



**Full Length Article**

## Variations in Leaf Stomatal and Vein Traits of *Oxyria sinensis* along an Elevational Gradient in the Hengduan Mountains, China

Zhenya Liu<sup>1,2</sup>, Derong Xiao<sup>1</sup>, Huijun Guo<sup>1\*</sup> and Mei Sun<sup>1,2</sup>

<sup>1</sup>College of Wetland, Southwest Forestry University, Kunming 650224, China

<sup>2</sup>Ecological Observatory Network of Dianchi in Yunnan Province, Jinning 650600, China

\*For Correspondence: hjguo1@sina.com

Received 08 June 2021; Accepted 03 August 2021; Published 28 September 2021

### Abstract

Leaf vein and stomatal traits are crucial for maintaining plant water balance and can reflect the adaptive strategies of plants to their environment. We studied vein and stomatal traits of *Oxyria sinensis* Hemsl. along an elevational gradient in the Hengduan Mountains to explore its adaptive strategies to environmental changes. The measured traits of *O. sinensis* were similar at the lowest (2287, 2390, 2542 and 2720 m) and highest (3157 and 3373 m) investigated elevations. Plants at both the lowest and highest elevations had significantly higher stomatal density, ratio of adaxial stomatal density to stomatal density, and vein density, but lower ratio of abaxial stomatal density to stomatal density and stomatal length than plants measured in the middle elevational band (2797, 2931 and 3005 m). Variations of stomatal and vein traits were mainly influenced by temperature and ultraviolet-B (UV-B) radiation. Stomatal density, ratio of adaxial stomatal density to stomatal density, and vein density were significantly positively correlated with the mean annual temperature (MAT), but the ratio of abaxial stomatal density to stomatal density and stomatal length were negatively correlated with MAT. Stomatal density and the ratio of adaxial stomatal density to stomatal density were negatively correlated with the intensity of daily mean maximum UV-B during the plant growing seasons, but the ratio of abaxial stomatal density to stomatal density was positively correlated with UV-B. It suggested that *O. sinensis* along this elevation gradient has variable water transporting capacities due to temperature and UV-B radiation, the capacity of plants to adapt to water deficits were enhanced at higher elevations. These findings provide a better understanding of the adaption of herbaceous plants to elevational change and how climate change affects their community dynamics. © 2021 Friends Science Publishers

**Keywords:** Adaptation; Temperature; UV-B; Leaf hydraulic balance; Hengduan Mountains

### Introduction

Variations in leaf vein, stomata and their coordinated relationships were known as indicators of plant physiological functions and adaptations (Blonder *et al.* 2017). Leaf veins are involved in sugar loading, transportation of photosynthates and water within leaves, and mechanical support (Song *et al.* 2015; Jensen *et al.* 2016). A lower photosynthetic rate requires a slower flow of water and thus sparse venation; it would couple with lower costs of construction for leaf growth (Jensen *et al.* 2016). Stomata are regulators between plants and the external atmosphere. Greater stomatal density and smaller stomatal size may increase the exchange of CO<sub>2</sub> and water vapor, thus increasing photosynthetic capacity and water transpiration (Franks and Beerling 2009). Stomata can be present as amphistomatous or on only either the abaxial (hypostomatous) or the adaxial (hyperstomatous) surface (Haworth *et al.* 2018). There is a link between stomatal

conductance and hydraulic conductance in water demand and supply to support continuity in photosynthesis (Brodribb and Jordan 2011). In general, plants can reach well balance between liquid and vapor water phases to avoid high water loss if the densities of veins and stomata are coordinated with the environment (Sun *et al.* 2014). Previous researches were focused on correlation between vein density and stomatal density at species and genus levels (Brodribb and Jordan 2011; Sun *et al.* 2014; Zhang *et al.* 2014b). However, it is unclear whether such correlation is affected by the environment.

Numerous studies have shown that the development of veins and stomata are regulated by environmental conditions (Soudzilovskaia *et al.* 2013; Hill *et al.* 2015; Stewart *et al.* 2017). For example, in the Australian native shrub *Dodonaea viscosa* subsp. *angustissima* has significant relationship was found between temperature and stomatal density, while no significantly correlation were detected between rainfall, stomatal density, and size (Hill *et al.* 2015).

*Nicotiana tabacum* plants grown in warmer conditions had significantly higher stomatal and vein densities compared with tobacco plants grown at lower temperatures (Huang *et al.* 2014). Under strong light, *Arabidopsis thaliana*, stomatal density and vein density are all enhanced (Stewart *et al.* 2017). The relationship between vein density and mean annual temperature is strong, while no relationships have been observed between any vein traits and precipitation (Blonder *et al.* 2017). Vein density increases with mean annual temperature (Sack and Scoffoni 2013), although sometimes contradictory or weak relationships are found (Dunbar-Co *et al.* 2009). Some researchers paid attention to the spatial variations in vein and stomatal traits within specific species or groups of species along environmental gradients, since addressing the questions of how and why these traits vary among spatial sites is the most important step towards understanding ecosystem properties (Soudzilovskaia *et al.* 2013).

