

Differences in light-harvesting, acclimation to growth-light environment, and leaf structural development between Swedish and Italian ecotypes of *Arabidopsis thaliana*

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Abstract

Main conclusion Leaf morphological differences have an impact on light distribution within the leaf, photosynthesis, and photoprotection in *Arabidopsis thaliana* ecotypes from near the limits of this species' latitudinal distribution in Europe.

Leaf morphology, photosynthesis, and photoprotection were characterized in two *Arabidopsis* ecotypes from near the limits of this species' latitudinal distribution in Europe (63°N and 42°N). The Swedish ecotype formed thicker leaves and upregulated photosynthesis more substantially than the Italian ecotype in high-light environments. Conversely, the smaller rosette formed, and lesser aboveground biomass accumulated, by the Swedish versus the Italian ecotype in low growth-light environments is consistent with a lesser shade tolerance of the Swedish ecotype. The response of the thinner leaves of the Italian ecotype to evenly spaced daily periods of higher light against a background of otherwise non-fluctuating low light was to perform the same rate of photosynthesis with less chlorophyll, rather than exhibiting greater rates of photosynthesis.

In contrast, the thicker leaves of the Swedish ecotype showed elevated photosynthetic performance in response to daily supplemental higher light periods in a low-light growth environment. These findings suggest significant self-shading in the lower depths of leaves of the Swedish ecotype by the chloroplasts residing in the upper portions of the leaf, resulting in a requirement for higher incident light to trigger photosynthetic upregulation in the lower portions of its thicker leaves. Conversely, photoprotective responses in the Italian ecotype suggest that more excess light penetrated into the lower depths of this ecotype's leaves. It is speculated that light absorption and the degree of utilization of this absorbed light inform cellular signaling networks that orchestrate leaf structural development, which, in turn, affects light distribution and the level of absorbed versus photosynthetically utilized light in a leaf.

Keywords Leaf thickness · Light response · NPQ · Photosynthesis · Tocopherol · Zeaxanthin

Abbreviations

A	Antheraxanthin
CBF	C-repeat-binding factor
LMA	Leaf mass per area
NPQ	Non-photochemical quenching of chlorophyll fluorescence
P_{\max}	Photosynthetic capacity (light- and CO ₂ -saturated rate of photosynthesis)
ROS	Reactive-oxygen species
V	Violaxanthin
VAZ	Xanthophyll cycle pigments violaxanthin, antheraxanthin, and zeaxanthin
Z	Zeaxanthin

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Introduction

Arabidopsis thaliana ecotypes from Sweden and Italy (Ågren and Schemske 2012) have received attention for the differential responsiveness of their foliar minor veins to temperature and the likely impact this has on foliar sugar-export capacity and, in turn, the leaf's capacity to upregulate photosynthesis. While leaves of both ecotypes exhibited a strong response to growth at low temperature under high light, consisting of a greater number of phloem cells per minor vein and record high rates of photosynthetic oxygen evolution (Cohu et al. 2013a, b), the Swedish ecotype had even more numerous minor vein phloem cells (Cohu et al. 2013a), more pronounced phloem parenchyma cell wall ingrowths (presumably providing for a greater number of phloem parenchyma cell membrane localized ATPases and sucrose-efflux transporters important for the active loading of sucrose into the sieve elements; Adams et al. 2014), and higher photosynthetic capacities (Cohu et al. 2013b; Adams et al. 2014) compared to the Italian ecotype when both were grown under moderate light at low temperature. Moreover, these two ecotypes, and hundreds of inbred lines derived from these two parents, have been used to dissect genes underlying the control of flowering and others involved in fitness and potential trade-offs in fitness (Ågren et al. 2013; Grillo et al. 2013; Dittmar et al. 2014; Oakley et al. 2014).

Furthermore, photosynthetic acclimation of *A. thaliana* to cold temperatures is supported by thicker leaves with more palisade cell layers (Gorsuch et al. 2010; Cohu et al. 2014a), a typical response in winter annuals (germinating in autumn, overwintering as a rosette of leaves, and bolting/flowering in spring). This responsiveness of winter annuals to cold temperature is in stark contrast to that of summer annuals (germinating in spring and completing their life cycle in autumn of the same year) that do not exhibit acclimation of leaf venation, leaf mesophyll tissue, or photosynthesis to growth at low compared to warm temperature (Dumlao et al. 2012; Cohu et al. 2014a; see also, e.g., Dahal et al. 2012). On the other hand, acclimation of both winter and summer annuals to high versus low-light levels typically involves thicker leaves in support of higher rates of photosynthesis (Amiard et al. 2005; Terashima et al. 2006).

The present study was undertaken to address several questions. Are leaves of the Swedish ecotype thicker than leaves of the Italian ecotype under common growth conditions? Does such a difference require cold temperature as a trigger, or are differences in leaf thickness between the ecotypes seen under warm growth temperature as well? What repercussions may result for the internal light environment of the leaf and its acclimation to growth-light

environment? Are there differences in photosynthetic acclimation to low light, high light, or fluctuating light between the ecotypes that may be associated with leaf morphological features? In order to address these questions, the two ecotypes were grown (1) in a naturally lit glasshouse with peak irradiance levels close to full sunlight, (2) under non-fluctuating low irradiance in growth chambers, and (3) under the latter low-light conditions supplemented with six daily, evenly spaced 5-min periods of higher light interspersed throughout each 9-h light period (the photoperiod).

The impact of these various growth conditions on the two ecotypes was assessed through characterization of leaf-anatomical metrics, transpiration and photosynthesis rates, foliar chlorophyll content, and photoprotection against the potentially harmful effects of excess light (i.e., greater levels of light absorbed than utilized in photosynthesis). Photoprotection was quantified as thermal energy dissipation of excess light via non-photochemical quenching (NPQ) of chlorophyll fluorescence coupled with assessment of photoprotective compounds (xanthophylls and tocopherols) that counteract formation of reactive-oxygen species and/or detoxify reactive-oxygen species and other reactive species. We quantified the levels of the high-light forms zeaxanthin and antheraxanthin formed in the violaxanthin, antheraxanthin, and zeaxanthin (VAZ) cycle that are associated with thermal dissipation of excess energy (and NPQ; Demmig-Adams et al. 2012) as well as the levels of tocopherols as antioxidant metabolites (Havaux and García-Plazaola 2014).

Materials and methods

Plant material

Two ecotypes of *A. thaliana* (L.) Heynhold originating from “near the northern [Sweden] and southern [Italy] limits of the native geographic range in Europe” (Ågren and Schemske 2012) were investigated. Mean annual air temperatures at these sites (between 2004 and 2010) were reported to be 14.4 °C in Italy and 4.9 °C in Sweden by the latter authors (Ågren and Schemske 2012). In addition, we estimated growing season (germination to seed set) temperatures for each native site, with average minimum, mean, and maximum temperatures of 5.5, 9.7, and 14 °C in Italy versus −4.4, −0.5, and 3.4 °C in Sweden (<http://www.worldclim.org/>; Hijmans et al. 2005). The plants used in the current study are isogenic progeny (sub-line 24) of line 12 for the Italian ecotype and isogenic progeny (sub-line 29) of line 47 of the Swedish ecotype. Lines 12 and 47 are available via *The Arabidopsis Information Resource* (TAIR; <https://www.>

arabidopsis.org). Seeds were incubated at 4 °C for 4 days and germinated in six-plug trays (50-mL volume). After cotyledons emerged, seedlings were transplanted individually into large pots (3.8 L) and allowed to acclimate under germination conditions for 2 days before transfer to final growth conditions (see below). All plants were grown in Canadian Growing Mix 2 (Conrad Fafard Inc., Agawam, MA, USA) with ample water (daily watering) and nutrient supply (nutrients every other day) that had been determined in previous experiments to be sufficient to allow maximal photosynthesis rates in all light environments used here. Five non-flowering plants that were at least 6-week old from each ecotype and each treatment were used for all measurements. From each plant, several fully expanded leaves that had been exposed to the respective growth-light intensity were used for the various parameters assessed, typically one leaf from each of the five plants for a given parameter (i.e., five biological replications).

