Spatial variations in leaf trichomes and their coordination with stomata in Quercus variabilis across Eastern Asia

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Abstract

Understanding leaf trait variation is critical for the prediction of plant responses to global climate changes. Leaf trichomes are derived from stomatal epidermal cells, which play critical roles in biotic defenses and against abiotic stress. Previous studies reported spatial variations in stomatal traits and their driving factors at the same sampling sites. However, it remains unclear how leaf trichome densities vary along large geographic gradients and whether trichomes and stomata co-vary under environmental changes. We measured the leaf trichome densities of 44 Quercus variabilis populations in situ across Eastern Asia (24 to 51.8 °N, 99 to 137 °E) and 15 populations grown in a common garden, and used structural equation modelling to evaluate their relationships with climatic factors and stomatal traits. Our results revealed that climatic factors not only had a direct effect on trichome density, but also had indirect effects on trichome density through stomatal density and leaf mass per area. Trichome density was positively correlated with stomatal density whether in situ or in the common garden, and both increased with drought, which implied that trichomes may impact stomatal behavior and both traits may co-vary under certain environmental conditions. Our results suggested that leaf trichomes possess highly adaptive variation and are in close coordination with stomata in response to climate changes. Our findings provide new insights toward elucidating the interactions between leaf traits and the adaptive strategies of plants under climate change.

Introduction

Leaf traits represent the functional strategies of plants, encompassing growth, carbon economies, and resource exploitation and conservation, which vary across species and environmental conditions (Deans et al. 2020, Xing et al. 2021). Understanding leaf trait variation is critical for the prediction of plant responses to global climate changes (Stotz et al. 2021). Leaf trichomes are derived from epidermal cells on the surfaces of leaves, which not only provide structural defenses against herbivores (Dalin et al. 2008), but also play multiple additional physiological and ecological roles (Ehleringer et al. 1976, Bickford 2016, Sack and Buckley 2020). Leaf trichomes can reduce the absorbance of solar radiation and UV damage, as well as decrease the leaf transpiration rate and temperature by increasing boundary layer resistance (Ripley et al. 1999, Sack and Buckley 2020). This, in turn, affects the photosynthesis rate and water use efficiency of plants (Ripley et al. 1999, Konrad et al. 2015), as well as determines plant fitness against adverse environmental conditions (Ehleringer 1988, Jon and Douglas 1994). Previous studies have examined the functions of leaf trichomes and their control factors, which have been extensively investigated under variable manipulation conditions and regional scales (Pérez-Estrada et al. 2000, Paulino et al. 2020). However, little is known about how leaf trichomes vary along a large geographic gradient in individual plant species, which can advance our understanding of spatial variations in leaf traits and plant adaptive strategies under climate change.

Leaf trichome densities can vary between plant species under localized environmental conditions and are affected by changes in light intensity, temperature, rainfall, CO_2 concentrations, UV-radiation, and soil

nutrients (Pérez-Estrada et al. 2000, Abdala-Roberts et al. 2016, Thitz et al., 2017, Moles et al. 2020). Leaf trichome densities typically increase under sunny and dry environments and decrease under shady and wet conditions (Kessler et al. 2007). In fact, leaf trichomes serve as an effective barrier against solar radiation (Skelton et al. 2012), which can increase light reflectivity to reduce photoinhibition and cool leaves to maintain optimal photosynthetic temperatures (Perez-Estrada et al. 2000). In addition, high trichome densities also may enhance resistance against drought by increasing the thicknesses of leaf boundary layers, reducing conductivity for water vapor diffusion, and limiting water loss through transpiration (Bickford 2016). Kenzo et al. (2008) found that the leaf trichomes of *Mallotus macrostachyus* contributed to high leaf water use efficiencies under drought conditions. However, other studies found drought stress did not induce trichome production (Piritta et al. 2010). Furthermore, the formation and development of trichomes can be significantly influenced by temperature (Skelton et al. 2012, Amada et al. 2020). However, it remains unclear exactly which environmental factors primarily drive spatial variations in leaf trichome densities.

