Thermal acclimation of the temperature dependence of the V_{Cmax} of Rubisco in quinoa

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Abstract

Changes in the temperature dependence of the maximum carboxylation capacity (V_{Cmax}) of Rubisco during thermal acclimation of P_N remain controversial. I tested for acclimation of the temperature dependence of V_{Cmax} in quinoa, wheat, and alfalfa. Plants were grown with day/night temperatures of 12/6, 20/14, and 28/22°C. Responses of P_N to substomatal CO_2 (C_i) and CO_2 at Rubisco (C_c) were measured at leaf temperatures of 10–30°C. V_{Cmax} was determined from the initial slope of the P_N *vs.* C_i or C_c curve. Slopes of linear regressions of $1/V_{Cmax}$ *vs.* 1/T [K] provided estimates the activation energy. In wheat and alfalfa the increases in activation energy with growth temperature calculated using C_i did not always occur when using C_c, indicating the importance of mesophyll conductance when estimating the activation energy. However, in quinoa, the mean activation energy approximately doubled between the lowest and highest growth temperatures, whether based on C_i or C_c.

Additional key words: carboxylation; mesophyll conductance; photosynthesis.

Introduction

Acclimation of photosynthesis (P_N) to temperature in higher plants has been extensively studied, and has primarily been related to changes in the maximum capacity of Rubisco (V_{Cmax}) per unit leaf area and to changes in the maximum capacity of photosynthetic electron transport (J_{max}) and/or the temperature dependence of J_{max} (*e.g.*, Bunce 2000, June *et al.* 2004, Onoda *et al.* 2005, Yamori *et al.* 2005, Hikosaka *et al.* 2006). Acclimation of P_N to growth temperature generally results in more similar rates of P_N under the various growth temperatures than expected from the short-term response to measurement temperature (Leuning 2002, Sage and Kubien 2007). This usually results at least partly from increases in V_{Cmax} at cooler growth temperatures. The temperature dependence of V_{Cmax} has often been considered to be quite uniform across species and growth conditions (*e.g.*, Farquhar *et al.* 1980, Bernacchi *et al.* 2003), although a slightly lower mean activation energy of V_{Cmax} has been found in C_3 species from cool compared to warm environments (Galmés *et al.* 2016). Deactivation of Rubisco at high temperatures occurs in some cases, and can confound the determination of activation energy (Kattge and Knorr 2007). However, Hikosaka *et al.* (2006) restricted their analysis of the literature to temperatures below those causing deactivation of Rubisco, and still found that lower growth temperatures often reduced the activation energy of V_{Cmax}. Most of the studies reviewed by Hikosaka *et al.* (2006) were for seasonal environmental changes, which involves both temperature and day length (Yamaguchi *et al.* 2016). Mesophyll conductance (g_m) to CO_2 movement from beneath the stomata to Rubisco may change strongly with temperature in species-specific patterns (von Caemmerer and Evans 2015), and could also affect the apparent temperature dependency of V_{Cmax}, if not accounted for. In this study, the analysis was restricted to temperatures below those resulting in deactivation of Rubisco, and g_m was measured at each temperature. I tested for the existence of thermal acclimation of P_N, and of the temperature dependence of the V_{Cmax} of Rubisco in three species, quinoa, alfalfa, and wheat, which grow well over a range of cool to moderate temperatures.

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Abbreviations: C_i – [CO_2] in the substomatal (intercellular) airspace; C_c – [CO_2] at Rubisco; g_m – mesophyll conductance to CO_2; J_{max} – the maximum rate of photosynthetic electron transport; K_{mCO2} – the Michaelis constant of Rubisco carboxylation; P_N – net photosynthetic rate; V_{Cmax} – the maximum rate of carboxylation of Rubisco; ΔH_a – activation energy.

