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A compendium of temperature responses of Rubisco kinetic traits: variability among and within photosynthetic groups and impacts on photosynthesis modeling

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Abstract

The present study provides a synthesis of the *in vitro* and *in vivo* temperature responses of Rubisco Michaelis–Menten constants for CO_2 (K_c) and O_2 (K_o), specificity factor ($S_{c,o}$) and maximum carboxylase turnover rate (k_{cat}^c) for 49 species from all the main photosynthetic kingdoms of life. Novel correction routines were developed for *in vitro* data to remove the effects of study-to-study differences in Rubisco assays. The compilation revealed differences in the energy of activation (ΔH_a) of Rubisco kinetics between higher plants and other photosynthetic groups, although photosynthetic bacteria and algae were under-represented and very few species have been investigated so far. Within plants, the variation in Rubisco temperature responses was related to species' climate and photosynthetic mechanism, with differences in ΔH_a for k_{cat}^c among C_3 plants from cool and warm environments, and in ΔH_a for k_{cat}^c and K_c among C_3 and C_4 plants. A negative correlation was observed among ΔH_a for $S_{c/o}$ and species' growth temperature for all data pooled, supporting the convergent adjustment of the temperature sensitivity of Rubisco kinetics to species' thermal history. Simulations of the influence of varying temperature dependences of Rubisco kinetics on Rubisco-limited photosynthesis suggested improved photosynthetic performance of C_3 plants from cool habitats at lower temperatures, and C_3 plants from warm habitats at higher temperatures, especially at higher CO_2 concentration. Thus, variation in Rubisco kinetics for different groups of photosynthetic organisms might need consideration to improve prediction of photosynthesis in future climates. Comparisons between *in vitro* and *in vivo* data revealed common trends, but also highlighted a large variability among both types of Rubisco kinetics currently used to simulate photosynthesis, emphasizing the need for more experimental work to fill in the gaps in Rubisco datasets and improve scaling from enzyme kinetics to realized photosynthesis.

Keywords: Activation energy, adaptation, carboxylation, meta-analysis, photosynthesis, temperature dependences.

Introduction

According to the Farquhar, von Caemmerer and Berry (1980) model of C_3 photosynthesis (FvCB model; Farquhar *et al.*, 1980; von Caemmerer, 2000), under physiologically relevant conditions, CO_2 fixation rates are limited by the carboxylation of ribulose-1,5-bisphosphate (RuBP). RuBP carboxylation in turn is either limited by the regeneration of RuBP (typically

by the rate of photosynthetic electron transport) or by the activity of the carboxylating enzyme, RuBP carboxylase/oxygenase (Rubisco). The limitations imposed by Rubisco result from its notorious catalytic inefficiencies, including slow catalysis and imperfect discrimination between CO_2 and O_2 (Whitney *et al.*, 2011). Due to these inefficiencies, plants need to accumulate high amounts of Rubisco, and lose significant amounts of previously fixed CO_2 and NH_3 in the process of photorespiration (Keys, 1986). Indeed, a slow rate of catalysis and competitive inhibition by O_2 not only limit the rate of CO_2 fixation, but also compromise the capacity of photosynthetic organisms for optimal use of water and nitrogen, the key limiting resources. Not surprisingly, Rubisco has been listed among the most obvious targets to improve the photosynthetic capacity of crops (Long *et al.*, 2006; Galmés *et al.*, 2014*a*). Theoretical estimations indicate that reducing these Rubisco inefficiencies could deliver increases in net photosynthesis of up to 60% in the mid-term (Murchie *et al.*, 2008; Zhu *et al.*, 2010).

The quantitative impacts of Rubisco inefficiencies depend on the environmental conditions during catalysis. For instance, under drought stress conditions, RuBP oxygenation is favored over carboxylation because of the lower concentration of CO_2 ($[CO_2]$) at the active sites of Rubisco due to reduced rate of CO_2 diffusion through stomata and leaf mesophyll (Chaves *et al.*, 2009; Cornic and Massacci, 2004; Flexas *et al.*, 2004; Niinemets and Keenan, 2014). Furthermore, at any given $[CO_2]$, Rubisco catalysis is also strongly affected by temperature. In particular, the maximum carboxylase turnover rate (k_{cat}^c) of Rubisco increases up to 50–55 ºC or even higher for some organisms from extreme environments (Galmés *et al.*, 2015 for a review), while the Rubisco specificity factor ($S_{c/o}$) decreases and the Michaelis–Menten constants for CO_2 (K_c) and O_2 (K_o) increase (Bernacchi *et al.*, 2001). Although the basic patterns of temperature-dependent variation in key Rubisco kinetic characteristics are well known and measured in a number of studies, there is surprisingly limited comparative information of the variability of temperature responses of Rubisco across different photosynthetic groups that evolved at different periods of time, as well as among photosynthetic organisms adapted to different environmental conditions.

Considerable variation exists in the catalytic properties of Rubisco among distant phylogenetic groups with different Rubisco types (Jordan and Ogren, 1981; Keys, 1986; Raven, 2000; Savir *et al.*, 2010; Whitney *et al.*, 2011), but also within closely related taxa (Galmés *et al.*, 2005, 2014*b*, *c*; Kubien *et al.*, 2008; Ishikawa *et al.*, 2009). Several lines of evidence suggest that the most likely factor shaping the specialization in Rubisco kinetics among higher plants is the availability of CO_2 at the active sites of the enzyme in the chloroplastic stroma (Delgado *et al.*, 1995; Raven, 2000; Young *et al.*, 2012; Galmés *et al.*, 2014*b*, *c*). Importantly, optimization of Rubisco kinetic traits to the prevailing $[CO_2]$ has inevitably to deal with the trade-off between Rubisco affinity for CO_2 and enzyme turnover rate (Badger and Andrews, 1987; Tcherkez *et al.*, 2006; Savir *et al.*, 2010). Thus, evolution in Rubisco catalytic properties in species with the C_4 carbon concentration mechanism has led to increased k_{cat}^c and

K_c (Ghannoum *et al.*, 2005; Kubien *et al.*, 2008). In contrast, C_3 species with lower $[CO_2]$ at the carboxylation site, especially species from dry, warm and saline habitats, are characterized by lower K_c and k_{cat}^c (Galmés *et al.*, 2005, 2014*c*).

Similar to the $[CO_2]$-driven evolution of Rubisco, other evidence suggests that the evolution of the enzyme's catalytic traits has also been driven by the prevailing growth temperature (Sage, 2002; Galmés *et al.*, 2005; Tcherkez *et al.*, 2006; Yamori *et al.*, 2006; Cavanagh and Kubien, 2013). A broad compilation of the temperature dependences of k_{cat}^c confirmed the existence of a notable natural variation in Rubisco thermal tolerance (Galmés *et al.*, 2015). More importantly, within the land plants, the energy of activation of k_{cat}^c was positively correlated with the species' thermal environment. A recent study further provided evidence that the evolutionary adjustment in the temperature sensitivity of Rubisco kinetic properties differed between C_3 and C_4 species of *Flaveria* (Perdomo *et al.*, 2015).

From a practical perspective, the accuracy of the FvCB model in simulating temperature responses of leaf photosynthesis for any given species requires information on the species-specific temperature dependences of the Rubisco catalytic constants, in particular, K_c, K_o, $S_{c/o}$ (or the photosynthetic CO_2 compensation point in the absence of mitochondrial respiration, Γ^*) and k_{cat}^c (von Caemmerer, 2000; Bernacchi *et al.*, 2001; Walker *et al.*, 2013). Indeed, recent modeling indicates that the temperature dependence of Rubisco kinetics dictates the optimum temperature for the photosynthetic rate (Galmés *et al.*, 2014*a*). So far, application of the FvCB model to leaf photosynthesis has used three main datasets of temperature dependences of Rubisco kinetics: Badger and Collatz (1977) for *Atriplex glabriuscula* (*in vitro* measurements, used also in the original FvCB model), Jordan and Ogren (1984) for *Spinacia oleracea* (*in vitro* measurements) and Bernacchi *et al.* (2001) for *Nicotiana tabacum* (determined from *in vivo* leaf gas-exchange measurements in transgenic lines with reduced Rubisco content where photosynthesis was assumed to be limited by Rubisco under all $[CO_2]$ and leaf temperature combinations). These three datasets are widely used in modeling photosynthesis of species, plant stands, landscapes and biomes, whereas the use of any one of the three datasets mainly reflects the historical roots of the given modeling community (e.g. Niinemets *et al.*, 2009*a*, *b*; Keenan *et al.*, 2010; Galmés *et al.*, 2011*a*; Bermúdez *et al.*, 2012; Bernacchi *et al.*, 2013; Bagley *et al.*, 2015; see also Niinemets *et al.*, 2015 for an analysis of the frequency of use of different Rubisco datasets across studies). Implicit in the use of a single species' temperature response of Rubisco kinetics is that the variability among these responses is small across species spanning biomes with extensive variations in temperature and water availability. However, already comparisons of the *in vitro A. glabriuscula* and *S. oleracea* data and *N. tabacum in vivo* data have indicated that the variability is profound (Bernacchi *et al.*, 2001). More recently, Walker *et al.* (2013) compared *in vivo* temperature dependences of Rubisco catalytic constants between *Arabidopsis thaliana* and *N. tabacum* and demonstrated that species-dependent differences in Rubisco kinetics do alter simulations of leaf photosynthesis.

Overall, the need for accurate estimations of the temperature dependences of Rubisco kinetic parameters has become apparent as mathematical modelers try to predict the impact of increasing global temperatures on plant productivity (Sage and Kubien, 2007; Gornall *et al.*, 2010). As the natural variation in temperature responses of k_{cat}^c has been analysed in a recent compilation (see above, Galmés *et al.*, 2015), temperature responses of k_{cat}^c can be included in models separately for different species groups from warm and cold habitats, but no such synthetic analysis exists for temperature responses of other key Rubisco characteristics, K_c, K_o and $S_{c/o}$. Construction of such integrated datasets has been difficult due to limited *in vivo* data and multiple complications with *in vitro* measurements. Among such complications for *in vitro* studies are study-to-study differences in the assay medium composition and in the values of physico-chemical characteristics used in the estimation of the concentrations of CO_2 and O_2 in the assay medium (e.g. Yokota and Kitaoka, 1985 for highlighting the problem). The present work aims to fill this gap and to provide a comprehensive analysis of the available temperature responses of Rubisco kinetics. The specific objectives of our analysis were: (i) to compile all available temperature responses of Rubisco, and to normalize the temperature parameters of different species to standard conditions for comparative purposes, (ii) to examine differences in the temperature parameters for the Rubisco kinetics from different species, (iii) to determine whether these differences are related to the phylogeny and/or the ecology of the species, (iv) to compare the temperature parameters of Rubisco kinetics derived from *in vivo* and *in vitro* measurements, and (v) to quantify the impact of these differences on model estimates of Rubisco-limited photosynthesis.

Methods

Data on the *in vitro* and *in vivo* Rubisco kinetic parameters, specificity factor ($S_{c/o}$) and Michaelis–Menten constants for CO_2 (K_c) and O_2 (K_o) at varying temperature were compiled from peer-reviewed literature identified by Thompson-Reuters ISI Web of Science (Philadelphia, PA, USA).

In vitro *data compilation*

The *in vitro* database consisted of the following information: article bibliographic data, species name, cultivar name for agricultural plants and strain name for bacteria (where reported), measurement temperature (T) and pH, the ionic composition of the assay buffer, headspace gas composition and, wherever available, the ionic strength of the assay solution and the acidity constant of dissolved CO_2 (pK_{a,CO_2}) used to estimate the CO_2 concentration in solution ($C_{CO_2,liq}$) from added bicarbonate concentration at given solution pH. Wherever relevant data were missing, article authors were contacted and obtained information was included in the database (see the Acknowledgements section).

The information about the assay buffer composition was needed to correct for study-to-study differences in the solution CO_2 and O_2 concentrations. In particular, the key issue with *in vitro* data is that the solubility of gases and the equilibrium coefficients of bicarbonate, which is commonly used as the source for CO_2, depend on solution temperature and composition (Yokota and Kitaoka, 1985). While early studies have often used the O_2, CO_2 and bicarbonate equilibrium characteristics for pure water, it was later realized that pK_{a,CO_2}

depends on solution ionic strength (Yokota and Kitaoka, 1985) with major implications for estimates of K_c. However, solution composition also affects the solubility of O_2 and thereby the estimation of K_o, and both differences in bicarbonate equilibrium and O_2 solubility affect estimation of $S_{c/o}$. Furthermore, equilibrium constants are needed to convert between gas- and liquid-phase equivalent values of K_c, K_o and $S_{c/o}$. This means that major differences among the estimates of these characteristics across studies can simply result from differences in the equilibrium constants used. Although the general importance of physico-chemical characteristics in Rubisco assays is well understood by the Rubisco research community, studies continue using different estimates of physico-chemical characteristics. In this study, the information on assay buffer characteristics was employed to correct for differences in Rubisco characteristics resulting from varying equilibrium coefficients used.

Across all analysed literature reporting *in vitro* data, the temperature response of $S_{c/o}$ was obtained from 12 different studies: Badger and Collatz (1977), Jordan and Ogren (1984), Lehnherr *et al.* (1985), Uemura *et al.* (1997), Zhu *et al.* (1998), Galmés *et al.* (2005), Haslam *et al.* (2005), Yamori *et al.* (2006), Gubernator *et al.* (2008), Boyd *et al.* (2015), Perdomo *et al.* (2015) and Hermida-Carrera *et al.* (2016). These studies provided estimates for 38 species (n=1 for Proteobacteria and Rhodophyta; n=2 for Cyanobacteria; n=4 for Bacillariophyta; n=30 for Spermatophyta).

The temperature response of *in vitro* K_c was obtained from the following studies: Laing *et al.* (1974), Badger and Collatz (1977), Badger (1980), Jordan and Ogren (1984), Lehnherr *et al.* (1985), Castrillo (1995), Wei *et al.* (1994), Boyd *et al.* (2015), Perdomo *et al.* (2015), Young *et al.* (2015) and Hermida-Carrera *et al.* (2016) providing information for 21 species (Cyanobacteria, n=1; Bacillariophyta, n=2; Spermatophyta, n=18). Finally, data on the temperature response of *in vitro* K_o for five species (all Spermatophyta) were obtained from Laing *et al.* (1974), Badger and Collatz (1977), Jordan and Ogren (1984), Lehnherr *et al.* (1985) and Boyd *et al.* (2015).

The specific temperatures at which the Rubisco kinetic parameters were measured differed among the original studies, but compiled studies reported measurements for at least three different temperatures, except the data from Jordan and Ogren (1984) for *Rhodospirillum rubrum*, and Young *et al.* (2015) for *Thalassiosira weissflogii* and *Fragilariopsis cylindrus*, with two assayed temperatures.

Initially, four additional datasets were incorporated in the database, but were ultimately not used in the analyses due to following reasons. In the case of *Triticum aestivum*, the data of Hall and Keys (1983) for the temperature response of $S_{c/o}$ and Mächler and Nösberger (1980) for the temperature response of K_c presented a large scatter. Analogously, K_c temperature response for *Agropyron smithii* in Monson *et al.* (1982) had a large scatter and evidence of non-monotonic temperature response (r of only 0.83 for the linear regression between measured and predicted values compared with r>0.95 for all other *in vitro* K_c data) and was therefore not included in comparison of K_c temperature responses. However, it was used in the comparison between *in vitro* and *in vivo* data to highlight the potential uncertainties among different types of data. The $S_{c/o}$ for *Thermococcus kodakariensis* of Ezaki *et al.* (1999) increased with increasing the measurement temperature contrary to all other data, and these data were therefore deemed unreliable.

The temperature responses of k_{cat}^c from Galmés *et al.* (2015) for 49 species (Archaea, n=1; Cyanobacteria, n=3; Proteobacteria, n=4; Rhodophyta, n=1; Chlorophyta, n=4; Spermatophyta, n=36) were also included for an integrated analysis of the relationships between the temperature dependence of the different kinetic parameters of Rubisco.