Growth conditions

Glasshouse-grown plants were grown from seed in a naturally lit glasshouse (for photographs of the plants, see Fig. S1). Seeds were germinated in a partially shaded portion of the glasshouse with a peak photon flux density (PFD) of 600 $\mu\text{mol m}^{-2} \text{s}^{-1}$ before transfer to their final growth location with a peak PFD of 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Temperature and relative humidity measurements were taken over a period of 43 days prior to plant characterization using an EL-USB-2 data logger (Lascar Electronics, Salisbury, UK). Mean (\pm standard deviation) daytime (between 12 and 4 pm) and nighttime (between 12 and 4 am) air temperatures were 21.9 ± 2.6 °C ($n = 157$) and 16.8 ± 0.7 °C ($n = 160$), respectively, and mean (\pm standard deviation) daytime and nighttime relative humidity levels were 27 ± 10 % ($n = 157$) and 34 ± 4 % ($n = 160$), respectively.

Another set of plants (for photographs of the plants, see Fig. S2) was grown from seed in temperature- and humidity-controlled (75 % relative humidity) growth chambers (Conviron E-15, Winnipeg, Canada) using growth conditions as described in Cohu et al. (2014b). Plants were grown under air temperatures of 25 °C/20 °C (light/dark) under a 9-h photoperiod (9 h light/15 h dark) of 200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, and final growth conditions included either the presence (+) or absence (–) of six evenly distributed, 5-min periods of 800 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (the first higher light period beginning 1 h after the photoperiod began, and the last higher light period ending 1 h before the end of the photoperiod). Plants thus experienced daily total photon fluxes of either 6.48 $\text{mol m}^{-2} \text{photoperiod}^{-1}$ without the higher light exposures or 7.56 $\text{mol m}^{-2} \text{photoperiod}^{-1}$ with the six periods of higher light.

Light- and CO₂-saturated photosynthesis and leaf thickness measurements

Measurements of P_{max} (photosynthetic capacity, as the light- and CO₂-saturated rate of oxygen evolution at 25 °C), leaf dry mass per unit leaf area (LMA), leaf and palisade mesophyll thickness, number of palisade mesophyll cell layers, and sample preparation for microscopy were conducted as previously described (Amiard et al. 2005; Dumlao et al. 2012; Muller et al. 2014). Photosynthetic oxygen evolution from excised leaf tissue was measured in a temperature-controlled leaf-disk oxygen electrode chamber in water-saturated air and 5 % CO₂, which eliminates cuticular, stomatal, and mesophyll resistance to CO₂ diffusion (Delieu and Walker 1981). Sample size for P_{max} and LMA was a total of five leaves (one leaf each from five different plants) for each ecotype from each treatment, while leaf thickness was determined from three cross sections from each of five leaves (from five different plants) per ecotype and species.

CO₂ and H₂O gas exchange

CO₂ uptake and transpiration rate were measured using an LCI Portable Photosynthesis System (ADC Bioscientific Ltd., Hoddesdon, Herts, England, UK). For measurements on plants grown in growth chambers under 200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, plants were removed from the growth chamber one at a time, the leaf clamped into the cuvette, and exposed to 200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ for 5 min before measurements were made. Measurements on growth chamber-grown plants occurred approximately 5 h into the photoperiod. To standardize environmental conditions prior to gas exchange measurements, glasshouse-grown plants were transferred to a light- and temperature-controlled growth chamber (25 °C, 200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) for 3 h, after which measurements were conducted following exposure of leaves to 1500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (corresponding to the midday peak light level in the naturally lit glasshouse) for 5 min in air. Because leaves of the *Arabidopsis* ecotypes used in this study did not fill the 6.25 cm² cuvette, total leaf area inside the cuvette was photographed prior to attaching the actinic light source using a Canon digital camera (EOS Rebel Series, Canon USA Inc., Melville, New York, USA) and quantified using ImageJ (Rasband W.S., ImageJ, US National Institute of Health, Bethesda, MD, USA, <http://imagej.nih.gov/ij/>, 1997–2012). Sample size for CO₂ and H₂O gas exchange measurements was a total of five leaves (one leaf each from five different plants) for each ecotype from each treatment.

Leaf pigments and tocopherols

For analysis by high-performance liquid chromatography, leaf disks of 0.325 cm² were collected from one leaf each of five different plants for each ecotype from each treatment. Leaf disks from glasshouse-grown plants were collected at the end of the night period and leaf disks from plants grown in growth chambers were collected both before onset and at the end (approximately 60 min after the final period of higher light) of the photoperiod. To prevent changes in pigment levels post-collection, each sample was immediately dropped into an aluminum envelope and submerged in liquid nitrogen until biochemical analysis. Since light, oxygen, and warmer temperatures lead to rapid destruction of the pigments, all steps in the following description were carried out under the lowest, indirect light possible (or in darkness where noted); extraction and storage of samples were carried out with pre-chilled glassware on ice and/or under refrigeration, and extracts were bubbled with nitrogen gas before sealing in pre-chilled autosampler vials or Eppendorf tubes. To extract pigments, leaf disks were masticated in an ice-cold glass grinder with a small amount of MgCO₃ and 0.2 mL of a refrigerated solution of 85 % acetone (15 % water), followed by multiple washes (85 % acetone to fully extract the more polar carotenoids, followed by 100 % acetone to fully extract the less polar carotenoids) and centrifugations (at 5 °C) as described in Adams and Demmig-Adams (1992). Total volume of the combined supernatant was determined with a syringe, passed through a filter (Minisart SRP4 Syringe Filters, 0.2 µm pore size, No. 17844K, Sartorius Stedim Biotech, Göttingen, Germany), bubbled with gaseous nitrogen before closing the autosampler vials, and kept on ice and/or refrigerated (at 4 °C in the refrigerated autosampler) until sampling. Pigments in each sample were separated and quantified using a high-pressure liquid chromatography (HPLC) system (Shimadzu Corporation, Kyoto, Japan) with a bonded silica Carotenoid YMCTM 5-µm column (YMC Co., Kyoto, Japan) maintained at 30 °C. A gradient of solvent A [86.7 % acetonitrile, 9.6 % methanol, 3.7 % 0.1 M Tris–HCl (pH 8.0), passed through a filter (Millex-GP Pump or Syringe-Driven Filter Unit, 0.22 µm pore size, No. SLGP05010, Millipore Corporation, Bedford, MA, USA)] to solvent B (80 % methanol, 20 % hexane) was used to elute all pigments except β-carotene. The gradient was followed by an isocratic elution of β-carotene using solvent B. All solvents were HPLC grade, and injection volumes for elutions were 20 µL for quantification of chloroplast pigments.

