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Contrasting altitudinal patterns of leaf UV reflectance and absorbance in four herbaceous species on the Qinghai–Tibetan Plateau

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Abstract

Aims

Alpine plants have to cope with intense ultraviolet (UV) radiation and its altitudinal changes. It has been argued that leaf UV reflectance and absorbance should play a central role in acclimation and adaptation to changes in UV radiation, but evidence is limited from high altitudinal ecosystems. In this study, we assessed whether leaf UV reflectance and leaf pigments jointly vary with altitude in alpine broadleaved herbaceous species. The primary hypothesis is that leaves with higher UV reflectance should have lower UV absorbance and/or lower contents of photosynthetic pigments.

Methods

Leaf UV reflectance, leaf UV absorbance and photosynthetic pigments (chlorophyll a and b, carotenoids) were examined in four broadleaved herbaceous species in relation to their habitat altitudes. The leaf surface reflectance and leaf extract absorbance at wavelengths of 305 and 360 nm were measured to examine the leaf optical and photochemical characteristics in the UV-B and UV-A bands, respectively. The species included *Saussurea katochaete* Maxim., *Saussurea pulchra* Lipsch., *Anaphalis lactea* Maxim. and *Rheum pumilum* Maxim., which are distributed along the same slope from 3 200 to 4 200 m in the Qilian Mountains, Qinghai–Tibetan Plateau.

Important Findings

The leaf UV absorbance was approximately twice as high at 305 nm (UV-B) than at 360 nm (UV-A) for all species except *R. pumilum*. Among the four species, the leaf UV absorbance was the highest and almost all values were within 2–6 Abs cm^{-2} (absorbance cm^{-2}) in *S. pulchra*, but the lowest (frequently <1 Abs cm^{-2}) were observed in *R. pumilum*. Only *R. pumilum* showed significantly higher values at higher elevations. Leaf UV reflectance was generally higher at higher elevations for all species except for *A. lactea,* and exhibited much larger altitudinal variations compared to leaf UV absorbance. *Anaphalis lactea* showed a very high UV reflectance even at low altitudes. Among the four species, photosynthetic pigments tended to decrease with an increase in leaf UV reflectance but increased with leaf UV absorbance. The study suggests that leaf UV reflectance, rather than leaf UV absorbance, plays a more active role in acclimation to altitudinal changes in UV radiation, and a high investment in leaf UV reflectance may limit the accumulation of photosynthetic pigments in alpine plants.

Keywords: altitudinal pattern, leaf UV reflectance, UV absorbance, photosynthetic pigment, UV environment

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INTRODUCTION

Ultraviolet (UV) radiation is an important environmental factor for alpine plants (Körner 2003). In general, UV radiation increases with increasing elevation. Depending on solar elevation and other sky conditions, UV radiation increases by ~15–25% per 1 000-m increase in elevation (e.g. Blumthaler 2012; Piazena 1996).

Terrestrial plants have evolved different mechanisms to cope with UV radiation, such as changes in leaf chemistry, leaf physical properties, canopy structure and other functional traits (Barnes *et al.* 2016b; Gupta *et al.* 2017; Klem *et al.* 2012). Among these, the changes in leaf UV absorbance and reflectance have been considered the most important mechanisms (Day and Demchik 1996; Guidi *et al.* 2016; Middleton and Teramura 1993).

Terrestrial plant leaves absorb UV radiation mainly through UV-absorbing compounds, which are a group of phenolic compounds that are located mainly in the epidermal cuticle layer of leaves. These compounds constitute a vital screen that protects the leaf from damage by UV radiation (Caldwell *et al.* 1983; Sumbele *et al.* 2012; Williamson *et al.* 2014). However, evidence on their correlation with UV radiation environments is controversial. Some experiments found that leaf UV-absorbing compounds increase with UV exposure (e.g. Barnes *et al.* 1987; Kataria *et al.* 2014; Robinson *et al.* 2005), and some observations show that the content of UV-absorbing compounds in leaves increases with increasing elevation, i.e. with an increase in UV radiation in a natural environment (González *et al.* 2002, 2007; McDougal and Parks 1984; Murai *et al.* 2009). With the close association of the UV environment and the content of UV-absorbing compounds, it has even been suggested that the content of leaf UV-absorbing compounds can be used as a reference index for UV radiation levels in nature (Rozema *et al.* 1997). However, other experimental studies reported that UV-B-resistant plants did not increase the accumulation of UV-absorbing compounds under increasing UV radiation (van de Staaij *et al.* 1995; Sullivan and Teramura 1989; Tosserams and Rozema 1995). Moreover, different groups of UV-absorbing compounds in one species can respond differently to increasing elevation (Bernal *et al.* 2013). It therefore remains unclear whether UV absorbance through UV-absorbing compounds is a general mechanism for alpine plants to cope with changes in UV radiation.

UV-absorbing compounds in the leaf are secondary plant metabolites, which consist mainly of carbon (Sumbele *et al.* 2012). An increase in UV-absorbing compounds should result in a relative reduction of the content of photosynthetic pigments (Barnes *et al.* 2015; Ibrahim *et al.* 2011). The biosynthesis of carotenoids was reported to decrease with an increase in leaf UV-absorbing compounds (Guidi *et al.* 2016). In addition, a recent meta-analysis showed that as UV-B radiation increased, a 4.7% increase in leaf UV-B-absorbing compounds was correlated with a 7.4% decrease in the chlorophyll content (Fu and Shen 2017). However, there is also evidence that enhanced UV-B radiation increases both carotenoids and UV-B-absorbing compounds (Middleton and Teramura 1993). Alpine plants usually have to face harsh environments with limited resources. It is of interest to determine how the leaves of alpine plants allocate their resources to photosynthetic pigments and UV-protecting compounds.