Correction of in vitro *data for differences in the equilibrium coefficients used*

In the case of K_c, the buffer composition-corrected value ($K_{c,c}$) and measured ($K_{c,m}$) value depend on the solution pH and used ($pK_{a,u}$) and buffer composition-corrected ($pK_{a,c}$) acidity constants of

dissolved CO_2. For the typical pH ranges used in Rubisco assays, the undissociated carbonic acid concentration is negligible ($<10^{-10}$ M), and $pK_{a,CO_2} = -\log([H^+][HCO_3^-]/C_{CO_2,liq})$, where $[H^+]$ is the hydrogen ion concentration and $[HCO_3^-]$ the bicarbonate concentration. Thus, $K_{c,c}$ is given as:

$$K_{c,c} = K_{c,m} \frac{\left(1 - \dfrac{10^{pH-pK_{a,c}}}{1+10^{pH-pK_{a,c}}}\right)}{\left(1 - \dfrac{10^{pH-pK_{a,u}}}{1+10^{pH-pK_{a,u}}}\right)} \qquad (1)$$

Yokota and Kitaoka (1985) have proposed an equation to estimate $pK_{a,c}$ values based on the solution ionic strength and temperature. However, their equation was based on only one study (Harned and Bonner, 1945), and included only three parameters such that it was accurate only over the temperature range of 10–35 °C (Yokota and Kitaoka, 1985). Because $K_{c,c}$ depends highly non-linearly on $pK_{a,c}$ (Eq. 1), we considered it essential to improve estimation of $pK_{a,c}$. Thus, we conducted a meta-analysis of published $pK_{a,c}$ values reported for different solution temperatures (0–50 °C) and ionic strengths (0–1.042 M). Altogether, 105 estimates of $pK_{a,c}$ were obtained (Shedlovsky and MacInnes, 1935; Harned and Davis, 1943; Harned and Bonner, 1945; Pocker and Bjorkquist, 1977; Schumacher and Smucker, 1983; Yokota and Kitaoka, 1985). To convert the molal concentrations reported in some studies, including Harned and Bonner (1945), to the corresponding molar concentrations, water density at different solute concentrations and temperatures was estimated using a polynomial equation based on data of Weast (1974).

Consistent with the Debye–Hückel theory of non-ideality of solutions, and as common in analytical chemistry studies fitting pK_a values of different buffer substances (e.g. Bates *et al.*, 1973; Roy *et al.*, 1998; Roy *et al.*, 2009), we have used a series of log and polynomial terms to describe the dependence of $pK_{a,c}$ on absolute temperature (T_k, K) and solution ionic strength (I_s, M) as:

$$\begin{aligned} pK_{a,c} &= \frac{a_1}{T_k} + a_2 \log(T_k) + a_3 T_k + a_4 + s_1 I_s \\ &\quad + s_2 \sqrt{I_s} + s_3 I_s^2 + s_4 I_s^3, \end{aligned} \qquad (2)$$

where the values of the empirical coefficients are: $a_1=16400$, $a_2=211.56$, $a_3=-0.1291$, $a_4=-533.63$, $s_1=0.3252$, $s_2=0.3830$, $s_3=-0.2692$, $s_4=-0.8503$. Equation 2 provided an excellent fit to the data ($r^2=0.9985$, mean squared error of 4.8×10^{-5}), i.e. a considerable improvement compared with the equation of Yokota and Kitaoka (1985) ($r^2=0.981$ for the T_k and I_s range over which the equation was valid).

The ionic strength of the assay buffer was calculated considering all the ionic species in the solution. For weak acids and bases, including buffer substances, their pK_a values were used to estimate the concentration of the ionic species in the solution. Again, multiple regression equations similar to Eq. 2 were developed for individual compounds to consider pK_a dependences on T_k and I_s based on published data (e.g. Bates *et al.*, 1973; Feng *et al.*, 1989; Roy *et al.*, 2006, 2009, 2011). However, in the case of zwitterionic buffers, e.g. for HEPES (Vega and Bates, 1976; Feng *et al.*, 1989; Roy *et al.*, 2009) and Bicine (Datta *et al.*, 1964; Azab *et al.*, 1994; Roy *et al.*, 2006), the effect of I_s was not always important (but see Bates and Hetzer, 1961; Durst and Staples, 1972; Bates and Robinson, 1973; Ramette *et al.*, 1977 for the I_s dependence of Tris). Given the pK_a dependence on I_s and the I_s dependence on pK_a, I_s and pK_a for the given buffer solution were ultimately calculated iteratively.

In the case of K_o estimations, the gas-phase oxygen concentration ($C_{O_2,gas}$, mol mol^{-1}) was typically varied to achieve variation in the liquid-phase oxygen concentration ($C_{CO_2,liq}$, mol m^{-3}). Thus, the key issue is how $C_{O_2,gas}$ has been converted in $C_{CO_2,liq}$. The concentrations in different phases are related through the Henry's law constant (H_{pc,O_2}, Pa m^3 mol^{-1}) as:

$$H_{pc,O_2} = \frac{C_{O_2,gas}}{PC_{O_2,liq}}, \qquad (3)$$

where P (Pa) is the air pressure. We note that the word 'constant' is misleading, because H_{pc} for the given compound depends on temperature and other solutes that can affect the solubility of the compound of interest (Sander, 2001; Staudinger and Roberts, 2001; Copolovici and Niinemets, 2007). Dependence of H_{pc} on the presence of solutes has been largely ignored by the Rubisco community. Here, we use different subscripts to clearly denote these effects.

Several different conversion factors taken from physical chemical reference sources had been used across the studies (data not shown), but all of these factors were based on pure water. However, oxygen solubility is importantly driven by the solute concentrations (Tromans, 2000; Gnaiger, 2001; Millero and Huang, 2003). The overall solubility in complex media such as biological assay buffers is difficult to predict due to partly non-additive effects of different solutes (e.g. Gros *et al.*, 1999). Thus, we have employed a simplified approach, and linked H_{pc} to total concentration of ions in solution. First, the value of H_{pc} for pure water, $H_{pc,0}$, was described in dependence on temperature using a polynomial equation in the form:

$$H_{pc,0} = c_1 T_k^3 + c_2 T_k^2 + c_3 T_k^3 + c_4, \qquad (4)$$

where the empirical coefficients have values $c_1=0.051816$, $c_2=-42.437$, $c_3=12977.3$, $c_4=-1388072.1$, which were derived from Millero and Huang (2003) and Millero *et al.* (2003). The value of H_{pc} corresponding to different solute concentrations, C_s ($H_{pc,s}$) was further described as:

$$H_{pc,s} = \frac{H_{pc,0}}{\exp\left[\left(d_1 + \dfrac{d_2}{T_k} + \dfrac{d_3}{\ln T_k}\right)C_s + \left(d_4 + \dfrac{d_5}{T_k} + d_6 \ln T_k\right)C_s^2\right]}, \qquad (5)$$

where d_1–d_6 are empirical coefficients that we initially derived for different electrolytes. As the differences among electrolytes were small and due to difficulties with the non-additivity mentioned above, in this analysis, we used the empirical coefficients $d_1=1.4565$, $d_2=-178.90$, $d_3=-6.0556$, $d_4=-0.7818$, $d_5=54.240$, $d_6=0.10796$ derived for KCl ($r^2=0.9983$ for the complete fit including both the temperature effects on $H_{pc,0,CO_2}$ described by Eq. 4 and the denominator) based on the data of Millero and colleagues (Millero *et al.*, 2002, 2003; Millero and Huang, 2003). The implication of Eq. 5 is that the O_2 solubility in ionic media is less, *ca* 3% at 0 °C and 1.5% at 50 °C, than in pure water (the salting-out effect, Table 1). Ultimately, the liquid-phase K_o values reported, $K_{o,m}$, were corrected for solute effects as:

$$K_{o,c} = K_{o,m} \frac{H_{pc,u,O_2}}{H_{pc,s,O_2}}, \qquad (6)$$

where $H_{pc,u}$ is the value of the Henry's law constant used at the given temperature in the original studies.

Estimates of the specificity factor, $S_{c/o}$, depend on both differences in pK_a values used for the acidity constant of dissolved CO_2 ($pK_{a,u}$) and Henry's law constant for O_2. Thus, the $S_{c/o}$ measurements, $S_{c/o,m}$, were converted to a common set of equilibrium coefficients as:

$$S_{c/o,c} = S_{c/o,m} \frac{\left(1 - \dfrac{10^{pH-pK_{a,u}}}{1+10^{pH-pK_{a,u}}}\right)H_{pc,u,O_2}}{\left(1 - \dfrac{10^{pH-pK_{a,c}}}{1+10^{pH-pK_{a,c}}}\right)H_{pc,s,O_2}}. \qquad (7)$$

In vivo *database*

In the case of *in vivo* estimates (only available for Spermatophyta), the database included the following: article bibliographic data,

Table 1. *Henry's law constants (Pa m³ mol⁻¹) for conversion of Rubisco kinetic characteristics among gas- and liquid-phase equivalent values (Eqs 8–10)*

Gas	Medium	Temperature (ºC)			
		15	**25**	**35**	**45**
CO_2	Pure water	2186	2982	3867	4777
CO_2	Chloroplast	2230	3041	3944	4873
O_2	Pure water	67 510	82 080	97 430	113 870
O_2	Chloroplast	69 260	83 950	99 370	115 840

Henry's law constant (H_{pc}) is the gas–liquid phase equilibrium partition coefficient and is given as the ratio of the gas partial pressure (Pa) and corresponding liquid-phase concentration (mol m⁻³, Eq. 3). Because the gas solubility depends on the presence of other solutes (salting-out effect), H_{pc} typically increases with increasing solute concentration. Equation 4 was used to estimate values of H_{pc} at different temperatures for pure water, and Eq. 5 for chloroplastic water. In the latter calculation, the dominant solute was assumed to be KCl and the solute concentration was taken as 0.11 M. Derivation of Eqs 4 and 5 with supporting references and review of chloroplast solute concentrations is provided in the Methods.

species name, cultivar name for agricultural plants, measurement temperature (T) and details of the measurement methods used (e.g. gas exchange combined with chlorophyll fluorescence, gas exchange and carbon isotopic discrimination, gas exchange and ¹⁴CO_2 uptake, indicating whether the leaf mesophyll conductance and mitochondrial respiration were considered when deriving Rubisco kinetic parameters). Typically, values of K_c, K_o and/or $S_{c/o}$ were reported for the gas phase ($K_{c,g}$, $K_{o,g}$, and $S_{c/o,g}$), but when available, liquid-phase equivalent values of these characteristics and corresponding values of Henry's law constants used were also included in the database. The gas-phase estimates of Rubisco characteristics estimated in *in vivo* studies themselves do not require any standardization, but as the Rubisco reaction takes place in the liquid phase, corresponding liquid-phase estimates are needed to compare Rubisco kinetics among species. The gas-phase estimates of Rubisco kinetic characteristics were converted to liquid-phase equivalent values ($K_{c,liq}$, $K_{o,liq}$, $S_{c/o,liq}$) using the following equations:

$$K_{c,liq} = \frac{K_{c,g}}{H_{pc,s,CO_2}} P \qquad (8)$$

$$K_{o,liq} = \frac{K_{o,g}}{H_{pc,s,O_2}} P \qquad (9)$$

$$S_{c,o,liq} = \frac{S_{c/o,g} H_{pc,s,CO_2}}{H_{pc,s,O_2}}, \qquad (10)$$

where the Henry's law constant for O_2, H_{pc,s,O_2}, is given by Eq. 5 and that for CO_2, H_{pc,s,CO_2}, was derived analogously (Table 1 for estimates of Henry's law constant at different temperatures). For pure water, the temperature dependence of $H_{pc,0,CO_2}$ was derived analogously to that for O_2 (Eq. 4) using an extensive set of values from published studies (van Slyke *et al.*, 1928; Markham and Kobe, 1940; Harned and Davis, 1943; Umbreit *et al.*, 1972; Rischbieter *et al.*, 1996) to estimate the empirical coefficients as $c_1=-0.01081$, $c_2=10.1188$, $c_3=-3065.93$ and $c_4=304097.1$ ($r^2=0.9994$). These reference sources were further employed to fit Eq. 5 to solute concentrations, and values of $d_1=-2.8858$, $d_2=-173.31$, $d_3=18.718$, $d_4=0.41177$, $d_5=-32.719$, $d_6=-0.050167$ were derived for KCl ($r^2=0.9993$ for the complete fit including both the temperature effects on $H_{pc,0,CO_2}$ as described by Eq. 4 and the denominator). KCl was used as K is the dominant

solute in plant cells (Gupta and Berkowitz, 1988; Schröppel-Meier and Kaiser, 1988), but analogous fits using other electrolytes such as NaCl were similar (data not shown). Given that ionic concentrations in chloroplasts of non-stressed leaves are on the order of 0.09–0.15 M in non-dissociated salt equivalents used in developing Eq. 5 (Gupta and Berkowitz, 1988; Schröppel-Meier and Kaiser, 1988), we have taken C_s as 0.11 M in this analysis. For comparison, an equivalent average value of 0.08 M was estimated across the *in vitro* studies (assuming a non-dissociated salt consisting of two monovalent ions). When *in vivo* studies reported liquid-phase equivalent values of Rubisco kinetic characteristics, they were again converted to a common set of equilibrium constants. In the case of K_c and K_o, Eq. 6 was used with corresponding Henry's law constant values for CO_2 and O_2, while the values of $S_{c/o}$ were standardized as:

$$S_{c/o,c} = S_{c/o,m} \frac{H_{pc,u,O_2} H_{pc,sCO_2}}{H_{pc,u,CO_2} H_{pc,s,O_2}}. \qquad (11)$$

In vivo data on the temperature response of $S_{c/o}$ were obtained from five different studies: Brooks and Farquhar (1985), Ghashghaie and Cornic (1994), Bernacchi *et al.* (2001), Viil *et al.* (2012) and Walker *et al.* (2013) providing information for six species. The temperature responses of *in vivo* K_c were obtained from the following studies: Monson *et al.* (1982), Harley *et al.* (1985), Bernacchi *et al.* (2001) and Walker *et al.* (2013) yielding data for four species. The temperature responses of *in vivo* K_o for three species were extracted from Harley *et al.* (1985), Bernacchi *et al.* (2001) and Walker *et al.* (2013). Across all these studies, mesophyll conductance (g_m) had been considered, and accordingly Rubisco kinetics based on chloroplastic CO_2 concentration had been derived, only in the study of Walker *et al.* (2013). In all other studies, Rubisco kinetics had been derived based on intercellular CO_2 concentration. Although Bernacchi *et al.* (2002) have reported the temperature kinetics of g_m, the Rubisco temperature characteristics of Bernacchi *et al.* (2001) cannot be readily converted to chloroplastic CO_2 concentration based estimates using these independent measurements of g_m. This is because at any given value of g_m, the CO_2 drawdown between the intercellular airspace and chloroplasts can vary due to differences in foliage anatomical characteristics and leaf photosynthetic capacity (Niinemets *et al.*, 2009a; Tomás *et al.*, 2013).

Species' phylogenetic and ecological characteristics grouping

Species were grouped into the main phylogenetic groups Archaea, Proteobacteria, Cyanobacteria, Rhodophyta, Chlorophyta, Bacillariophyta and Spermatophyta. The average optimum growth temperature (T_{growth}) for each species was obtained from the literature or assigned according to the species' climatic range as in Galmés *et al.* (2015). Spermatophytes were further classified according to their photosynthetic mechanism and T_{growth} as C₃ plants from warm environments ($T_{growth} \geq 25$ ºC), C₃ plants from cool environments ($T_{growth} < 25$ ºC) and C₄ plants. The arbitrary threshold of 25 ºC was based on analogous studies (Sage, 2002; Galmés *et al.*, 2015).

Fitting the temperature responses

The temperature response curves of the kinetic parameters obtained from the original data were fitted for each individual temperature response dataset by an Arrhenius-type temperature response function:

$$f(T) = e^{c - \Delta H_a / RT} \qquad (12)$$

where c is the scaling constant for the parameter, ΔH_a (J mol⁻¹) is the activation energy, T (K) is the temperature and R is the universal gas constant (8.314 J mol⁻¹ K⁻¹). Equation 12 was fitted to the data by iteratively minimizing the sum of squares between the measured and predicted values of each kinetic parameter using the Microsoft Excel Solver function.