Elevational gradients are usually used to study how plant traits respond to environmental changes, even if a close range can lead to large climatic gradients along with the elevation changes (Körner 2007). Variation in stomatal traits are an important component of plant adaptation to increasing elevation (Li *et al.* 2006; Wang *et al.* 2014; Shi *et al.* 2015), because the changes in air temperature, CO<sub>2</sub> partial pressure, vapor pressure deficit, wind speed, and UV irradiance may affect stomatal development (Wang *et al.* 2014). However, some studies have reported fewer stomata at high elevations (Schoettle and Rochelle 2000) and no changes (Bucher *et al.* 2016; Zhao *et al.* 2016), or non-linear (Li *et al.* 2006) variations in stomatal density or size with increasing elevation. Thus, the variation of stomatal traits with environmental gradients is not fully understood. Moreover, studies are limited on the variations of vein traits and the relationships between stomata and venation along an elevation gradient (Zhao *et al.* 2016) and most investigations have focused on woody plants but not herbaceous species (Bucher *et al.* 2016). Since woody and herbaceous species may act differently in water-use strategies (Blonder and Enquist 2014), studies on herbaceous plants are important to further understand plant adaptation to environmental change.

The Hengduan Mountains are located on the southeastern boundary of Qinghai-Tibet Plateau (QTP). It is an ideal study site to investigate the response of plants to environmental changes because of its large elevational gradient and sensitivity to climate change (Sun *et al.* 2016). *Oxyria sinensis* Hemsl. is a typical herbaceous species that belongs to *Oxyria* of Polygonaceae (Editorial Board of Flora of China 1998). It is commonly found on mountains from 1,600 m to 3,800 m above sea level in this region (Wu and Chen 2000). *O. sinensis* shows a strong ecological adaptability, thus it can be used as a model plant for exploring plant adaptation to environments. Previous studies have focused on the economic value of *O. sinensis* for ornamental, consumption, and medicinal purposes as well as

heavy metal pollution (Luo *et al.* 2017). To our knowledge, no study has explored the variations in functional traits of this species across an elevation gradient or in varied environments. In this study, we used *O. sinensis* to investigate the variation in vein and stomatal traits with elevation and environmental factors at nine elevational sites in the central Hengduan Mountains. Furthermore, we explored the relationships between vein and stomatal traits under varying environments.

## Materials and Methods

### Sampling sites

The leaves of *O. sinensis* were collected from nine sampling sites along an elevational gradient (from 2,287 to 3,373 m a.s.l.) in middle of Hengduan Mountains in southwestern China. All of the collecting sites were at forest edges. Because our sampling sites were set in a large elevational range from 2,287 to 3,373 m a.s.l., but short longitudinal (98.627°–98.854°) and latitudinal ranges (28.478–29.189°) on the study site (Table 1), the leaf traits were mainly shaped by the environmental changes along the elevational gradients.

The climate in the sampling area is dominated by East Asian monsoon and Indian Ocean monsoon, with a well-defined contrast between the wet (May to October) and dry (November–April) seasons; approximately 85% of the total precipitation occurs during the wet season. The mean annual temperature (MAT), mean annual precipitation (MAP) and annual mean relative humidity (RH) of each site was extracted from a global gridded climate dataset (precision: 0.16°×0.16°; <http://www.paleo.bris.ac.uk/>) (Table 1). Daily maximum UV-B radiation (280–315 nm) was obtained from a Tropospheric UV and Visible Radiation Model ([http://cprm.acd.ucar.edu/Models/TUV/Interactive\\_TUV/](http://cprm.acd.ucar.edu/Models/TUV/Interactive_TUV/)). UV-B (W m<sup>-2</sup>) was calculated as the daily mean maximum UV-B intensity (Table 1). The soil type in the sampling area was brown forestry soil that was formed by erosion of sandy slate and shale. The pH of the soil ranged from 6 to 7. The dominant woody plants in the region were *Quercus* sect. *Heterobalanus*, alpine conifers, and *Rhododendron*.

### Experimental design

All plants in the present study were native populations and we conducted our measurements during the wet season (from July to September) in 2016. We randomly selected four healthy individuals at each site. From each individual, we collected one fresh, undamaged, mature leaf. The size of plot meets the experimental design of investigations and the requirements of statistical analyses (Baraloto *et al.* 2010). The sampled leaves were harvested, preserved in FAA (formalin, glacial acetic acid, ethanol, and distilled water; 10:5:50:35; v:v:v:v) and then taken to the laboratory to measure vein and stomatal traits.

**Table 1:** The elevations, latitudes, longitudes and climatic factors of the nine sampling sites in this study

Sampling sites	Elevation (m)	Latitude (°)	Longitude (°)	MAT (°C)	MAP (cm)	RH (%)	UV-B ( $W m^{-2}$ )
1	2287	28.980	98.627	5.337	64.904	53.313	1.733
2	2390	28.534	98.800	5.445	69.911	55.026	1.757
3	2542	28.514	98.812	5.125	70.265	55.080	1.771
4	2720	29.121	98.638	4.284	64.365	52.845	1.766
5	2797	28.489	98.823	4.239	70.669	54.996	1.795
6	2931	29.144	98.648	3.958	64.406	52.855	1.784
7	3005	28.478	98.854	4.586	70.296	55.407	1.814
8	3157	29.168	98.652	4.729	63.193	53.174	1.802
9	3373	29.189	98.627	5.050	62.585	53.505	1.818