Changes in leaf reflectance also play an important role through which alpine plants adapt or acclimate to changes in UV radiation (Barnes *et al.* 2015; Filella and Penuelas 1999; Holmes and Keiller 2002). Leaf reflectance at photosynthetically active radiation (PAR) wavelengths seems to be mainly a result of leaf internal scattering and absorption, but the UV reflectance appears to be due more to the characteristics of the leaf surface (Grant 1987; Grant *et al.* 2003). The magnitude of leaf UV reflectance in many tree species is largely a function of the morphology and distribution of epicuticular wax (Grant *et al.* 2003).

A central concern in plant physiological ecology is to understand how plants function in different environments. Some recent studies suggest that UV protection “strategies” differ among different plant species (Barnes *et al.* 2016a, 2017). However, field evidence is lacking for any significant generalization on the possible strategies. Most of the evidence concerning leaf UV reflectance and absorbance in relation to natural UV environments is limited to a lower altitudinal range, usually <3 200 m (González *et al.* 1993, 2002, 2007). Moreover, observations appear to be lacking on how leaf UV reflectance in alpine herbaceous species changes with altitudinal changes in UV radiation. Alpine plants are able to change their leaf UV reflectance and/or leaf UV absorbance to cope with changes in UV radiation.

We thus addressed the following questions in this study. How do leaf UV reflectance and absorbance change with altitude in alpine environments? Since both leaf traits contribute to UV protection, which trait is more flexible in response to the altitudinal gradient? Moreover, as biomass investment into leaf structure and biochemistry can often be limited by lower biomass production in alpine environments, we hypothesized that leaves with a higher UV reflectance will have a lower UV absorbance and/or lower photosynthetic pigment content under a similar biomass cost, which will result in the same UV protection effect among these traits.

The Qinghai–Tibetan Plateau, with an average altitude >4 000 m, provides a unique natural environment for examining changes in leaf UV characteristics in relation to the natural variations in a UV radiation environment (Beckmann *et al.* 2014; Cui *et al.* 2008; Norsang *et al.* 2009). We understand that altitudinal gradients differ, not only with respect to UV radiation but also in terms of other environmental factors, including PAR, temperature, precipitation, and atmospheric pressure (Körner 2003). When we focus on only leaf reflection and absorption of UV radiation, the large variation of UV gradients along altitudes can be used for efficient examination of the acclimation and adaptation of plants to UV environments (Nybakken *et al.* 2004; Ruhland *et al.* 2013; Ziska *et al.* 1992).

In particular, observations from the largest altitudinal gradient range and the highest average altitude on the Qinghai–Tibetan Plateau are expected to address the knowledge gap on how plants cope with the strongest terrestrial UV environment in the world.

METHODS AND MATERIALS

Study sites and plant materials

Four herbaceous species, *Saussurea katochaete* Maxim., *Saussurea pulchra* Lipsch., *Anaphalis lactea* Maxim. and *Rheum pumilum* Maxim., were used to measure UV reflectance, UV-absorbing compounds and photosynthetic pigments in relation to altitude because of their wide altitudinal distribution. The detailed information for each species is shown in Table 1 and Fig. 1. We collected leaf samples for measurements of the physical and chemical properties of the leaves of the four species from the mountain Lenglong, which is located nearby the Haibei Alpine Meadow Ecosystem Research Station (37°29′N, 101°12′E), at the northeastern edge of the Qinghai–Tibetan Plateau. We sampled 16 individual leaves per species at each elevation along the slope during the period from 26 July to 4 August 2016. To reduce the effects of diurnal UV change, we collected the leaf samples during the period from 10:00 to 14:00 local time. Moreover, we only sampled mature and healthy leaves in the open to reduce the effects of shading from neighboring leaves.

Leaf UV absorbance

To measure UV-absorbing compounds, 16 leaf disks of 0.283 cm^2 were punched with a cork borer from four plants per species at each elevation. These disks were extracted at 4°C in the dark for 24 h in an acidified methanol solution (10 ml of 70% methanol, 29% H_2O and 1% HCl) after oven drying (Barnes *et al.* 2008). The absorbance of the leaf extracts was measured using a UV-2601 (Beifen-Ruili Analytical Instrument Co., Ltd.) with a measurement range from 190 to 1 100 nm. The leaf UV absorbance of each individual plant was the average measurement from four leaves, and the absorbance was expressed as the absorbance per unit leaf area at 305 and 360 nm (Abs cm^{-2} 10 ml).

Chlorophyll and carotenoid contents

To obtain chlorophyll and carotenoid contents, we collected another 16 disks from the same leaves using the same procedure as that used for UV-absorbing compound analysis. The chlorophylls and carotenoids were extracted from the leaf disks that were soaked in 3 ml dimethylformamide (DMF) at 6–10°C in the dark for 48 h immediately after punching until the disks became colorless. The concentration of pigments was calculated following the equations of Porra *et al.* (1989) and Wellburn (1994). The content of pigments was expressed as the pigment weight per unit leaf area (μg cm^{-2}).