Peak areas captured from the photodiode array detector (at 445 nm for carotenoids and at 654 nm for chlorophylls) were converted from µAu to total carotenoids or chlorophylls based on standards generated by us from leaves in the dark state (VAZ cycle pigments present predominantly as violaxanthin) and from leaves exposed to high light and low

CO₂ (VAZ cycle pigments present predominantly as antheraxanthin and zeaxanthin). Leaves in these two states were frozen in liquid nitrogen to preserve pigments. Four to five leaf disks were then ground together and extracted as described above using the same minimal volumes to yield a concentrated extract. Under the lowest light possible, each chilled extract was applied to a thin layer plate (Number 5553/7, Silica gel 60 on aluminum sheets; EM Science, Gibbstown, NJ, USA) in a line 2 cm from the bottom of the plate and separated in a chamber (in darkness) using a solvent composed of 90 mL petroleum ether (boiling point 37–57 °C), 70 mL chloroform, and 10 mL isopropyl alcohol. Once the solvent front was just below the top of the plate (after 50–60 min of running time), each pigment band was quickly scraped off, funneled into a Pasteur pipette packed (at the constricted end) with a small plug of nonabsorbent cotton on top of which was layered approximately 0.5 cm of acid washed sand (to capture most of the silica gel), and eluted (using a bulb to apply gentle pressure) with a small amount of chilled HPLC grade 100 % ethanol (for carotenoids) or 100 % acetone (for chlorophylls) into test tubes on ice. Each eluted pigment was drawn into a syringe, filtered as described above into chilled Eppendorf tubes (1.5 mL), bubbled with nitrogen gas, capped, and kept in a darkened ice bucket. The concentration of each pigment was determined spectroscopically at the wavelength for each pigment's absorption maximum using previously established extinction coefficients (Gillam 1935; MacKinney 1940; Zscheile et al. 1942; Goodwin 1955; Davies 1965, 1976; Hager and Meyer-Bertenrath 1966; Liaaen-Jensen and Jensen 1971; Stransky 1978), after which the samples were bubbled with nitrogen gas in autosampler vials, sealed, and kept on ice/refrigerated in darkness until injection into the HPLC system (three different volumes of each pigment sample) in order to provide the calibration for each pigment.

The extraction process for tocopherols was the same as that of chloroplast pigments, except that no MgCO₃ was added during the initial mastication. Extinction coefficients supplied by Sigma-Aldrich for each standard were used along with a dilution series for the calibration of tocopherol standards, α-tocopherol, β-tocopherol, γ-tocopherol, and δ-tocopherol (Sigma-Aldrich, St. Louis, MO, USA). Tocopherols were detected (absorbance wavelength of 292 nm) using the HPLC system (injection volume of 40 µL) and running protocol described above.

Total light-exposed rosette area per plant and aboveground biomass

Photographs were taken of the intact plants from directly overhead on the day before the harvest using a Canon digital camera. Using a ruler at the approximate plane of the rosette as a scale, total light-exposed leaf area was calculated using

ImageJ. Sample size for rosette area and biomass was five different plants for each ecotype from each treatment. The results for dry aboveground biomass are qualitatively and quantitatively similar to those reported from a similar treatment of these two ecotypes under 200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ with and without added 5-min periods of 800 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (Cohu et al. 2014b), demonstrating the reproducibility of this treatment.

Chlorophyll fluorescence

NPQ ($[F_m(\text{unquenched})/F_m(\text{quenched})] - 1$) was calculated as described in Demmig-Adams et al. (1996). Chlorophyll fluorescence from leaves in the low-light growth chambers was obtained with a portable PAM-2000 fluorometer (Walz, Effeltrich, Germany), and fluorescence yields were recorded on a chart recorder (Kipp & Zonen B.V., Delft, The Netherlands). For measurements under 800 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, plants were removed from the growth chamber one at a time and exposed for 5 min to 800 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ from an actinic light source (Hansatech, King's Lynn, UK) during the approximate time of the scheduled fourth higher light period of that day. Light-saturated NPQ capacities were ascertained by exposing excised leaf disks to 2425 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ under conditions (2 % O_2 , 0 % CO_2 , balance N_2) preventing high rates of linear electron transport in a modified leaf-disk oxygen electrode chamber (Hansatech) maintained at 25 °C. Sample size for NPQ measurements was a total of five leaves (one leaf each from five different plants) for each ecotype from each treatment.

Statistical analyses

All statistical analyses were conducted using JMP software (Pro 11.0.1, SAS Institute Inc., Cary, NC, USA). Student's *t* test was used for comparison of two means (for the comparison of the two ecotypes grown in the glasshouse), while two-way analysis of variance (ANOVA) was used for comparison of multiple means (i.e., for the effect of growth-light environment, ecotype, and the degree of ecotype response to light environment in the growth chamber treatments of the two ecotypes under two different light environments).

Results

Plants grown in a glasshouse under natural sun exposure

Light- and CO_2 -saturated maximal rates of photosynthetic oxygen evolution (P_{max}) on a leaf-area basis were

significantly greater in the Swedish versus the Italian ecotype (Fig. 1a) grown in a naturally lit glasshouse. Likewise, rates of CO_2 uptake and of water loss by transpiration on a leaf-area basis under midday peak irradiance and ambient CO_2 levels were also significantly greater in the Swedish versus the Italian ecotype (Fig. 1b, c), with similar ratios of CO_2 uptake to water loss (water-use efficiency of photosynthesis) under ambient conditions (Fig. 1d). The greater photosynthesis rates on a leaf-area basis in the Swedish versus the Italian ecotype were,

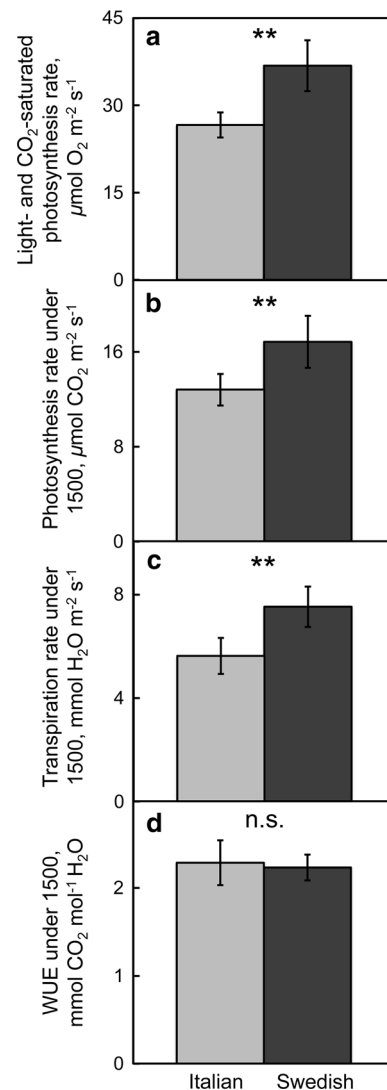


Fig. 1 Light- and CO_2 -saturated rate of oxygen evolution measured at 25 °C (a), CO_2 uptake (b), and transpiration rate (c), all expressed on a leaf-area basis, as well as (d) water-use efficiency of photosynthesis (ratio of CO_2 uptake to transpirational water loss) (d) from the Italian (light gray columns) and Swedish (dark gray columns) ecotypes of *A. thaliana* grown in a naturally lit glasshouse. Gas exchange measurements (b–d) were obtained under 1500 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ and ambient CO_2 . Mean values \pm SD; statistically significant differences between ecotypes are indicated with asterisks (** $P < 0.01$, n.s. not significantly different)

