Spatial variations in stomatal traits and their coordination with leaf traits in *Quercus variabilis* across Eastern Asia

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HIGHLIGHTS

- Stomatal traits have high environmental plasticity.
- Stomatal traits are highly coordinated with other leaf traits.
- Trait-trait coordination is formed through long-term evolutionary adaptions.

GRAPHICAL ABSTRACT
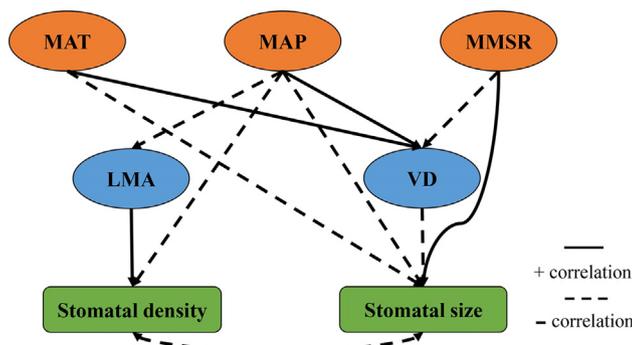
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ABSTRACT

The stomatal traits influence ecosystem carbon-water fluxes and play essential roles that enable plants to adapt to changing environmental conditions. However, how stomatal traits vary along a large climate gradient and whether stomatal traits coordinated with other leaf functional traits in response to environmental changes remain unclear. We investigated the stomatal density (SD), stomatal size (SS), and leaf traits (leaf area (LA), leaf mass per area (LMA), and vein density (VD)) of 44 in situ *Quercus variabilis* populations across Eastern Asia (24 to 51.8°N, 99 to 137°E) and 15 populations grown in a common garden, and evaluated their relationships with environmental factors. Stepwise multiple regression showed that the SD was significantly associated with mean annual precipitation (MAP), LMA, and VD, and the SS with latitude, mean annual temperature (MAT), mean monthly solar radiation (MMSR), and VD. The SD was positively correlated with the LMA, while the SS was negatively correlated with the VD. The SD and LMA increased with decreasing precipitation, which indicated that they may coordinate to commonly enhance plant resistance against drought. The SS decreased; however, the VD increased with temperature. This implied that plants might further reduce their SS by increasing VD limitations under global warming. In the common garden, plants exhibited a higher SD and VD and lower SS and LA compared to those in the field; however, no relation between the stomatal and leaf traits was observed. Our results suggested that stomatal traits have high environmental plasticity and are highly coordinated with other leaf functional traits in response to environmental changes. Nevertheless, this coordination may have been formed through long-term adaptations, rather than over short time spans.

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1. Introduction

Stomata play a critical role in the control of global water and carbon cycles (Hetherington and Woodward, 2003; Wang et al., 2020), and some stomata functions models have been integrated into land surface models to estimate these fluxes (Franks et al., 2017). As two important stomatal traits, stomatal density (SD) and stomatal size (SS) are different between plant species (Bertolino et al., 2019; Liu et al., 2018), which vary with environmental factors, such as water, temperature, light irradiance, atmospheric CO_2 concentrations, and soil nutrients, etc. (Buckley, 2019; Durand et al., 2020; Franks and Beerling, 2009; Liu et al., 2018; Yan et al., 2017). Thus, investigating the responses of stomata to environmental changes is crucial toward predicting ecosystem carbon-water fluxes, while elucidating plant adaptive strategies in the context of climate change (Lawson and Vialet-Chabrand, 2019; Yan et al., 2017).

However, the impacts of environmental factors on stomatal development and plasticity are quite complex (Bertolino et al., 2019; Driesen et al., 2020; Hetherington and Woodward, 2003). The SD typically increases with greater light intensity (Gay and Hurd, 1975) and decreases with elevated CO_2 concentrations (Lammertsma et al., 2011). Under water-stress conditions, some plants may increase their SD and/or decrease their SS (Xu and Zhou, 2008) against water loss to enhance water use efficiencies, while rice (Caine et al., 2019) and barely (Hughes et al., 2017) reduce their SD. Further, no consistent conclusions have been drawn as to the spatial effects of SD or experimentally higher temperatures, where increases, decreases, or even no changes were observed (Hill et al., 2015; Urban et al., 2017; Wu et al., 2018). Yan et al. (2017) also demonstrated that different factors were not independent of each other in the control of stomatal plasticity. Thus, the relationships between stomatal traits and environmental factors still need to be further examined. Furthermore, previous studies have been primarily based on short-term experimental observations under controlled conditions at small-scales (Durand et al., 2020; Lei et al., 2018). It remains unclear how plants, particularly individual tree species, adjust their stomata over extensive geographical gradients, which can deepen our understanding of plant adaptive strategies under intensifying global warming.