Measurements of leaf UV reflectance

To understand leaf UV and PAR reflectance, we measured the spectral characteristics of the leaves at UV (280–400 nm) and PAR (400–700 nm) wavelengths at the same locations along the same slope and in the same period of the following year except for *A. lactea*, which was sampled at corresponding elevations at a mountain ~20 km from Lenglong. The distance can be ignored since we focused on only the potential altitudinal effects of UV radiation.

We collected one leaf from five individual plants per species at each elevation. The leaves were removed and transported to Haibei Station by a dark refrigerator for the reflectance measurements, which were performed inside a dark room. We used a spectrometer (AvaSpec-ULS2048XL, AVANTES B.V., Netherlands) with an integrating sphere (AvaSphere-50-REFL, AVANTES B.V.) to measure the reflectance of the adaxial leaf surface. Since the active diameter of the sphere was only 50 mm, we obtained 10 readings from five leaves, i.e. two measured values of one leaf from each of five individual plants at each elevation. The reflectance measured at 305 and 360 nm was used to represent the reflectance of the UV-B and UV-A bands, respectively, and reflectance measured at 664, 647 and 480 nm, which are the absorbing peaks of DMF extract in chlorophylls a and b and carotenoids, was used to represent the reflectance of red and blue light at PAR wavelengths.

Table 1: altitudinal distribution, leaf surface and UV-absorbing properties of four alpine herbaceous species

Species	*Saussurea katochaete*	*Saussurea pulchra*	*Rheum pumilum*	*Anaphalis lactea*
Altitudinal range (m)	2 230–4 700	1 920–2 800 (4 200)*	2 800–4 500	2 000–3 400 (3 800)*
Plant height (cm)	No stem	8–27	10–25	10–40
Adaxial leaf surface	Green and glabrous	Green and glaucous	Waxy, leathery and smooth	Visible white and pallid tomentose
Leaf UV absorbance*	Relatively high, no altitudinal change	High, tends to increases at higher altitudes	Very low, increases linearly at higher altitudes	Low, no significant altitudinal pattern
Leaf UV reflectance*	Low but increases rapidly at higher altitudes	Low but increases rapidly at higher altitudes	High and increases linearly with altitude	Very high at low altitude, but no altitudinal pattern

Data source: The altitudinal range, plant height and leaf surface properties were derived from *Flora of China* (http://www.efloras.org/) and *Flora Reipublicae Popularis Sinicae*, Science Press, except for the data marked with an asterisk *, which are obtained in this study.

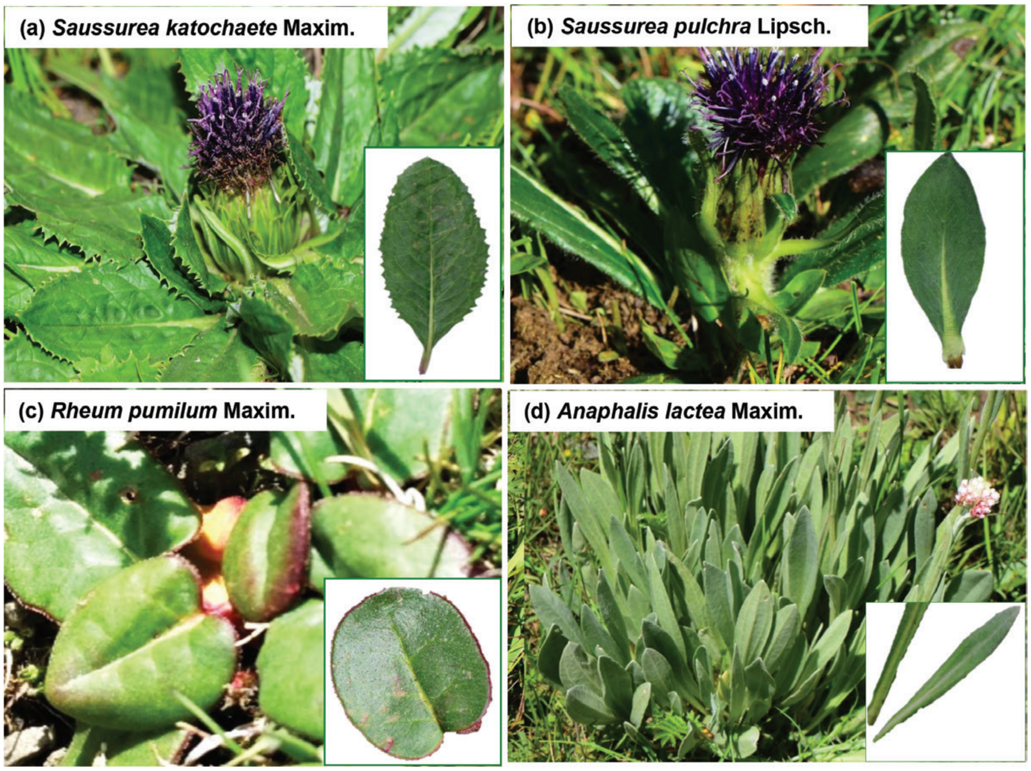

Figure 1: photographs showing leaf morphology and canopy structure of the four experimental species: (a) *Saussurea katochaete*, (b) *Saussurea pulchra*, (c) *Rheum pumilum* and (d) *Anaphalis lactea*. The leaf surface features are detailed in Table 1.