The temperature response curves of the kinetic parameters were also fitted by second- and third-order polynomial equations in the form of:

$$f(T) = a + bT + cT^2 \qquad (13)$$

$$f(T) = a + bT + cT^2 + dT^3 \qquad (14)$$

As the actual measurement temperatures differed across studies, the Arrhenius-type and polynomial equations (Eqs 12–14) were further used to calculate the standardized values of each parameter at 5, 15, 25, 35 and 45 ºC for each species. These values were used to obtain the Q_{10} value over the temperature intervals of 5–15, 15–25, 25–35 and 35–45 ºC. We also reanalysed the k_{cat}^c temperature data described in Galmés *et al.* (2015) to calculate the Q_{10} values.

To compare the different functions, the correlation coefficient (*r*) was calculated for linear regressions for predicted *vs.* measured values by SigmaPlot 11.0 (Systat Software, Inc., San Jose, CA, USA) as a measure of goodness of the fits.

Simulation of temperature responses of Rubisco-limited photosynthesis

We used the photosynthesis model of Farquhar *et al.* (1980) to quantify the importance, in terms of Rubisco-limited CO_2 gross assimilation rate ($A_{Rubisco}$), of different thermal sensitivities of Rubisco kinetics in C_3 plants from cool and warm environments. $A_{Rubisco}$ only provides the potential estimate of photosynthesis rate supported by a given set of Rubisco characteristics under RuBP-saturated conditions (Farquhar *et al.* 1980). The extent to which this potential is realized depends on the rate of RuBP regeneration, which in turn is driven by the actual light level, the capacity of RuBP regeneration (typically determined by the capacity for photosynthetic electron transport) and the temperature dependence of RuBP regeneration (Farquhar *et al.* 1980; Galmés *et al.* 2014a). Although this complicates interpretation of differences in $A_{Rubisco}$ temperature responses, we note that over the ambient temperature range of 15–40 ºC, Rubisco characteristically operates in RuBP-saturated conditions at higher light (photosynthetic quantum flux density greater than *ca* 400 µmol m^{-2} s^{-1}) and lower CO_2 concentrations (chloroplastic CO_2 concentration, C_c, less than *ca* 200 µmol mol^{-1}) with the actual crossover between Rubisco-limited and RuBP-limited conditions depending on combinations of light, C_c, temperature and temperature responses of $A_{Rubisco}$ and RuBP regeneration (Farquhar *et al.* 1980; Galmés *et al.* 2014a). In these simulations, group-specific average temperature parameters for $S_{c/o}$, K_c and k_{cat}^c were used (Table 3), while the temperature dependence parameters of K_o were considered invariable among plant functional types. They were obtained as the average of the four reported *in vitro* values for C_3 plants ($c=9.9$ and $\Delta H_a=9.7$ kJ mol^{-1}, see Table 2 for single species data). A value of k_{cat}^c of 2.5 s^{-1} at 25 ºC and a leaf Rubisco content of 2 g m^{-2} (equivalent to a concentration of 29 µmol catalytic sites m^{-2}) were used for all plant functional types. The values of the deactivation energy (ΔH_d) and the entropy term (ΔS) for k_{cat}^c used for the different plant functional types were taken from Galmés *et al.* (2015): C_3 plants from cool habitats, $\Delta H_d=305$ kJ mol^{-1}, $\Delta S=929$ J mol^{-1} K^{-1}; C_3 plants from warm habitats, $\Delta H_d=220$ kJ mol^{-1}, $\Delta S=664$ J mol^{-1} K^{-1}; C_3 average, $\Delta H_d=258$ kJ mol^{-1}, $\Delta S=782$ J mol^{-1} K^{-1}.

Analogous simulations were conducted to compare the average C_3 *in vitro* temperature response functions developed here and three different temperature functions widely used in the literature in simulating photosynthesis, i.e. *in vivo* Rubisco temperature responses for *Nicotiana tabacum* (Bernacchi *et al.*, 2001; Walker *et al.*, 2013) and *in vitro* Rubisco temperature responses for *Spinacia oleracea* (Jordan and Ogren, 1984). To quantitatively compare different simulated temperature response curves, warm *vs.* cool C_3 plants and temperature response curves currently in use in the modeling community, mean absolute (σ_A) and root mean squared (σ_S) differences between

different model estimates (Willmott and Matsuura, 2005; Niinemets *et al.*, 2013) were calculated through the modeled temperature range of 5–50 ºC. The mean absolute difference was calculated as:

$$\sigma_A = \frac{1}{n}\sum_{i=1}^{n}\left|A_{Rubisco,f1}(T_i) - A_{Rubisco,f2}(T_i)\right|, \qquad (15)$$

where $A_{Rubisco,f1}(T_i)$ is the estimated $A_{Rubisco}$ for the first function at temperature T_i and $A_{Rubisco,f2}(T_i)$ is the corresponding $A_{Rubisco}$ value for the second function. The root mean squared differences was further calculated as:

$$\sigma_S = \sqrt{\frac{1}{n}\sum_{i=1}^{n}\left[A_{Rubisco,f1}(T_i) - A_{Rubisco,f2}(T_i)\right]^2}. \qquad (16)$$

Statistical analysis and tests for phylogenetic signals and trait correlations

Conventional statistical analyses consisted of one-way ANOVA and correlation and linear regression analyses. For all the parameters studied, a univariate model of fixed effects was assumed. The univariate general linear model for unbalanced data (Proc. GLM) was applied and significant differences among groups of species were revealed by Duncan's test. To avoid type II errors due to limited data, only groups with at least five species were statistically compared. In particular, the limited data available on the *in vitro* temperature dependence of K_o impeded the comparative analysis among groups of species for temperature responses of this characteristic. Modeled temperature responses of $A_{Rubisco}$ were compared by pairwise *t*-test over temperature ranges of 5–20 and 30–50 ºC. In addition, paired *t*-tests were used to compare the mean absolute and root mean squared differences in model estimates among different groups of model datasets (*in vitro* warm *vs.* cool C_3 dataset developed in this study *vs.* three currently widely used model datasets, comparisons conducted for four different simulations with chloroplastic CO_2 concentrations of 120, 150, 200 and 400 µmol mol^{-1}). These analyses were conducted with the IBM SPSS Statistics 20 software package (IBM, Armonk, NY, USA).

In order to test phylogenetic signal strength on trait correlations (the theoretical background of these analyses followed the framework set in Galmés *et al.* (2015)), complete phylogeny was assembled for all the species in this study. For this, we used *RbcL* and 16S ribosomal RNA (for species with no available *RbcL*) sequences from GenBank (http://www.ncbi.nlm.nih.gov). Where genetic data were not available for the given species, we obtained data from GenBank for functionally similar species from the same genus that had overlapping distribution. Phylogeny was constructed in MEGA6 (Tamura *et al.*, 2013), using standardized methods of aligning multiple sequences: Muscle (Edgar, 2004) and constructing maximum likelihood phylogenetic tree (Chor and Tuller, 2005). Phylogenetic independent contrasts, indicative of the strength of phylogenetic signal, were calculated in R using packages 'ape' (Paradis *et al.*, 2004), 'nlme' (Pinheiro *et al.*, 2014) 'geiger' (Harmon *et al.*, 2008) and 'phytools' (Revell, 2012). The effects of phylogenetic signal on trait correlations were assessed by analysis of covariance (ANCOVA) and by calculating Pagel's lambda (λ) based on phylogenetic independent contrasts values (Pagel, 1999). All statistical differences were considered significant at $P<0.05$.

Results

Standardization of in vitro *data*

The default value of acidity constant of dissolved CO_2, pK_{a,CO_2}, for pure water used in early studies was commonly taken as 6.35–6.37 at 25 °C (with reference to e.g. Harned

Table 2. *Temperature dependence parameters of in vitro-measured Rubisco catalytic constants*

Group	Species	Reference	T_{meas} (°C)	c	ΔH_a (kJ mol⁻¹)	r	Q_{10}^{15-5}	Q_{10}^{25-15}	Q_{10}^{35-25}	Q_{10}^{45-35}	T_{growth} (°C)
$S_{c/o}$											
Proteobacteria	Rhodospirillum rubrum	Jordan and Ogren (1984)[a]	2–25	−5.2	−18.8	n.d.	0.75	0.77			33
Cyanobacteria	Thermosynechococcus elongatus BP-1	Gubernator et al. (2008)	15–45	−13.0	−43.0	0.998	0.52	0.55	0.57	0.59	56
	Synechococcus lividus	Zhu et al. (1998)	15–35	−0.9	−11.7	0.999	0.84	0.85	0.86	0.87	45
Rhodophyta	Galdieria partita Tokara	Uemura et al. (1997)	15–45	−10.3	−38.7	0.991	0.56	0.58	0.60	0.62	45
Bacillariophyta	Chaetoceros socialis	Haslam et al. (2005)	10–25	−3.2	−18.7	0.975	0.76	0.77	0.78	0.80	10
	Skeletonema costatum	Haslam et al. (2005)	10–25	−7.1	−28.1	0.992	0.66	0.67	0.69	0.71	20
	Thalassiosira antarctica	Haslam et al. (2005)	10–25	−3.8	−20.3	0.963	0.74	0.75	0.77	0.78	−0.5
	Thalassiosira hyalina	Haslam et al. (2005)	10–25	−2.9	−18.1	0.991	0.76	0.78	0.79	0.80	2
Spermatophyta (C₃ plants from cool habitats)	Atriplex glabriuscula	Badger and Collatz (1977)[b]	15–35	−0.1	−12.1	0.984	0.76	0.81	0.90	1.05	20
	Avena sativa cv. Forridena	Hermida-Carrera et al. (2016)[c]	15–35	−2.9	−18.4	0.998	0.76	0.77	0.79	0.80	20
	Hordeum vulgare ssp. vulgare cv. Morex	Hermida-Carrera et al. (2016)[c]	15–35	−2.5	−17.4	0.997	0.77	0.78	0.80	0.81	20
	Lysimachia minoricensis	Galmés et al. (2005)	15–35	−4.2	−21.4	0.989	0.73	0.74	0.76	0.77	20
	Mentha aquatica	Galmés et al. (2005)	15–35	−4.2	−21.4	0.996	0.73	0.74	0.76	0.77	20
	Spinacea oleracea	Uemura et al. (1997)	15–35	−3.5	−19.8	0.996	0.74	0.76	0.77	0.78	16
	Spinacea oleracea	Zhu et al. (1998)	15–35	−2.6	−17.6	0.999	0.77	0.78	0.79	0.81	16
	Spinacea oleracea	Jordan and Ogren (1984)	7–25	−3.8	−20.5	0.999	0.77	0.75	0.74	0.74	16
	Spinacea oleracea cv. Torai	Yamori et al. (2006)[d]	5–45	−1.4	−14.7	0.987	0.80	0.81	0.83	0.84	16
	Spinacea oleracea	Average		−3.3	−19.3		0.77	0.78	0.78	0.79	16
	Triticum aestivum	Haslam et al. (2005)	10–25	−3.7	−20.5	0.997	0.74	0.75	0.76	0.78	20
	Triticum aestivum	Hermida-Carrera et al. (2016)[c]	15–35	−3.4	−19.7	0.999	0.74	0.76	0.77	0.79	20
	Triticum aestivum	Average		−3.6	−20.0		0.74	0.75	0.77	0.78	20
	Urtica atrovirens ssp. bianorii	Galmés et al. (2005)	15–35	−4.1	−21.1	0.999	0.73	0.74	0.76	0.77	20
Spermatophyta (C₃ plants from warm habitats)	Beta maritima ssp. marcosii	Galmés et al. (2005)	15–35	−4.4	−22.0	0.999	0.72	0.73	0.75	0.76	25
	Beta maritima ssp. maritima	Galmés et al. (2005)	15–35	−4.5	−22.4	0.994	0.71	0.73	0.75	0.76	25
	Diplotaxis ibicensis	Galmés et al. (2005)	15–35	−5.6	−24.9	0.998	0.69	0.71	0.72	0.74	25
	Flaveria cronquistii	Perdomo et al. (2015)	10–40	−3.5	−19.5	0.985	0.75	0.76	0.77	0.79	30
	Flaveria pringlei	Zhu et al. (1998)	15–35	−3.0	−18.9	0.999	0.75	0.77	0.78	0.79	30
	Flaveria pringlei	Perdomo et al. (2015)	10–40	−4.0	−20.8	0.991	0.73	0.75	0.76	0.77	30
	Flaveria pringlei	Average		−3.5	−19.8		0.7	0.8	0.8	0.8	30
	Hypericum balearicum	Galmés et al. (2005)	15–35	−4.7	−22.6	0.999	0.71	0.73	0.74	0.76	25
	Kundmannia sicula	Galmés et al. (2005)	15–35	−4.9	−23.2	0.996	0.71	0.72	0.74	0.75	25
	Limonium gibertii	Galmés et al. (2005)	15–35	−5.1	−24.1	0.999	0.70	0.71	0.73	0.74	25
	Limonium magallufianum	Galmés et al. (2005)	15–35	−5.2	−24.3	0.998	0.69	0.71	0.73	0.74	25
	Pistacia lentiscus	Galmés et al. (2005)	15–35	−4.4	−22.0	0.999	0.72	0.74	0.75	0.76	25
	Rhamnus alaternus	Galmés et al. (2005)	15–35	−4.7	−22.8	0.998	0.71	0.73	0.74	0.76	25
	Rhamnus ludovici-salvatoris	Galmés et al. (2005)	15–35	−5.2	−24.0	0.993	0.70	0.71	0.73	0.74	25
	Trifolium repens (native ecotype)	Lehnher et al. (1985)	10–25	−2.8	−18.0	0.983	0.76	0.78	0.79	0.80	25
	Urtica membranacea	Galmés et al. (2005)	15–35	−3.7	−20.4	0.998	0.74	0.75	0.77	0.78	25
	Flaveria angustifolia (C₃–C₄)	Perdomo et al. (2015)	10–40	−4.3	−21.6	0.978	0.72	0.74	0.75	0.77	30
	Flaveria floridana (C₃–C₄)	Perdomo et al. (2015)	10–40	−3.8	−20.4	0.989	0.74	0.75	0.77	0.78	30

Table 2. *Continued*

Group	Species	Reference	T_{meas} (°C)	c	ΔH_a (kJ mol⁻¹)	r	Q_{10}^{15-5}	Q_{10}^{25-15}	Q_{10}^{35-25}	Q_{10}^{45-35}	T_{growth} (°C)
Spermatophyta (C₄ plants)	*Amaranthus hybridus*	Jordan and Ogren (1984)	5–35	−7.0	−27.9	0.998	0.66	0.68	0.69	0.71	30
	Flaveria bidentis	Perdomo et al. (2015)	10–40	−3.8	−20.0	0.994	0.74	0.76	0.77	0.78	30
	Flaveria trinervia	Perdomo et al. (2015)	10–40	−4.3	−21.4	0.999	0.73	0.74	0.76	0.77	30
	Saccharum officinarum	Hermida-Carrera et al. (2016)[c]	15–35	−4.9	−23.0	0.997	0.71	0.72	0.74	0.75	30
	Setaria viridis	Boyd et al. (2015)	10–40	−4.6	−21.3	0.950	0.73	0.74	0.76	0.77	25
	Zea mays cv. Carella	Hermida-Carrera et al. (2016)[c]	15–35	−3.6	−20.1	0.999	0.74	0.75	0.77	0.78	30
K_c											
Cyanobacteria	*Anabaena variabilis* M3	Badger (1980)	15–40	20.8	38.8	0.989	1.79	1.72	1.66	1.61	35
Bacillariophyta	*Fragilariopsis cylindrus*	Young et al. (2015)[e]	0–20	17.7	34.9	n.d.	1.69	1.63			5
	Thalassiosira weissflogii	Young et al. (2015)[e]	0–20	21.1	43.0	n.d.	1.91	1.83			22
Spermatophyta (C₃ plants from cool habitats)	*Atriplex glabriuscula*	Badger and Collatz (1977)	5–35	15.9	32.4	0.987	1.63	1.57	1.53	1.49	20
	Avena sativa cv. Forridena	Hermida-Carrera et al. (2016)[c]	15–35	20.2	44.2	0.999	1.94	1.86	1.78	1.72	20
	Espeletia schultzii	Castrillo (1995)[f]	5–35	11.9	23.7	0.988	1.50	1.38	1.31	1.26	20
	Hordeum vulgare ssp. *vulgare* cv. Morex	Hermida-Carrera et al. (2016)[c]	15–35	16.2	34.6	0.999	1.68	1.62	1.57	1.53	20
	Spinacia oleracea	Jordan and Ogren (1984)	7–35	22.4	50.2	0.994	2.12	2.02	1.93	1.85	16
	Triticum aestivum cv. Cajeme	Hermida-Carrera et al. (2016)[c]	15–35	19.0	41.3	0.990	1.86	1.78	1.72	1.66	20
Spermatophyta (C₃ plants from warm habitats)	*Flaveria cronquistii*	Perdomo et al. (2015)	10–40	22.9	51.8	0.996	2.18	2.07	1.97	1.89	30
	Flaveria pringlei	Perdomo et al. (2015)	10–40	17.7	38.6	0.983	1.78	1.72	1.66	1.60	30
	Glycine max cv. Wayne	Laing et al. (1974)	15–35	17.8	37.0	0.963	1.74	1.68	1.62	1.57	25
	Oryza sativa indicaxjaponica hybrid	Wei et al. (1994)	20–40	25.6	58.4	0.978	2.40	2.26	2.15	2.05	25
	Trifolium repens (native ecotype)	Lehnherr et al. (1985)	10–25	22.5	48.9	0.999	2.08	1.98	1.90	1.82	25
	Flaveria angustifolia (C₃–C₄)	Perdomo et al. (2015)	10–40	20.0	44.0	0.998	1.93	1.85	1.78	1.71	30
	Flaveria floridana (C₃–C₄)	Perdomo et al. (2015)	10–40	19.9	44.1	0.999	1.94	1.85	1.78	1.72	30
Spermatophyta (C₄ plants)	*Flaveria bidentis*	Perdomo et al. (2015)	10–40	15.4	31.6	0.993	1.61	1.56	1.51	1.47	30
	Flaveria trinervia	Perdomo et al. (2015)	10–40	15.4	31.7	0.992	1.61	1.56	1.51	1.47	30
	Saccharum officinarum	Hermida-Carrera et al. (2016)[c]	15–35	17.8	35.8	0.998	1.71	1.65	1.60	1.55	30
	Setaria viridis	Boyd et al. (2015)	10–40	24.7	51.8	0.990	2.17	2.06	1.97	1.89	25
	Zea mays cv. Carella	Hermida-Carrera et al. (2016)[c]	15–35	12.6	22.9	0.971	1.41	1.38	1.35	1.32	30