Changes in environmental conditions not only influence stomatal traits but also alter additional leaf functional traits (Cui et al., 2020; Wright et al., 2004). Leaf size is essential for leaf thermoregulation (Fauset et al., 2018), and can vary significantly with temperature and precipitation (Wright et al., 2017). Leaf vein density (VD), which determines water transport efficiency, decreases at higher latitudes for *Quercus variabilis* (Zhu et al., 2012), whereas higher temperatures and increasing drought events likely enhance leaf VD (Dunbar-Co et al., 2009; Zhu et al., 2012). The leaf mass per area (LMA), which reflects the investment of structural tissues per area, generally increases with drought for most studied plants (Poorter et al., 2009). Several investigations have reported that there is a negative relationship between the VD and SS (Zhang et al., 2012) and a positive one between the LMA and SD (Loranger and Shipley, 2010). However, whether stomatal traits can co-vary with these leaf traits in response to environmental changes has received less attention. High VD supports high water transport efficiency and water loss rates (Brodribb and Jordan, 2011), meanwhile it can limit stomatal distribution on leaf surfaces (Fiorin et al., 2016). Both high LMA (Wu et al., 2020) and SD (Yan et al., 2017) are potentially linked to a plant's resistance to water limitations. Therefore, as anticipated, stomatal traits and these functional leaf traits may be highly coordinated in response to environmental changes (Brodribb and Jordan, 2011; Fiorin et al., 2016).

Stomata respond to environmental changes across time scales that can range from minutes to millennia (Hetherington and Woodward, 2003), including short-term behaviors (opening or closing) and the long-term adaptation of morphological traits (e.g., size and density) (Lake et al., 2001; Casson and Hetherington, 2010), which may occur concurrently. *Arabidopsis thaliana* can quickly transmit environmental signals from mature to new leaves, which induces SD development in the new leaves (Lake et al., 2001). Trait-based ecological scaling theory predicts that plant traits will be modified in response to climate change (Enquist et al., 2017). Generally, plants under long-term selection will produce certain morphological and physiological adaption to the local environment, but when environmental conditions change, these functional traits will exhibit to some extent plasticity (Lusk et al., 2008; Ramírez-Valiente et al., 2010). For example, Pyakurel and Wang (2014) collected samples from 16 birch populations across Canada, as well as those grown in a common garden, and found that leaf morphologies and stomatal characteristics are altered, and that these changes could be correlated to the climate of the population's origin and to plant age (Mediavilla and Escudero, 2003). Under a rapidly changing climate when stomatal traits are quickly transformed (Lake et al., 2001), whether other leaf traits follow suit and trait-trait relationships take hold, remain unclear.

The deciduous oriental oak (*Q. variabilis*) is one of the most widely distributed trees across Eastern Asia (Chen et al., 2012), with important ecological and economical value (Aldrich and Cavender-Bares, 2011). This provides an ideal experiment material to study the responses of stomata and leaf functional traits to environmental changes. Previous studies have investigated spatial variations in leaf vein density (Zhu et al., 2012) and the stoichiometric traits of leaves (Sun et al., 2015). For this study, we investigated the stomatal traits and leaf functional traits of 44 in situ *Q. variabilis* populations across Eastern Asia, and evaluated their relationships with environmental factors. Meanwhile, a common garden was established with 15 of the 44 populations at the middle latitude. Our aims were to (1) Investigate how the SD and SS varied along a large climate gradient, which spanned subtropical to temperate biomes; (2) Examine whether a high coordination existed between stomatal traits and other leaf functional traits in response to environmental changes; (3) If so, to explore whether this coordination might be sustained under new environmental conditions. We hypothesized that (1) spatial variations in stomatal traits are highly environmental plasticity, (2) stomatal traits and other leaf functional traits are highly coordinated in response to environmental changes, and (3) this coordination is formed based on long-term local adaptation rather than a short time.

2. Materials and methods

2.1. Plant materials and sampling methods

In this study, we collected 44 populations in situ across the whole of East-Asia from 2007 to 2009, including mainland China, Taiwan Island, the Zhoushan Islands, Peninsular Korea, and Japan (Fig. 1). The sampled sites spanned latitudes from 24°N to 51.8°N, and longitudes from 99°E to 137°E, with mean annual temperatures (MAT) ranging from 8.7 to 22.8 °C, mean annual precipitation (MAP) from 495 to 2600 mm, and mean monthly solar radiation (MMSR) from 298.2 to 541.6 (MJ m^{-2}) (Table S1). Furthermore, we collected the seeds of 15 of 44 sampling sites in 2008, which were grown in a common garden in Shanghai (located at 4 m above sea-level, and 31°02′N and 121°26′E), where MAT and MAP was 15.5 °C and 1149.8 mm, respectively. Different populations were followed a randomized complete block design (RCBD) in a common garden, where there were five replicates for each population (Sun et al., 2016).