MAT, mean annual temperature; MAP, mean annual precipitation; RH, annual mean relative humidity; UV-B, and daily mean maximum UV-B intensity during the growing season

### Measurements of vein and stomatal traits

The leaves were divided into upper, middle, and lower sections in the laboratory and subsequently cut into 1×1 cm squares. These squares were later immersed in 7% NaOH aqueous solution for 5–8 days until the color of the solution remained unchanged. The leaf squares were prepared for microscopy using the following procedure: the squares were soaked in distilled water for 30 min, bleached in 5% sodium hypochlorite for 20 min, soaked in distilled water for another 30 min, soaked in 150% chloral hydrate solution for 12 h and soaked in distilled water for the other 30 min. The squares were then stained with 1% toluidine blue for 3 min, decolorized by an alcohol series (25, 50, 70 and 95%) for 3 min per concentration, mounted on slides with the abaxial side up and photographed under the light microscope (DM2500, Leica Inc., Bensheim, Germany).

Thirty digital images with clear abaxial stomata (Fig. 1a) and another 30 digital images with clear venation (Fig. 1c) for each site were measured for abaxial stomatal traits and vein length, respectively, using the ImageJ software (v.1.48; Wayne Rasband, National Institutes of Health, USA; available on the website: <http://rsb.info.nih.gov/ij/>). Abaxial stomatal density ( $SD_{down}$ , no.  $mm^{-2}$ ) was measured as the number of abaxial stomata per area and was calculated as the mean value of 30 digital images. The length of abaxial stomata ( $SL_{down}$ ,  $\mu m$ ) was averaged from 30 randomly selected stomata for each site. Vein density ( $VD$ ,  $mm mm^{-2}$ ) was measured by the total vein length per area and was calculated using the mean value of the 30 digital images.

After photographing the abaxial sides of the leaf squares, 30 digital images with clear adaxial stomata (Fig. 1b) were also measured for adaxial stomatal traits with ImageJ software. Adaxial stomatal density ( $SD_{up}$ , no.  $mm^{-2}$ ) was measured as the number of adaxial stomata per area and was calculated as the mean value of 30 digital images. Adaxial stomatal length ( $SL_{up}$ ,  $\mu m$ ) was averaged from 30 randomly selected stomata at each site. The stomatal density ( $SD$ , no.  $mm^{-2}$ ) was calculated as the sum of  $SD_{down}$  and  $SD_{up}$ . The stomatal length ( $SL$ ,  $\mu m$ ) was determined as the

average of  $SL_{down}$  and  $SD_{up}$ . The ratios of adaxial stomatal density to the total stomatal density ( $SD_{up}/SD$ ) and the ratios of abaxial stomatal density to the total stomatal density ( $SD_{down}/SD$ ) were also calculated.

### Data analysis

All statistical analyses were performed with the R statistical program (v.3.01; R Development Core Team, Vienna, Austria; available at: <http://ftp.ctex.org/mirrors/CRAN/>). A cluster analysis with the “complete,” “average,” “mcquitty,” and “ward” methods were used to realize the clustering conditions of the sites. Since the cluster analysis divided the sites into two elevational bands, the differences of the detected traits between these bands were analyzed by independent sample *t*-tests. Pearson-bivariate correlations were used to explore the variations of leaf traits along the elevational gradient and with environmental factors. Correlations between vein density and stomatal density traits were also established by Pearson-bivariate method. Statistically significant level was  $p < 0.05$ .

## Results

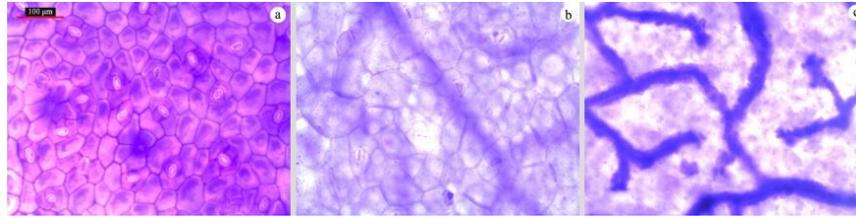
### Variations of vein and stomatal traits

The data from the nine sampling sites were divided into two groups based on the cluster analyses of vein and stomatal traits. The first group included four sites at the lowest elevations (2287, 2390, 2542 and 2720 m) and the two sites at the upper elevations (3157 and 3373 m); the second group included three sites in the middle elevations (2790, 2931 and 3005 m) (Fig. 2). Consistent with the results obtained from the cluster analysis, the values for the tested traits in the plants at the lowest elevations were similar to those at the upper elevations. The lower and upper elevational bands had significantly higher values of  $SD$ ,  $SD_{up}/SD$  and  $VD$ , but lower values of  $SD_{down}/SD$  and  $SL$  compared with the middle elevational band (Fig. 3).