Previous studies have primarily focused on the isolated roles of given traits (Poorter et al. 2009; Li et al. 2020). However, environmental stresses are often combined; thus, a given functional trait can provide resistance against multiple stressors, whereas multiple functional traits may also coordinate in response to a given stress (Read et al. 2014, Sack and Buckley 2020, Xing et al. 2021). Leaf size is essential for leaf thermoregulation, where the leaf mass per area (LMA) reflects the investment of structural tissues per area and leaf veins determine water transport efficiencies, all of which are variable under environmental changes (Fauset et al. 2018, Du et al. 2021). Stomata regulate water and gas exchange between plants and the ambient atmosphere (Hetherington and Woodward 2003), whereas stomatal guard and trichome cells share the same development origins and are distributed at specific distances across the epidermal surfaces of leaves (Holroyd et al. 2002). Negative correlations between the trichome and stomatal densities (SD) have also been observed in other studies (Galdon-Armero et al. 2018). The coordinated development of trichomes and stomata may influence plant water use efficiencies and environmental adaptations (Galdon-Armero et al. 2018; Bertolino et al. 2019), which may reflect a trade-off between the proportions of cells that possess trichomes and guard cell fates (Simon et al. 2020). Yet, it remains unclear whether the relationships between leaf trichomes and other functional traits may co-vary with stomata in response to environmental changes.

For this work, we investigated the leaf trichome density of 44 in situ Q. variabilis populations across Eastern Asia and evaluated their associations with environmental factors and other functional leaf traits (e.g., leaf area (LA), LMA, vein density (VD), stomatal size (SS), and SD) using a piecewise structural equation model (SEM). Furthermore, a common garden was established with 15 of the 44 populations at the middle latitude to further examine adaptive variation of leaf trichomes. We hypothesized that leaf trichomes possess highly environmental variation and can coordinate with stomata in response to environmental changes. To test the hypotheses, we investigated how leaf trichome density varied along an extensive climate gradient (which spanned subtropical to temperate biomes) and its driving factors. Further, the relationships between trichome density and functional leaf traits in response to environmental changes were examined, particularly for stomata.

Materials and methods

Plant materials

The deciduous oriental oak (Q. variabilis) is one of the most widely distributed trees across Eastern Asia (Chen et al. 2012), which provides ideal experimental samples to explore the spatial variation and driving factors of functional plant traits under climate change. In the present study, the sampled sites spanned latitudes from 24degN to 51.8degN, and longitudes from 99degE to 137degE (Fig. 1). The mean annual temperatures (MAT) ranged from 8.7 to 22.8degC, mean annual precipitation (MAP) from 495 to 2600 mm, and mean monthly solar radiation (MMSR) from 298.2 to 541.6 (MJ m⁻²) (Du et al. 2021). In previous studies, spatial variations in leaf vein density (Zhu et al. 2012) and stomatal traits (Du et al. 2021) have been examined in oriental oak.

Field investigation: spatial variations in leaf trichomes and their relationships with environ-

mental factors

We collected 44 populations in situ across the whole of Eastern Asia from 2007 to 2009, including mainland China, Peninsular Korea, Japan, the Zhoushan Islands and Taiwan Island. At each population, five dominant trees were selected, after which 20 healthy and mature leaves per tree were collected from three twigs in the upper central south-facing crown. Leaf samples were collected from between 9:00 am to 12:00 noon every day from August to September. The sampled leaves were divided into two sections for the measurement of trichome and stomatal densities and other leaf functional traits.

Common garden experiment: adaptive variation of leaf trichomes and their relationships with stomatal traits

Moreover, we collected the seeds of 15 of 44 sampling sites in 2008, which were grown in a common garden. The common garden located in Shanghai at 4 m above sea-level, 31deg02'N and 121deg26'E, where MAT and MAP was 15.5 degC and 1149.8 mm, respectively. Different populations in the common garden followed a randomized complete block design (RCBD), where there were five replicates for each population. Leaf samples in the common garden were collected in 2011. For each replicate, three healthy seedlings were selected, and at least 10 healthy and mature leaves were collected from the middle of the south-facing crown.

Trichome and leaf functional trait measurements

The measurement of trichome density in oriental oak was similar to stomatal measurement that reported in previous study (Du et al. 2021). Firstly, the trichomes at the right side of the midrib in the central section of the lower leaf surface were gently removed with a blade under an anatomical lens, and then one 0.5 x 0.5 cm² section was selected as a sample. Subsequently, the samples were fixed in 2.5% glutaraldehyde and stored in a refrigerator at 4oC. Prior to being imaged, the samples were in turn dehydrated using an ethanol gradient, freeze-dried, and gold sputtered, after which three graphs for each sample were collected at 1000x magnification with a FEI Sirion 200 ultrahigh resolution Schottky field emission scanning electron microscope (FEI Company, Hillsboro, OR, USA). The trichome density was calculated by averaging 45 images per population with a 0.056 mm² viewing field.