For each of the 44 in situ sampling sites, their coordinates were recoded via a geographical positioning system (Thales, USA), including latitude, longitude, and altitude. Leaf samples were collected from August to September. At each site, five dominant trees were selected in a 20 m × 20 m plot, after which 20 mature and healthy leaves per tree were collected from three twigs in the middle of the south-facing crown. The leaf samples were collected from between 9:00 am and 12:00 noon every day. The leaf samples in the common garden were
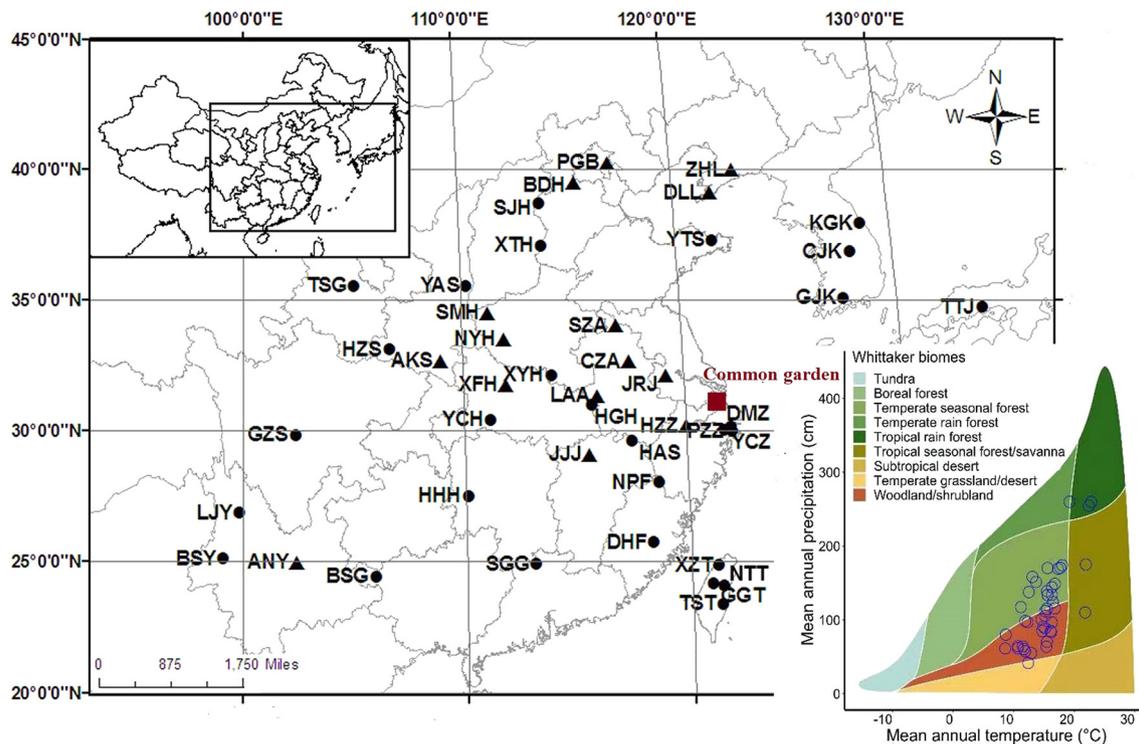
Fig. 1. Distribution of sampling sites of oriental oak across Eastern Asia. The circles and triangles represent 44 in situ populations; triangles represent the 15 original sites of common garden populations, and the square represents the location of the common garden.

collected in 2011 (3-year seedlings). For each replicate, three healthy seedlings were selected, and at least 10 mature and healthy leaves were collected in the upper central south-facing part of crown. The collected leaf samples were divided into two sections for the measurement of stomata and leaf functional traits.

2.2. Stomata and leaf trait measurements

The stomata of oriental oak were distributed only in the abaxial leaves and covered with trichomes (Kim et al., 2011). Prior to measuring the SD and SS, the trichomes were gently removed with a blade under an anatomical lens. Subsequently, one 0.5×0.5 cm^2 section was selected at the right side of the midrib in the central section of lower leaf surface, and then fixed in 2.5% glutaraldehyde and stored at 4 °C in a refrigerator. These sections were in turn dehydrated using an ethanol gradient, freeze-dried, and gold sputtered before being imaged. Three micrographs for each section were obtained at 1000× magnification using a FEI Sirion 200 ultrahigh resolution Schottky field emission scanning electron microscope (FEI Company, Hillsboro, OR, USA). These images were randomly taken at no overlapping locations on the sections, from between the midrib of the lamina and its edge, and the middle of lamina length (Russo et al., 2010). The stomata density was calculated by averaging 45 images per population with a 0.056 mm^2 viewing field. Furthermore, ten open stomata were selected within each viewing field to quantify the stomatal length and width; thus, a total of 450 stomata per site were measured. The stomatal length was defined as the distance along the longer axis of the guard cells, and stomatal width as the distance along the shorter axis of two guard cells (Willmer and Fricker, 1996). The SS was calculated as the stomatal length multiplied by the stomatal width (Franks and Beerling, 2009).

The leaf area (LA) was measured using WinFolia software (Regent Instrument Inc. Quebec, Canada), whereas the leaf dry mass was obtained after drying at 70 °C for 48 h. The leaf dry mass per area (LMA) was defined as the dry mass per unit leaf area. To determine the leaf vein density (VD), a 1 cm^2 sample was excised from the adaxial and middle of leaf right side. Leaf veins were recorded using a Leica DM 2500 microscope (Leica Microsystems, Wetzlar, Germany). The leaf vein density (mm mm^{-2}) was expressed as the lengths of leaf veins per area, which were determined using Image J software (Abràmoff et al., 2004), with the detailed methodology reported by Zhu et al. (2012).