Table 2. *Continued*

K_o

Group	Species	Reference	T_{meas} (°C)	c	ΔH_a (kJ mol^{-1})	r	Q_{10}^{15-5}	Q_{10}^{25-15}	Q_{10}^{35-25}	Q_{10}^{45-35}	T_{growth} (°C)
Spermatophyta (C$_3$ plants from cool habitats)	*Atriplex glabriuscula*	Badger and Collatz (1977)	15–35	19.7	34.6	0.996	1.68	1.62	1.57	1.53	20
Spermatophyta (C$_3$ plants from warm habitats)	*Spinacia oleracea*	Jordan and Ogren (1984)[g]	7–35	6.2	0.0		1.00	1.00	1.00	1.00	16
	Glycine max cv. Wayne	Laing et al. (1974)	15–35	3.7	−5.5	0.989	0.92	0.93	0.93	0.94	25
	Trifolium repens (native ecotype)	Lehnherr et al. (1985)	10–25	10.1	9.3	0.927	1.15	1.14	1.13	1.12	25
Spermatophyta (C$_4$ plants)	*Setaria viridis*	Boyd et al. (2015)	10–40	4.5	−4.0	0.738	0.94	0.95	0.95	0.95	25

Species were assigned to different phylogenetic groups. One phylogenetic group, Spermatophyta, was further divided into warm- and cool-temperature species according to their optimum growth temperature (T_{growth}). The two C$_3$–C$_4$ intermediate species *Flaveria angustifolia* and *F. floridana* were assigned to the group of C$_3$ plants from warm habitats because they present C$_3$-like Rubisco kinetics (Perdomo et al., 2015). The optimum growth temperature (T_{growth}) for each species was either obtained from literature or assigned according to their climate of origin. For *Spinacea oleracea*, *Triticum aestivum* and *F. pringlei*, individual reports' values and average values for $S_{c/o}$ of different reports are given. c, scaling constant; ΔH_a, activation energy; K_o, Michaelis–Menten constant for CO$_2$; K_o, Michaelis–Menten constant for O$_2$; r, correlation coefficient for linear regressions between measured *vs.* predicted (Microsoft Excel Solver function) values of each kinetic parameter at the assayed temperatures; Q_{10}, coefficient over the temperature intervals of 5–15 °C (Q_{10}^{15-5}),

15–25 °C (Q_{10}^{25-15}), 25–35 °C (Q_{10}^{35-25}) and 35–45 °C (Q_{10}^{45-35}); $S_{c/o}$, Rubisco specificity factor for CO$_2$/O$_2$; T_{meas}, range of measurement temperature.

n.d.: r was not determined because measurements consisted in only two assay temperatures.

[a] Data from Jordan and Ogren (1984) for *Rhodospirillum rubrum* consisted of only two measurement temperatures (2 and 25 °C) and, therefore, Q_{10}^{35-25} and Q_{10}^{45-35} were not calculated.

[b] Due to poor convergence in the Excel Solver (low degree of explained variance), c and ΔH_a for this report were not considered in determining the group averages, and Q_{10} values were obtained from second-order polynomial fits. Due to high scatter at higher temperature, values of Q_{10}^{35-25} and Q_{10}^{45-35} from polynomial fits were also unreliable and were therefore not considered for group averages.

[c] Data from Hermida-Carrera (2016) consisted of measurements at three temperatures (15, 25 and 35 °C), and the assays were performed following the procedures described in Galmés et al. (2014b).

[d] Low adjustment of the Excel Solver; c and ΔH_a for this report were not considered in calculating the averages for *Spinacea oleracea*.

[e] Young et al. (2015) consists of only two measurement temperatures (0 and 20 °C); Q_{10}^{35-25} and Q_{10}^{45-35} were not calculated.

[f] Low adjustment of the Excel Solver; c and ΔH_a of this report were not considered for group averages.

[g] r is not provided given the large scattering between measured *vs.* predicted values.

and Bonner, 1945; Umbreit *et al.*, 1972). Across the studies, the average ionic strength of the assay medium (I_s) at 25 ºC was 0.117 ± 0.006 M (range 0.066–0.165 M), and the predicted true value of pK_{a,CO_2} (Eq. 2) corresponding to this average estimate of I_s is 6.112, while the equation of Yokota and Kitaoka (1985) suggests a value of 6.118. Given further that the average pH used in K_c assays at 25 ºC was 8.17 ± 0.04 (range 8.0–8.5), using the pK_{a,CO_2} estimates of pure water would overestimate K_c by 1.75-fold (and 1.015-fold for the equation of Yokota and Kitaoka (1985)). At 35 °C, the pK_{a,CO_2} for pure water is 6.32 (Harned and Bonner, 1945), while the predicted true value for the average I_s is 6.067 (predicted overestimation by 1.78-fold), and the value predicted according to Yokota and Kitaoka (1985) is 6.076 (predicted overestimation 1.021-fold). In our analysis, across all data (different species and temperatures pooled) the average error ($\pm$SE) in K_c estimates ($K_{c,c}$ for standardized and $K_{c,m}$ for measured K_c values), $100(K_{c,c}-K_{c,m})/K_{c,m}$ was $-19.8 \pm 1.6\%$ (range -50 to 10%). For comparison, the overall variation in $K_{c,c}$ values across species and temperatures was 206-fold (average$\pm$SE$=31 \pm 5$ μM, coefficient of variation of 179%).

In the case of K_o, differences among the estimates can result both from the effects of solutes on H_{pc} for oxygen and from differences in $H_{pc,0}$ values used among the studies to estimate O_2 concentration in solutions (Eqs 4–6), while differences in $S_{c/o}$ can include both differences in pK_{a,CO_2} and oxygen solubility calculations (Eq. 7). Equations 4 and 5 predict an H_{pc,s,O_2} value of 83950 Pa m^3 mol^{-1} at 25 ºC for the average ion concentration observed in our study, while the typical value of $H_{pc,0,O2}$ used in original studies was 80040 Pa m^3 mol^{-1}. As Eq. 6 indicates, the use of the default value of Henry's law constant would lead to 4.9% underestimation in K_o at 25 ºC. In our database, across all species and temperature combinations, the average error ($\pm$SE) in K_o estimates was $-4.6 \pm 0.3\%$ (range -2.4 to -6.6%), whereas the variation in standardized K_o values across species and temperatures was 28-fold (average$\pm$SE$=560 \pm 100$ mM, coefficient of variation of 119%). In the case of $S_{c/o}$ where both bicarbonate equilibrium and O_2 solubility play a role, average estimate deviation for all species and temperature combination was $-3.5 \pm 0.7\%$ (range -10.2 to 66.3%), whereas the variation in standardized $S_{c/o}$ values across species and temperatures was 32-fold (average$\pm$SE$=92.3 \pm 2.6$ mol mol^{-1}, coefficient of variation of 39%).

Comparison of different functions in capturing the Rubisco temperature responses

Both the exponential and the polynomial functions used to fit the temperature responses of Rubisco characteristics (Eqs 12–14) provided a good fit to the data, with most *r* values (for predicted *vs.* measured trait values) for individual relationships greater than 0.950 (Tables 2 and 4, and data not shown). With some exceptions indicated in Table 2, differences between *r* values from second- and third-order polynomial equations (Eqs 13–14) and the Arrhenius-type function (Eq. 12) were minor (for instance, average *r* values for *in vitro* K_o were 0.951, 0.974 and 0.913, respectively). For all kinetic parameters, significant

correlation was found between polynomial- and Arrhenius-derived values of Q_{10}^{25-15} and Q_{10}^{35-25} (data not shown). To the contrary, the relationship between polynomial- and Arrhenius-derived values of Q_{10}^{15-5} and Q_{10}^{45-35} was non-significant for some kinetic parameters. This fact indicates that second- and especially third-order polynomial equations are problematic in predicting kinetics values out of the range of assayed temperatures. Therefore, we suggest using the Arrhenius-type function in capturing the Rubisco temperature responses.

Overall variability of the Rubisco *in vitro temperature response parameters for* $S_{c/o}$, K_c *and* K_o

Among all 38 species, the energy of activation (ΔH_a) for $S_{c/o}$ ranged between -43.0 kJ mol^{-1} for the *Thermosynechococcus elongatus* and -11.7 kJ mol^{-1} for *Synechococcus lividus* (Table 2). With regard to K_c, *Zea mays* had the lowest ($\Delta H_a=22.9$ kJ mol^{-1}) and *Oryza sativa* the highest ($\Delta H_a=58.4$ kJ mol^{-1}) temperature-dependent increases in K_c among the 20 species (Table 2). For both $S_{c/o}$ and K_c, a high correlation was observed between ΔH_a and the Q_{10} coefficients calculated at specific ranges of measurement temperatures ($r>0.995$), so that the extremes of the range for Q_{10} were generally represented by the same species as for ΔH_a (Table 2).

The *in vitro* temperature response of K_o is the least documented trait of Rubisco with data only available for five land plant species (Table 2). Moreover, the values of the temperature dependence parameters for these five species are contradictory. Hence, Badger and Collatz (1977) for *Atriplex glabriuscula*, and Lehnherr *et al.* (1985) for *Trifolium repens* reported positive values of ΔH_a for K_o (i.e. K_o increasing with temperature of measurement); Laing *et al.* (1974) for *Glycine max*, and Boyd *et al.* (2015) for *Setaria viridis* reported negative values of ΔH_a for K_o (i.e. K_o decreasing with increases of the temperature of measurement); and Jordan and Ogren (1984) reported that *Spinacia oleracea* K_o was insensitive to temperature of measurement ($\Delta H_a=0$ kJ mol^{-1}). Data standardization did not change the direction of K_o temperature responses in any of the cases.

Comparison of the *in vitro temperature response parameters for* $S_{c/o}$, K_c *and* k_{cat}^c *among groups of species*

Across all the measurement temperatures analysed, average $S_{c/o}$ values for Spermatophyta were lower than those for Rhodophyta (*Galdieria partita*), higher than those for Proteobacteria (*Rhodospirillum rubrum*), and similar to Bacillariophyta and Cyanobacteria (Fig. 1A). The divergence between the two Cyanobacteria species, *Synechococcus lividus* and *Thermosynechococcus elongates*, resulted in large standard errors at ≤25 ºC for this group. In all phylogenetic groups, Rubisco $S_{c/o}$ decreased with increasing the assay temperature, but the extent of such decrease differed among groups. Hence, average ΔH_a for $S_{c/o}$ of Spermatophyta (-21.5 kJ mol^{-1}) was similar to the values reported for Proteobacteria and Bacillariophyta, and 44% lower compared with
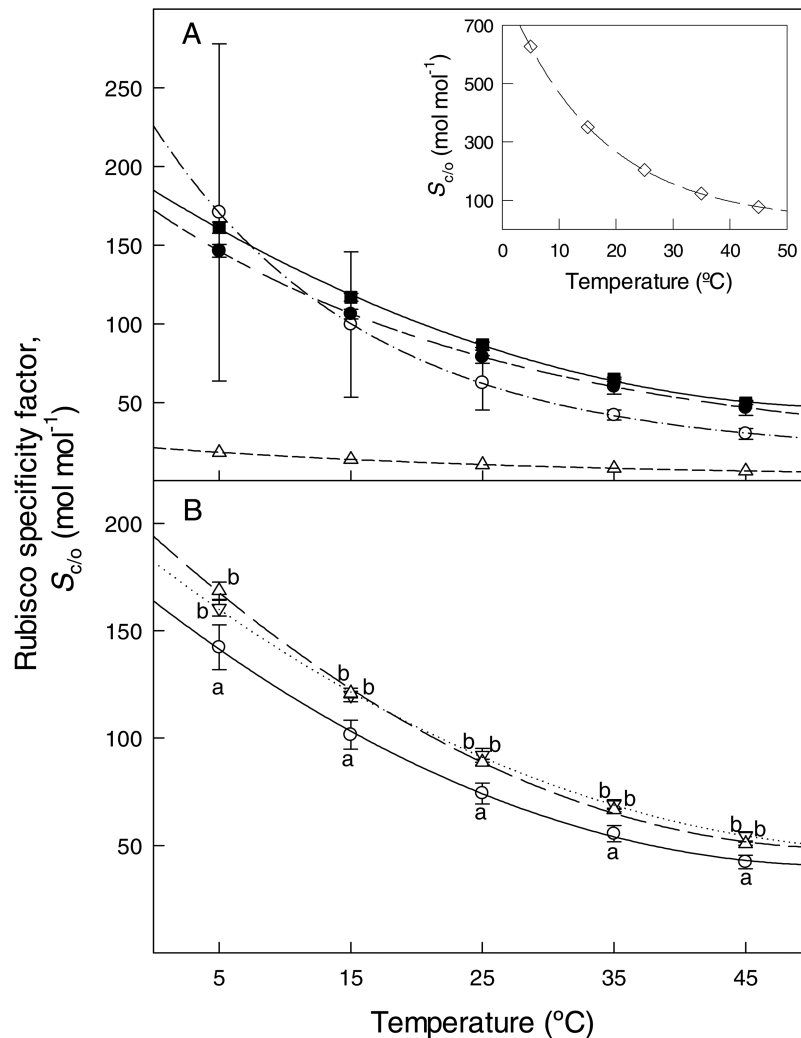
Fig. 1. Values of the Rubisco specificity factor for CO_2/O_2 ($S_{c/o}$) in liquid phase at a range of temperatures in different phylogenetic groups of photosynthetic organisms (A) and in land plants only (B). (A) Open upward triangles and short-dashed line, Proteobacteria; open circles and dash-dotted line, Cyanobacteria; filled circles and long-short-dashed line, Bacillariophyta (diatoms); empty diamond and long-dashed line, Rhodophyta (red algae); filled squares and solid line, Spermatophyta (plants). Sample number n=4 for Bacillariophyta and n=30 for Spermatophyta; no replication was available for Proteobacteria, Cyanobacteria and Rhodophyta. The inset in shows the values of $S_{c/o}$ for Rhodophyta. (B) Open downward triangles and dotted line, C_3 plants from cool habitats (n=8); open upward triangles and long-dashed line, C_3 plants from warm habitats (n=16); open circles and solid line, C_4 plants (n=6). Different letters denote statistically significant differences by Duncan's analysis (P<0.05) among plant functional and photosynthetic groups. All data for $S_{c/o}$ correspond to *in vitro* measurements at discrete temperatures from data shown in Table 3 after applying Eq. 12, and were standardized to a common set of liquid-phase CO_2 and O_2 physico-chemical characteristics by Eqs 1–7. For CO_2, these equations correct for study-to-study differences in assumed bicarbonate equilibrium as dependent on solution pH, temperature and ionic strength and when pertinent study-to-study differences in the value of Henry's law constant used. For O_2, these equations standardize for differences in the value of Henry's law constant used. Means and standard errors are provided when n≥2. Table 1 provides Henry's law constants that can be used to convert the Rubisco kinetic characteristics to gas-phase equivalent values.