The methods of measurement of the SD and SS were described in Du et al. (2021). The LA was determined with WinFolia software (Regent Instrument Inc. Quebec, Canada), whereas the leaf dry mass was measured after drying at 70oC for 48h. The LMA was obtained as the dry mass per unit leaf area. Leaf veins were measured with a Leica DM 2500 microscope (Leica Microsystems, Wetzlar, Germany), and VD were defined as the lengths of leaf veins per area, which were measured with Image J software (Zhu et al. 2012).

Data analyses

The meteorological data was obtained from http://cdc.cma.gov.cn/home.do, where the mean value of 30 years (1979-2009) was used for analysis. The piecewise SEM (Lefcheck 2016) were employed to evaluate the direct and indirect effects of predictor variables (including geographic and climatic factors and leaf functional traits) on trichome density. A backward stepwise selection procedure was used to simplifying the model until the AIC value began to increase and P value was above 0.05. The sampled sites were considered as a random effect with linear mixed models (LMMs) in our analyses (Lefcheck 2016, Ali et al 2020). For all endogenous variables, the conditional R^2c (all factors, including the random effect) and marginal R^2m values were estimated their variations (Lefcheck 2016). In the piecewise SEM, the effect of each predictor on the endogenous variables was accounted for through their standardized path coefficients. The piecewise SEM and LMM models were separately implemented with the piecewise SEM package (Lefcheck 2016) and the nlme R package. To avoid the model complexity, all geographic variables (latitude, longitude and altitude) and climatic variables (MAT, MAP, aridity index (AI), potential evapotranspiration (PET), and MMSR) were clustered by principal component analysis (PCA). All variables, used in piecewise SEM, were log-transformed and then standardized. Moreover, one-way ANOVA was employed to test the differences in trichome density between the field samples and common garden population samples. To determine the direction of trait variation in response to environmental changes, linear regressions were performed to correlate environmental factors with trichome density. The above analyses were carried out with R version 3.6.1 (R Development Core Team, available from www.r-project.org/, accessed 2019) and SigmaPlot 10.0 (Systat Software, Inc., R.ichmod, CA, USA).

Results

Spatial variations of leaf trichomes and their relationships with climatic factors across Eastern Asia

The mean value of trichome density in situ was 459.78 trichome mm⁻² within the range of from 325.79 to 552.38 trichome mm⁻², and the coefficient of variations of trichome density was 0.16 (Fig. 1). The first two of PCA axes of 3 geographic factors explained 57% and 27% of the total variation in geographic gradients of 44 populations across eastern Asia (Fig. 2). The first axis of PCA (Geo_{PC1}) generally explained longitude and elevation, and the second axis (Geo_{PC2}) mainly related to latitude. The PCA axes of 5 environmental factors accounted for 72% and 16% of the total variation in climate. The first axis of PCA (Clim_{PC1}) mainly explained the variability of precipitation and temperature-related climatic factors, including MAP, AI, and MAT. The second axis of PCA (Clim_{PC2}) best explained the variability of solar radiation (i.e. MMSR).

The piecewise SEM explained 36% of the spatial variation in trichome density. Geo_{PC1} and Geo_{PC2} showed indirect effects on trichome density through $Clim_{PC1}$ and other leaf functional traits, including LMA, SD, SS, and VD. $Clim_{PC1}$ had both direct and indirect effects on trichome density through four pathways (Fig. 2): one direct from $Clim_{PC1}$ to trichome density (path coefficient 0.21), three indirect including from $Clim_{PC1}$ via LMA to trichome density (path coefficient 0.13, 0.64x0.20), from $Clim_{PC1}$ via SD to trichome density (path coefficient 0.06, 0.17x0.35), and from $Clim_{PC1}$ through LMA and SD to trichome density (path coefficient 0.08, 0.64x0.37x0.35).

Moreover, leaf functional traits could have both direct and indirect effects on trichome density. We found that LMA, SD, and SS had direct effects on trichome density (path coefficient 0.20, 0.35, and -0.15, respectively), whereas LMA and VD had indirect effects on trichome density through SD (path coefficient 0.13, 0.37x0.35) and SS (path coefficient 0.05, -0.31x-0.15), respectively (Fig. 2). Clim_{PC2} and LA had no significant effects on trichome density resulting in the removal of these pathways from the model. In addition, we further evaluated the correlation of trichome density with climatic factors in the Clim_{PC1} , showing leaf trichome density was positively correlated with AI and negatively with MAT and MAP (Fig. 3).