2.3. Data analysis

The definition of biome was followed the method of Whittaker (1970). The meteorological data was obtained from http://cdc.cma. gov.cn/home.do, and the mean value of 30 years (1979–2009) was used for analysis. One-way ANOVA was employed to test the differences in stomatal traits (SD and SS) and leaf functional traits (LA, LMA, and VD) between the field samples and common garden population samples. Linear regressions were performed to correlate environmental factors with the stomatal and leaf traits. Stepwise multiple regressions were used to examine the effects of geographic factors (latitude, longitude, and altitude), climatic factors (MAT, MAP, and MMSR) and leaf traits on SD and SS, respectively All tests were performed using R version 3.6.1 (R Development Core Team, available from www.r-project. org/, accessed 2019) and SigmaPlot 10.0 (Systat Software, Inc., Richmond, CA, USA).

3. Results

3.1. Spatial patterns of stomatal traits in oriental oak across East-Asia

The mean SD and SS values of in oriental oak were 962.79 stomata mm^{-2} and 310.69 μm^2, with the range from 565.48 to 1236.48 stomata mm^{-2} for the SD, and from 218.41 to 391.69 μm^2 for the SS, respectively. The coefficient of variations of the SD and SS were 0.14 and 0.09, respectively, showing a higher variation in the SD in contrast to the SS (Fig. S1). The SD decreased significantly with longitude (from inland to coastal areas), whereas the SS increased with latitude and longitude, while the altitude had no significant impacts on the SD or SS (Fig. 2).
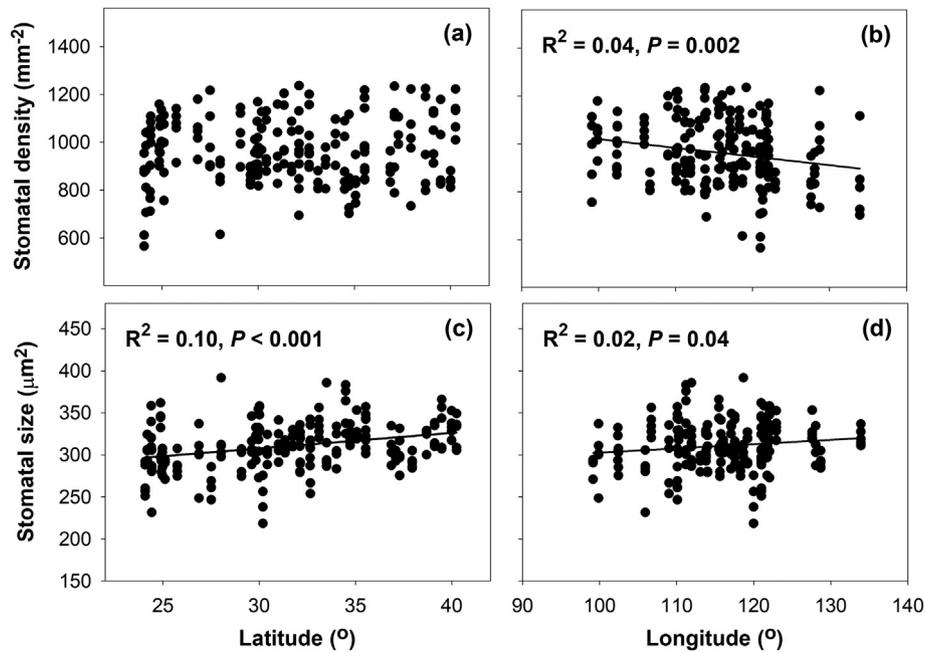
Fig. 2. Spatial patterns of stomatal traits in the oriental oak across Eastern Asia.

3.2. Effects of environmental factors on stomatal traits and their relationships with other leaf functional traits

Stepwise multiple regression analyses indicated that a 24% variation in the SD and 17% variation in the SS were accounted for by environmental factors and leaf traits. The SD was significantly associated with MAP, LMA, and VD, and the SS with LAT, MAT, MMSR, and VD (Table 1). Generally, the SD and SS increased considerably with reduced precipitation, whereas the SS decreased with higher temperature but increased with the MMSR (Fig. 3). The LA and LMA increased with lower precipitation, while VD increased with higher temperature but decreased with lower precipitation and higher MMSR (Fig. 4). Moreover, there was a significant positive relationship between the SD and LMA, and a negative relationship between the SS and VD (Fig. 5), while there was no relationship in the SD and SS with LA. Furthermore, there was a significant negative relationship between the SD and SS (Fig. 5).

3.3. Long-term adaptation and short-term plasticity of stomata and other leaf functional traits

In the common garden, plants exhibited higher SD and lower SS compared to those in the field ($p < 0.05$) (Fig. 6). In terms of leaf traits,

plants exhibited a lower LA and higher VD in the common garden ($p < 0.05$), while there was no difference in the LMA between the common garden and in situ samples. Interestingly, the SD and SS had no relation to leaf traits in the common garden (Table S2), which was totally different from the findings for the in situ samples. The SD was negatively associated with SS regardless of the plant source (Table S2). Moreover, there was a positive relationship between the SS and the precipitation of original sites in the common garden, while this was opposite for the field sample (Fig. 7).