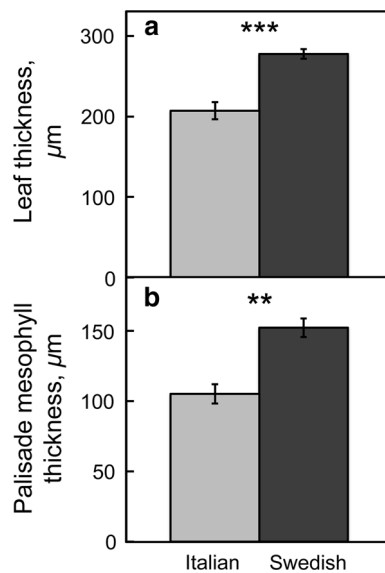


Fig. 2 Leaf thickness (a) and palisade mesophyll thickness (b) from the Italian (*light gray columns*) and Swedish (*dark gray columns*) ecotypes of *A. thaliana* grown in a naturally lit glasshouse. Mean values \pm SE; statistically significant differences between ecotypes are indicated with an asterisk (** $P < 0.01$, *** $P < 0.001$)

furthermore, associated with thicker leaves (Fig. 2a), resulting mainly from a thicker layer of the palisade cells under the upper leaf surface (Fig. 2b). Although not statistically different, the mean number of palisade cell layers (\pm standard error, SE) were slightly greater for the Swedish (3.3 ± 0.2) versus the Italian (2.8 ± 0.2) ecotype, as were the lengths of the palisade cells (data not shown).

The thicker leaves of the Swedish ecotype also contained more chlorophyll per leaf area than the thinner leaves of the Italian ecotype (Fig. 3a). Chlorophyll content on a leaf-volume basis—taking the different leaf thicknesses into account as the third dimension—was, however, similar in the two ecotypes (Fig. 3b), as was P_{\max} expressed on a chlorophyll basis (Fig. 3c). Furthermore, means for leaf dry mass (DW) per area (\pm standard deviation, SD) were also significantly greater ($P < 0.001$) for the Swedish (29.8 ± 2.2 g DW m^{-2}) versus the Italian (18.6 ± 4.0 g DW m^{-2}) ecotype, while light- and CO_2 -saturated P_{\max} (\pm SD) was not significantly different between the two ecotypes when expressed on a dry mass basis (1.5 ± 0.3 and 1.2 ± 0.1 $\mu\text{mol O}_2 \text{ g}^{-1} \text{ DW s}^{-1}$ for the Italian and Swedish ecotypes, respectively).

In addition to chlorophyll levels, carotenoid levels were obtained for the two ecotypes grown in the naturally lit glasshouse. When expressed on a leaf-area basis, all leaf carotenoids (just like chlorophyll; Fig. 3a) were present at greater levels in the thicker leaves of the Swedish ecotype compared to the thinner leaves of the Italian ecotype [see Table 1 for the carotenoids participating in the VAZ cycle,

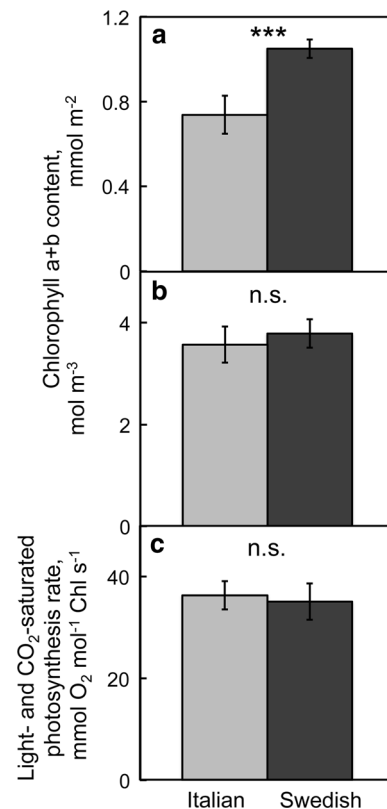


Fig. 3 Chlorophyll content on a leaf-area basis (a) and a leaf-volume basis (b), and light- and CO_2 -saturated rate of oxygen evolution measured at 25 °C on a chlorophyll basis (c) from the Italian (*light gray columns*) and Swedish (*dark gray columns*) ecotypes of *A. thaliana* grown in a naturally lit glasshouse. Mean values \pm SD; statistically significant differences between ecotypes are indicated with an asterisk (*** $P < 0.001$, n.s. not significant)

violaxanthin (V), antheraxanthin (A), and zeaxanthin (Z); β -carotene, lutein, and neoxanthin not shown]. However, when expressed on a chlorophyll basis, the only carotenoids present at different levels between the ecotypes were those of the VAZ cycle (Table 1). The level of V + A + Z on a chlorophyll basis was actually greater in the thinner leaves of the Italian ecotype compared to the thicker leaves of the Swedish ecotype (Table 1). Likewise, the level of the high-light components of the VAZ cycle (Z + A) retained in leaves overnight was greater in the thinner leaves of the Italian ecotype compared to the thicker leaves of the Swedish ecotype (Table 1).

Plants grown in growth chambers under low PFD with and without short periods of higher PFD

Even under low growth PFD in a temperature-, humidity-, and photoperiod-controlled growth chamber, where both ecotypes exhibited thinner leaves than when grown in the naturally lit glasshouse (cf. Figs. 2a, 4a), the leaves of the

Table 1 Predawn leaf xanthophyll cycle composition of the Italian and Swedish ecotypes of *A. thaliana* grown in a naturally lit glasshouse expressed on either a leaf-area basis or a chlorophyll basis

	Italian	Swedish	Level of significance
Z + A ($\mu\text{mol m}^{-2}$)	9.7 ± 0.9	7.7 ± 2.3	*
Z + A (mmol mol^{-1} Chl <i>a</i> + <i>b</i>)	13.2 ± 1.2	7.4 ± 2.2	***
V + A + Z ($\mu\text{mol m}^{-2}$)	42.3 ± 7.2	51.5 ± 5.5	*
V + A + Z (mmol mol^{-1} Chl <i>a</i> + <i>b</i>)	57.2 ± 3.7	49.7 ± 4.8	**
(Z + A)/(V + A + Z)	0.23 ± 0.03	0.15 ± 0.03	***

Mean values \pm standard deviation

A antheraxanthin, V violaxanthin, Z zeaxanthin

Statistically significant differences between the ecotypes are indicated with asterisks (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$)

Swedish ecotype were still significantly thicker than those of the Italian ecotype (Fig. 4a; see Table 2 for 2-way ANOVA results). The addition of six daily 5-min periods of higher light (200 + P) did not cause leaf thickness to be significantly greater (Fig. 4a; Table 2) in either ecotype than under non-fluctuating low growth PFD (200). Such additional daily high-light exposures did, however, result in a lower chlorophyll content on both a leaf area (Fig. 4b) and leaf volume (Fig. 4c) basis in both ecotypes, while these latter response to increased light availability did not differ between the two ecotypes (Table 2).