Materials and methods

Quinoa (*Chenopodium quinoa* Willd.) cv. Cherry Vanilla, alfalfa (*Medicago sativa* L.) cv. Arc, and wheat (*Triticum aestivum* L.) cv. Jamestown were grown with day/night temperatures of 12/6, 20/14, and 28/22°C in indoor controlled environment chambers. Three sets of plants of each species were grown at each temperature, with the growth temperature treatments rotated among two growth chambers. Light at 1,000 μmol(photon) m^{-2} s^{-1} PPFD was provided 14 h per day, which provides a daily total photon flux similar to summer values in Beltsville. The CO_2 concentration in the chamber was controlled at 380 μmol mol^{-1} during the light, and 410 μmol mol^{-1} in the dark by addition of pure CO_2 or air scrubbed of CO_2. CO_2 concentrations were monitored with *WMA-4* infrared analyzers (*PP Systems*, Amesbury, MA, USA). The chamber dew point temperatures were 4°C below the night time temperatures for all growth regimes. Plants were grown in plastic pots filled with vermiculite and flushed daily with a complete nutrient solution containing 14.5 mM N. Leaf gas-exchange measurements were conducted on fully expanded main stem leaves within a few days after reaching maximum area. Fifth main stem leaves were measured in alfalfa and quinoa, and eighth leaves in wheat.

Leaf gas-exchange measurements of responses of P_N to CO_2 concentration were conducted in a darkened controlled environment chamber, whose temperature was set to match that of the measured leaf. The use of the darkened chamber prevented possible dehydration of plants grown at low temperatures when exposed to higher evaporative demand at high temperatures. Steady-state responses of P_N to five CO_2 concentrations from 50 to 800 μmol mol^{-1} were measured at PPFD = 2,000 μmol m^{-2} s^{-1} at leaf temperatures of 10, 15, 20, 25, and 30°C, using a *CIRAS-3* portable photosynthesis system (*PP Systems*, Amesbury MA, USA) with light, temperature, CO_2, and humidity control. The leaf to air water vapor pressure differences were between 0.8 and 1.8 kPa at all measurement temperatures. Mesophyll conductance (g_m) was estimated independently of this data, using different plants, and a different leaf gas exchange system. A *Li-Cor 6400* system (*Li-Cor, Inc*., Lincoln, NB, USA) was used to determine g_m from the oxygen sensitivity of photosynthesis (Bunce 2009) at substomatal CO_2 (C_i) values of about 150 μmol mol^{-1}, at a PPFD of 2,000 μmol m^{-2} s^{-1} at leaf temperatures of 10, 15, 20, 25, and 30°C for three leaves of each species at each growth and measurement temperature. Mean values of g_m for each species, measurement and growth temperature were used to calculate the CO_2 concentration at Rubisco (C_c) from P_N and C_i, using $C_c = (C_i - P_N)/g_m$. V_{Cmax} was then determined from the initial slope of the P_N *vs.* C_i or C_c for each leaf and measurement temperature, with V_{Cmax} calculated in two different ways: taking g_m into account (C_c), and alternatively, by assuming infinite g_m (C_i). Linear regressions of $1/V_{Cmax}$ *vs.* 1/T [K] provided estimates of the activation energy (ΔH_a) of V_{Cmax} (Medlyn *et al.* 2002, Kattge and Knorr 2007), and standard errors of regression. There were no cases in which measurements made at the highest temperature (30°C) deviated substantially from a linear regression developed from measurements at lower temperatures. Therefore deactivation of Rubisco at high measurement temperature did not occur in this data, and no deactivation term was used in estimating ΔH_a.

For each growth condition, ΔH_a was determined with and without g_m included, for three or four leaves of each species. Analysis of variance was used to test for effects of growth temperature on the ΔH_a of V_{Cmax} for each species, using V_{Cmax} estimated with or without g_m.

Results

Overall, thermal acclimation of P_N was evident in the shifts in the temperature response of P_N measured at C_i = 250 μmol mol^{-1}. In all of these cases, P_N was limited by V_{Cmax} rather than J_{max} at C_i below about 300 μmol mol^{-1}. In quinoa, all three growth temperatures produced different P_N *vs*. temperature curves (Fig. 1*A*). Acclimation to temperature in quinoa resulted in higher rates of P_N at the daytime growth temperature in plants acclimated to lower temperatures than would have occurred without acclimation (Fig. 1*A*). In wheat, the highest growth temperature resulted in lower P_N at low temperatures and higher P_N at high temperatures than the two lower growth temperatures (Fig. 1*B*). Alfalfa had low P_N at all temperatures when grown at the lowest temperature (Fig. 1*C*). As with wheat, in alfalfa the highest growth temperature reduced P_N at the lowest measurement temperatures and increased P_N at the highest measurement temperature (Fig. 1*C*).