4. Discussion

4.1. Spatial variations of stomatal traits in Quercus variabilis and driving factors

The SD is linked to stomatal conductance, such that when the SD increases, the stomatal conductance of water and CO_2 will be enhanced (McElwain et al., 2016; Sakoda et al., 2020; Yin et al., 2020). Meanwhile, smaller stomatal pores also facilitated rapid closing to reduce water loss during dry periods, and opening, to ease CO_2 exchange under conditions of favorable water availability (Aasamaa et al., 2001). Therefore, compared with other plants (Abrams et al., 1994; Bresson et al., 2011; Willmer and Fricker, 1996), the higher SD and smaller SS of the oriental oak, to some extent, reflect its high adaptability to environmental change, which may account for why this plant is so widely distributed. Furthermore, the CV of the SD was higher than that of the SS (Fig. S1), which indicated that the SD may have a relatively higher plasticity compared with the SS in response to environmental fluctuations.

Geographic factors drives climate change (Table S3), thereby affecting stomatal plasticity (Fig. 3). The effects of water limitations on stomatal traits have been widely examined in previous studies (Hughes et al., 2017; Xu and Zhou, 2008; Yan et al., 2017). Our results indicated that both the SD and SS increased with lower precipitation (Fig. 3). In earlier studies; however, under drought conditions Populus trichocarpa (with a smaller SS and higher SD) and B. papyrifera (with a larger SS and lower SD) were observed (Pyakurel and Wang, 2014). These results indicated that various plants have different stomata regulation strategies when they suffer from drought. In this study, an increase in the SD with drought may have been due to higher SD under dry conditions, which might increase stomatal conductance; thus, enhancing water use

Table 1
Simple stepwise multiple regression of stomatal density (SD) and stomatal size (SS) against mean environmental factors and leaf functional traits, and full model describing SD and SS in terms of latitude (LAT), longitude (LON), altitude (ALT), mean annual temperature (MAT), mean annual precipitation (MAP), mean monthly solar radiation (MMSR), leaf area (LA), leaf mass per area(LMA), vein density (VD) ($n = 44$).

Methods	Equation	R^2	p
SD			
Full model	SD = 809.45 + 2.19 × LAT-2.21 × LOG-0.02 × ALT-0.05 × MAT-0.07 × MAP-0.003 × MMSR-1.28 × LA + 20.41 × LMA + 46.42 × VD	0.25	<0.001
Stepwise	SD = 557.58–21.09 × LMA-0.08 × MAP + 46.70 × VD	0.24	<0.001
SS			
Full model	SS = 5.95 + 2.75 × LAT + 1.12 × LOG+0.01 × ALT+4.72 × MAT-0.01 × MAP + 0.05 × MMSR+0.26 × LA + 1.66 × LMA-6.32 × VD	0.21	<0.001
Stepwise	SS = 146.58 + 3.12 × LAT + 4.47 × MAT+0.11 × MMSR-8.92 × VD	0.17	<0.001

Fig. 3. Relationships between environmental factors and stomatal density (a, b, and c) and stomatal size (d, e, and f) in the oriental oak across Eastern Asia. MAT, mean annual temperature; MAP, mean annual precipitation; MMSR, mean monthly solar radiation.

efficiency (data not shown), which was in line with previous studies (Xu and Zhou, 2008). Smaller SS could open and close more rapidly, which may augment the resistance of the plants against drought; however, our results were inconsistent in this regard. In our study, the SS increased with decreasing precipitation, which aligned with the findings for *B. papyrifera* (Pyakurel and Wang, 2014). This was likely due to greater SS linked to increased stomatal conductance (Maherali et al., 2002), which facilitated the diffusion of CO_2 into leaves (Parkhurst, 1994). In general, the results indicated that different plants could engage in variable trade-off strategies between the SS and SD in response to drought. However, for the oriental oak, larger and denser stomata may serve as an adaptive plasticity strategy against drought.

Since temperature influences cell division, stomatal development may be altered by elevated temperatures (Driesen et al., 2020). In the present study, the SS was decreased with higher temperatures (Fig. 3). This was

likely due to the low requirement of stomatal conductance in regions at low latitudes, where the temperature is higher and accompanied by greater precipitation, which may cause low vapor pressure deficits; thus, reducing the SS (Song et al., 2021). An alternate interpretation relates to VD limitations, as we found that there was a positive relationship between temperature and the VD (Fig. 4), meaning that the VD could increase with temperature, which may have enhanced the limitations for stomatal distribution; thus, lowering the SS. Furthermore, we found that there was no linkage between temperature and the SD, which was in contrast to the study of Yan et al. (2017), who found that elevated temperature may increase the abaxial SD, but aligned with the study of Sadras et al. (2012). Our results suggested that the SS was more sensitive than the SD when subjected to elevated temperatures.