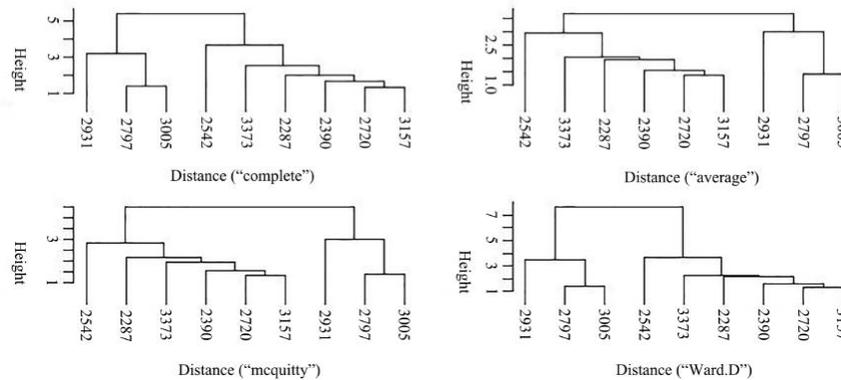
Variations of stomatal and vein traits in this study were mainly influenced by temperature and UV-B radiation; precipitation and air relative humidity had limited effect on the stomatal and vein traits.  $SD$ ,  $SD_{up}/SD$ ,  $VD$  were positively correlated with MAT, but  $SD_{down}/SD$  and  $SL$  were negative correlated with MAT (Fig. 4).  $SD$  and  $SD_{up}/SD$  were negatively correlated with UV-B, but  $SD_{down}/SD$  was positively correlated with UV-B (Fig. 4).

### Correlations between stomatal density traits and vein density

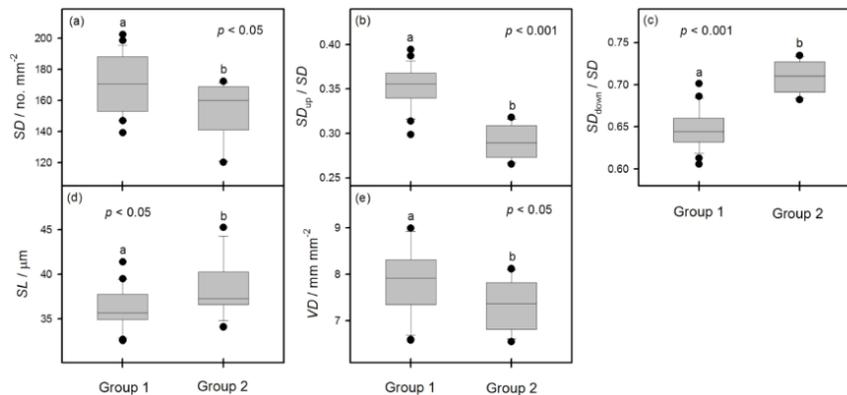
The  $SD$  and  $VD$  showed a significantly positive correlation across all the collecting sites (Fig. 5).  $SD_{up}/SD$  was also significantly positively correlated with  $VD$ , while  $SD_{down}/SD$  was negatively correlated with  $VD$  (Fig. 5).



**Fig. 1:** Anatomical images of abaxial stomata (a), adaxial stomata (b) and veins (c) in this study



**Fig. 2:** Cluster analysis of the sampling sites in this study. The numbers at the end of the cluster branches are the elevations (m) at each site