The effect of measurement temperature on the initial slope of the P_N *vs.* C_i curves was in all cases less at lower growth temperatures than at the highest growth temperature, as illustrated for quinoa in Fig. 2 and for wheat in Fig. 3. The V_{Cmax} based in C_i at low measurement temperatures was higher in quinoa plants grown at 12/6°C than in plants grown at 28/22°C, but the reverse pattern occurred at high measurement temperatures (Fig. 4). These patterns indicate that the activation energy of V_{Cmax} based on C_i was in all cases highest at the highest growth temperature.

Mesophyll conductance varied substantially with measurement temperature in all of the species, in ways which depended on the growth temperature (Fig. 5). In general, low growth temperature reduced the increase in

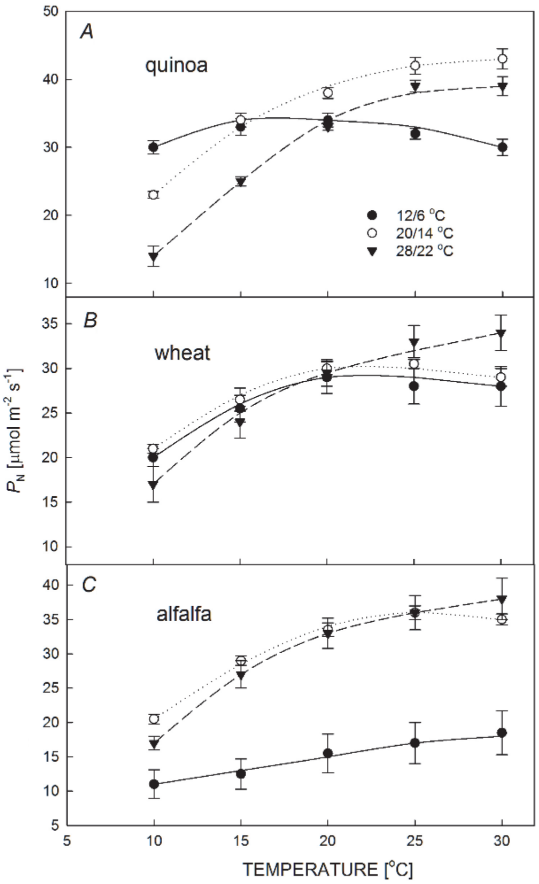

Fig. 1. The temperature responses of P_N measured at a photosynthetic photon flux density of 2,000 μmol m^{-2} s^{-1}, at a substomatal CO$_2$ concentration of 250 μmol mol^{-1} for quinoa (*A*), wheat (*B*), and alfalfa (*C*) grown at day/night temperatures of 12/6, 20/14, and 28/22°C. Error bars indicate standard errors for three or four leaves.

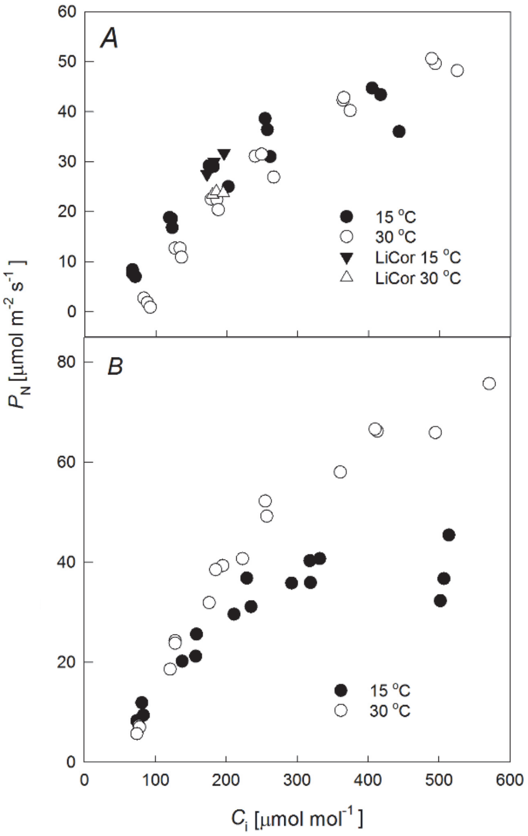

Fig. 2. Responses of P_N to substomatal CO$_2$ concentrations measured at 15 or 30°C, for quinoa grown at day/night temperatures of 12/6 (*A*) or 20/14°C (*B*). *Circles* represent values from the *Ciras-3* instrument, and *triangles* represent values obtained with the *LiCor* instrument on different plants obtained during the course of estimating mesophyll conductance.

g_m at the high measurement temperatures, which occurred in all of the species when grown at the highest temperature (Fig. 5). In the case of alfalfa and wheat grown at the lowest temperature, g_m was reduced at the high measurement temperatures.