Light intensity had a positive influence on the SD, as increased SD under high irradiance conditions may improve stomatal conductance
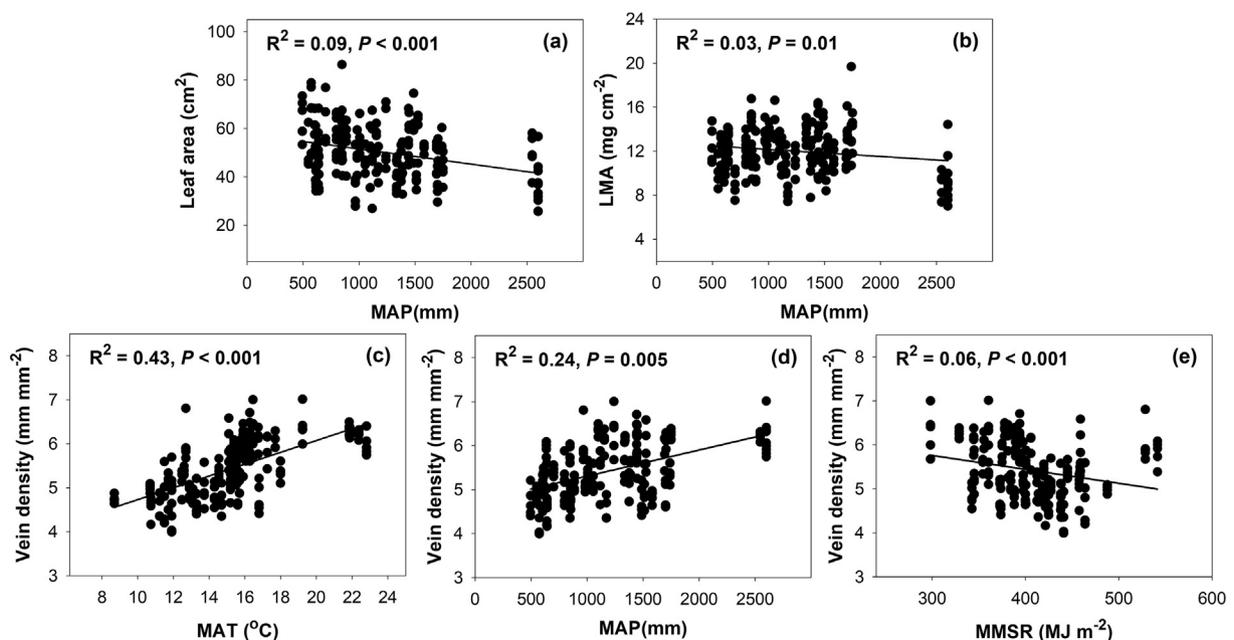
Fig. 4. Relationships between environmental factors and leaf functional traits in oriental oak across Eastern Asia.
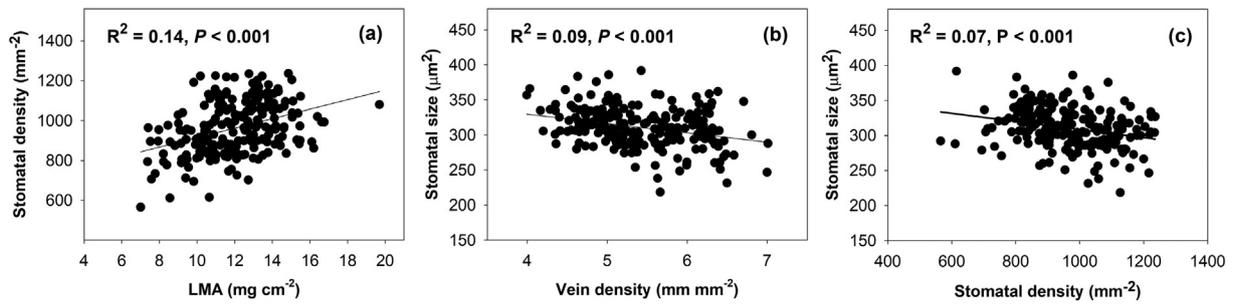
Fig. 5. Relationships between leaf functional traits and stomatal traits in the oriental oak.

(Shimazaki et al., 2007) and assists plants with maintaining favorable leaf temperatures (Lu et al., 1994). However, this was different from our study, as we found that there was no relation between light intensity and the SD (Fig. 4), but a positive correlation between light intensity and the SS. This indicated that the oriental oak adapts to different light intensities by changing the SS rather than SD. Interestingly, the present study suggested that spatial variation in the SD was related only to MAP, while the SS was associated with the MAT, MAP, and MMSR, which implied that the SS may be more sensitive to environmental changes compared with the SD. This was likely due stomatal populations that are established at an early stage of leaf development (Furukawa, 1998); thus, maintaining high SS plasticity may be more conducive for plants to adapt to changing environments during the growing season. Furthermore, our results illustrated that changing environmental factors cannot only directly determine stomatal traits, but also indirectly influence stomatal traits through the modification of other leaf functional traits.

4.2. High coordination between stomatal traits and other leaf functional traits in response to environmental change

The present study revealed that both the SD and LMA increased with lower precipitation (Figs. 3, 4), and there was a positive relationship between the SD and LMA (Fig. 5), which was consistent with the study of Loranger and Shipley (2010). This positive relationship was likely due to the fact that leaves with higher LMA generally have lower mesophyll conductance (Wu et al., 2020), thus they need to be coupled with high SD to increase stomatal conductance and reduce leaf temperatures. The results indicated that the development of SD and LMA in the

oriental oak is highly coordinated in response to drought conditions, which helps plants to enhance their resistance against drought.