Rhodophyta (in absolute values, Table 3). The comparison of average ΔH_a for $S_{c/o}$ of Spermatophyta with that of Cyanobacteria depended on the species (1.8-fold higher compared with *Synechococcus lividus* and 50% lower compared with *Thermosynechococcus elongatus*).

Within Spermatophyta, C_4 plants had lower $S_{c/o}$ values than C_3 plants from cool and warm habitats at all assay temperatures (between 5 and 45 °C), while non-significant differences were found between C_3 plants from cool and warm habitats (Fig. 1B). The temperature response of $S_{c/o}$ also differed within Spermatophyta, with higher thermal sensitivity of $S_{c/o}$ (i.e. more negative ΔH_a and lower Q_{10}) in C_4 plants compared with C_3 plants from cool habitats (Table 3). C_3

plants from warm habitats presented intermediate values of ΔH_a for $S_{c/o}$, and similar Q_{10} values to C_4 plants (Table 3).

Rubisco from Spermatophyta had a higher affinity for CO_2 (i.e. lower K_c) than Rubiscos from Bacillariophyta and Cyanobacteria throughout the range of temperatures of measurement (Fig. 2A). For the three phylogenetic groups with available data, K_c values from *in vitro* measurements increased with the temperature of measurement, and the values of ΔH_a and Q_{10} for K_c were similar among the groups (Table 3). Within land plants, Rubisco from C_4 plants displayed higher K_c values compared with Rubiscos from C_3 plants, at all temperatures of measurement except 45 °C (Fig. 2B). Differences in K_c between C_3 plants from cool and

Table 3. *Average temperature dependence parameters of the* in vitro-*measured Rubisco specificity factor for CO$_2$/O$_2$ ($S_{c/o}$), the Michaelis–Menten constant for CO$_2$ (K_c) and the Rubisco maximum carboxylase turnover rate (k_{cat}^c)*

Group	n	c	ΔH_a (kJ mol^{-1})	Q_{10}^{15-5}	Q_{10}^{25-15}	Q_{10}^{35-25}	Q_{10}^{45-35}	T_{growth} (ºC)
$S_{c/o}$								
Proteobacteria	1	−5.2	−18.8	0.75	0.77			33
Cyanobacteria	2	−6.9±6.0	−27.4±15.6	0.68±0.16	0.70±0.15	0.71±0.14	0.73±0.14	50.5±5.5
Rhodophyta	1	−10.3	−38.7	0.56	0.58	0.60	0.62	45
Bacillariophyta	4	−4.2±1.0	−21.3±2.3	0.73±0.02	0.74±0.02	0.76±0.02	0.77±0.02	7.8±4.6
Spermatophyta	30	−4.2±0.2	−21.5±0.4	0.73±0.01	0.74±0.01	0.75±0.01	0.77±0.01	25.0±0.7
Spermatophyta (C$_3$ plants)	24	−4.1±0.2	−21.4±0.4	0.73±0.01	0.75±0.01	0.76±0.01	0.77±0.01	24.0±0.8
Spermatophyta (C$_3$ plants from cool habitats)	8	−3.5±0.3[b]	−19.9±0.6[b]	0.74±0.01[b]	0.77±0.01[b]	0.78±0.01[b]	0.79±0.01[b]	19.5±0.5[a]
Spermatophyta (C$_3$ plants from warm habitats)	16	−4.4±0.2[a]	−22.0±0.5[ab]	0.72±0.01[a]	0.74±0.01[a]	0.75±0.01[a]	0.76±0.01[a]	26.3±0.6[b]
Spermatophyta (C$_4$ plants)	6	−4.8±0.5[a]	−22.3±1.2[a]	0.72±0.01[a]	0.73±0.01[a]	0.75±0.01[a]	0.76±0.01[a]	29.2±0.8[c]
K_c								
Cyanobacteria	1	20.8	38.8	1.79	1.72	1.66	1.61	35
Bacillariophyta	2	19.4±1.7	39.0±4.1	1.80±0.11	1.73±0.10			13.5±8.5
Spermatophyta	18	19.2±0.9	41.1±2.3	1.85±0.06	1.77±0.06	1.70±0.05	1.64±0.05	25.3±1.1
Spermatophyta (C$_3$ plants)	12	20.0±0.8	43.8±2.2	1.91±0.07	1.82±0.06	1.75±0.06	1.68±0.06	23.9±1.4
Spermatophyta (C$_3$ plants from cool habitats)	6	18.8±1.2[a]	40.5±3.2[ab]	1.79±0.09[a]	1.71±0.09[a]	1.64±0.09[a]	1.58±0.08[a]	19.3±0.7[a]
Spermatophyta (C$_3$ plants from warm habitats)	7	20.9±1.1[a]	46.1±2.8[b]	2.01±0.09[a]	1.92±0.08[a]	1.84±0.07[a]	1.77±0.06[a]	27.9±1.0[b]
Spermatophyta (C$_4$ plants)	5	17.2±2.0[a]	34.7±4.7[a]	1.70±0.13[a]	1.64±0.11[a]	1.59±0.10[a]	1.54±0.09[a]	29.0±1.0[b]
k_{cat}^c								
Archaea	1	15.2	37.2	1.75	1.68	1.63	1.58	85.0
Proteobacteria	4	18.5±1.5	45.9±4.1	2.00±0.13	1.91±0.11	1.83±0.10	1.76±0.09	33.8±5.5
Cyanobacteria	3	16.3±3.5	40.1±8.9	1.86±0.26	1.78±0.23	1.71±0.21	1.66±0.19	46.7±7.3
Rhodophyta	1	30.8	76.3	3.14	2.91	2.71	2.55	57.0
Chlorophyta	4	10.8±0.4	26.7±0.9	1.49±0.02	1.45±0.02	1.42±0.02	1.39±0.02	15.5±5.5
Spermatophyta	36	23.5±0.7	58.1±1.7	2.43±0.07	2.28±0.06	2.16±0.05	2.06±0.05	24.9±0.9
Spermatophyta (C$_3$ plants)	26	24.3±0.9	60.2±2.3	2.51±0.09	2.35±0.08	2.23±0.07	2.11±0.06	23.1±1.0
Spermatophyta (C$_3$ plants from cool habitats)	12	22.3±0.8[a]	55.3±2.0[a]	2.30±0.07[a]	2.18±0.06[a]	2.07±0.05[a]	1.98±0.05[a]	18.3±0.8[a]
Spermatophyta (C$_3$ plants from warm habitats)	14	26.0±1.4[b]	64.5±3.5[b]	2.68±0.15[b]	2.51±0.13[b]	2.36±0.11[b]	2.23±0.10[b]	27.2±0.7[b]
Spermatophyta (C$_4$ plants)	10	21.3±0.5[a]	52.8±1.3[a]	2.22±0.05[a]	2.10±0.04[a]	2.00±0.03[a]	1.91±0.03[a]	29.6±0.4[b]

The original data for $S_{c/o}$ and K_c were taken from Table 2 and those for k_{cat}^c from Galmés *et al*. (2015). For *Spinacea oleracea*, *Triticum aestivum* and *Flaveria pringlei*, average values from Table 2 were used. The values are means±SE, except when n=1. Within Spermatophyta, significant differences among C$_3$-cool, C$_3$-warm and C$_4$ species (P<0.05 according to one-way ANOVA followed by Duncan's test) are denoted by different letters. The optimum growth temperature (T_{growth}) for each species is shown in Table 2. c, scaling constant; ΔH_a, activation energy; Q_{10} coefficient over the temperature intervals of 5–15 ºC (Q_{10}^{15-5}), 15–25 ºC (Q_{10}^{25-15}), 25–35 ºC (Q_{10}^{35-25}) and 35–45 ºC (Q_{10}^{45-35}).

warm habitats were non-significant across the range of temperatures of measurement. The temperature dependence of K_c varied within Spermatophyta, with Rubisco from C$_4$ plants presenting lower values of ΔH_a compared with C$_3$ plants from warm habitats, while differences between C$_3$ plants from cool and warm habitats were non-significant (Table 3). Differences in Q_{10} for K_c among higher plants groups were non-significant.

Reanalysing the k_{cat}^c data compilation of Galmés *et al*. (2015) in terms of Q_{10}, we note that in the comparisons of the temperature response of k_{cat}^c among Spermatophyta, Rubisco from C$_4$ plants presented lower values of ΔH_a and Q_{10} than C$_3$ plants from warm habitats (Table 3). Similarly to the $S_{c/o}$ temperature dependence, k_{cat}^c of C$_3$ plants from warm environments was more sensitive to increases in temperature (i.e. higher ΔH_a or Q_{10}) than those of C$_3$ plants from cool environments (Table 3).

The calculated values for ΔH_a integrate all data of the temperature response curve, while Q_{10} values refer to specific thermal ranges of the curve. With the exception of ΔH_a *vs*. Q_{10} for K_c in the comparison among higher plant groups, the trends observed for Q_{10}, in terms of species or groups comparison, are identical to those described for ΔH_a (Table 3) in all kinetic parameters. Due to the changes in scaling exponent of the temperature response of Rubisco kinetics, values of Q_{10} for $S_{c/o}$ increase from Q_{10}^{15-5} to Q_{10}^{45-35}, while they decrease for K_c and k_{cat}^c, in all groups of species.

Phylogenetic signals were not significant in ANCOVA models (P-values for $S_{c/o}$, K_c and k_{cat}^c within Spermatophyta were 0.519, 0.114 and 0.742, respectively). This fact indicates that when corrected for the phylogenetic signal, the comparison of $S_{c/o}$ and K_c values at given temperatures (Figs 1B and 2B), as well as the differences in temperature dependence
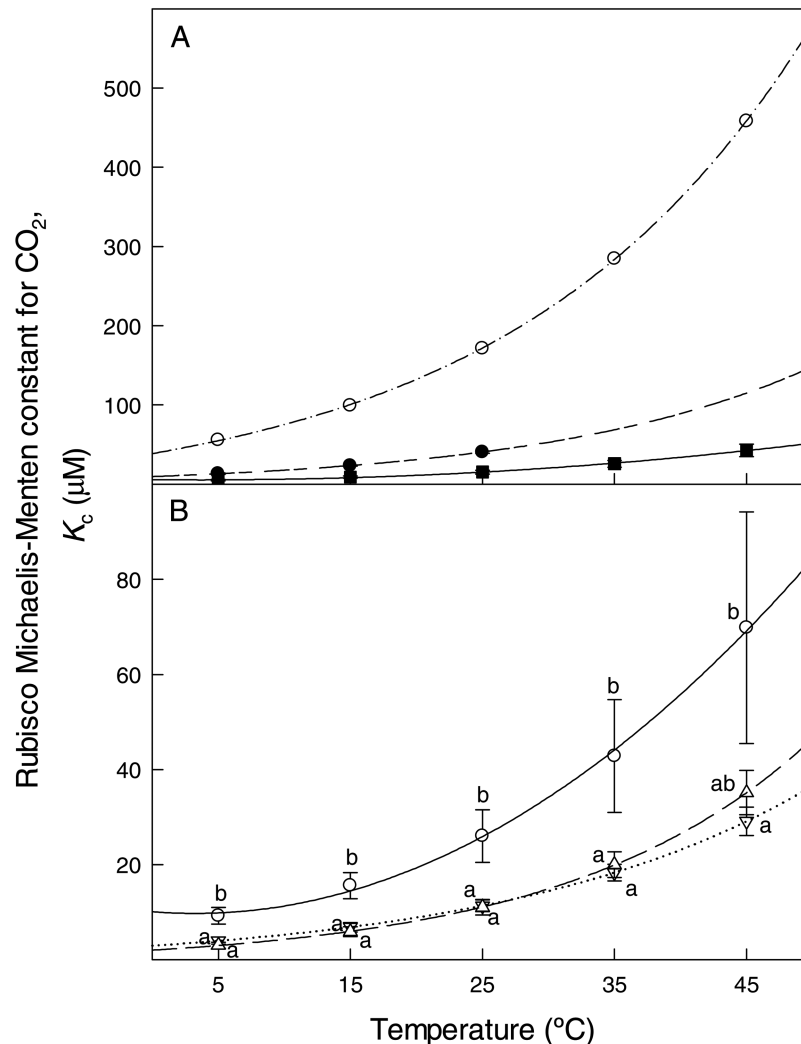
Fig. 2. Values of the Rubisco Michaelis–Menten constant for CO_2 (K_c) in liquid phase at a range of temperatures in different phylogenetic groups (A) and in land plants only (B). (A) Open circles and dash-dotted line, Cyanobacteria; filled circles and dashed line, Bacillariophyta (diatoms); filled squares and solid line, Spermatophyta (plants). Sample number $n=2$ for Bacillariophyta and $n=17$ for Spermatophyta; no replication was available for Cyanobacteria. (B) Open downward triangles and dotted line, C_3 plants from cool habitats ($n=5$); open upward triangles and dashed line, C_3 plants from warm habitats ($n=7$); open circles and dotted line, C_4 plants ($n=5$). Values for K_c correspond to *in vitro* measurements at discrete temperatures from data shown in Table 3 after applying Eq. 12, and were standardized to a common set of CO_2 liquid-phase physico-chemical characteristics as explained in Fig. 1 (Table 1 for Henry's law constants for CO_2 and O_2 that can be used to convert the values reported to gas-phase equivalents). Data presentation as in Fig. 1.

parameters for $S_{c/o}$, K_c and k_{cat}^c among Spermatophyta groups (Table 3), were qualitatively identical to the conventional statistics (ANOVA).

Relationship between the energies of activation of Rubisco catalytic traits and the species optimum growth temperature

An inverse relationship was found between ΔH_a for $S_{c/o}$ and the species average optimum growth temperature (T_{growth}), suggesting that $S_{c/o}$ values of Rubisco from species inhabiting hot environments present a higher sensitivity to changes in temperature (Fig. 3A). Although this relationship was significant and not affected by the species' phylogeny (Pagel's $\lambda=0.575$), it was substantially influenced by the values of the thermophiles *Thermosynechococcus elongatus* (Cyanobacteria) and *Galdieria partita* (Rhodophyta), which presented the highest T_{growth} and the lowest ΔH_a for $S_{c/o}$.

Furthermore, *Synechococcus lividus*, with the same T_{growth} as *Galdieria partita* (45 °C), had the highest ΔH_a for $S_{c/o}$ among all the species studied. Nevertheless, when the data were reanalysed without these three species with $T_{growth}>40$ °C, there was still a significant correlation between ΔH_a for $S_{c/o}$ and T_{growth} ($r^2=0.175$, $P<0.02$ for a second-order polynomial regression). Clearly, there is evidence of common trends in temperature scaling of $S_{c/o}$ across disparate phylogenetic groups, indicating that convergent evolution has led to similar functional responses. In contrast, the relationships between T_{growth} and ΔH_a for K_c and k_{cat}^c were non-significant (Figs 3B, C). The relationships were analogous with Q_{10} values (data not shown).