The activation energy of V_{Cmax} increased with growth temperature over the whole range of temperatures in quinoa, whether V_{Cmax} was calculated using C_i or C_c (Fig. 6). In wheat, activation energies increased from the medium to the highest growth temperature whether calculated using C_i or C_c, but increased significantly at the highest growth temperature compared with the lowest temperature only when based on C_i, not when based on C_c (Fig. 6). In alfalfa, activation energy increased at the highest growth temperature when based on C_i, but not when calculated using C_c (Fig. 6).

Discussion

Although responses of P_N to C_i were obtained on different plants and by different instruments than those used to measure g_m, both sets of plants and instruments produced very similar rates of P_N at a given C_i, as illustrated for quinoa (Fig. 2*A*). This supports the validity of both sets of measurements.

By the usual criterion of changes in the value of P_N assayed at a common moderate temperature (*e.g.*, 20°C) after growth at different temperatures, acclimation of P_N to growth temperature did not occur in wheat (Fig. 1). In quinoa, the intermediate growth temperature produced leaves with the highest V_{Cmax}, suggesting that acclimation to the intermediate growth temperature occurred compared with the highest growth temperature, but that photosynthetic acclimation to the lowest growth temperature was unsuccessful, as in wheat (Fig. 1). That this is too narrow a criterion for the existence of acclimation of P_N to growth temperature is most clearly evident in the case of quinoa, where P_N at the lowest measurement temperature was increased by growth at the lowest temperatures (Fig. 1*A*), despite the reduction in V_{Cmax} assayed at 20°C. Higher P_N at the lowest measurement temperature in this

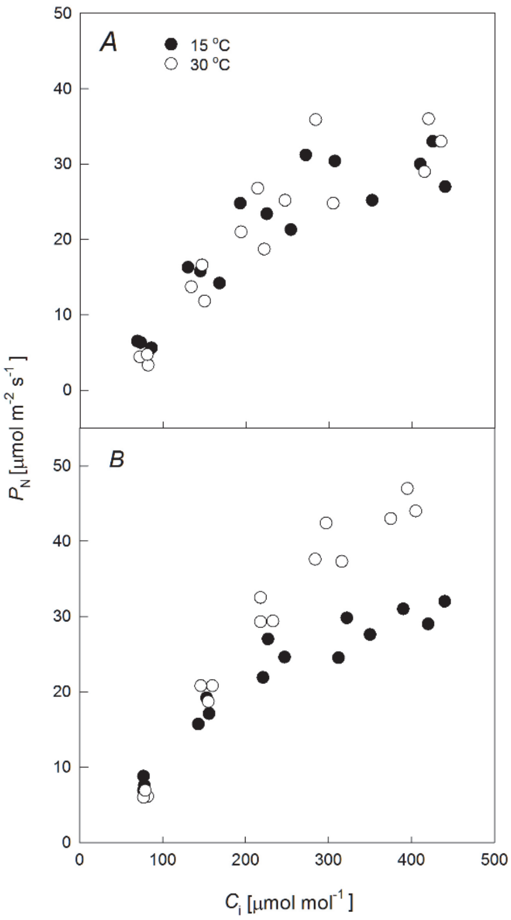

Fig. 3. Responses of P_N to substomatal CO_2 concentrations measured at 15 or 30°C, for wheat grown at day/night temperatures of 20/14 (*A*) or 28/22°C (*B*).