The leaf VD was positively correlated with the MAT and MAP, and negatively with the MMSR (Fig. 4), which indicated that the VD was extremely sensitive to environmental change, akin to the SS. Previous studies also demonstrated that plants possessed high VD plasticity in response to different environmental factors (Fiorin et al., 2016; Lei et al., 2018), meaning that when the environment changes, plants are able to control the development of leaf veins to adapt to changing photosynthetic and hydraulic demands (Brodribb and Jordan, 2011). A strong, linear correlation between the VD and temperature was reported in a study by Zhu et al. (2012) that aligned with our results. This was likely due to the reduced leaf water transpiration and flow rate of plants at low temperature, which resulted in a low VD requirement. In addition, our results revealed VD and SS responses to environmental factors that were completely converse, which matched their negative relationships, as demonstrated in *Paphiopedilum* spp. leaves (Zhang et al., 2012). Our findings suggested that the development of veins and stomata may also be highly coordinated in response to environmental change. This coordinated development is critical for maintaining the hydraulic balance of supply and demand (Brodribb and Jordan, 2011). It is well known that global warming has been rapidly increasing over the last century; a trend that continues to accelerate. Meanwhile, the frequency and intensity of drought has also been enhanced due to increasing temperatures to some extent (Naumann et al., 2018; Wang et al., 2011). Our results indicated that with higher temperatures, the SS might decrease in the future, while with increased drought events, both the SD and SS may increase. For contradictory results on decreasing or increasing SS, stepwise multiple regression analyses indicated that precipitation
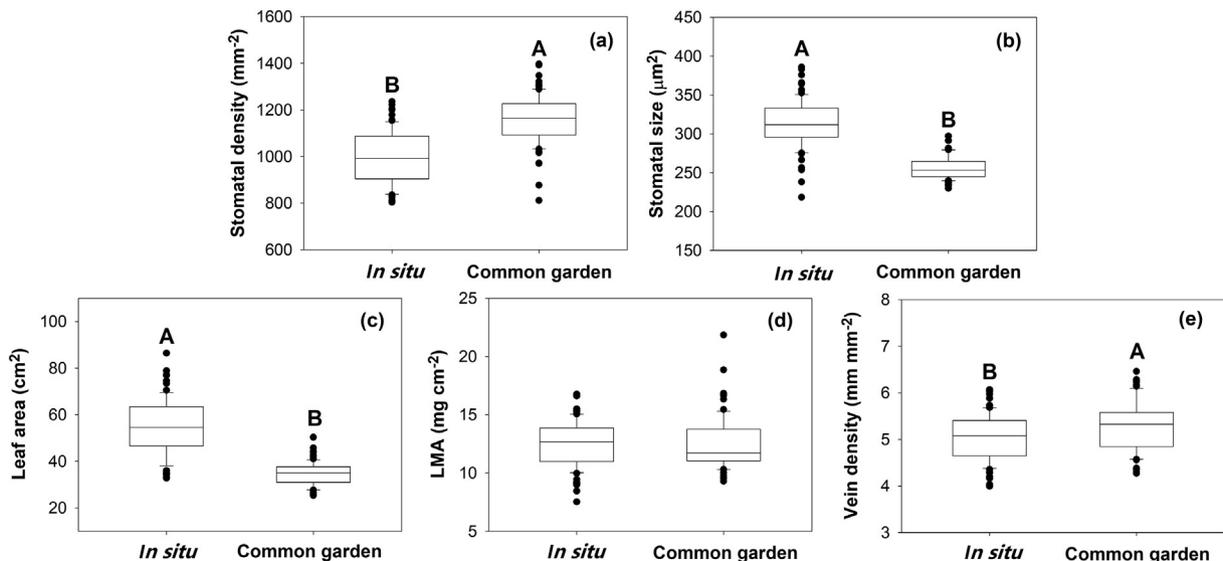
Fig. 6. Differences in stomatal and leaf traits of the in situ and common garden samples ($n = 15$).
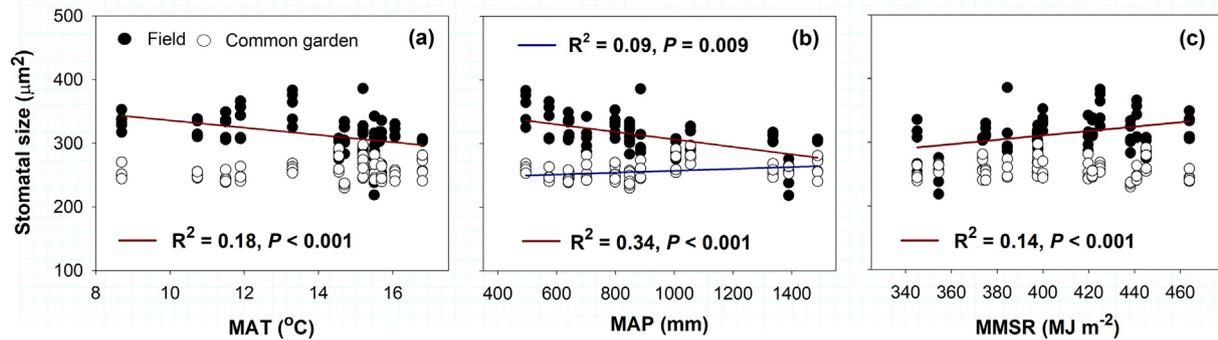
Fig. 7. Relationships between climatic factors and stomatal traits in the common garden (n = 15) and in situ samples (*n* = 15).

could play a more important role in determining the SS compared to temperature. Moreover, with the exception of the direct effects of environmental factors on the SD and SS, elevated temperatures may indirectly decrease the SS by increasing the VD, whereas increasing drought may indirectly affect the SD and SS by increasing the LMA and reducing the VD, as shown in our results (Fig. 8). Generally, our results suggested that different environmental factors are not independent of each other in influencing stomatal traits (Larcher et al., 2015), and different functional traits may co-vary in response to climate change. Further, our results provided new insights regarding the incorporation of stomatal traits into mechanistic vegetation models (for predicting plant adaptation and ecosystem functionality), and into land surface models to obtain more accurate estimations of global carbon-water cycling.