When considering group averages, a negative relationship was found between ΔH_a for $S_{c/o}$ and ΔH_a for k_{cat}^c in all groups except the Cyanobacterium *T. elongatus* (Fig. 4). This relationship was significant both when all plants were averaged and when plant functional and photosynthetic groupings
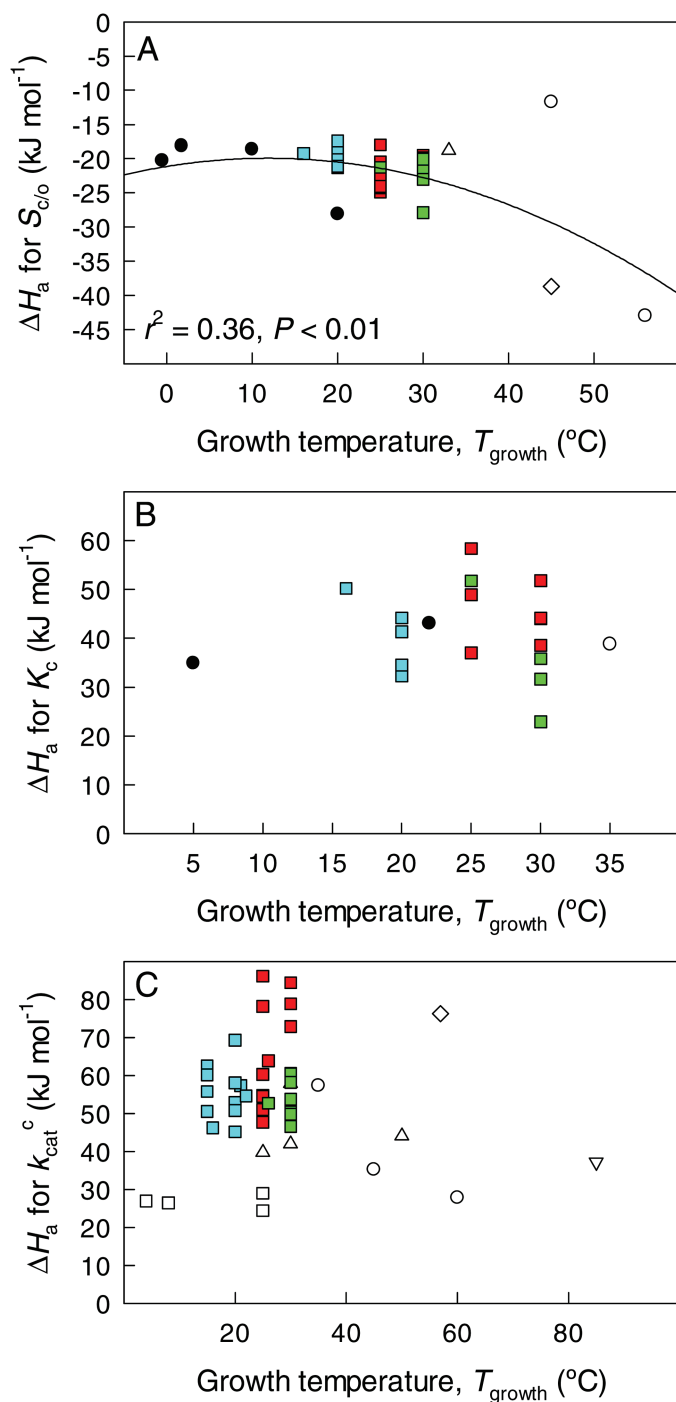
Fig. 3. The relationship between the growth temperature (T_{growth}) and the energy of activation (ΔH_a) for (A) the Rubisco specificity factor for CO_2/O_2 ($S_{c/o}$) in liquid phase, (B) the Rubisco Michaelis–Menten constant for CO_2 (K_c) in liquid phase, and (C) the Rubisco maximum carboxylase turnover rate (k_{cat}^c). Each symbol corresponds to individual species (Table 2 for data sources). Open upward triangles, Proteobacteria; open circles, Cyanobacteria; black circles, Bacillariophyta (diatoms); open squares, Chlorophyta (green algae); open diamond, Rhodophyta (red algae); blue squares, C_3 plants from cool habitats; red squares, C_3 plants from warm habitats; green squares C_4 plants. *In vitro* estimates at discrete temperatures were standardized for study-to-study differences in physico-chemical characteristics for CO_2 and O_2 used as in Figs 1 and 2 and the temperature responses were fitted by Eq. 12. In (A), the data were fitted by a non-linear equation in the form $y=-20.911+0.207x-0.009x^2$. In (B) and (C), data fits by linear and different monotonic non-linear equations were statistically not significant (best r^2 values were 0.090 for (B) and 0.115 for (C), $P>0.1$ for both).
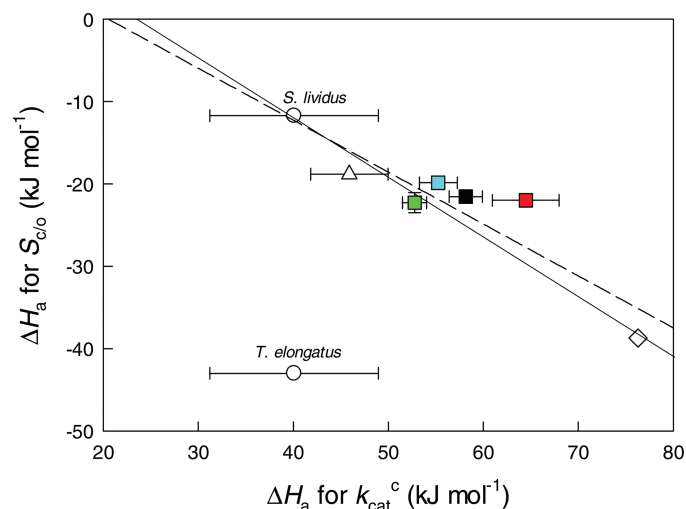
Fig. 4. The relationship between the energies of activation (ΔH_a) for the Rubisco maximum carboxylase turnover rate (k_{cat}^c) and the Rubisco specificity factor for CO_2/O_2 ($S_{c/o}$) in liquid phase across domains of life and photosynthetic and ecological groupings of plants (symbols as in Fig. 3). Data were separately fitted by linear regressions across domains of life (all plants averaged; solid line, $r^2=0.952$, $P<0.01$) and across all groupings (plant functional and photosynthetic groupings, C_3 cool and warm and C_4, separately considered; dashed line, $r^2=0.846$, $P<0.01$). In Rhodophyta, the value of ΔH_a for $S_{c/o}$ is from *Galdieria partita*, while that of ΔH_a for k_{cat}^c is from *Cyanidium caldarium*. For the other phylogenetic groups, data correspond to averages±SE from different numbers of species (Table 3 for data sources). Data for *Thermosynechococcus elongatus* (Cyanobacteria) with vastly different Rubisco kinetics (Figs 1 and 2) were not considered in the regression analysis.

were separately considered, and was not affected by the phylogenetic signal (Pagel's λ=−0.936, and ANCOVA $P=0.21$, respectively).

Temperature dependence of in vivo-estimated Rubisco kinetics and the relationship with the temperature parameters derived from in vitro measurements

In the six species with available data, *in vivo*-estimated $S_{c/o}$ decreased with increasing temperature, although important differences existed among the species in the rate of decrease (Fig. 5A). As a consequence, ΔH_a for $S_{c/o}$ varied 2-fold between *Arabidopsis thaliana* (−20 kJ mol^{-1}) and *Epilobium hirsutum* (−40.8 kJ mol^{-1}; Table 4). The average values of *in vitro* $S_{c/o}$ for C_3 plants fell within the lower range of the *in vivo*-estimated values, and the *in vitro*-based ΔH_a for $S_{c/o}$ (−21.4 kJ mol^{-1}, Table 3) was similar to that estimated *in vivo* for *A. thaliana* and *S. oleracea* (Fig. 5A).

An increase in the *in vivo* K_c with increasing temperature of measurement was observed for all the species (Fig. 5B), but the ΔH_a for K_c varied three-fold between 17.1 kJ mol^{-1} for *Agropyron smithii* (Monson *et al.*, 1982) and 59.5 kJ mol^{-1} for *Nicotiana tabacum* (Bernacchi *et al.*, 2001) (Table 4). The average (±SE) energy of activation for *in vitro* K_c in C_3 plants was 43.8 ± 2.2 kJ mol^{-1} (Table 3), i.e. higher than all the *in vivo*-based values except that for *N. tabacum* described in Bernacchi *et al.* (2001).

The temperature response of *in vivo*-estimated K_o has been reported for *N. tabacum*, *A. thaliana* and *Glycine max*
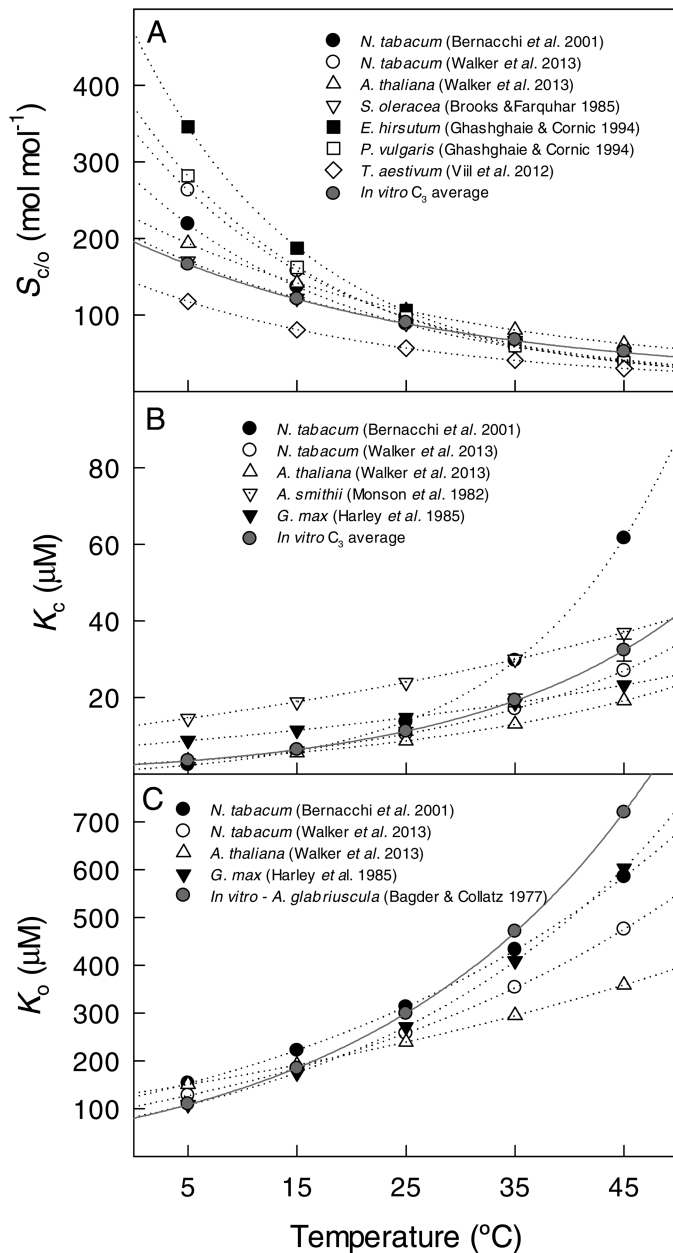
Fig. 5. Values of the Rubisco specificity factor for CO_2/O_2 ($S_{c/o}$) (A) and the Michaelis–Menten constants for CO_2 (K_c) (B) and O_2 (K_o) (C) at a range of temperatures. Values for these parameters were obtained at discrete temperatures from *in vivo* gas-phase data (shown in Table 4) after applying Eq. 12 and converted to the liquid phase by Eqs 8–10 (Table 1 for corresponding Henry's law constants to convert between liquid-phase and gas-phase equivalents). For comparative purposes, *in vitro* C_3 average values for $S_{c/o}$ and K_c have been also included (using data shown in Table 3). In (C), *in vitro* K_o data for *Atriplex glabriuscula* (Badger and Collatz 1977, shown in Table 2) that have been widely used to model leaf photosynthesis (see Introduction) have been also included.

(Table 4). In contrast to some *in vitro* data (Table 2), all *in vivo* data exhibited a positive scaling of K_o with temperature (Fig. 5C). Compared with the *in vivo* K_o values, the *in vitro* K_o of the C_3 plant *Atriplex glabriuscula* (Badger and Collatz, 1977) presented lower values at temperatures below 25 °C and higher values at temperatures above 25 °C. The

higher thermal sensitivity of the *in vitro* K_o of *A. glabriuscula* resulted in a higher ΔH_a for K_o (34.6 kJ mol^{-1}, Table 2) compared with the *in vivo*-based values for ΔH_a of other plants (Table 4).

In the case of *in vivo* data, only the study of Walker *et al.* (2013) considered the leaf mesophyll conductance, i.e. used the 'true' chloroplastic CO_2 concentration in the derivation of Rubisco characteristics by inverting the FvCB photosynthesis model. Indeed, $S_{c/o}$ and K_c temperature characteristics for *A. thaliana* estimated in this study are closer to C_3 average *in vitro* data than most of the other estimates (Fig. 5A, B). However, in *N. tabacum*, $S_{c/o}$ *in vivo* temperature characteristics estimated without mesophyll conductance in the study of Bernacchi *et al.* (2001) are actually closer to C_3 average estimates than *N. tabacum in vivo* characteristics estimated by considering mesophyll conductance in the study of Walker *et al.* 2013 (Fig. 5).

For several species, the temperature responses of Rubisco characteristics had been reported both *in vitro* and *in vivo*, whereas all these *in vivo* analyses had been conducted without considering mesophyll conductance. As for the temperature response of $S_{c/o}$, *in vitro* and *in vivo* data were available for *Spinacia oleracea* and *Triticum aestivum* (Fig. 6A, B). In *S. oleracea*, *in vitro* (average from different references) and *in vivo* (Brooks and Farquhar, 1985) data for $S_{c/o}$ were similar over the range of measurement temperatures (Fig. 6A). In *T. aestivum*, *in vitro* values (average from different references) were higher than the *in vivo* ones (Viil *et al.*, 2012) at all temperatures of measurement (Fig. 6B). It should be noted that differently from all other *in vivo* studies, Viil *et al.* (2012) used *in vivo* $^{14}CO_2$ leaf uptake for $S_{c/o}$ derivation.

In vitro and *in vivo* data on the temperature response of K_c have been published for *Glycine max* and *Agropyron smithii* (Fig. 6C, D). In *G. max*, *in vitro* (Laing *et al.*, 1974) and *in vivo* (Harley *et al.*, 1985) reported data were similar over the temperature range 5–25 °C, but at higher temperatures of measurement *in vitro* values were higher than *in vivo* values (Fig. 6C). In *A. smithii*, *in vivo* estimations for K_c were higher than *in vitro* values at all temperatures of measurement (Fig. 6D). *Glycine max* is the unique species for which the temperature response of K_o has been examined both *in vitro* and *in vivo* (Fig. 6E). Differently from other comparisons between *in vitro* and *in vivo* values for $S_{c/o}$ and K_c that were at least qualitatively similar, the *in vivo* values of K_o (Harley *et al.*, 1985) increased exponentially with increasing temperature, but the *in vitro* values (Laing *et al.*, 1974) tended to decrease.

Variation in the temperature responses of Rubisco kinetics: implications for modeling photosynthesis

The simulation analysis combining all different Rubisco kinetic characteristics indicated that the temperature responses of Rubisco kinetics of C_3 plants from cool environments resulted in higher simulated Rubisco-limited gross photosynthesis rate ($A_{Rubisco}$) at lower temperatures than in C_3 species from warm environments, while the latter species performed better at higher temperatures (Fig. 7), although the overall differences among the cool and warm C_3 species were moderate.