**Fig. 3:** Comparisons of five stomatal and vein traits between the elevational bands based on a cluster analysis

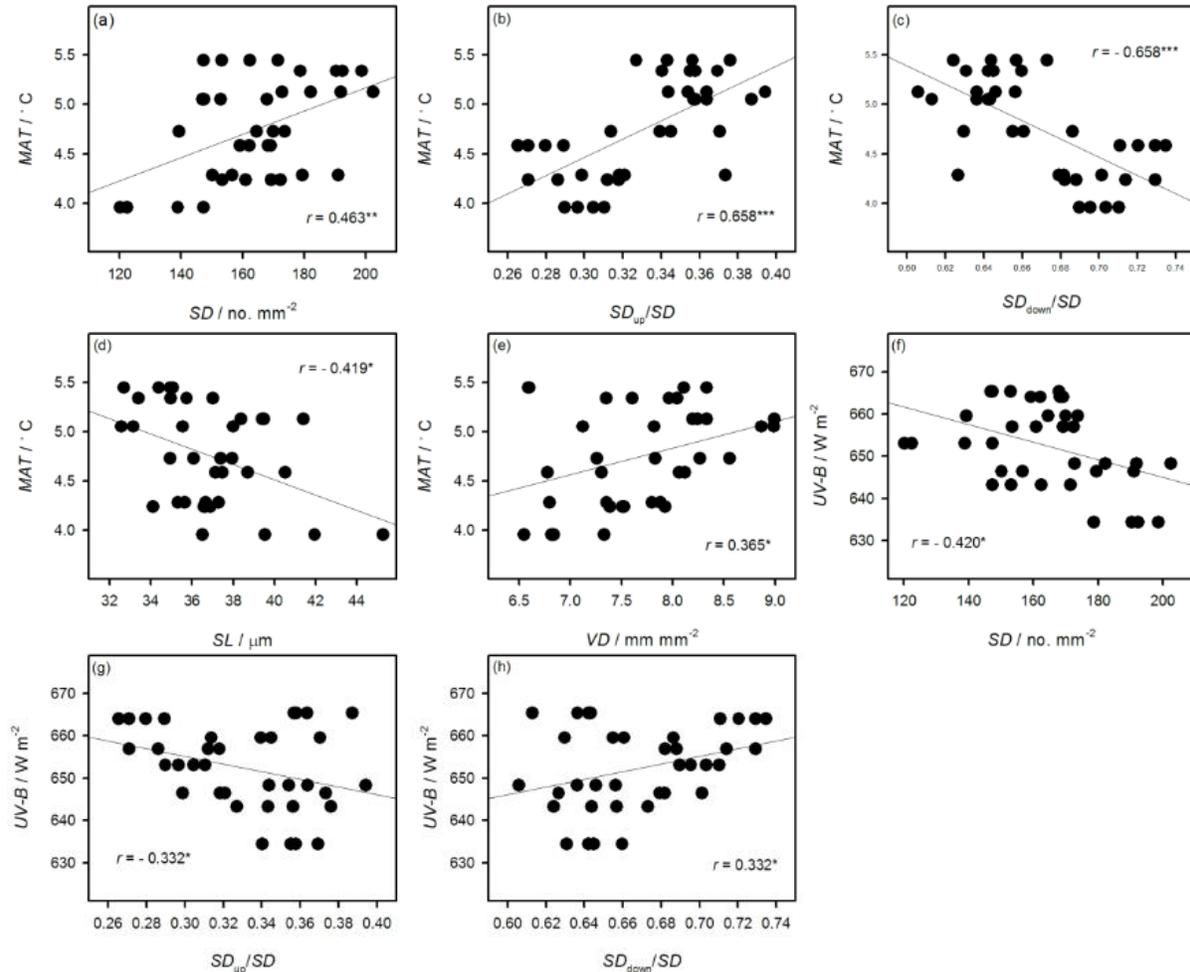
*SD*, stomatal density; *SD<sub>ap</sub>/SD*, ratio of adaxial stomatal density to stomatal density; *SD<sub>down</sub>/SD*, ratio of abaxial stomatal density to stomatal density; *SL*, stomatal length; *VD*, vein density. Different letters above boxes indicate significant differences in each trait among sites ( $p < 0.05$ )

## Discussion

Functional traits affect plant performance, plant fitness, and overall survival in a given environment (Blonder and Enquist 2014). For example, a previous study conducted on 22 species growing at an elevation range of 700 to 1,800 m a.s.l. in the northern Alps showed that most of the species had an increase in stomatal densities (Bucher *et al.* 2016). Moreover, in *Arabidopsis thaliana*, both stomatal density and size increased with the increasing elevation ranging from 50 to 1,260 m a.s.l. (Caldera *et al.* 2016). The stomatal density of 150 woody plant species initially increased and then decreased, while elevation-related differences in

stomatal size were not statistically significant in the Changbai Mountain, China (Wang *et al.* 2014). These inconsistent results might be due to species-specific adaptations and/or different study sites. Plant species has genetic homeostasis in limited range of distribution and velocity of tolerance to environmental changes (Corlett and Westcott 2013). The variations in environmental factors along an elevational gradient may be different in different regions.

Temperature and UV-B were the two key factors influencing stomatal and vein traits (Fig. 4), indicating the major role of these factors in adaptation and distribution of *O. sinensis* in the Hengduan Mountains. External conditions

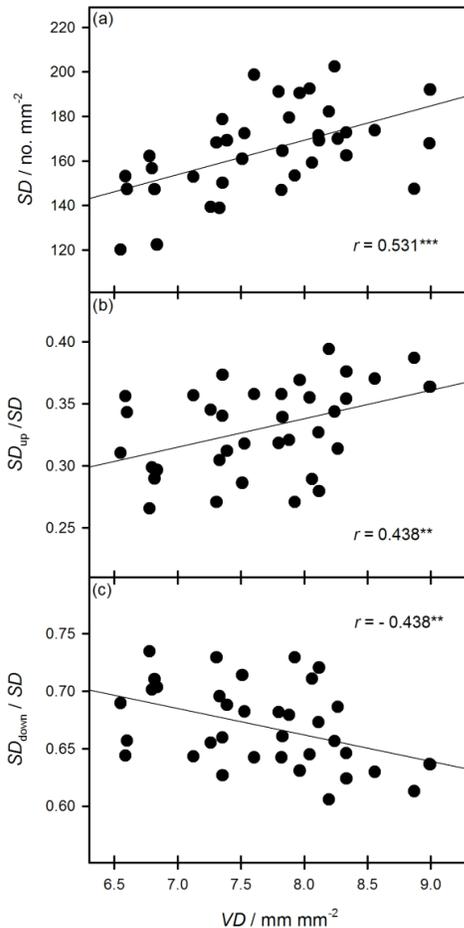


**Fig. 4:** Correlations between environmental factors and leaf traits in this study  
 SD, stomatal density;  $SD_{up}/SD$ , ratio of adaxial stomatal density to stomatal density;  $SD_{down}/SD$ , ratio of abaxial stomatal density to stomatal density significant at: \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$