Statistical analyses

The data are expressed as X ± SE (average value and standard error). All the statistical analyses were performed with SPSS 18 software. Figures were drawn using SigmaPlot 12.5.

RESULTS

Leaf UV absorbance

The leaf UV absorbance per unit area at 305 nm, which is an indicator of UV-B absorbance, was generally 2–4 times higher than that at 360 nm (UV-A absorbance indicator) in the observed species except for *R. pumilum,* in which the absorbance was similar at the two wavelengths (Fig. 1). The leaf UV absorbance per unit area was almost the same for *S. katochaete, S. pulchra* and *A. lactea* across the elevations. Regarding the leaves of *R. pumilum*, however, the UV absorbance was significantly higher at higher altitudes (Fig. 2, $R^2 = 0.86$, $P < 0.05$).

Leaf UV reflectance

The leaf UV reflectance was generally higher at higher elevations, except for *A. lactea,* and exhibited much larger altitudinal variations compared with leaf UV absorbance (Fig. 3). On average, leaves reflected 5–28% of UV-B compared with 5–20% of UV-A depending on species and habitat elevations. The leaves often showed a dramatic increase in the UV reflectance when the habitat elevation was higher than 4000 m. The leaf UV reflectance of *A. lactea* was very high, even at low altitudes, but showed no significant altitudinal variation due to the limited distribution range of this species.

Photosynthetic pigments

The total chlorophyll a and b content per unit leaf area tended to increase at higher altitudes, especially in *A. lactea*; however, the chlorophyll content of *S. katochaete* decreased significantly at higher elevations (Fig. 4). The carotenoid content showed similar altitudinal patterns as the chlorophyll, but no statistical correlation was found.

Leaf UV absorbance and reflectance in relation to photosynthetic pigments

The total photosynthetic pigment content tended to increase with an increase in leaf UV absorbance (Fig. 5, see online supplementary Fig. S1); in particular, the leaf carotenoid content showed a very close linear correlation with either UV-A or UV-B absorbance.

However, in general, a lower content of total photosynthetic pigments, varying between 30 and 76 μg cm^{-2}, was measured under higher leaf UV reflectance among species and within the species *S. katochaete* (Fig. 6). The total photosynthetic pigment content decreased by 8.3 and 12.1 μg cm^{-2} with a 10% increase in UV-B and UV-A reflectance, respectively. A greater decrease in the total photosynthetic pigment content was observed in response to an increase in PAR reflectance (see online supplementary Fig. S2).

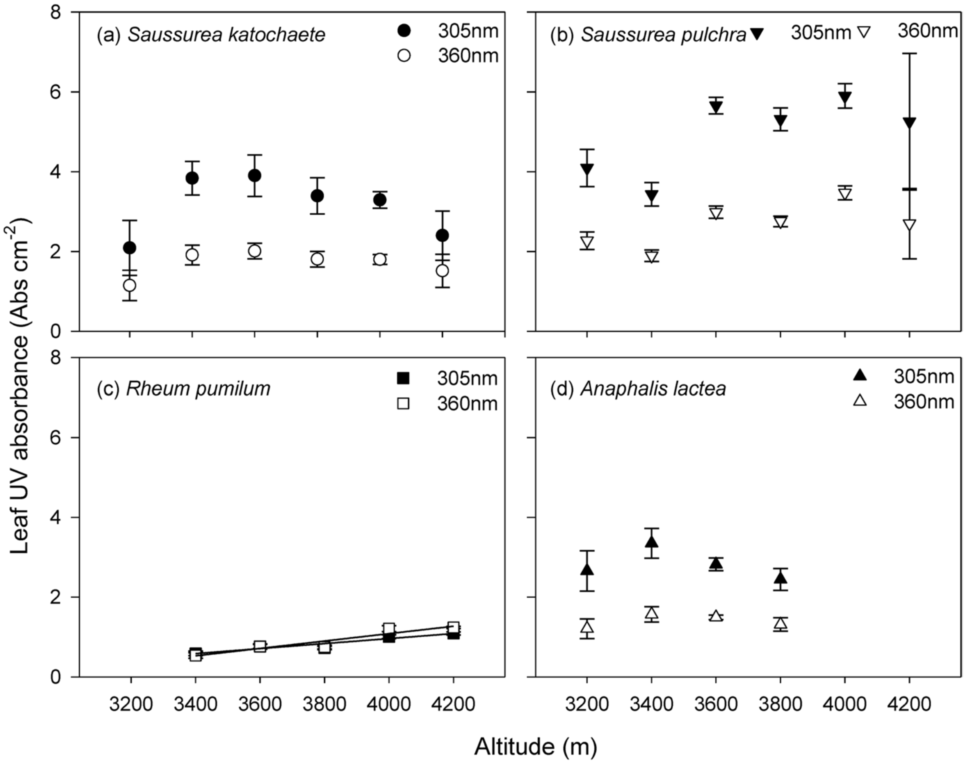

Figure 2: leaf UV-A and UV-B absorbance in relation to the habitat altitude for the four herbaceous species along the same slope in Haibei, Qilian Mountains, Qinghai–Tibetan Plateau. In this and the following figures, the circles, squares, triangles and inverted triangles represent *Saussurea katochaete* (a), *Rheum pumilum* (c), *Anaphalis lactea* (d) and *Saussurea pulchra* (b), respectively; the closed ones indicate leaf UV-B absorbance at 305 nm and the open ones indicate leaf UV-A absorbance at 360 nm. Data are shown as the mean ± SE of 16 leaf samples from four individual plants. The linear equations for *Rheum pumilum* (c) are $Y = 6 \times 10^{-4}X - 1.55$; $R^2 = 0.90$; $P < 0.05$ for UV-B at 305 nm and $Y = 9 \times 10^{-4}X - 2.62$; $R^2 = 0.89$; $P < 0.05$ for UV-A at 360 nm.