Relationship between leaf trichomes and stomatal traits and their adaptive variation in the common garden

The leaf trichome density was positively correlated with LMA and SD, but negatively with SS (Fig. 4) in situ. When different populations were grown in the common garden, leaf trichome density was significantly lower compared with in situ (Fig. 5). The trichome density in the common garden was positively associated only with SD (Fig. 6), while it had no relation with other leaf functional traits. Furthermore, no correlations between the trichome density and environmental factors at the original sites were observed.

Discussion

Effects of climatic factors on leaf trichome density across a large geographic gradient

We investigated spatial variations in leaf trichomes in oriental oak and evaluated their associations with environmental factors and other functional leaf traits across Eastern Asia. Our results revealed that differences in latitudinal and longitudinal factors drive changes in climate such as temperature and precipitation, which inevitably direct or indirect impact the spatial patterns of leaf trichome traits (Fig. 2). Our results indicated that the density of trichomes was reduced with temperature, which did not align with the study of Skelton et al. (2012), who found that there was positive relationship between temperature and trichome density, but did with an investigation by Perez-Estrada et al. (2000). This was likely the result of a reduced trichome density via temperature increases that reduced the thickness of the boundary layer; thus, lowering the water diffusion resistance to cool leaf temperatures (Gasparini et al. 2020). Since temperature and precipitation were coupled in our study area, the temperature was higher and accompanied by greater precipitation (Du et al. 2021); thus, plants at low latitudes (high temperature and precipitation) were induced to lower their trichome density to enhance the diffusion of water to maintain an optimal water vapor balance and leaf temperature.

The effects of water limitations on the density of leaf trichomes have been widely investigated in previous studies (Kessler et al. 2007, Cach-Perez et al. 2016, Amada et al. 2019), most of which indicated that trichome densities increase under restricted water conditions, as demonstrated by our results (Fig. 3). On one hand, dense trichomes can collect dew droplets above stomatal pores (Fernandez et al. 2014) that reduce the water potential gradient between the leaf interior and ambient air (Konrad et al. 2015), which can store water and delay the leaf drying process. Conversely, higher trichome densities under drought conditions can increase the leaf boundary layer thickness, which can reduce the plant transpiration rate and improve water use efficiencies (Bickford 2016). The transpiration rate was decreased with minimal reductions in photosynthetic rates, thereby improving the plant WUE, as demonstrated by previous studies of *Arctotheca populifolia*(Ripley et al. 1999) and *Mallotus macrostachyus* (Kenzo et al. 2008). In general, our results suggested that the trichome density possessed highly adaptive variation to environmental changes, which may contribute to higher plant tolerances against future drought pressures.

Leaf trichomes are highly coordinated with stomata in response to climate changes

Stomata control gas exchange between plants and the environment and serve as the main conduit for the loss of transpiration water and the influx of CO_2 (Hetherington and Woodward 2003). Our results revealed that leaf trichomes were positively correlated with stomatal density and negatively so with stomatal size (Fig. 4), which suggested that the development of leaf trichomes may impact stomatal behavior. In a previous study, Du et al. (2021) reported that stomatal density was related to the LA and LMA, whereas the stomatal size was associated with LA and VD in field populations, while there was no relationship between stomatal and leaf traits in the common garden. These results implied that the coordination between stomatal and leaf traits may be due to long-term local adaptations. However, in the present study the trichome density not only showed a significant correlation with mature trees in the field, but also showed the same results as seedlings in the common garden (Figs. 4 and 6). These results suggested that there was an evolving intimate relationship between the leaf trichomes and stomata. This signified that leaf trichomes may closely coordinate with stomata guard cells and collectively influence plant transpiration, photosynthesis, and water use efficiencies, as demonstrated by Galdon-Armero et al. (2018) and Paulino et al. (2020).

In effect, stomatal guard cells and trichome cells have shared developmental origins (Holroyd et al. 2002), where trichome formation can lead to changes in stomatal density. Simon et al. (2020) reported that glabrous leaves had a greater stomatal density compared with hairy leaves in Arabidopsis halleri. Other studies found there was a negative relationship between trichome and stomata densities in Phaseolus vulgaris (Silva et al. 1999) and Solanum lycopersicum (Galdon-Armero et al. 2018). These studies indicated that there might be a trade-off between trichome formation and stomatal development. However, the results of our experiments were distinct from these previous studies, as they indicated that there was a positive relationship between trichome and stomata densities regardless of whether the plants were grown in the field or in the common garden. One possible explanation was that there was a synergic relationship that existed between the stomata and trichome in response to environment changes, rather than trade-off relationships reported in earlier studies (Cach-Perez et al. 2016, Simon et al. 2020). This was likely due to the requirement of high stomata densities for corresponding trichomes that covered the surfaces of the stomatal pores to assist plants with the joint control of water-gas exchange. For example, we observed that when the stomata density increased under drought conditions, the trichome density also needed to be increased accordingly (Fig. 3) (Du et al. 2021). These results implied that the level of trichome development appeared to be coupled with that of stomatal development, where both were likely impacted by environmental signals.