strongly shape vein and stomatal traits along the elevational gradients (Hill *et al.* 2014; Bucher *et al.* 2016). Our findings suggested that *O. sinensis* along this elevation gradient has variable water transporting capacities due to temperature and UV-B radiation. Among our study sites, MAT typically decreased with the increasing elevation and UV-B radiation was generally more intense at higher elevations (Table 1). Because MAT and UV-B radiation are the main impacting factors to determine the behavior of vein and stomatal traits in this study (Fig. 4), lower SD,  $SD_{up}/SD$  and VD and higher  $SD_{up}/SD$  and SL suggested the adaptive strategies of *O. sinensis* to lower temperatures and higher UV-B intensities at the higher elevations. Lower temperatures decrease the water flow and higher solar radiation may cause transpiration to increase, which together may induce a water deficit condition (Hovenden and Brodrribb 2000; Guo *et al.* 2013). Thus, the fluctuations of stomatal density, adaxial stomatal density, and vein density may reflect the capacity of plants to adapt to water deficits was enhanced at

higher elevations.

The lower SD,  $SD_{up}$  and VD at lower temperatures and higher UV-B radiations may be attributed to two factors. First, colder temperatures and stronger solar radiation might affect the development of leaf anatomy because lower temperature and stronger radiation might limit cell or leaf expansion, especially in high mountain regions (Sack *et al.* 2012). Leaf expansion occurs over both a slow phase and a rapid phase of growth (Sack *et al.* 2012). Substantial leaf expansion of *O. sinensis* may continue after most of its stomatal and vein procambium is formed, thus limited leaf expansion during the rapid phase could induce greater stomatal and vein densities. A second possible reason is that increased evaporative demand might induce acclimation of plants to higher temperatures. More adaxial stomata and veins shorten the distance between stomata and veins, thus accelerating the cooling by efficient water flow and corresponding evaporative water loss (Brodrribb *et al.* 2007). However, in this process, abaxial stomata density



**Fig. 5** Correlations between stomatal density and vein density of *O. sinensis*

SD, stomatal density;  $SD_{up}/SD$ , ratio of adaxial stomatal density to stomatal density;  $SD_{down}/SD$ , ratio of abaxial stomatal density to stomatal density; VD, vein density. Significant levels: \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$

decreased (Fig. 4c), indicating an energy balance. Plant species might input a consistent amount of energy to produce stomata, thus the increase of the abaxial stomatal density should correspond to the decrease of adaxial stomatal density at higher elevations.

The SD and  $SD_{up}/SD$  were positively correlated with VD across all the collecting sites (Fig. 5), suggesting that the capacity of leaf water transport in *O. sinensis* was sufficient to match potential transpirational demands along elevations (Brodribb *et al.* 2013). The homeostatic balance between the liquid and gas phase of hydraulic conductance can be achieved by a close coupling of VD and  $SD_{up}$  along an elevational gradient (Brodribb and Jordan 2011). Higher stomatal conductance enables higher rates of carbon assimilation (Brodribb *et al.* 2007); selecting against plants with certain water-use or carbon-gain strategies could result in environmental filtering on a suite of venation network traits. Therefore, covariation between venation and stomata plays an important role in optimizing the

trade-off between photosynthetic benefit and evaporative cost (Feild *et al.* 2011).

Environmental changes across the studied elevations may affect the correlation between vein and stomatal densities. Along the elevation gradient in the Hengduan Mountains that we selected for our study site, the higher transpiration potentially induced by higher temperature is likely to be one of the main factors by which leaf temperature is decreased, sustaining enzyme activity and maintaining leaf physiological functions. This could explain why high temperature drives plants to increase vein and stomatal densities. Higher vein density indicates that leaf vascular structures can connect to more mesophyll cells (Sack and Frole 2006), whereas a higher stomatal density means that leaves has more transpiration sites, especially on the adaxial surface (Franks and Beerling 2009). Higher vein and stomatal densities can decrease the distance over which water is transmitted from venation to stomata, and improve leaf hydraulic conductance and the transpiration rate (Brodribb *et al.* 2007; Franks and Beerling 2009).

The selected sites covered the entire elevational distribution of *O. sinensis*, representing almost all of the environmental conditions of this species in middle of Hengduan Mountains (Wu and Chen 2000). The outstanding variability in stomatal and vein traits reflected their plasticity and our findings demonstrated their important effect about this species in the adaptability to alpine environments (Sun *et al.* 2016). The temperature has increased 14.9% in the central Hengduan Mountains from 1958, showing a significantly faster rate of temperature increase than other parts of Yunnan Province, China (Fan *et al.* 2008, 2010; Zhang *et al.* 2014a). Because of this climate warming, many alpine species have had to "move up the mountain" meaning that their range has expanded to higher elevations (Moseley 2006; Duputié *et al.* 2011). As an herbaceous species that has a wide distribution over an elevational gradient, *O. sinensis* responded strongly to temperature variations, thus may also expand to higher elevations in this region. Given significant plasticity, the vein and stomatal traits should contribute to adaptation at higher elevations when the upper limit of this species moves further up the mountain.

## Conclusion

Stomatal and vein traits varied across elevations. Plants at both the lowest and highest elevations had similar stomatal and vein traits compared to the middle elevational band. Temperature and UV-B were the two key factors influencing stomatal and vein traits, which had major role in adaptation and distribution of *O. sinensis* in the Hengduan Mountains. The stomatal density and ratio of adaxial stomatal density to stomatal density were positively correlated with vein density across all the collecting sites, suggesting that the capacity of leaf water transport in *O. sinensis* was sufficient to match potential transpirational demands along the elevations.