In addition, the specific leaf weight of most species increased significantly with elevation (see online supplementary Fig. S4).

DISCUSSION

Is the leaf UV reflectance more sensitive than the leaf UV absorbance in response to the altitudinal variation in UV radiation?

Both leaf UV reflectance and absorbance are important for alpine plants to cope with UV radiation. This study suggests that leaf UV reflectance plays a more active role in helping plants cope with the altitudinal gradient of UV radiation as compared with leaf UV absorbance. Firstly, the leaf UV reflectance showed a much larger altitudinal variation than the leaf UV absorbance within the same elevation range across all species (Figs 2 and 3). Secondly, the leaf UV reflectance varied more closely with altitude than the leaf UV absorbance except for *A. lactea*, which had a lower and narrower altitudinal distribution but a very high UV reflectance in comparison with the three other species at similar altitudes. Thirdly, it should be emphasized that the tendency for the photosynthetic pigment content to decrease with increasing leaf UV reflectance seems to exist among species and within the species *S. katochaete* (Fig. 6). All these differences indicate that the leaf UV reflectance should be more flexible and active in response to habitat changes in UV radiation.

There is only very limited information available for determining the spectrum reflectance in relation to leaf surface features, particularly in terms of UV radiation (Grant *et al.* 2003; Holmes and Keiller 2002; Richardson and Berlyn 2002). UV reflectance was <10% for most leaves, but can reach as high as 70% in some species (see citations from Holmes and Keiller 2002). In the current study, the leaf UV reflectance of *S. katochaete* changed from 5% to 25% across an elevation range from 3 200 to 4 200 m (Fig. 3). The reflectance change may be due mainly to the epicuticular wax since there was no visible leaf pubescence (Table 1). Holmes and Keiller (2002) assessed the relative contribution of leaf pubescence, i.e. hairs and glaucousness (presence of epicuticular wax), to leaf UV and PAR reflectance. They reported that both leaf pubescence and glaucousness affected total leaf reflectance, but pubescent leaves tended to reflect wavelengths longer than UV radiation (Holmes and Keiller 2002). Leaf wax layers are effective reflectors of both UV and visible light, which can partly explain the reflectance changes in the three species of our study, but not in *A. lactea*. The leaves of *A. lactea*, which have very thick pubescence, showed much higher reflectance