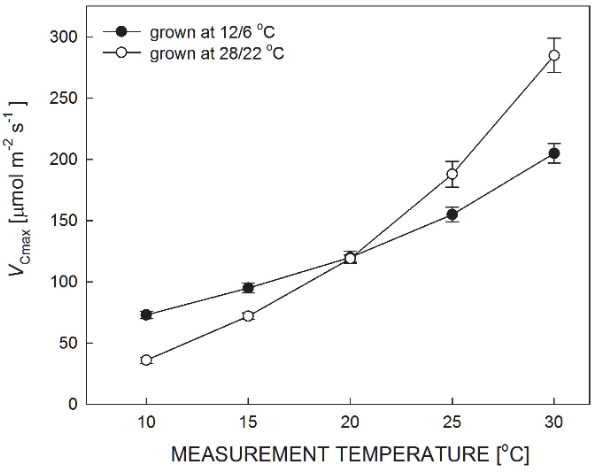

Fig. 4. Responses of the maximum carboxylation capacity of Rubisco (V_{Cmax}) to measurement temperature in quinoa grown with day/night temperatures of 12/6 or 28/22°C. V_{Cmax} was calculated assuming infinite mesophyll conductance. Error bars represent standard errors.

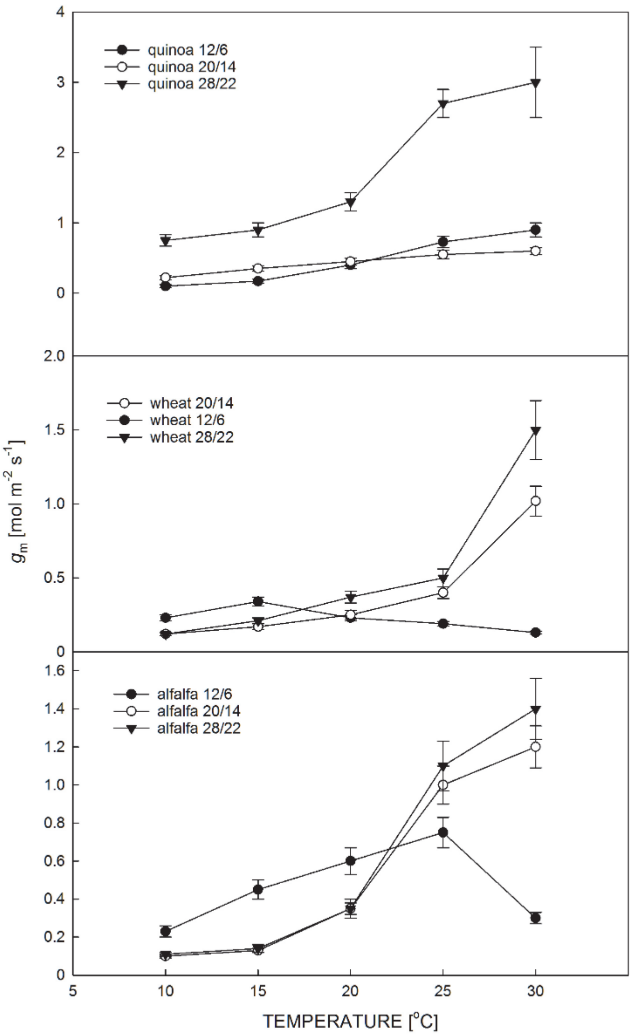

Fig. 5. Responses of mesophyll conductance (g_m) to measurement temperature for quinoa, wheat, and alfalfa grown at three temperatures.

case was clearly the result of the reduction in the activation energy of V_{Cmax} (Figs. 4, 5) during growth at low temperature.

In quinoa grown at the lowest temperature, P_N at C_i = 250 µmol mol^{-1} decreased at measurement temperatures above 20°C (Fig 1*A*). This also occurred, to a smaller extent, for wheat grown at the lowest two growth temperatures (Fig. 1*B*). In these cases, the reductions in P_N at high temperature were attributable to their low values of the ΔH_a of V_{Cmax}, rather than the more usual cause of limitation by J_{max} at high temperatures. For these growth conditions and species, the low ΔH_a of V_{Cmax} apparently result in a smaller increase in V_{Cmax} with temperature than the increase in the Michaelis constant of Rubisco carboxylation (K_{mCO2}), since J_{max} was found to be not limiting below C_i = 300 µmol mol^{-1}. Busch and Sage (2017) also found that V_{Cmax} rather than J_{max} limited P_N at high