## Acknowledgements

We acknowledge the team of National Plateau Wetland Research Center, University of Southwest Forestry, who help a lot for various lab analyses in this study. This study was supported by the National Science Foundation of China (31760115) and the Science Research Foundation of the Yunnan Provincial Department of Education of China (2019Y0142).

## Author Contributions

ZY Liu and M Sun planned the study, ZY Liu and M Sun contributed in conducting of study, data analysis and manuscript write up, M Sun contributed to interpreted the result, HJ Guo supervised the study.

## Conflicts of Interest

All authors declare no conflict of interest.

## Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

## Ethics Approval

Not applicable in this paper.

## References

- Baraloto C, CE Paine, S Patino, D Bonal, B Herault, J Chave (2010). Functional trait variation and sampling strategies in species-rich plant communities. *Funct Ecol* 24:208–216
- Blonder B, BJ Enquist (2014). Inferring climate from angiosperm leaf venation networks. *New Phytol* 204:116–126
- Blonder B, N Salinas, BL Patrick, A Shenkin, PP Chambi, YV Tejeira, C Violle, NM Fyllas, GR Goldsmith, RE Martin (2017). Predicting trait-environment relationships for venation networks along an Andes-Amazon elevation gradient. *Ecology* 98:1239–1255
- Brodribb TJ, GJ Jordan (2011). Water supply and demand remain balanced during leaf acclimation of *Nothofagus cunninghamii* trees. *New Phytologist* 192:437–448
- Brodribb TJ, GJ Jordan, RJ Carpenter (2013). Unified changes in cell size permit coordinated leaf evolution. *New Phytol* 199:559–570
- Brodribb TJ, TS Field, GJ Jordan (2007). Leaf maximum photosynthetic rate and venation are linked by hydraulics. *Plant Physiol* 144:1890–1898
- Bucher SF, K Auerswald, S Tautenhahn, A Geiger, J Otto, A Müller, C Römermann (2016). Inter- and intraspecific variation in stomatal pore area index along elevational gradients and its relation to leaf functional traits. *Plant Ecol* 217:229–240
- Caldera HIU, WAJMD Costa, FI Woodward, JA Lake, SMW Ranwala (2016). Effects of elevated carbon dioxide on stomatal characteristics and carbon isotope ratio of *Arabidopsis thaliana* ecotypes originating from an altitudinal gradient. *Physiol Plant* 159:74–92
- Corlett RT, DA Westcott (2013). Will plant movements keep up with climate change? *Trends Ecol Evol* 28:482–488
- Dunbar-Co S, MJ Sporck, L Sack (2009). Leaf trait diversification and design in seven rare taxa of the Hawaiian *Plantago* radiation. *Intl J Plant Sci* 170:61–75
- Duputié A, J Salick, D McKey (2011). Evolutionary biogeography of *Manihot* (Euphorbiaceae), a rapidly radiating Neotropical genus restricted to dry environments. *J Biogeogr* 38:1033–1043
- Editorial Board of Flora of China (1998). *Oxyria sinensis* Hemsl. *Flora Chin* 25:145
- Fan ZX, A Bräuning, KF Cao (2008). Annual temperature reconstruction in the central Hengduan Mountains, China, as deduced from tree rings. *Dendrochronologia* 26:97–107
- Fan ZX, A Brauning, QH Tian, B Yang, KF Cao (2010). Tree ring recorded May–August temperature variations since A.D. 1585 in the Gaoligong Mountains, southeastern Tibetan Plateau. *Palaeogeogr Palaeoclimatol Palaeoecol* 296:94–102
- Feild TS, TJ Brodribb, A Iglesias, DS Chatelet, A Baresch, GR Upchurch, B Gomez, BA Mohr, C Coiffard, J Kvacsek, C Jaramillo (2011). Fossil evidence for Cretaceous escalation in angiosperm leaf vein evolution. *Proc Natl Acad Sci USA* 108:8363–8366
- Franks PJ, DJ Beerling (2009). Maximum leaf conductance driven by CO<sub>2</sub> effects on stomatal size and density over geologic time. *Proc Natl Acad Sci USA* 106:10343–10347
- Guo X, WH Guo, YJ Luo, XF Tan, N Du, RQ Wang (2013). Morphological and biomass characteristic acclimation of *trident maple* (*Acer buergerianum* Miq.) in response to light and water stress. *Acta Physiol Plantarum* 35:1149–1159
- Haworth M, CP Scutt, C Douthe, G Marino, MTG Gomes, F Loreto, J Flexas, M Centritto (2018). Allocation of the epidermis to stomata relates to stomatal physiological control: Stomatal factors involved in the evolutionary diversification of the angiosperms and development of amphistomaty. *Environ Exp Bot* 151:55–63
- Hill K, RS Hill, JR Watling (2014). Do CO<sub>2</sub>, temperature, rainfall and elevation influence stomatal traits and leaf width in *Melaleuca lanceolata* across southern Australia? *Aust J Bot* 62:666–673
- Hill KE, GR Guerin, RS Hill, J Watling (2015). Temperature influences stomatal density and maximum potential water loss through stomata of *Dodonaea viscosa* subsp. *angustissima* along a latitude gradient in southern Australia. *Aust J Bot* 62:657–665
- Hovenden MJ, T Brodribb (2000). Altitude of origin influences stomatal conductance and therefore maximum assimilation rate in Southern *Beech*, *Nothofagus cunninghamii*. *Funct Plant Biol* 27:451–456
- Huang W, H Hu, J Hu, SB Zhang, QY Yang (2014). Effects of temperature on leaf hydraulic architecture of tobacco plants. *Planta* 240:489–496
- Jensen KH, K Berg-Sørensen, H Bruus, NM Holbrook, J Liesche, A Schulz, MA Zwieniecki, T Bohr (2016). Sap flow and sugar transport in plants. *Rev Mod Phys* 88; Article 035007
- Körner C (2007). The use of ‘altitude’ in ecological research. *Trends Ecol Evol* 22:569–574
- Li C, X Zhang, X Liu, O Luukkanen, F Berninger (2006). Leaf morphological and physiological responses of *Quercus aquifolioides* along an altitudinal gradient. *Silva Fenn* 40:5–13
- Luo X, QJ Hu, PP Zhou, D Zhang, Q Wang, RJ Abbott, JQ Liu (2017). Chasing ghosts: Allopolyploid origin of *Oxyria sinensis* (Polygonaceae) from its only diploid congener and an unknown ancestor. *Mol Ecol* 26:3037–3049
- Moseley RK (2006). Historical landscape change in northwestern Yunnan, China. *Mount Res Dev* 26:214–219
- Sack L, C Scoffoni (2013). Leaf venation: Structure, function, development, evolution, ecology and applications in the past, present and future. *New Phytol* 198:983–1000
- Sack L, C Scoffoni, AD McKown, K Frole, M Rawls, JC Havran, H Tran, T Tran (2012). Developmentally based scaling of leaf venation architecture explains global ecological patterns. *Nature Commun* 3; Article 837
- Sack L, K Frole (2006). Leaf structural diversity is related to hydraulic capacity in tropical rain forest trees. *Ecology* 87:483–491
- Schoettle AW, SG Rochelle (2000). Morphological variation of *Pinus flexilis* (Pinaceae), a bird-dispersed pine, across a range of elevations. *Amer J Bot* 87:1797–1806