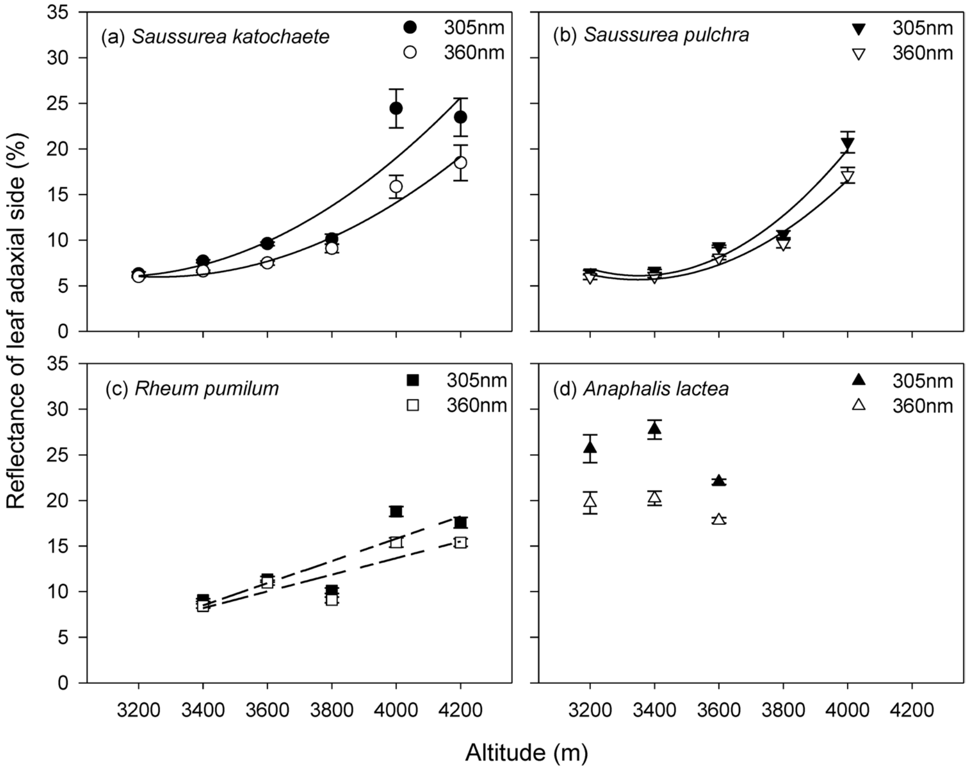

Figure 3: reflectance of UV-A and UV-B from the adaxial leaf surface in relation to the habitat altitude for four herbaceous species along the same slope in Haibei, Qilian Mountains, Qinghai–Tibetan Plateau. Symbols of species are the same as described in Fig. 2, but the closed ones indicate leaf UV-B reflectance at 305 nm and the open ones indicate leaf UV-A reflectance at 360 nm. Data are shown as the mean ± SE of 10 readings from five leaves, i.e. two measured values of one leaf from each of five individual plants at each elevation. The equations of the fitting curves for *Saussurea katochaete* (a) are $Y = 1.69 \times 10^{-5} X^2 - 0.11X + 170.68$; $R^2 = 0.86$; $P = 0.054$ for UV-B at 305 nm, and $Y = 1.50 \times 1\ 0^{-5} X^2 - 0.10X + 165.66$; $R^2 = 0.96$; $P < 0.01$ for UV-A at 360 nm; for *Saussurea pulchra* (b), are $Y = 3.29 \times 10^{-5} X^2 - 0.22X + 376.43$; $R^2 = 0.95$; $P < 0.05$ for UV-B at 305 nm, and $Y = 2.57 \times 10^{-5} X^2 - 0.17X + 294.13$; $R^2 = 0.97$; $P < 0.05$ for UV-A at 360 nm; and for *Rheum pumilum* (c), are $Y = 0.012X - 33.09$; $R^2 = 0.74$; $P < 0.06$ for UV-B at 305 nm, and $Y = 0.009X - 23.01$; $R^2 = 0.74$; $P = 0.06$ for UV-A at 360 nm.

under both PAR and UV radiation than the three other species, which have no or very little leaf pubescence (Fig. 6, see online supplementary Fig. S2).

Leaf UV reflectance and absorbance should not vary independently in response to environmental changes in UV radiation. Grant *et al.* (2003) reported the influence of cellular pigments on the subsurface contribution to reflectance, but there are no other studies to clarify the relationship. From the evidence presented in this study (Figs 2 and 3), we propose that leaf UV reflectance may increase in importance in leaf UV acclimation and adaptation in intense UV radiation environments. Further evidence is needed to address the assumption.

Is there a trade-off in the biomass investment between leaf UV reflectance and photosynthetic pigments?

Changes in leaf UV reflectance, which is mainly determined by leaf surface structure including leaf pubescence and waxy cuticle, and in photosynthetic pigment content have been considered as important morphological and biochemical processes for plants to cope with environmental changes, especially environmental light variation (Jansen *et al.* 2015; Randriamanana *et al.* 2015). Biomass is needed in the synthesis of leaf surface substances and photosynthetic pigments. In alpine environments, plant biomass production is usually limited because of harsh physical conditions such as low temperature and low water availability, and/or short growing seasons (Körner 2003). It is unclear how plants allocate limited resources to leaf surface structure for UV protection and to leaf pigments for photosynthesis. The data in our study provide some insights into addressing this question. As shown in Fig. 6, plants, either in terms of species or in terms of population at different altitudes within some species such as *S. katochaete*, tended to have a lower total photosynthetic pigment content at the higher leaf UV reflectance (Fig. 6). This seems mainly due to the high negative correlation between the chlorophyll content and the leaf UV reflectance (see online supplementary Fig. S3). These results suggest that there is probably a trade-off for investing biomass to leaf UV-reflectance and to photosynthetic pigments. It should be noticed that many biochemical components assumed to be involved in leaf UV reflectance have a smaller molecular weight than chlorophylls (see online supplementary Table S1). Since a smaller molecular should often cost less

