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Stomatal traits relate to habitat preferences of herbaceous species in a temperate climate

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ABSTRACT

Stomata enable plants to balance uptake of CO_2 with water loss via transpiration. Previous studies have shown that stomatal size and density trade-off with one another and are related to a suite of environmental factors (light, atmospheric CO_2 concentrations, moisture and temperature) which influence gas exchange. We examined the hypothesis that the habitat preferences of species are reflected in stomatal traits. Thus, we examined the relation of stomatal size and density of 36 herbaceous species along elevational gradients in relation to Ellenberg indicator values for light and moisture. The light value is a proxy for the transpiration demand due to exposure to radiation and air mass exchange, the moisture value reflects water supply. Additionally we measured carbon isotope discrimination ($\Delta^{13}C$), a proxy for intrinsic water use efficiency and internal CO_2 concentration. Stomatal size changed in parallel on both sides of a leaf, whereas changes in density differed between sides depending on species identity. There was an increase in absolute variation of sizes and densities with increasing mean size and density respectively, but not in relative variation. Species with few but large stomata tended to adjust stomatal size across environmental gradients, whereas species with small but many stomata mainly adjusted stomatal density. A higher Ellenberg indicator value for light and a lower value for moisture was associated with equal distribution of stomata between leaf sides. The carbon isotope discrimination data indicated that amphistomatic species, which illustrated a preference for high radiation and high air mass exchange as well as for dry habitats, had higher water use efficiency. We conclude that stomatal traits such as size, density or the distribution between the two sides of the leaf are indicators of how species optimize carbon uptake and balance water loss and radiation gain.

1. Introduction

The appearance of stomata more than 400 million years ago is considered as key event in the evolution of terrestrial plant species, since it enabled plants to conquer terrestrial land surfaces (Hetherington and Woodward, 2003; Raven, 2002). The main function of stomata is to regulate the balance between uptake of CO_2, which is essential for photosynthesis, and water loss via transpiration (Chaerle et al., 2005; Roelfsema and Hedrich, 2005). The fossil record suggests that long-term adaptations of maximum leaf conductance for CO_2 and H_2O in response to changes in abiotic conditions (mainly variation in atmospheric CO_2 concentration) appear to involve coordinated and opposing changes in stomatal size and density (Beerling and Woodward, 1997; Edwards et al., 1998; Franks and Beerling, 2009 Hetherington and Woodward, 2003; Raven, 2002). The optimization of stomatal traits such as size or density has important consequences for the productivity, competitive strength and survival of a plant (Vatén and Bergmann, 2012).

Abbreviations: $\triangle^{13}C$, discrimination of ^{13}C; c_i, CO_2 concentration in the substomatal cavity; c_a, external CO_2 concentration; WUE, water use efficiency.

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So far it is known that stomatal development is controlled by both, light and water supply. Other important factors are gene expression (Nadeau and Sack, 2002) and prevailing environmental conditions such as CO_2 concentration, temperature, humidity and nutrient availability (Casson and Gray, 2008; Chaerle et al., 2005; Gindel, 1969; Lockheart et al., 1998; Woodward and Bazzaz, 1988; Woodward et al., 2002). Stomatal size was found to remain rather constant within species but to be highly species-specific, whereas stomatal density seems to be more responsive to abiotic conditions (Ashton and Berlyn, 1994; Bucher et al., 2016; Kumekawa et al., 2013; Richardson et al., 2001; Römermann et al., 2016). Stomatal size is closely linked to genome size, with plants possessing larger genomes having larger guard cells (Beaulieu et al., 2008; Franks and Beerling, 2009; Jordan et al., 2015). Stomata can be either located on the adaxial or abaxial side of a leaf or on both sides. Usually, in most herbaceous species the abaxial side displays more stomata while in grasses the adaxial side is dominant. It is not known whether species change size and density of stomata in parallel on either sides, or whether such changes can occur independently.

The trade-off between stomatal size and density is a substitutable effect (Brodribb et al., 2013; Franks and Beerling, 2009 Hetherington and Woodward, 2003). High densities of small stomata offer the highest maximum leaf diffusive conductance and therewith highest photosynthesis rates (Drake et al., 2013). At the same time, many but small stomata allow greater stomatal resistance and stomatal control, as well as a rapid response during drought conditions (Aasamaa et al., 2001; Brodribb et al., 2013; Drake et al., 2013; Franks and Beerling, 2009; Franks and Farquhar, 2007 Hetherington and Woodward, 2003). Most of these studies focussing on the trade-off between stomatal size and density used fossil records or herbarium specimen and long-term data (Franks and Beerling, 2009; Franks et al., 2012). In our study we tested for evidence of trade-offs in the interaction between stomatal size and density along broad environmental gradients. We further examined how the distribution of stomata varies on the two sides of the leaf.