4.3. Long-term adaptation and short-term plasticity of stomata and other leaf functional traits

Variations in stomatal traits and other leaf functional traits are linked to the sequestration and utilization of resources, as well as plant adaptations to environmental changes. Our study revealed that the SD of different sample populations in a common garden was higher than that of in situ populations, while the SS showed an opposite trend (Fig. 6). In previous studies, smaller SS and denser SD were observed to initiate the rapid opening and closing of stomata, which was considered to improve the adaptability and resistance of plants against drought (Drake et al., 2013; Hetherington and Woodward, 2003). However, our results indicated that this pattern may be more conducive for early plant growth rather than enhancing plant resistance against drought, as
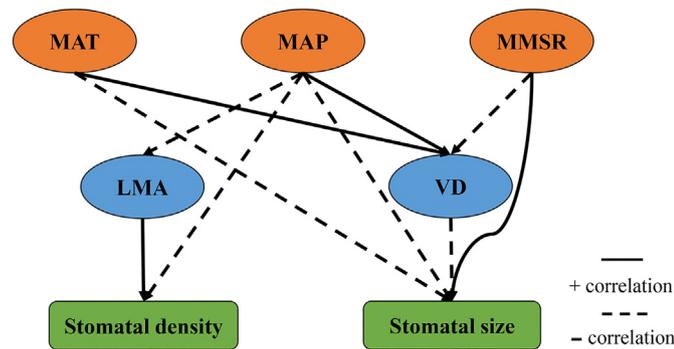
demonstrated by the study of Mediavilla and Escudero (2003). They found that the seedlings with smaller SS and denser SD compared with mature trees can rapidly increase in stomatal conductance and maximize CO_2 diffusion into the leaf, which can improve larger photosynthetic efficiency and cause plant greater growth.

The impacts of environmental factors on the SS in 15 selected field sites were consistent with a total of 44 field sites. Interestingly, there was a positive relationship between the SS and precipitation at original sites in the common garden, which was opposite to the results of the in situ field samples. This meant that under new environmental conditions, plants from arid regions likely make larger adjustments in SS, and indicated that the memory of precipitation signals by plants may affect the adaptability of offspring. In the common garden, the slope of the equation was small and even close to 1, while in the field, the slope of the equation was greater. This result suggested that the SS of the oriental oak had a convergent response when it was moved to the common garden.

In the common garden, with the exception of changes in stomatal traits, the leaf traits also changed, showing a lower LA and greater VD compared with the in situ field samples (Fig. 6). This indicated that the variations in leaf functional traits were primarily environmentally plastic, akin to stomatal traits. Moreover, leaf morphology and physiology should be coordinated at each life stage (Ishida and Yazaki, 2005). In the field samples, the SD in the mature trees was related to the LA and LMA, and the SS was associated with the LA and VD. However, there was no relation between the stomatal and leaf traits as seedlings in the common garden, which was completely different from the field observations. These findings suggested that the coordinated relationship between stomatal and leaf traits may be the result of long-term local adaptations. As expected, with the development of plant growth, these functional traits will also show high coordination, which requires further investigation.

5. Conclusion

Our study provides a comprehensive picture to describe the spatial variations in the stomatal and leaf functional traits in the oriental oak and their relationships with environmental factors across Eastern Asia. Our conclusions were: (1) Stomatal traits possess highly adaptive plasticity to environmental changes (as expected) with global climate change, stomatal density may increase with reduced precipitation, while stomata size may be declined with temperature; (2) Stomatal traits and other leaf functional traits may be highly coordinated with environmental changes; (3) The coordination between leaf stomatal and functional traits may be formed through long-term local adaptations. Our findings not only advance our understanding of plant adaptation strategies and carbon-water fluctuation processes under climate change, but also provide new insights for the incorporation of stomatal traits into mechanistic vegetation and land surface models.

Fig. 8. Conceptual model describing the coordination relationships between stomatal and leaf functional traits in response to environmental change. Red ellipses, blue ellipses, and green rectangles represent environmental factors, leaf functional traits, and stomatal traits, respectively. The model is constructed based on the results of Figs. 3–5.

CRediT authorship contribution statement

Baoming Du: Software, Formal analysis, Writing – original draft.
Yanhua Zhu: Investigation, Software, Writing – original draft.
Hongzhang Kang: Conceptualization, Methodology, Investigation,
Funding acquisition. **Chunjiang Liu:** Conceptualization, Resources,
Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial
interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.
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