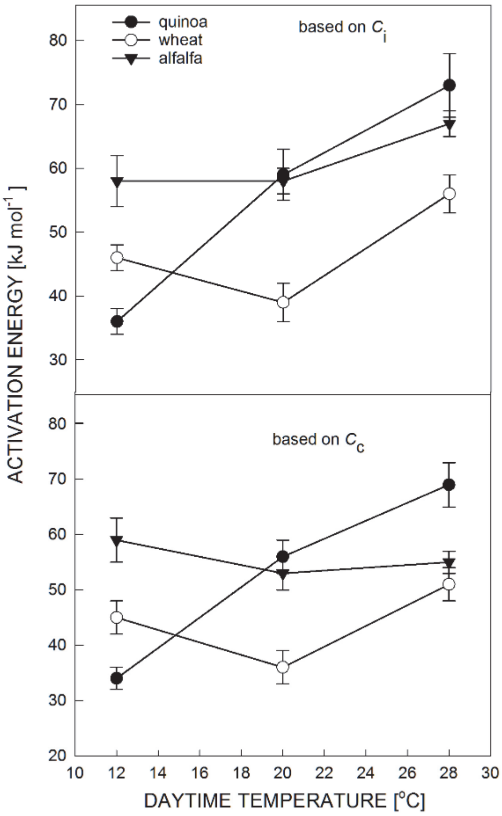

Fig. 6. The activation energy of the maximum carboxylation capacity of Rubisco (V_{Cmax}) of Rubisco for quinoa, wheat, and alfalfa grown at three temperatures. Activation energies were based either on P_N *vs*. substomatal CO_2 concentration (C_i) or P_N *vs*. CO_2 at Rubisco (C_c) response curves. Error bars represent standard errors of regression for determination of activation energies. *See* the text for details.

temperatures in sweet potato. A low ΔH_a of V_{Cmax} would result in a decrease in P_N at high temperatures despite P_N being still limited by V_{Cmax}. Although the ΔH_a of the K_{mCO2} was not measured here, it averages about 44 kJ mol^{-1} in C_3 Spermatophyta (Galmés *et al*. 2016). It would be interesting to know whether other cases of low optimum temperature of P_N in air are caused by low values of ΔH_a of V_{Cmax} compared with the ΔH_a of K_{mCO2}.

For the quinoa and wheat, thermal acclimation of the temperature dependence of V_{Cmax} was evident whether based on P_N *vs*. C_i or P_N *vs*. C_c curves. However, in alfalfa and wheat different conclusions would be reached depending on whether C_i or C_c was used to estimate the ΔH_a of V_{Cmax}. Other species have shown similar responses of g_m to temperature as found here, ranging from large increases with temperature to having a maximum at an intermediate temperature (von Caemmerer and Evans 2015). Sensitivity of the method of estimating g_m from the O_2 sensitivity of P_N to uncertainty or variation in Michaelis constants of Rubisco for CO_2 and O_2, the CO_2 compensation point and mitochondrial respiration are not large (Bunce 2009). Significant intraspecific variation in g_m has been documented in several species, including wheat (Barbour *et al*. 2016), so conclusions about thermal acclimation of the ΔH_a of V_{Cmax} cannot be considered reliable unless g_m has been measured in the same plant material. Variation in g_m with C_i has sometimes been reported, but remains controversial. The C_i values used in the estimate of g_m in this report were in the middle of the C_i range used to determine V_{Cmax}, which would minimize the impact of any possible variation in g_m with C_i on these estimates of V_{Cmax}.

The pattern of lower activation energy of V_{Cmax} based on C_c after acclimation to a low growth temperature found here in quinoa also occurred in collard (Bunce 2008), another cool-adapted species, so is certainly not unique to quinoa. However, seasonal changes in activation energy (based on C_i) observed in older, field-grown alfalfa plants of the same cultivar (Bunce 2007) were quite different from those found here where growth temperature was the only variable. It is interesting that in oak leaves, seasonal changes in the activation energy of V_{Cmax} (based on C_i) were poorly correlated with changes in the optimum temperature of P_N (Yamaguchi *et al*. 2016, Fig. 4). Clearly, changes in the activation energy of V_{Cmax} with growth temperature, when they occur, are only one aspect of overall temperature acclimation of P_N.

The molecular/biochemical basis of variation in the ΔH_a of the V_{Cmax} of Rubisco with growth temperature is not yet known, but may possibly relate to changes in *rbcS* gene expression (Cavanagh and Kubien 2014). Quinoa may be a useful species in which to study such relationships, because of its large capacity for the thermal acclimation of the temperature dependence of the V_{Cmax} of Rubisco.

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