How distinct given traits such as LMA, stomata, and trichome density vary with environment changes has been widely examined (Buckley 2019, Bhusal et al. 2021), yet it remains unclear whether diverse functional traits can coordinate in response to specific environmental conditions (Sack and Buckley 2020). In the present study, the modelling results revealed that precipitation may be the major driving factor for the spatial variation in leaf trichomes (Figs. 2 and 3). In combination with previous work (Du et al. 2021), these results showed that the trichome density, stomata density, and LMA increased with lower precipitation, which was consistent with the study of Salgado-Negret et al. (2015), who found that *Aextuxion punctatum* growing under drier conditions had higher LMA, trichome, and stomata densities compared to that under wet environments. Interestingly, there was a correlation between the trichome densities, as well as LMA and stomata densities. One possible interpretation was that the leaves with higher LMA generally had lower mesophyll conductance (Wu et al. 2020); thus, they needed to be coupled with higher stomata densities (Du et al. 2021) to increase the stomatal conductance, which determines leaf temperatures and photosynthesis. Correspondingly, higher trichome densities are required to cover the surface of the stomata to increase the resistance of the boundary layer, thereby reducing transpiration related water loss and increasing leaf wettability (Konrad et al. 2015). Our results suggested that plants with higher LMA and trichome and stomata density may be an important adaptation strategy against drought. Importantly, our results revealed that multiple functional traits may co-vary and coordinate in response to a given environmental pressure.

Conclusions

Our study provides a comprehensive picture to describe the spatial variations in leaf trichomes in oriental oak, as well as their relationships with environmental factors and other leaf traits across Eastern Asia. Our conclusions included that: (1) leaf trichomes may possess highly adaptive variation to changes in climate, which may contribute to the tolerances of plants against different potential environmental stresses; (2) leaf trichomes and other leaf functional traits (such as LMA and SD) may be highly coordinated in response to environmental changes; (3) leaf trichomes do indeed affect stomatal behavior, which may further influence water-gas exchange and photosynthesis. Our findings contribute new insights toward elucidating the interactivities that occur between the functional traits of leaves and adaptive plant strategies under climate change.

Conflict of interest – The authors declare no conflict of interest.

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Lists of figures and tables

Fig. 1 Distribution of sampling sites (a) and spatial patterns (b and c) of trichome density in oriental oak across Eastern Asia. The circles and triangles represent 44 in situ populations, triangles represent 15 original sites of common garden populations, and the square represents the location of the common garden. The picture on the right represents the morphology of trichome in oriental oak. N, sample number; Max, maximum; Min, minimum; SE, stand error; CV, coefficient of variation.

Fig. 2 The piecewise structural equation model (SEM) for testing the effects of environmental factors and leaf functional traits on leaf trichome density. Geo_{PC1} and Geo_{PC2} represent the first two principal components of the 3 geographic variables, Clim_{PC1} and Clim_{PC2} represent the first two principal components of the 5 climatic variables, and eigenvector scores for two axes of principal component analysis (PCA) are also given. The standardized regression coefficient is shown for each significant path (P < 0.05). The conditional (c) and marginal (m) R² are provided for each exogenous variable (R²_c = both fixed and random effects, and R²_m = fixed effects). Black arrows denote positive relationships, red arrows negatives ones. Blue arrow denotes the correlation between SD and SS. LMA, leaf mass per area; SD, stomatal density; SS, stomatal size; VD, vein density.

Fig. 3 Relationships between trichome density and environmental factors for oriental oak (n = 44).

Fig. 4 Relationships between trichome density and leaf functional traits for oriental oak.

Fig. 5 Difference in trichome density between the common garden and in situ. The common garden is located in Shanghai (at 4 m above sea-level, and 31deg02'N and 121deg26'E), where MAT and MAP is 15.5 degC and 1149.8 mm, respectively. The letters above bars represent a significant difference at the P < 0.05 level, by Duncan's test (n = 15).

Fig. 6 Relationships between trichome density and leaf functional traits in the common garden (n = 15) and in situ (n = 15).





Fisher's C = 37.29, df = 28, *P*-value = 0.113, AIC = 97.29 Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05