Table 4. *Temperature dependence parameters of* in vivo-*measured Rubisco catalytic constants for Spermatophyta*

Group	Species	Reference	T_{meas} (°C)	c	ΔH_a (kJ mol^{-1})	r	Q_{10}^{15-5}	Q_{10}^{25-15}	Q_{10}^{35-25}	Q_{10}^{45-35}	T_{growth} (°C)
$S_{c/o}$											
C$_3$ plants from cool habitats	*Epilobium hirsutum*	Ghashghaie and Cornic (1994)	16–28	−11.8	−40.8	0.978	0.54	0.57	0.59	0.61	18.0
	Spinacia oleracea hybrid 102	Brooks and Farquhar (1985)	15–30	−4.5	−22.3	0.993	0.72	0.73	0.75	0.76	16.0
	Triticum aestivum cv. Saratovskaya 29	Viil *et al.* (2012)	5–41	−6.1	−25.2	0.997	0.69	0.70	0.72	0.73	20.0
	Arabidopsis thaliana cv. Columbia	Walker *et al.* (2013)	15–35	−3.8	−20.9	0.956	0.73	0.75	0.76	0.77	22.0
C$_3$ plants from warm habitats	*Nicotiana tabacum* cv. W38	Bernacchi *et al.* (2001)	10–40	−8.2	−31.4	0.997	0.62	0.64	0.66	0.68	25.0
	Nicotiana tabacum	Walker *et al.* (2013)	15–35	−9.2	−34.2	0.950	0.60	0.62	0.64	0.66	25.0
	Phaseolus vulgaris	Ghashghaie and Cornic (1994)	12–32	−10.3	−36.8	0.973	0.58	0.60	0.62	0.64	25.0
K_c											
C$_3$ plants from cool habitats	*Arabidopsis thaliana* cv. Columbia	Walker *et al.* (2013)	15–35	14.8	31.4	0.946	1.60	1.55	1.51	1.47	22
C$_3$ plants from warm habitats	*Agropyron smithii*	Monson *et al.* (1982)	10–40	10.1	17.1	0.933	1.29	1.27	1.25	1.23	25
	Glycine max cv. P61–22	Harley *et al.* (1985)	20–40	10.0	18.0	0.993	1.31	1.29	1.27	1.25	25
	Nicotiana tabacum cv. W38	Bernacchi *et al.* (2001)	10–40	26.6	59.5	0.995	2.44	2.30	2.18	2.07	25
	Nicotiana tabacum	Walker *et al.* (2013)	15–35	17.5	37.6	0.991	1.76	1.69	1.64	1.59	25
K_o											
C$_3$ plants from cool habitats	*Arabidopsis thaliana* cv. Columbia	Walker *et al.* (2013)	15–35	11.9	16.0	0.734	1.27	1.25	1.23	1.22	22
C$_3$ plants from warm habitats	*Nicotiana tabacum* cv. W38	Bernacchi *et al.* (2001)	10–35	15.7	24.6	0.991	1.45	1.41	1.38	1.35	25
	Nicotiana tabacum	Walker *et al.* (2013)	15–35	15.3	24.1	0.935	1.44	1.40	1.37	1.34	25
	Glycine max cv. P61–22	Harley *et al.* (1985)	20–40	18.3	31.5	0.988	1.60	1.55	1.51	1.47	25

Species were classified as C$_3$ and C$_4$ species, and C$_3$ species were further divided among warm- and cool-temperature species according to their optimum growth temperature (Table 2). *c*, scaling constant; ΔH_a, activation energy; K_c, Michaelis–Menten constant for CO$_2$; K_o, Michaelis–Menten constant for O$_2$; Q_{10}, coefficient over the temperature intervals of 5–15 °C (Q_{10}^{15-5}), 15–25 °C (Q_{10}^{25-15}), 25–35 °C (Q_{10}^{35-25}) and 35–45 °C (Q_{10}^{45-35}); *r*, correlation coefficient for linear regressions between measured *vs.* predicted (Microsoft Excel Solver function) values of each kinetic parameter at the assayed temperatures; $S_{c/o}$, specificity factor for CO$_2$/O$_2$; T_{meas}, range of measurement temperature.

The mean absolute difference (σ_A, Eq. 15) between cool and warm C$_3$ species was 0.39 µmol m^{-2} s^{-1} at chloroplastic CO$_2$ concentration (C_c) of 120 µmol mol^{-1}, and 2.9 µmol m^{-2} s^{-1} at C_c=400 µmol mol^{-1}, and corresponding root mean squared differences (σ_S, Eq. 16) were 0.20 µmol m^{-2} s^{-1} at C_c=120 µmol mol^{-1} and 12.5 µmol m^{-2} s^{-1} at C_c=400 µmol mol^{-1}.

The predicted quantitative differences in simulated $A_{Rubisco}$ between C$_3$ plants from cool and warm environments depended on C_c. Hence, at lower temperature, the higher simulated $A_{Rubisco}$ for C$_3$ plants from cool environments, compared with that for C$_3$ plants from warm environments, was more evident at C_c of 120 µmol mol^{-1} (for the temperature range of 5–20 °C, average±SE $A_{Rubisco}$ was 7.7±0.2 µmol m^{-2} s^{-1} for C$_3$ cool *vs.* 7.3±0.3 µmol m^{-2} s^{-1} for C$_3$ warm; means are significantly different at $P<0.001$ according to paired samples *t*-test). At higher temperature, the enhancement in simulated $A_{Rubisco}$ for C$_3$ plants from warm environments was greater at C_c of 400 µmol mol^{-1} (Fig. 7; for the temperature range of 30–50 °C, average±SE $A_{Rubisco}$ was 36.0±0.4 µmol m^{-2} s^{-1} for C$_3$ cool *vs.* 40.8±0.7 µmol m^{-2} s^{-1} for C$_3$ warm; means are significantly different at $P<0.001$ according to paired samples

t-test). Of course, this simulation is only based on two groups of species, and it is further important that there is a significant within group variability in any of the Rubisco temperature traits that is not related to growth temperature (Fig. 3).

An analogous modeling exercise was conducted to compare the potential effects of using the average temperature parameters of C$_3$ Rubiscos (taken from the *in vitro* compilation, Tables 2 and 3) and any of the three datasets widely used in photosynthesis modeling (Fig. 8). As with the C$_3$ cool *vs.* warm comparison, the differences among simulated $A_{Rubisco}$ by different Rubisco temperature responses depended on the temperature range and C_c, but in general, the *in vitro* datasets, i.e. *in vitro* C$_3$ average from the present study and that of *S. oleracea* from Jordan and Ogren (1984), yielded higher simulated $A_{Rubisco}$ than the *in vivo* datasets based on *N. tabacum* from Bernacchi *et al.* (2001) and Walker *et al.* (2013). Irrespective of C_c, simulated $A_{Rubisco}$ of the *in vitro* C$_3$ average was similar to simulated $A_{Rubisco}$ of *N. tabacum* from Walker *et al.* (2013) at the lower temperature range (<15 °C), and similar to that of *S. oleracea* from Jordan and Ogren (1984) at the higher temperature range (>40 °C). Between 15 and 40 °C,
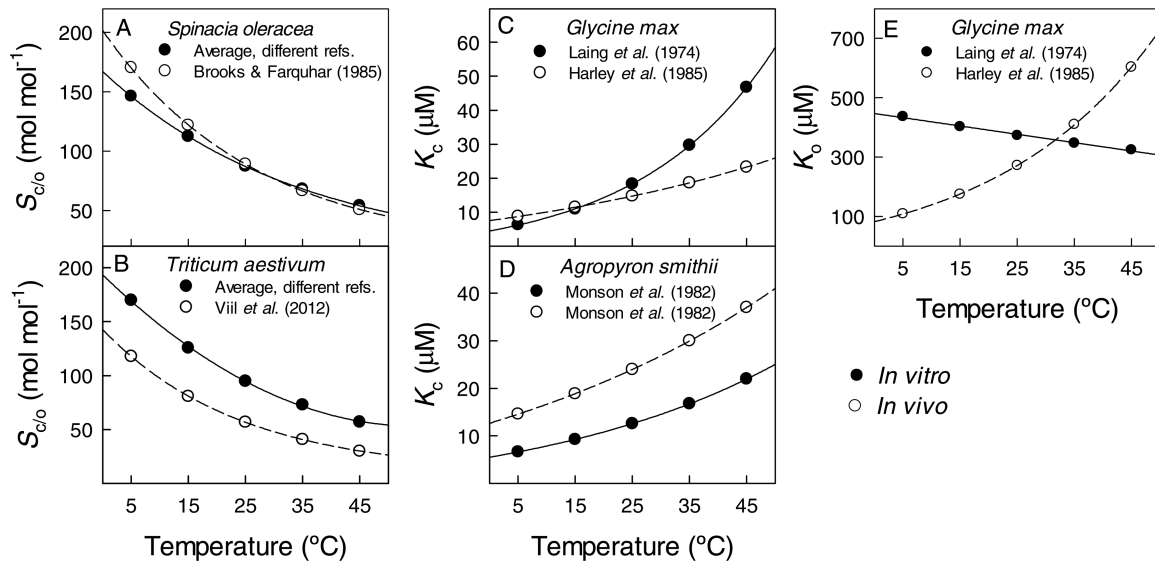
Fig. 6. Comparisons between *in vitro* (filled symbols) and *in vivo* (open symbols) values of the Rubisco specificity factor for CO_2/O_2 ($S_{c/o}$) (A, B) and the Michaelis–Menten constants for CO_2 (K_c) (C, D) and O_2 (K_o) (E) at a range of temperatures for species with available data. Equation 12 was used to derive estimates for these parameters at discrete temperatures from data in Tables 2 and 4. The *in vitro* liquid-phase data were standardized for a common set of physico-chemical characteristics of CO_2 and O_2 as explained in Figs 1 and 2, while the *in vivo* gas-phase data were converted to the liquid phase as explained in Fig. 5 (Table 1 for pertinent Henry's law constant to convert between liquid- and gas-phase equivalents). $S_{c/o}$ data for *Spinacia oleracea* are averages for the studies Jordan and Ogren (1984), Uemura *et al.* (1997), Zhu *et al.* (1998) and Yamori *et al.* (2006). $S_{c/o}$ data for *Triticum aestivum* are averages for studies Haslam *et al.* (2005) and Hermida-Carrera *et al.* (2016).

simulated $A_{Rubisco}$ of the *in vitro* C3 average was lower than that of *S. oleracea* (Jordan and Ogren, 1984) and higher than that of *N. tabacum* (Bernacchi *et al.*, 2001; Walker *et al.*, 2013). At all C_c values used in the simulations, differences between simulated $A_{Rubisco}$ of the *in vitro* C3 average and *in vivo* estimates (Bernacchi *et al.*, 2001; Walker *et al.*, 2013) became larger at higher temperatures (Fig. 8, according to paired samples *t*-tests, $P<0.001$ for all these comparisons mentioned above).

The differences in predicted $A_{Rubisco}$ temperature relationships from the three datasets of Rubisco kinetics were extensive. For 12 comparisons with temperature response curves derived for four C_c (120, 150, 200 and 400 µmol mol^{-1}), average $\sigma_A \pm SE = 4.5 \pm 0.8$ µmol m^{-2} s^{-1} and $\sigma_S = 31 \pm 12$ µmol m^{-2} s^{-1}. These differences were much greater than the differences in predicted $A_{Rubisco}$ temperature relationships among cool and warm C3 species (Fig. 7 *vs*. Fig. 8; see above for σ_A and σ_S values), and the differences in predicted $A_{Rubisco}$ temperature responses for cool and warm C3 species from the $A_{Rubisco}$ responses predicted by the mean *in vitro* data for C3 species (for these eight comparisons, average $\sigma_A = 0.65 \pm 0.18$ µmol m^{-2} s^{-1} and average $\sigma_S = 0.93 \pm 0.48$ µmol m^{-2} s^{-1}). Indeed, the average σ_A and σ_S values for $A_{Rubisco}$ predictions using C3 *in vitro* kinetics and the average σ_A and σ_S values for $A_{Rubisco}$ predictions using the three selected Rubisco kinetics datasets were significantly different ($P<0.005$ according to pairwise *t*-tests).

Discussion

Rubisco in vitro *temperature relationships: caveats and potentials*

To our knowledge, the present study provides the most complete dataset of temperature dependences of Rubisco kinetic characteristics including altogether 17 *in vitro* and seven *in vivo* studies providing information for 49 species from most autotrophic kingdoms of life (Tables 2 and 4). As with any meta-analysis, creating such a synthetic summary inevitably requires analysing the effects of different experimental protocols on the observed trait values. In the case of Rubisco, one should be particularly careful when comparing data from different labs, due to differences in the assay conditions and the calculation of CO_2 concentration in the assay medium.

In our analysis, we have considered all the assay medium conditions and developed appropriate corrections to standardize for study-to-study differences in assay conditions (Eqs 1–7). In addition to the effects of assay medium composition on the equilibrium CO_2 concentration, which is known to affect K_c estimations (Yokota and Kitaoka, 1985, Eqs 1 and 2), we have further highlighted the potential problems with O_2 solubility that affect K_o estimation (Eqs 3–6). Furthermore, appropriate corrections were provided for $S_{c/o}$ (Eq. 7), which is potentially affected by both bicarbonate equilibrium and O_2 solubility. Although the corrections of K_c, K_o and $S_{c/o}$ values at any given temperature were large in several cases, the temperature effect on these corrections was relatively weak, such that the absolute trait values at all temperatures were similarly affected. Thus, the overall temperature sensitivity as characterized by the activation energy (ΔH_a) and Q_{10} values was much less affected by the applied corrections (in most cases <5%).

Although part of the compiled variation may still be due to differences in the methodology from the different reports, we argue that with the corrections applied we have overcome discrepancies in the concentration of CO_2 and O_2 in the assay media. Furthermore, data were filtered as explained in the Methods, with the most problematic reports being excluded
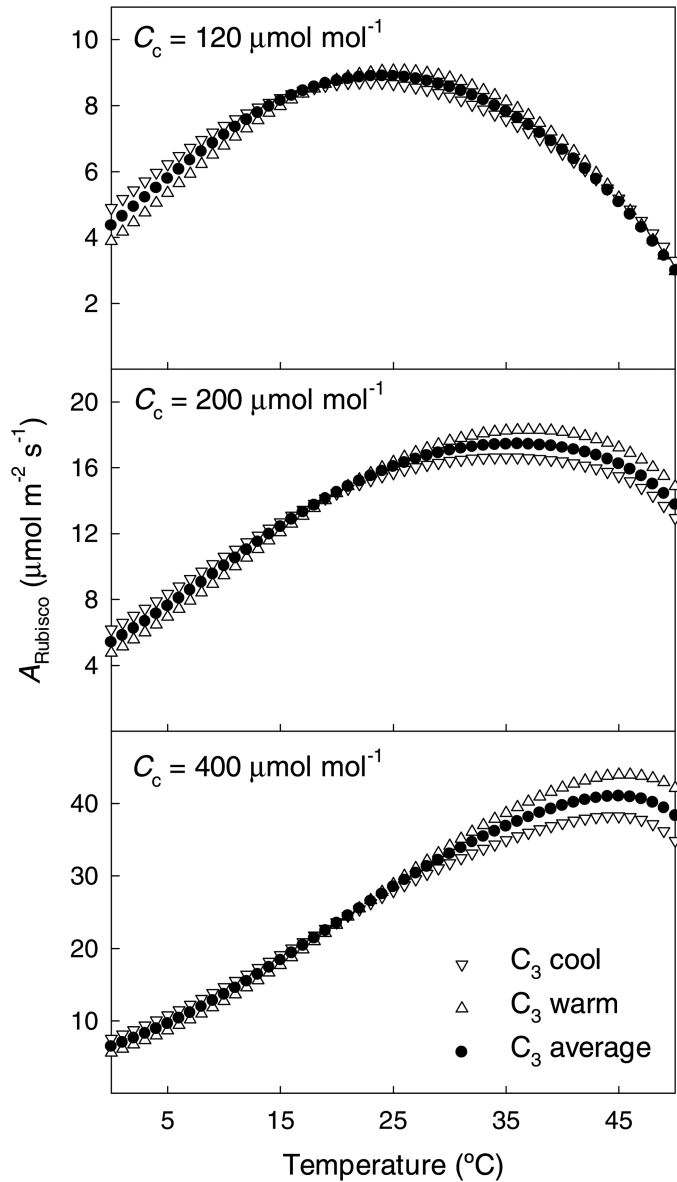
Fig. 7. Modeling effect of the different temperature responses of Rubisco kinetic parameters from C₃ plants from cool habitats (open downward triangles), C₃ plants from warm habitats (open upward triangles) and C₃ average (open circles) on the Rubisco-limited gross assimilation rate ($A_{Rubisco}$) at chloroplastic CO_2 concentrations (C_c) of 120, 200 and 400 µmol mol⁻¹. To model $A_{Rubisco}$ at different temperatures, the values for the temperature dependence parameters of $S_{c/o}$, K_c and k_{cat}^c were taken from Table 3 (see Methods for further details). We simulated gross assimilation here to avoid confounding effects of mitochondrial respiration.