- Shi K, X Li, H Zhang, GQ Zhang, YR Liu, YH Zhou, XJ Xia, ZX Chen, JQ Yu (2015). Guard cell hydrogen peroxide and nitric oxide mediate elevated CO<sub>2</sub>-induced stomatal movement in tomato. *New Phytol* 208:342–353
- Song LQ, CM Hu, XL Hou, L Shi, L Liu, JC Yang, CD Jiang (2015). Relationship between photosynthetic characteristics and leaf vein density in *Sorghum bicolor* and *Perilla frutescens*. *Chin Bull Bot* 50:100–106
- Soudzilovskaia NA, TG Elumeeva, VG Onipchenko, LL Shidakov, FS Salpagarova, AB Khubiev, DK Tekeev, JHC Cornelissen (2013). Functional traits predict relationship between plant abundance dynamic and long-term climate warming. *Proc Natl Acad Sci USA* 110:18180–18184
- Stewart JJ, SK Polutchko, WW Adams, B Demmig-Adams (2017). Acclimation of Swedish and Italian ecotypes of *Arabidopsis thaliana* to light intensity. *Photosynth Res* 134:215–229
- Sun M, SJ Yang, JL Zhang, M Bartlett, SB Zhang (2014). Correlated evolution in traits influencing leaf water balance in *Dendrobium* (Orchidaceae). *Plant Ecol* 215:1255–1267
- Sun M, T Su, S Zhang, SF Li, J Anberree-Lebreton, ZK Zhou (2016). Variations in leaf morphological traits of *Quercus guyavifolia* (Fagaceae) were mainly influenced by water and ultraviolet irradiation at high elevations on the Qinghai-Tibet Plateau, China. *Intl J Agric Biol* 18:266–273
- Wang RL, GR Yu, NP He, QF Wang, FC Xia, N Zhao, ZW Xu, JP Ge (2014). Elevation-related variation in leaf stomatal traits as a function of plant functional type: Evidence from Changbai Mountain, China. *PLoS One* 9; Article e115395
- Wu Z, S Chen (2000). *Vegetation in China*. Science Press, Beijing, China
- Zhang K, SPan, L Cao, Y Wang, Y Zhao, W Zhang (2014a). Spatial distribution and temporal trends in precipitation extremes over the Hengduan Mountains region, China, from 1961 to 2012. *Quat Intl* 349:346–356
- Zhang SB, M Sun, KF Cao, H Hu, JL Zhang (2014b). Leaf photosynthetic rate of tropical ferns is evolutionarily linked to water transport capacity. *PLoS One* 9; Article e84682
- Zhao WL, YJ Chen, TJ Brodribb, KF Cao (2016). Weak co-ordination between vein and stomatal densities in 105 angiosperm tree species along altitudinal gradients in Southwest China. *Funct Plant Biol* 43:1126–1133