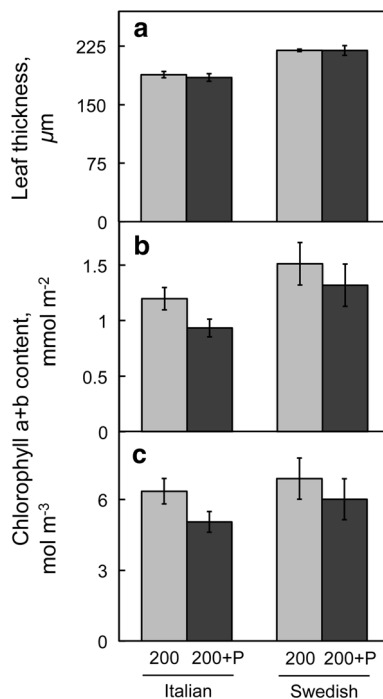


Fig. 4 Leaf thickness (a) and chlorophyll content on a leaf-area basis (b) and a leaf-volume basis (c) from the Italian and Swedish ecotypes of *A. thaliana* grown at 200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ in either the absence (200; light gray columns) or the presence (200 + P; dark gray columns) of six periods of higher light (800 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) of 5 min duration each interspersed evenly during the 9-h photoperiod. Mean values \pm SE for a and \pm SD for b and c

Concerning photosynthesis rates on a leaf-area basis, the response to growth in the presence of daily high-light exposures did not differ significantly between the two ecotypes for the rates of CO_2 uptake ascertained under 200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ in air (Fig. 5a; Table 2). However, rates were significantly greater in the Swedish versus the Italian ecotype for P_{max} ascertained under experimental exposure to saturating light and CO_2 levels in an oxygen electrode (Fig. 5b; Table 2). While the Swedish ecotype responded more strongly, both ecotypes did exhibit significantly greater P_{max} on a leaf-area basis when grown in the presence of daily high-light exposures (Fig. 5b; Table 2). It should be noted that this increase in P_{max} (Fig. 5b, c) in response to daily high-light exposures occurred without increases in leaf thickness (Fig. 4).

However, when expressed on a chlorophyll basis (Fig. 5c) or a leaf-volume basis (Fig. 5d), P_{max} increased similarly in response to daily high-light exposures in both ecotypes (Table 2). The absence of an ecotype-dependent difference in P_{max} on a chlorophyll basis for plants grown in the presence of daily supplemental exposures to higher light (Fig. 5c) resulted from a cancelation of the significantly lower P_{max} on a leaf-area basis in the Italian ecotype (Fig. 5b) by the also significantly lower chlorophyll content on either a leaf area or volume basis (Fig. 4b, c).

Consistent with the similar maximal photosynthetic capacities on a chlorophyll basis—and thus similar photosynthesis rates relative to absorbed light—between the two ecotypes (Fig. 5c), levels of thermal dissipation of excess light absorbed in photosystem II antennae (estimated from non-photochemical quenching, or NPQ) were also not significantly different between the two ecotypes (Fig. 6a) during experimental exposure to a light level corresponding to that of the daily supplemental periods of higher light (800 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) that some plants received during growth. For both ecotypes, plants exposed to daily supplemental periods of 800 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ during growth exhibited less NPQ under 800 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ —consistent with their higher P_{max} on a chlorophyll basis (Fig. 5c)—than plants grown

Table 2 Results of two-way analysis of variance (ANOVA) for the effect of ecotype, light environment, and the degree of ecotype response to the light environment for all data from growth chamber experiments (Figs. 4, 5, 6, 7, 8, 9)

Metric	Ecotype	Light	Ecotype x light
Leaf thickness (μm)	***	n.s.	n.s.
Chlorophyll $a + b$ (mmol m^{-2})	***	***	n.s.
Chlorophyll $a + b$ (mol m^{-3})	**	***	n.s.
Photosynthesis rate under 200 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	*	n.s.	n.s.
Light- and CO_2 -saturated photosynthesis rate ($\mu\text{mol O}_2 \text{ m}^{-2} \text{ s}^{-1}$)	***	**	*
Light- and CO_2 -saturated photosynthesis rate ($\text{mmol O}_2 \text{ mol}^{-1} \text{ Chl } a + b \text{ s}^{-1}$)	n.s.	***	n.s.
Light- and CO_2 -saturated photosynthesis rate ($\text{mmol O}_2 \text{ m}^{-3} \text{ s}^{-1}$)	**	**	n.s.
NPQ under 800 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ($F_m/F'_m - 1$)	n.s.	**	n.s.
Light-saturated NPQ ($F_m/F'_m - 1$)	***	***	**
$Z + A$ ($\mu\text{mol m}^{-2}$)	***	***	***
$Z + A$ ($\text{mmol mol}^{-1} \text{ Chl } a + b$)	***	***	***
$(Z + A)/(V + A + Z)$	***	***	***
Tocopherols ($\mu\text{mol m}^{-2}$)	***	n.s.	**
Tocopherols ($\text{mmol mol}^{-1} \text{ Chl } a + b$)	***	n.s.	***
Rosette area (cm^2)	***	n.s.	n.s.
Aboveground biomass ($\text{g dry weight plant}^{-1}$)	***	***	**

Statistically significant effects are indicated with asterisks (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, n.s. not significant)

without supplemental light (Fig. 6a; Table 2). In contrast, light-saturated NPQ capacity (ascertained under experimental exposure to 2425 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) was much greater for all treatments than NPQ levels measured at 200 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$, and the response to daily high-light exposures was significantly greater in the Swedish versus the Italian ecotype (Fig. 6b; Table 2).

Figure 7 shows the level of $Z + A$ expressed on a leaf-area basis (Fig. 7a), a chlorophyll basis (Fig. 7b), or relative to the total VAZ pool (Fig. 7c). For all reference bases, daily supplemental high-light exposures resulted in significantly greater $Z + A$ levels for both ecotypes (Table 2). The total VAZ pool on a chlorophyll basis was significantly larger in both ecotypes in the presence versus absence of daily periods of higher light (39 ± 3 versus $35 \pm 3 \text{ mmol mol}^{-1}$ in the Italian ecotype and 40 ± 3 versus $35 \pm 1 \text{ mmol mol}^{-1}$ in the Swedish ecotype), while there was no difference between the VAZ pools in the two ecotypes or in the degree of ecotype response to supplemental light. However, $Z + A$ levels on all three references bases were greater in the thinner leaves of the Italian ecotype compared to the thicker leaves of the Swedish ecotype (Fig. 7a–c; Table 2), especially in plants grown with daily supplemental high-light exposures.

Daily high-light exposures caused a depletion of the levels of the antioxidant tocopherols on either a leaf-area basis (Fig. 8a) or a chlorophyll basis (Fig. 8b) at the end of the photoperiod in leaves of the Italian ecotype but not the Swedish ecotype (Table 2). Over the course of the

nocturnal dark period, however, tocopherol levels recovered again in leaves of the Italian ecotype (data not shown).

Total rosette area per plant was significantly smaller in the Swedish versus the Italian ecotype, irrespective of whether plants were grown under non-fluctuating low-light or received six daily high-light exposures (Fig. 9a; Table 2). Similarly, total aboveground dry biomass accumulation was less in the Swedish ecotype versus the Italian ecotype under low non-fluctuating light (Fig. 9b; Table 2). Furthermore, only the Swedish ecotype exhibited increased biomass accumulation in response to daily high-light exposures (Fig. 9b; Table 2).