The role of stomatal traits as indicators of changes in abiotic conditions is of great interest in ecological research (Aasamaa et al., 2001; Beerling and Chaloner, 1993; Bucher et al., 2016; Drake et al., 2013; Kardel et al., 2010; Li et al., 2015; Yang et al., 2014). The quasi-metric Ellenberg indicator values, which are based on field observations and expert knowledge, are an indirect assessment of a speciesí 'ecological behaviour' under field conditions (Bartelheimer and Poschlod, 2015; Diekmann, 2003; Ellenberg, 1974; Ellenberg et al., 1991) and can be used to classify its habitat niche (Silvertown et al., 2006). We focused on Ellenberg indicator values for light (called L-value hereafter), which is also a proxy for the transpiration demand due to exposure to radiation and air mass exchange, and moisture (called F-value hereafter; Ellenberg, 1974; Ellenberg et al., 1991; Penman, 1948). Since the light-dependence of stomatal occurrence is well documented e.g. by Hanson (1917), we tested whether stomatal characteristics such as size, density or the distribution between the two leaf sides change with changing L-values, which range from 1 (species adapted to low light conditions) to 9 (species exhibiting high light demand) and for F-values which range from 1 (species adapted to low moisture conditions) to 12 (water plants). We used carbon isotope discrimination $\Delta^{13}C$ to indicate intrinsic water use efficiency (WUE) and internal CO_2 concentrations (Drake et al., 2013; Farquhar et al., 1989; Guehl et al., 1995; Hetherington and Woodward, 2003; Osório et al., 1998; Raven, 2014). We expect that small stomata react more rapid to changes in abiotic conditions and are faster in their movements than bigger ones which then could lead to higher intrinsic WUE in plants with small stomata.

We measured changes in stomatal size and density of 32 herbaceous plant species at 22 sites located along elevational gradients in the Northern Limestone Alps in two consecutive years. This dataset was complemented by data on stomatal size and density of five co-occurring species on 14 European dry grasslands. We analysed the relationship between stomatal parameters, L- and F-values as well as stable carbon discrimination ($\Delta^{13}C$). Using these datasets, we specifically focussed on the following questions:

(1) Do stomatal size and density change similarly on the adaxial and abaxial sides of leaves in response to environmental gradients?
(2) Is there a coordinated trade-off between stomatal size and density?
(3) What are the ecological interpretations of the trends identified in (1) and (2)?

2. Material and methods

2.1. Study sites and species selection

We selected 32 herbaceous plant species (cf. Table 1; nomenclature follows Wisskirchen and Haeupler, 1998) occurring over a wide elevational range along two south facing elevational gradients near Garmisch-Partenkirchen (47° 29′ 30" N, 11° 5′ 43" E) in the Northern Limestone Alps ranging from 700 to 1800 m a.s.l. and from 800 to 1700 m a.s.l. respectively. We studied 20 species in two consecutive years, 2012 and 2013, whereas 12 species were studied only once (either in 2012 or in 2013, see Table 1) as we started with a subset in the first year and extended this subset as results were promising. Samples were collected every 100 m in elevation on 22 sites (for an overview see Table 1). To examine whether the elevational patterns are consistent with latitudinal patterns, we analysed five species from calcareous grasslands at 14 sites in Europe which were sampled during the growing season of 2011 (unpublished dataset; for location of sites see Fig. A1 in Appendix A). One of the species (*Centaurea jacea* L.) was present in both, the elevational and the latitudinal gradient data. Fully developed sun-leaves of five individuals per site were selected, their guard cell length as a measure of (hereafter referred to as) stomatal size and densities were measured and $\Delta^{13}C$ was determined. In the case of plants collected along elevational gradients, stomatal density and abundance on the adaxial side was also determined. To test whether stomatal size and density relate to genome size in our dataset, we extracted 1C-DNA values, ploidy levels and chromosome numbers from Kew garden database (Bennett and Leitch, 2012) for all focal species included therein (20 species).

2.2. Stomatal size and density

Two imprints from the abaxial surface and one from the adaxial surface of each leaf were taken on the elevational gradients, and two stomatal imprints from the abaxial side were taken on the latitudinal gradient using the clear nail polish method as described by Hilu and Randall (1984). Sample position within the leaves was kept constant in each species to minimize the effect of within-leaf variation of stomatal size and density (Poole et al., 1996). Samples were analysed using a light microscope (Axiostar Plus, Carl Zeiss, Jena, Germany) equipped with a scale grid at 400-fold magnification. Stomata were counted in two fields of view per stomatal imprint; stomatal size was recorded on two closed stomata per field of view. Fields of view were selected so as to exclude veins since stomata are absent there (Martin and Glover, 2007). We calculated the stomatal ratio, i.e. the distribution of stomata between the two leaf sides as

$$\text{Stomatal ratio} = \frac{\min(\text{adaxial},\text{abaxial})}{\max(\text{adaxial},\text{abaxial})}$$