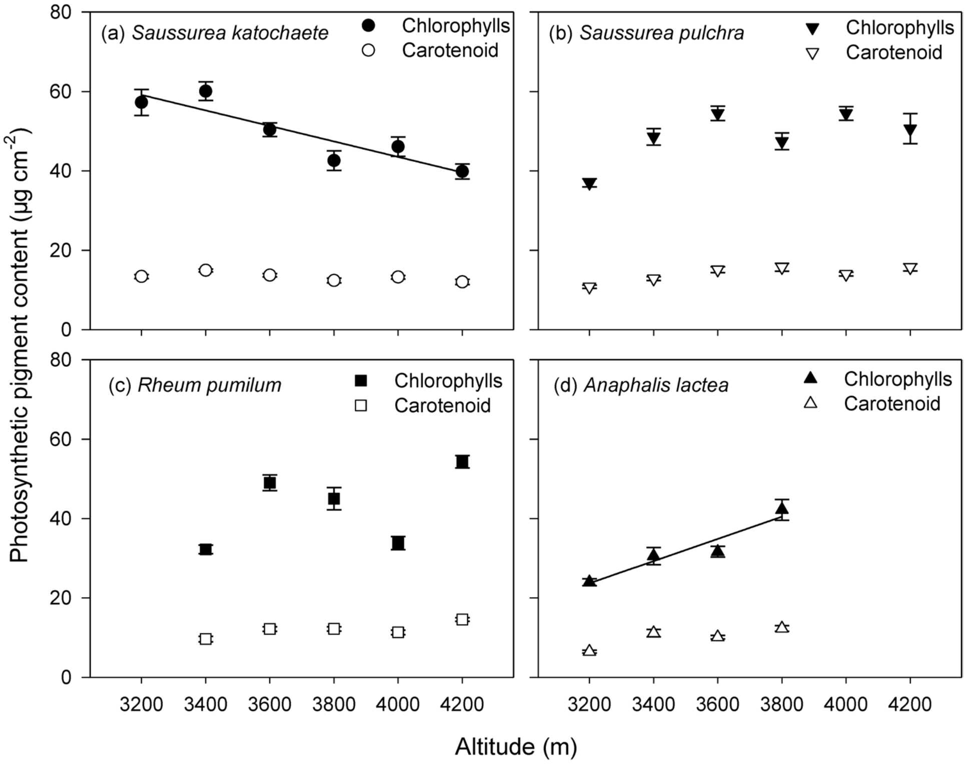

Figure 4: leaf chlorophyll a and b and carotenoids in relation to the habitat altitude for four herbaceous species along the same slope in Haibei, Qilian Mountains, Qinghai–Tibetan Plateau. Symbols of species are the same as described in Fig. 2, but the closed ones indicate the content of chlorophylls and the open ones indicate carotenoid content. The data are shown as the mean ± SE of 16 leaf samples from four individual plants at each elevation. The equations of the fitting curves for chlorophyll a and b are $Y = -0.02X + 121.58$; $R^2 = 0.82$; $P < 0.05$ for *Saussurea katochaete* (a) and $Y = -0.03X - 65.86$; $R^2 = 0.91$; $P < 0.05$ for *Anaphalis lactea* (d).

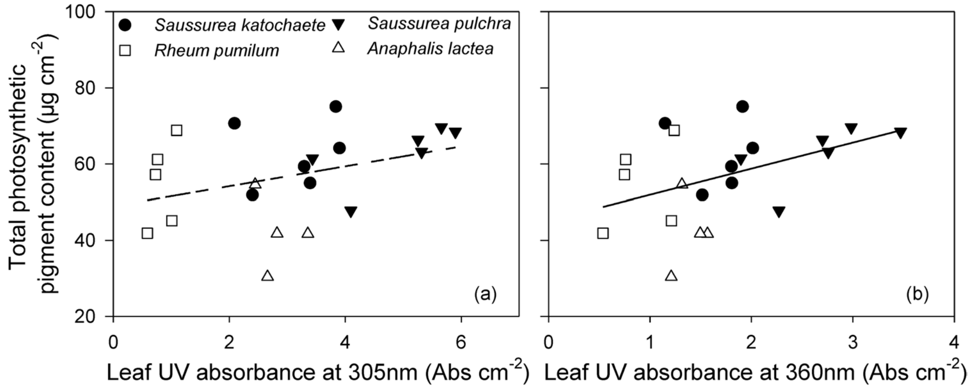

Figure 5: the total photosynthetic pigment content (chlorophyll a and b + carotenoids) in relation to the leaf UV absorbance for the four experimental species indicated as different symbols (closed circle: *Saussurea katochaete*, open square: *Rheum pumilum*, closed inverted triangle: *Saussurea pulchra* and open triangle*: Anaphalis lactea*) along the same slope in Haibei, Qilian Mountains, Qinghai–Tibetan Plateau. The data are shown as the means of the absorbance values at 305 and 360 nm from Fig. 2 and the means of the total photosynthetic pigment content from Fig. 4. The equations of the fitting curves are $Y = 2.61X + 49$; $R^2 = 0.13$; $P < 0.11$ for the total photosynthetic pigment in relation to the UV-B absorbance at 305 nm (a) and $Y = 6.84X + 45.10$; $R^2 = 0.20$; $P < 0.05$ for the total photosynthetic pigment in relation to the UV-A absorbance at 360 nm (b).

biomass, it is expected for alpine plants to increase biomass investment to leaf UV reflectance using limited resources.

It is unclear whether the trade-off between the investment in leaf UV reflectance and photosynthetic pigments is a common strategy for alpine plants to cope with UV environments. Further evidence from field observations and biochemical mechanisms is needed to address this question in the future. On the other hand, we found that the leaf UV absorbance exhibited a fairly positive correlation with the photosynthetic pigment contents. We assume that photosynthetic pigments may partly function as UV-absorbing pigments since both chlorophylls and carotenoids showed relatively high absorbance in the UV wavelengths (Mohr and Schopfer 1995).

Different strategies of leaf UV reflectance and absorbance in alpine plants

Among the four species examined, we found contrasting patterns in the leaf UV reflectance and absorbance in relation to altitudinal gradients. Although the evidence is still very limited, acclimation and adaptation strategies are assumed to exist for alpine plants to cope with environmental UV changes through the adjustment of leaf UV reflectance and absorbance (Fig. 7, Table 1).

Among the four species examined in this study, *A. lactea* was insensitive in terms of both leaf UV reflectance and absorbance in response to the altitudinal variation (Fig. 7). The thick leaf pubescence associated with high UV and PAR reflectance (see online supplementary Fig. S3) may prevent effective photosynthetic light utilization, which perhaps partly contributes to the relatively small altitudinal range and low altitude of the species distribution (Table 1).