Discussion

Leaf morphology and photosynthesis as affected by ecotype and growth environment

We here report differences for *A. thaliana* ecotypes from areas near the northern (Sweden) and southern (Italy) limits of the native geographic distribution of this species in Europe with respect to leaf morphology (especially leaf thickness) that is modulated by growth environment and is associated with leaf functional traits (photosynthetic performance, chlorophyll content, and photoprotection). For many species, acclimation to high-light levels involves thicker leaves (see, e.g., Givnish 1988; Amiard et al. 2005; Terashima et al. 2006). Furthermore, for species differing

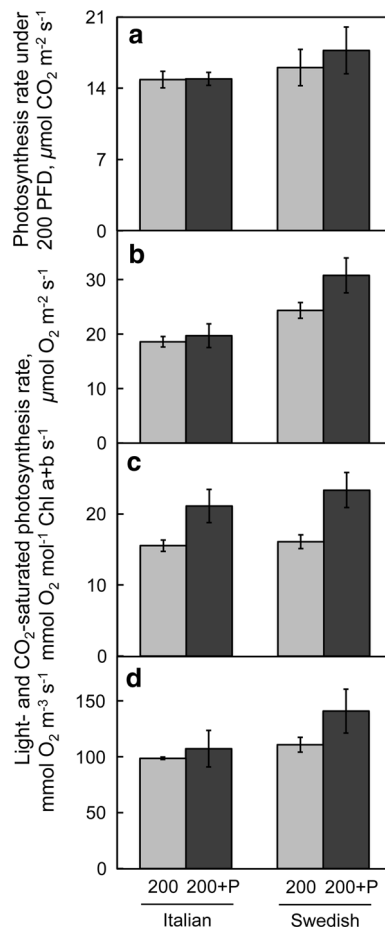


Fig. 5 CO₂ uptake (a) under 200 μmol photons m⁻² s⁻¹ (200 PFD) in air and light- and CO₂-saturated rate of oxygen evolution determined at 25 °C on a leaf-area basis (b), a chlorophyll basis (c), and a leaf-volume basis (d) from the Italian and Swedish ecotypes of *A. thaliana* grown at 200 μmol photons m⁻² s⁻¹ in the absence (200; light gray columns) or presence (200 + P; dark gray columns) of six periods of higher light (800 μmol photons m⁻² s⁻¹) of 5 min duration each interspersed evenly during the 9-h photoperiod. Leaf volume is the product of leaf area and leaf thickness. Mean values ± SD

in their tolerance to low versus high-light levels, differences in the response to growth PFD have been reported, e.g., high-light-demanding/shade-intolerant species typically exhibit thicker leaves and a higher maximal (light-saturated) rate of photosynthesis (Hanba et al. 2002). Ecological studies likewise uncovered trends for sun-tolerant (shade-intolerant) species to have higher maximal rates of photosynthesis and to require higher growth-light levels to achieve a positive carbon balance and net growth, while shade-tolerant species exhibit lower maximal rates of photosynthesis and lower light-compensation points (Bazzaz 1979). There is thus clearly considerable plasticity in these leaf traits during acclimation to different growth environments as well as genetic adaptation. We here

explored both plasticity and apparent genetic constraints in the foliar response of Swedish and Italian ecotypes of *A. thaliana*.

Anderson et al. (1995) noted that, “growth at low temperature mimics high light acclimation” and suggested that similar triggers (associated with cellular redox-signaling networks; see below) are involved (for a recent review, see Hüner et al. 2012). Increases in leaf thickness in response to cold growth temperature have been reported for several biennial and winter annual species (Boese and Huner 1990; Gorsuch et al. 2010; Dumlao et al. 2012; Cohu et al. 2014a; Muller et al. 2014). Various changes in foliar structure and function in response to cold temperature are coordinated by the transcription factor family CBF (for C-repeat-binding factor) in *Arabidopsis* and other species (Thomashow 1999, 2001, 2010; Carvallo et al. 2011; Dong et al. 2011; Dahal et al. 2012; Lee and Thomashow 2012; see also Agarwal et al. 2006; Medina et al. 2011). These transcription factors induce a host of morphological, physiological, and biochemical leaf features associated with cold acclimation, including increased leaf thickness, lengthening of palisade cells, higher chlorophyll content, and greater maximal rates of photosynthesis (see, e.g., Pino et al. 2008; Dahal et al. 2012). The upregulation of leaf thickness has been discussed extensively in the context of increased freezing tolerance (see, e.g., Dahal et al. 2012). Leaf thickness is also greater in the Swedish versus the Italian ecotype under cool growth temperatures at intermediate PFDs and under moderate growth temperatures at very high growth PFDs in growth chambers (our unpublished data). The present study explores the implications of changes in internal light environment for acclimation to the external (growth) light environment more fully. The findings of the present study show that cool temperature is not needed as a trigger for the greater response of the Swedish ecotype in leaf thickness and P_{max} .

While acclimation to high-light levels typically involves thicker leaves (Givnish 1988; Amiard et al. 2005; Terashima et al. 2006), shade acclimation often involves larger leaf areas (Givnish 1988; Niinemets and Tenhunen 1997; Evans and Poorter 2001). These findings suggest that, in thicker leaves, chloroplasts (and chlorophyll) are packed vertically, which presumably requires high-light intensities for light saturation of photosynthesis of self-shaded chloroplasts in the lower portion of the leaf, whereas in larger leaves chloroplasts (and chlorophyll) can be displayed horizontally thus minimizing self-shading.

Overall, it appears that the Swedish ecotype is adapted to upregulate photosynthesis in high-light environments more substantially than the Italian ecotype by virtue of having a propensity to form thicker leaves. However, our comparison of the response of the two ecotypes to full sunlight (in a naturally lit greenhouse) versus just a few added high-light

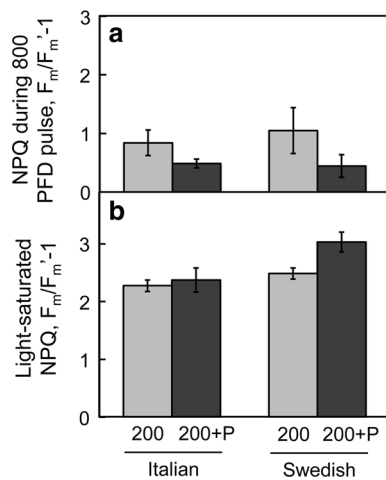


Fig. 6 NPQ determined after 5 min exposure to 800 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ under ambient CO_2 conditions (a) and light-saturated (under 2425 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) maximal NPQ capacity (b) (in 2 % O_2 , balance N_2) from leaves of the Italian and Swedish ecotypes of *A. thaliana* grown under 200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ in the absence (200; light gray symbols) or presence (200 + P; dark gray symbols) of six periods of higher light (800 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) of 5 min duration each interspersed evenly during the 9 h photoperiod. Mean values \pm SD

periods against the background of low non-fluctuating growth PFD shows that the Swedish ecotype exhibits a greater upregulation of P_{max} than the Italian ecotype even when the additional light availability is not enough to result in greater leaf thickness. This finding suggests a greater propensity of the Swedish ecotype for both anatomical and biochemical adjustments associated with a high P_{max} . We speculate that one of the mechanistic features involved in this difference between the Swedish and Italian ecotypes could be the recently reported mutation in one of the members of the CBF family of transcription factors, CBF2, in the Italian ecotype (Oakley et al. 2014).

