0k). Transection of (B) middle rib of leaf ; (C) isobilateral leafat 600 m (×400); bifacial leaf at (D) 800 m, (E) 1000 m, (F) 1200 m; (G) 1400 m; (H) 1600 m; and (I) 1800 m. All images ×400 magnification. a, stratum corneum; b, trichome; c, upper epidermis; d, collenchyma; e, xylem; f, phloem; g, parenchyma; h, lower epidermis; i, mesophyll cell; j, sclerenchyma strands; k, palisade parenchyma; l, spongy parenchyma; m, stomata; n, intercellular space; o, vein.

Short-wave radiation increases with increasing altitudes, causing the palisade cells of leaves to become protective structures that can help prevent the temperature of the leaf surface from increasing to a point that would damage the leaf. The coefficient of palisade cells of leaves of *L. caerulea* var. *edulis* at high elevation was high, indicating that there is a barrier between mesophyll cells and the environment because the palisade parenchyma is located on the side irradiated by direct solar radiation in the mesophyll parenchyma (Li & Wang, 1993). P/S was lower at 1800 m and 1600 m, revealing a more aerated parenchyma compared with below 1600 m, comprising interstitial spaces among the parenchyma cells. Such a parenchyma structure is an adaption that might improve photosynthetic efficiency given the deficiency of O_2 and CO_2 in thin air, and favoring the growth and development of plants in a short time (Zeng & Li, 2000). The variation in mesophyll parenchyma structure has a direct effect on photosynthesis. P/S exists at optimal values in specific environments. Thus, it appears that the photosynthesis rate was higher at higher altitudes because the value of P/S was also higher (Higuchi *et al*., 1999). The thicknesses of the palisade and spongy parenchymas of *L. caerulea* var. *edulis* varied irregularly. However, P/S on average increased with increasing altitude, although the number of layers showed no obvious change. PpT of *L. caerulea* var. *edulis* correlated positively with S, and PsT correlated positively with MT and D (Table 4). Thus, changes in mesophyll thickness along elevation gradients resulted mainly from changes in the size of mesophyll cells. The volume and interstitial space of mesophyll cells were smaller at the low altitudes with relatively higher temperatures and less precipitation, whereas the palisade parenchyma was more developed than the spongy parenchyma at high altitudes with relatively lower temperatures and higher precipitation. These specialized structures enable gases, such as CO_2, to be transmitted from stomatal chambers to sites of photosynthesis to overcome the reduction in conductivity of CO_2 resulting from closed stomata and changes in the mesophyll structure, also preventing water from being lost. Meanwhile, cell walls of smaller mesophyll cells show more flexibility (Castro-Diez *et al.*, 2000; Chartzoulakis *et al.*, 2002; Zuo *et al*., 2015). These characteristics not only improve the photosynthesis rate and water utilization rate of plants, but also efficiently maintain the tension of cells and stomata. Moreover, they prevent unnecessary water loss in response to environmental factors.

Table 10. Correlation analysis between leaf ecology characteristics and environmental factors.

Traits	Annual average temperature (°C)	Annual precipitation (mm)	>5°C accumulated temperature (°C)	Precipitation from June to September (mm)	Average January temperature (°C)	No, of frost-free days	No. o frost days	Dryness index	Humidity index
SW	-0.15*	0.17**	-0.11	0.17**	-0.15*	-0.13*	0.15*	-0.12	0.15*
LB	-0.06	0.09	-0.02	0.09	-0.06	-0.04	0.06	-0.03	0.06
SLA	-0.43**	0.41**	-0.48**	0.41**	-0.43**	-0.45**	0.43**	-0.47**	0.43**
LMA	0.22**	-0.21**	0.24**	-0.21**	0.22**	0.23**	-0.22**	0.24**	-0.22**
SWC	-0.21**	0.20**	-0.24**	0.20**	-0.21**	-0.23**	0.21**	-0.24**	0.22**
SW/LB	-0.21**	0.20**	-0.24**	0.20**	-0.21**	-0.23**	0.21**	-0.24**	0.22**

Note: ** Indicates $p<0.01$; *** Indicates $p<0.001$

LB, SLA and LMA are important ecological characteristics of leaves, reflecting the ability of the plant to accumulate dry matter and to respond to water, heat, light and soil indexes. The large biomass, small specific leaf area and significant specific leaf indicate that the photosynthesis ability of the leaves is strong. Some studies report that the specific leaf area is positively correlated with the relative growth rate of plant seedlings and the net photosynthetic rate of leaves, but negatively correlated with the growth life of leaves, whereas, the specific leaf area can reflect the ability of plants to obtain resources, with plants with a low specific leaf area being better adapted to barren and arid environments (Lambers *et al.*, 1992; Grime *et al*., 1997; Wilson *et al.*, 1999). There were significant differences in SW, LB, SLA, LMA, SWC, SW/LB and other ecological indicators of *L. caerulea* var. *edulis* at the seven different altitudes in the Changbai Mountains. The leaf area and leaf biomass were highest at 1800 m, whereas the specific leaf area was highest at 1600 m. The saturated weight, leaf biomass and specific leaf weight were lowest at 1200 m. The leaf area, specific leaf area and SW/LB were lowest and the specific leaf weight was highest at 800 m. The SWC and SW/LB were highest at 600 m. The relationship between environmental factors and leaf traits was complicated by the changes in altitude. The results showed that leaf biomass was highest at 1800 m, and the leaf specific area and leaf weight were lowest at 800 m, indicating that the photosynthetic accumulation capacity of *L. caerulea* var. *edulis* was higher at both 1800 m and 800 m.

The water indexes of leaves, such as SW, SWC and SW/LB, reflect the utilization of water by plants. The SWC of *L. caeruleavar. edulis* was highest at 1400 m, and was the highest at 600 m. Thus, the leaf utilization efficiency was higher at mid and low altitudes. Water and temperature conditions are likely to be more suitable at these levels to enable improved plant growth and development compared with higher altitudes, also reflecting by increased leaf biomass, water content and other indicators.

Conclusions

In conclusion, ecological factors at different altitudes have effects of different degrees on the morphological structure and ecological properties of *L. caerulea* var. *edulis*. There were significant differences in SW, LB, SLA, LMA, SWC, SW/LB and other ecological indicators of *L. caerulea*var. *edulis* across the seven altitudes investigated. LA and LB were highest at 1800 m, whereas SLA was highest at 1600 m. SW, LB and LMA were lowest at 1200 m. LA, SLA and SW/LB were lowest and LMA was highest at 800 m. SWC and SW/LB were highest at 600 m. The occurrence of several different tissue forms of *L. caerulea* var. *edulis* reflects the influence of environmental factors as well as the adaptability of this species to varied environments. Thus, *L. caerulea* var. *edulis* is able to modify its morphological structure to adapt to alpine environments.

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