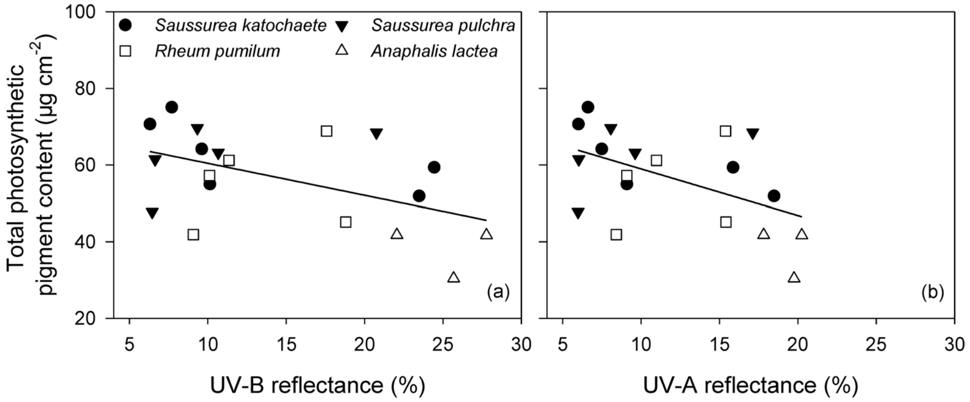

Figure 6: the total photosynthetic pigment content (chlorophyll a and b + carotenoids) in relation to the leaf UV reflectance for the four experimental species indicated as different symbols (closed circle: *Saussurea katochaete,* open square: *Rheum pumilum,* closed inverted triangle: *Saussurea pulchra* and open triangle*: Anaphalis lactea*) along the same slope in Haibei, Qilian Mountains, Qinghai–Tibetan Plateau. The data are shown as the means of the reflectance values at wavelengths of 305 and 360 nm from Fig. 3 and the mean values of the total photosynthetic pigment content from Fig. 4. The equations of the fitting curves are $Y = -0.83X + 68.77$; $R^2 = 0.25$; $P < 0.05$ for UV-B reflectance (a) and $Y = -1.21X + 71.10$; $R^2 = 0.26$; $P < 0.05$ for UV-A reflectance (b).

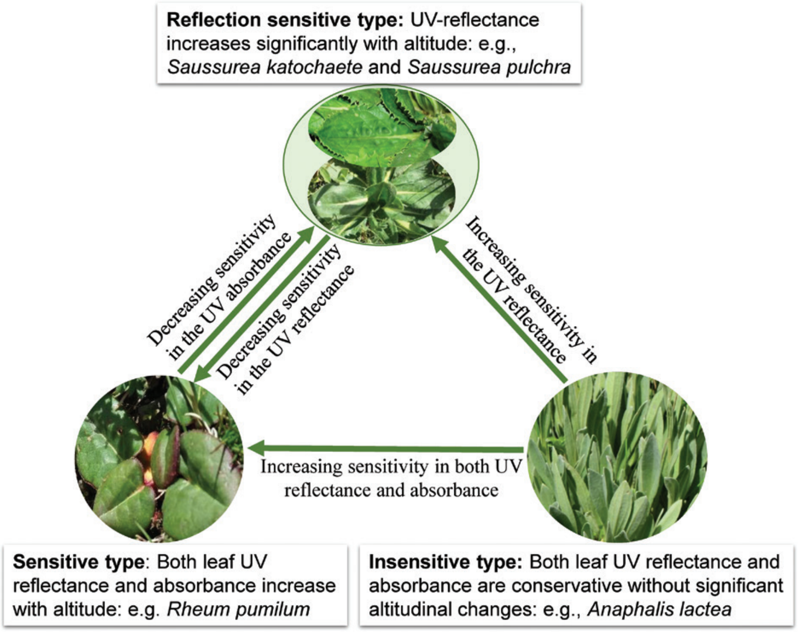

Figure 7: illustration showing a set of strategies proposed for alpine plants to acclimate and adapt to UV radiation by adjusting leaf UV reflectance and UV absorbance.

In *S. katochaete* and *S. pulchra*, the leaf UV reflectance increased with altitude, but the leaf UV absorbance showed no significant change with altitude. It seems that these two species are very flexible in terms of leaf UV reflectance at high elevations (Fig. 3). Previous studies also emphasized the importance of UV reflection in the contribution to protect plants from UV-B radiation injury (Caldwell *et al.* 1983; Filella and Penuelas 1999; Robberecht and Caldwell 1978).

Rheum pumilum, however, showed a completely different strategy from that exhibited by *A. lactea*. Both the leaf UV reflectance and absorbance increased with altitude (Fig. 7). The leaf absorbance was very low among all altitudes but showed a close correlation with altitude as compared with the three other species in this study.

As indicated in Fig. 7, we proposed a preliminary summary for the UV protection strategy in alpine plants to acclimate and/or adapt to the variation in UV radiation. Three types of strategies can be recognized in terms of leaf reflectance and absorbance: UV reflectance and absorbance insensitive type, UV reflectance and absorbance sensitive type, and UV reflectance sensitive type. A UV absorbance sensitive type may also exist. Further studies are thus necessary for clarifying both the biological mechanisms and ecological consequences involved in the diversity of acclimation and adaptation of alpine plants to UV environments, especially in ecosystems with strong UV radiation, such as the Tibetan Plateau.

SUPPLEMENTARY DATA

Supplementary material is available at *Journal of Plant Ecology* online.

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