Conversely, the smaller rosette formed, and the lesser degree of aboveground biomass accumulation, by the Swedish versus the Italian ecotype in low growth-light environments (see also Cohu et al. 2014b) is consistent with a lesser shade tolerance of the Swedish ecotype. In response to growth PFD, plants typically increase their shoot-to-root ratio (ratio of aboveground to belowground biomass) in the shade, thus allocating more resources to light capture rather than soil resource acquisition (for a review, see Bloom et al. 1985). It is likely that both ecotypes follow this trend, but we cannot exclude differences in shoot-to-root ratio between the two ecotypes under common conditions. To further explore the overall performance of these ecotypes, both aboveground and belowground biomass accumulation would need to be assessed as well as seed production (for reproductive fitness).

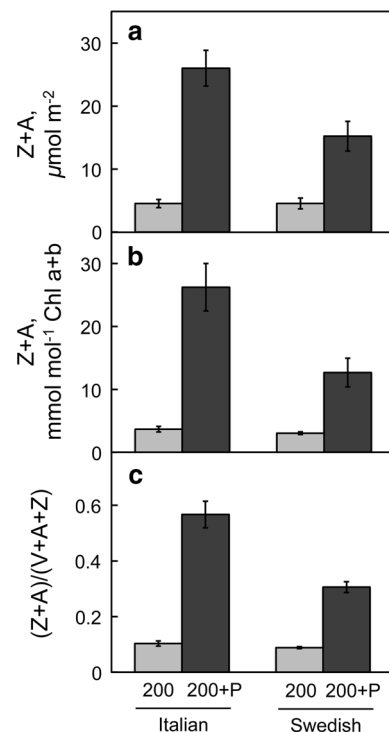


Fig. 7 Foliar levels of zeaxanthin and antheraxanthin (Z + A) on a leaf-area basis (a), a chlorophyll basis (b), and as a fraction of the xanthophyll cycle pool of violaxanthin + antheraxanthin + zeaxanthin (V + A + Z) (c) from the Italian and Swedish ecotypes of *A. thaliana* grown under 200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ in the absence (200; light gray symbols) or presence (200 + P; dark gray symbols) of six periods of higher light (800 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) of 5-min duration each interspersed evenly during the 9-h photoperiod. Leaf disks were taken at the end of the photoperiod. Mean values \pm SD

For an annual weedy species like *Arabidopsis*, growing naturally in sun-exposed sites in a cold climate like Sweden, and being adapted to strongly increase leaf thickness and maximal photosynthetic capacities in response to cool temperature and high-light levels, may allow the plant to take advantage of solar heating on cold days. Such solar insolation could facilitate the melting of snow, with the provision of liquid water permitting intermittent resumption of photosynthesis and carbon gain during relatively warmer periods in the winter (see Adams et al. 2001). Moreover, while flowering in the Italian ecotype starts in early spring (day 40 of the year) in its native habitat in Italy, flowering in the Swedish ecotype is shifted to early summer (day 120–130 of the year) in its native habitat in Sweden (Ågren and Schemske 2012). The natural photoperiod, and thus total daily light availability, is much greater before and during flowering of the Swedish (16.5 h) versus the Italian (10 h) ecotype in their respective native habitats. The greater P_{max} of the Swedish ecotype presumably not only allows this ecotype to take full advantage of this greater light availability, but, due to its thicker

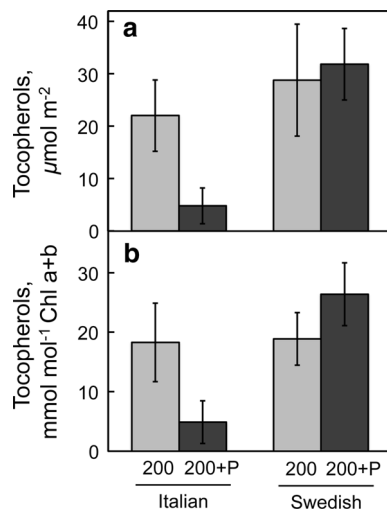


Fig. 8 Tocopherol levels (including α -, γ -, β -, and δ -tocopherols) on a leaf-area basis (a) or a chlorophyll basis (b) from the Italian and Swedish ecotypes of *A. thaliana* grown under $200 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ in the absence (200; light gray symbols) or presence (200 + P; dark gray symbols) of six periods of higher light ($800 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) of 5-min duration each interspersed evenly during the 9-h photoperiod. Leaf disks were taken at the end of the photoperiod. Mean values \pm SD

leaves, the Swedish ecotype actually *requires* higher light levels to penetrate into the self-shaded lower portions of the leaf.

Importance of the reference basis for expressing photosynthesis

Our study illustrates the importance of reference basis (e.g., leaf area versus chlorophyll basis) to capture the response of photosynthesis and other parameters. While both photosynthesis rates and chlorophyll levels are higher on a leaf-area basis in thicker versus thinner leaves, expression of photosynthesis rates on a chlorophyll basis indicates that the two parameters (photosynthesis rate and chlorophyll content) change in proportion. Previous studies on the regulation of photosynthesis by sink demand of the whole plant (Krapp and Stitt 1995) similarly showed that sink demand concomitantly modulates *both* chlorophyll content [via gene regulation of chlorophyll (a + b)-binding proteins] and CO_2 fixation rates (via gene regulation of the small subunit of ribulose-bisphosphate carboxylase oxygenase, Rubisco). Furthermore, studies on CBF overexpression showed that, once again, chlorophyll levels, photosynthesis rates, and leaf thickness are modulated *in concert* (Savitch et al. 2005; Pino et al. 2008; see also Dahal et al. 2012). Future studies should address the involvement of sink regulation (and of the CBF transcription factor family) in the leaf morphological and

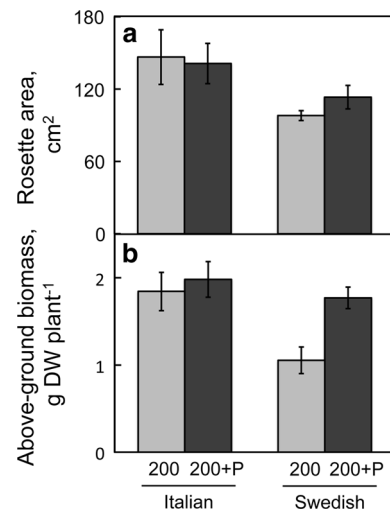


Fig. 9 Total light-exposed rosette area per plant (a) and total aboveground dry biomass (b) of the Italian and Swedish ecotypes of *A. thaliana* grown under $200 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ in the absence (200; light gray symbols) or presence (200 + P; dark gray symbols) of six periods of higher light ($800 \mu\text{mol photons m}^{-2} \text{s}^{-1}$) of 5-min duration each interspersed evenly during the 9-h photoperiod. Total light-exposed rosette area was quantified from photographs taken of the intact plants from directly overhead the day before the harvest. Mean values \pm SD

physiological differences between the two ecotypes characterized here. Our results highlight the utility of expressing photosynthesis on multiple reference bases, including a leaf-area basis in addition to a chlorophyll basis, to capture the full response. Hüner et al. (2012) presented a detailed discussion of the fact that cold acclimation involves “a system-wide change in morphology, physiology, and biochemistry of cold-tolerant crop plants,” and Dahal et al. (2012) pointed out that increased photosynthesis rates on a leaf-area basis, but not on a dry weight basis, in cold-tolerant crop varieties grown under cold temperatures indicate that cold acclimation increases the ratio of plant biomass, or plant energy content, to plant volume to “divert energy from vegetative growth to storage for successful overwintering.”

Our findings that the Swedish ecotype exhibits a stronger response of maximal light- and CO_2 -saturated photosynthesis on a leaf-area basis to a few daily periods of high light than the Italian ecotype (Table 2) in the absence of concomitant leaf thickness increases in either ecotype, show that both leaf-anatomical and chloroplast-based processes respond more strongly in the Swedish ecotype. This message can only be gleaned when expressing P_{max} on a leaf-area basis since P_{max} on a chlorophyll basis does not increase more strongly in response to daily periods of high-light exposure in the Swedish ecotype compared to the Italian ecotype (Table 2).

Consequences of leaf morphology for internal light environment and plant response to growth-light environment

The findings reported here illustrate that differences in leaf morphology affect light distribution within the leaf and apparently drive different responses of the two *Arabidopsis* ecotypes from contrasting latitudes to growth-light environment. The present report thereby adds to the understanding of the structure–function relationship of leaves and the role of geographic origin in influencing this structure–function relationship.

Growth in high-light environments favors higher photosynthesis rates on a leaf-area basis in the Swedish versus Italian ecotype, while growth under low light greatly diminishes this effect (leaves of both ecotypes were thinner with lower maximal photosynthetic capacities when grown under low PFD). The close relationship between leaf thickness and photosynthesis demonstrated in the present study was established using light- and CO₂-saturated photosynthesis (P_{\max}), thus eliminating effects of leaf architecture on CO₂ fluxes within the leaf that have been studied by others (cf. Terashima et al. 2006). It is attractive to assume that the greater P_{\max} on a leaf-area basis in the Swedish versus the Italian ecotype is due to the greater palisade thickness, allowing packaging of more chloroplasts and more chlorophyll into the leaf column associated with a given leaf area.

Furthermore, availability of a few daily periods of higher light against the background of low light—as often experienced in nature by plants growing underneath a forest canopy—is exploited to a greater extent by the Swedish compared to the Italian ecotype. The response of the thinner leaves of the Italian ecotype to six periods of higher light against otherwise non-fluctuating low light in the growth environment was to maintain the same rate of photosynthesis with less chlorophyll, rather than exhibiting net increases in photosynthesis. In contrast, the thicker leaves of the Swedish ecotype had an increased P_{\max} in response to the supplemental periods of high light in a low-light growth environment.

These findings suggest that sufficient light already reaches into the lower depths of the thinner leaves of the Italian ecotype in a low-light growth environment and that additional available light from daily supplemental periods of high light is not utilized for additional photosynthesis. In contrast, the thicker leaves of the Swedish ecotype are likely subject to significant self-shading of the lower leaf portions by the chlorophyll/chloroplasts residing in the upper half of the leaf, and it thus apparently takes at least intermittent exposure to higher incident light to trigger acclimatory engagement (presumably via photosynthetic upregulation) of the full depth of the thicker leaf in photosynthesis. Conversely, the

differences in Z + A and tocopherol levels between the Swedish versus the Italian ecotype suggest that daily periods of higher light against the background of low growth light lead to the experience of a greater level of excess light in the thinner leaves of the Italian ecotype versus the thicker leaves of the Swedish ecotype. This apparent greater level of excess light experienced was coupled with a greater engagement of photoprotection via Z + A and tocopherols and an exhaustion of the tocopherol pool in the Italian ecotype.

It is important to note that Z + A levels represent the average conversion of the VAZ pool to the excess-light forms (Z + A) for the entire depth of a leaf (including top leaf portions presumably exposed to similar levels of excess light in the two ecotypes and lower leaf portions presumably exposed to lesser levels of excess light in the thicker leaves of the Swedish ecotype). In contrast, thermal energy dissipation (NPQ) levels in the two ecotypes under growth conditions presumably represent predominantly the responses of the top leaf portions receiving similar levels of excess light in the two ecotypes.

Interplay between chloroplast modulators in the context of leaf architecture

The foliar redox-signaling network controls many aspects of plant growth and development (see, e.g., Demmig-Adams et al. 2014, and references cited therein). Hüner et al. (2012) have reviewed the synergism between light and cold temperature in modulating whole-plant phenotypic plasticity via redox-sensing networks. The chloroplast, through the balance between absorbed light and light utilized in photosynthesis, provides critical input into the cellular redox-signaling network (Li et al. 2009), with several chloroplast processes acting synergistically to maintain this balance between absorbed and photosynthetically utilized light. These balance-restoring processes include, e.g., zeaxanthin-associated thermal energy dissipation (Demmig-Adams et al. 2012) that counteracts the production of oxidants (reactive-oxygen species, or ROS) and antioxidants like tocopherols that detoxify ROS or membrane lipids oxidized by ROS. The xanthophyll zeaxanthin has a dual role in thermal dissipation and in antioxidation (Havaux and Niyogi 1999). The two main lipophilic antioxidants in biological membranes are zeaxanthin and tocopherols that act synergistically in protection against photosensitized lipid peroxidation mediated by singlet oxygen (Wrona et al. 2003; Havaux and García-Plazaola 2014); zeaxanthin, however, is formed only under excess light, while tocopherols are constitutively present.

The finding of greater levels of Z + A formation along with diurnal decreases in the tocopherol pool in the Italian ecotype suggests that more excess light penetrates into the lower depths of the leaf in this ecotype. Why does the leaf

of the Italian ecotype not lower its Chl level even more and/or convert even more V to Z + A to prevent such a decrease in tocopherol level? Decreased tocopherol levels at the end of the photoperiod and a lesser maximal NPQ capacity under experimental high-light exposure in the Italian ecotype suggest the possibility that this ecotype may be genetically programmed to allow a greater generation of redox-dependent signals, resulting from both less detoxification of ROS (via tocopherol) and less preemptive counteraction of ROS formation (via thermal dissipation). Future studies should explore the possibility that redox-dependent signaling networks provide input for genetic differences in leaf development in the Swedish versus the Italian ecotypes (Cohu et al. 2013a, b; Adams et al. 2014) and that a possible difference in the set-point of ROS formation (perhaps via differential expression of the NPQ-controlling PsbS protein, the protein S of photosystem II, required for rapidly reversible dissipation of excess absorbed light [Li et al. 2009] and/or genes in tocopherol biosynthesis) between these two ecotypes may be one of the mechanisms of ecotypic adaptation to the environment.

Multiple feedback loops thus apparently exist between photosynthetic light-harvesting and leaf morphology. Light absorption and the degree of utilization of this absorbed light inform cellular redox-signaling networks that help orchestrate leaf structural development, which, in turn, affects light distribution and the level of absorbed versus photosynthetically utilized light in a leaf.

Author contribution statement BDA and WWA conceived this comparative study of *Arabidopsis* ecotypes and wrote the manuscript, with input from all other authors. BDA, WWA, CMC, and JJS designed the experiments, with input from EML. CMC and JJS conducted all experiments and analyses, with assistance from SKP and EML.

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Compliance with ethical standards

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Conflict of interest The authors declare that they have no conflict of interest.

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