Fig. 8. Comparison of the Rubisco-limited gross photosynthesis ($A_{Rubisco}$) among average of *in vitro* data reported for C₃ plants and three widely cited datasets at chloroplastic CO_2 concentrations (C_c) of 120, 200 and 400 µmol mol⁻¹. The temperature dependence parameters of $S_{c/o}$, K_c, k_{cat}^c and K_o for *in vitro* average C₃ plants (shown in Table 3) were the same as in Fig. 7, while those for *in vivo Nicotiana tabacum* (Bernacchi *et al.*, 2001; Walker *et al.*, 2013) and *in vitro Spinacia oleracea* (Jordan and Ogren, 1984) were obtained from the original papers (shown in Tables 2 and 4). Bernacchi *et al.* (2001), Walker *et al.* (2013) and Jordan and Ogren (1984) did not report values of the deactivation energy (ΔH_d) and the entropy term (ΔS) for k_{cat}^c, and the simulation assumed identical values to those used for the *in vitro* average C₃ plants (indicated in the Methods). Note that Bernacchi *et al.* (2001) *in vivo* values have been derived without considering mesophyll conductance, while mesophyll conductance has been included in the *in vivo* estimates of Walker *et al.* (2013).

from the analyses. Analysis of the data also indicates that (i) independent measurements for the same species yielded similar ΔH_a and Q_{10} values (see $S_{c/o}$ for *Spinacia oleracea* and *Flaveria pringlei* in Table 2), and (ii) the variability within the main groups of species was low (Tables 2 and 3), suggesting that the approach developed for *a posteriori* standardization of Rubisco characteristics was sound. Thus, we consider that the temperature responses reported here are robust, and that the data in Tables 2 and 3 mainly reflect biological differences.

We note that, although up to seven phylogenetic groups had temperature responses of Rubisco kinetics, data for some
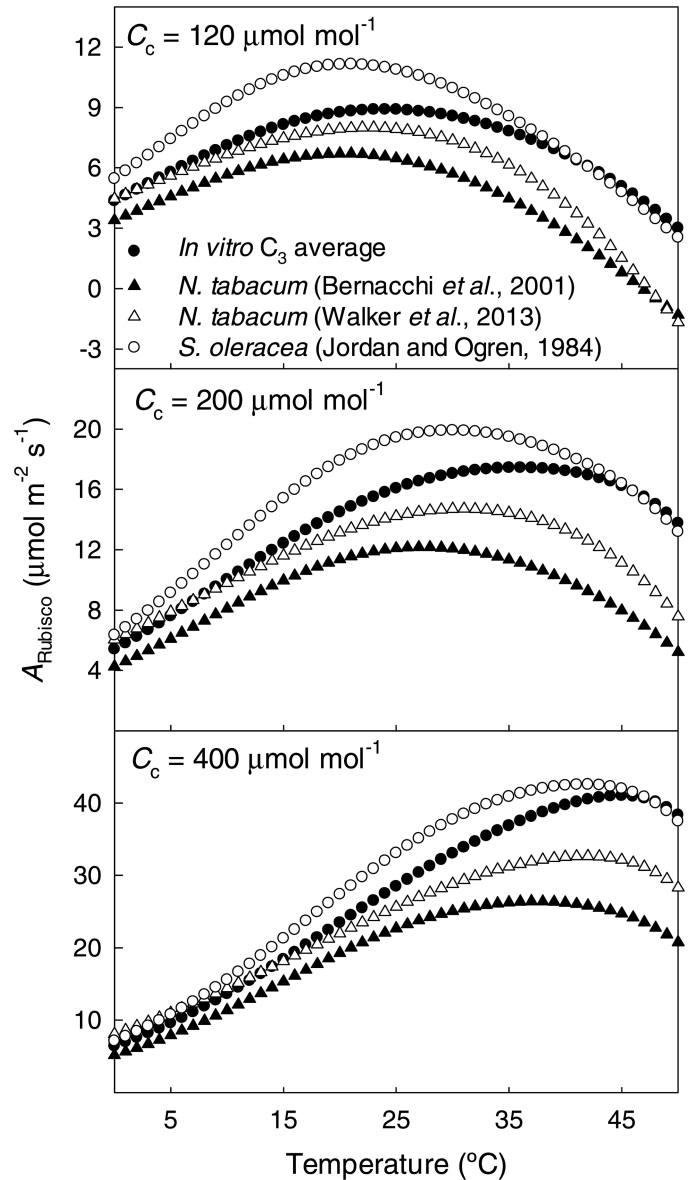
phylogenetic groups (in particular Archaea, Proteobacteria, Cyanobacteria and Rhodophyta) were represented by very few species. We therefore suggest that more species of these phylogenetic groups should be surveyed in the future to confirm the trends observed in the present database. Future surveys should include the analysis of the temperature responses

of Rubisco kinetics from non-vascular plant groups (liverworts, hornworts and mosses) and ferns, which have not been studied so far. This would allow gaining deeper insight into the evolutionary trends in the temperature response of Rubisco catalysis.

What do we learn from the compiled dataset? Important species variations in the temperature dependence of Rubisco kinetics suggest adaptation to the thermal environment and photosynthetic mechanism of the species

Among *in vitro* data, $S_{c/o}$ (the present study) and k_{cat}^c (Galmés *et al.*, 2015) are the best documented traits, compared with K_c and especially K_o, in terms of its temperature response (Table 2). With the exception of the excluded study by Ezaki *et al.* (1999), showing an increase in $S_{c/o}$ of *Thermococcus kodakariensis* with increasing temperatures, all compiled reports agree that $S_{c/o}$ decreases with increasing temperature, while k_{cat}^c and K_c increase (Figs 1 and 2; Galmés *et al.*, 2015).

Despite general trends, the data indicate that the temperature response of Rubisco kinetics varies among phylogenetic groups (Figs 1A and 2A; Galmés *et al.*, 2015). In this sense, Spermatophyta present higher ΔH_a for $S_{c/o}$ (in absolute values) than Proteobacteria and lower values compared with Rhodophyta (Table 3). A similar trend has been observed in the comparative analysis of ΔH_a for k_{cat}^c among these phylogenetic groups (Galmés *et al.*, 2015), indicative of adjustment in the temperature sensitivity of Rubisco kinetics. As for $S_{c/o}$, this adjustment seems to be related to the T_{growth} where the species evolved, with those species from warmer environments presenting a higher thermal dependence of $S_{c/o}$ (Fig. 3A).

The lack of correlation between ΔH_a for k_{cat}^c and T_{growth} apparently contradicts a co-adjustment of Rubisco kinetics to the prevailing environmental conditions (Fig. 3C), and may be attributed to the different set species included in each correlation. Actually, considering all species, the range of variation in ΔH_a for these two parameters is similar, about 3.5-fold (Table 2 and Galmés *et al.* 2015). Moreover, excluding the data for the cyanobacterium *Thermosynechococcus elongatus*, with vastly different Rubisco kinetics, the results suggest a significant correlation between ΔH_a for $S_{c/o}$ and k_{cat}^c (Fig. 4). In other words, those Rubiscos with a large increase in k_{cat}^c with temperature also present a large decrease in $S_{c/o}$. This finding may be related to the widely described trade-off between $S_{c/o}$ and k_{cat}^c at 25 ºC (Tcherkez *et al.*, 2006), suggesting that this trade-off is also valid at other temperatures. It should be noted that this correlation was made using average data for the different groups, and that different species were used to obtain the group averages. When the same correlation was analysed for the subset of species for which both $S_{c/o}$ and k_{cat}^c have been measured at different temperatures (nine Spermatophytes), a non-significant relationship was found (data not shown).

Within Spermatophyta, ΔH_a for k_{cat}^c and K_c of Rubiscos from C_4 plants was lower compared with C_3 plants from warm habitats (Table 3), in agreement with recent observations in congeneric species of *Flaveria* with contrasting photosynthetic mechanism (Perdomo *et al.*, 2015). Differences between C_3 plants from cool and warm habitats were significant in ΔH_a for k_{cat}^c, but not in ΔH_a for K_c. On average, the ratio between ΔH_a for k_{cat}^c and ΔH_a for K_c was 1.37, 1.40 and 1.52 for C_3 plants from cool and warm habitats and C_4 plants, respectively. This observation indicates that the carboxylase catalytic efficiency (k_{cat}^c/K_c) varies with temperature and suggests that k_{cat}^c/K_c increases more steeply with temperature in C_4 plants than in C_3 plants. Perdomo *et al.* (2015) reported the same trend between C_3 and C_4 *Flaveria* species, and related these differences to specific structural changes in the C_4 Rubisco causing a conformational change that favors product formation (i.e. higher k_{cat}^c) at the expense of weaker substrate affinity (i.e. higher K_c).

In spite of the trend for increased ΔH_a for $S_{c/o}$ (in absolute values) in C_3 plants from warm habitats as compared with C_3 plants from cool habitats, differences between these two groups averages were non-significant (Table 3). This result is in agreement with Galmés *et al.* (2005), who reported a tendency for higher temperature dependence for $S_{c/o}$ in Mediterranean C_3 species from warmer habitats, although with some exceptions (e.g. *Lysimachia minoricensis* from wettest and coolest environments displaying also a high activation energy). On the other hand, the non-significant difference in ΔH_a for $S_{c/o}$ between C_3 and C_4 plants is in line with the observations of Perdomo *et al.* (2015). Overall, differences in the temperature sensitivity between $S_{c/o}$ and k_{cat}^c/K_c suggest that the temperature dependences for k_{cat}^c/K_c and k_{cat}^o/K_o also differ among C_3 and C_4 plants.

Given the observed variability, we suggest using the corrected species-specific functions describing the temperature dependence of Rubisco kinetic parameters (shown in Table 2) to model photosynthesis in the different species. If the temperature response of Rubisco kinetic parameters has not been described for the species under study, we recommend using the groups' average values (presented in Table 3) to increase the accuracy of photosynthesis models in the different functional groups.

The temperature dependence of K_o: apparent contradictions among in vitro *data*

The temperature response of K_o based on *in vitro* data is available for five Spermatophyta species (Table 2), with contradictory results. A quantitatively similar value of ΔH_a for K_o to those of ΔH_a for K_c in Spermatophyta has been reported only for *Atriplex glabriuscula* (Badger and Collatz, 1977). The overall difficulty with K_o estimations is that Rubisco is much less specific for O_2 than for CO_2, and the oxygenase reaction of Rubisco is not saturated even in a 100% O_2 atmosphere. Estimation of K_o over an O_2 concentration range exhibiting only a slight non-linearity is extremely sensitive to any errors in gas concentrations. According to the compiled evidence, the temperature dependence of K_o for the other three species is very low compared with that for K_c, with values of ΔH_a for K_o close to zero, or even negative for *Glycine max* (Laing *et al.*, 1974) and *Setaria viridis* (Boyd *et al.*, 2015). Apparently, this discrepancy is not due to the specific methods used by the different studies as qualitatively different temperature scaling

functions have been observed using the same methodologies, and similar responses with different methodologies: Badger and Collatz (1977) and Lehnherr *et al.* (1985) measured oxygenase activity using an oxygen electrode, Jordan and Ogren (1984) obtained K_o from the inhibition of carboxylase activity at 50% O_2 (i.e. $K_{c,app}$), Laing *et al.* (1974) measured the oxygenase activity at different O_2 concentrations by spectrophotometric determination of a P-glycolate derivate, and Boyd *et al.* (2015) used membrane inlet mass spectrometry to measure the temperature responses of Rubisco kinetics.

Common trends and discrepancies between in vitro *and* in vivo*-based data on the temperature dependence of Rubisco kinetics*

In vivo data on the temperature response of K_o agree in that values of ΔH_a for K_o are positive (i.e. K_o increasing with assay temperature, Fig. 5C), although with lower values compared with the *in vitro*-based trend described for *Atriplex glabriuscula* (Badger and Collatz, 1977) (Tables 2 and 4). The comparison of the *in vitro* and *in vivo* measurements of the temperature response of K_o in *Glycine max* exemplifies how the discrepancies between *in vitro* and *in vivo* are particularly important for this kinetic parameter (Fig. 6E). Presence of some CO_2 is inevitable in *in vivo* measurements due to mitochondrial respiration, which could be affected by $[O_2]$ itself. Typically, only two, seldom three, O_2 concentrations have been used in K_o estimations, but given the high sensitivity of these measurements to O_2 concentration, we argue that both *in vivo* and *in vitro* measurements of K_o can be improved by using more O_2 concentrations in the assays. Overall, K_o (or the Michaelis–Menten constant of Rubisco for CO_2 at atmospheric conditions, K_c^{air}) is needed to model photosynthesis responses to temperature, and therefore, more *in vitro* and *in vivo* data on the temperature dependence of K_o are required in a larger number of species to confirm observed trends and solve discrepancies.

Contrary to K_o, *in vitro* and *in vivo* determinations of the temperature response of $S_{c/o}$ and K_c describe similar shapes, with the *in vitro* averages lying intermediate between *in vivo* estimates (Fig. 5). In this case, methodological issues associated with *in vitro* assays and *in vivo* estimations may determine the degree of agreement between *in vitro* and *in vivo* reports. For instance, *in vitro* and *in vivo* data on the temperature response of $S_{c/o}$ in *Spinacia oleracea* match very well between 15 and 45 ºC (Fig. 6A), with minor differences in the ΔH_a (Tables 2 and 4). The values of ΔH_a for $S_{c/o}$ in *Triticum aestivum* are also similar between *in vitro* (-19.7 kJ mol^{-1}) and *in vivo* (-25.2 kJ mol^{-1}), although absolute values of *in vitro* $S_{c/o}$ are approximately 1.6-fold higher than *in vivo* ones across the range of measurement temperatures (Fig. 6B).

Both *in vitro* and *in vivo* data on Rubisco kinetics present concerns implicitly linked to the specific methodologies. Since Monson *et al.* (1982), the first study discussing the possible reasons for discrepancies between *in vitro* and *in vivo* data, other studies have added to the debate on whether *in vitro* or *in vivo* data are more reliable and should be used in photosynthesis models (Ghashghaie and Cornic, 1994; von Caemmerer

et al., 1994; Rogers *et al.*, 2001; Galmés *et al.*, 2011*b*; Díaz-Espejo, 2013; Walker *et al.*, 2013). The main concerns related to *in vitro* measurements are that assay conditions may not be representative of the solute-rich environment of a chloroplast stroma where Rubisco operates, and that the extraction may not be fully efficient in terms of enzyme recovery and activation (Monson *et al.*, 1982; Rogers *et al.*, 2001; Galmés *et al.*, 2011*b*; Díaz-Espejo, 2013). The latter, however, will have no consequences for $S_{c/o}$, K_c and K_o assays since they are independent of Rubisco concentration. Furthermore, as we have observed, the differences in assay conditions have only a moderate effect on ΔH_a and Q_{10} values. In turn, difficulties in the *in vivo* estimations are mainly related to the quantification of the actual concentration of CO_2 at the site of carboxylation (C_c) and the rate of mitochondrial respiration in the light (R_{light}), in addition to the concerns associated with the leaf mesophyll heterogeneity in terms of photosynthetic capacity, CO_2 concentration and light reaching the chloroplasts (Ghashghaie and Cornic, 1994; von Caemmerer *et al.*, 1994; Díaz-Espejo, 2013; Walker *et al.*, 2013). The accurate quantification of C_c depends on the estimation of the leaf mesophyll conductance (g_m), which relies on a series of assumptions (see for instance Tholen *et al.*, 2012; Busch *et al.*, 2013; Loriaux *et al.*, 2013; Gu and Sun, 2014). The incorporation of g_m in photosynthesis models has resulted in an improvement of the *in vivo* estimates of Rubisco kinetics and partially reconciled *in vitro* and *in vivo* data (von Caemmerer *et al.*, 1994; Walker *et al.*, 2013). In addition to technical difficulties in both *in vitro* and *in vivo* measurements, we note that there has not been much attention on how to conduct valid comparisons among *in vivo* and *in vitro* data. As our methodological analysis demonstrates, the comparison is not trivial because the conversion of kinetic characteristics among gas (*in vivo* data) and liquid (*in vitro* data) phases is strongly dependent on equilibrium constants used (Eqs 8–10).

A principal problem with *in vivo* measurements is that derivation of Rubisco kinetics using inverse modeling techniques becomes increasingly challenging at the physiological limits, e.g. leaf temperatures above 35–40 °C and below 10–15 °C, and especially in conditions leading to heat (above *ca* 45 °C) and cold stress (below *ca* 5 °C). Under higher temperatures, enhanced mitochondrial respiration, inactivation of Rubisco, closure of stomata and unclear response of g_m preclude any accuracy of derivation of Rubisco kinetics (Crafts-Brandner and Salvucci, 2000; Atkin and Tjoelker, 2003; von Caemmerer and Evans, 2015). At lower temperatures, the challenges are associated with overall low gas-exchange fluxes due to low stomatal conductance and enzymatic capacities and again unclear effects of g_m (Ensminger *et al.*, 2012). Even if g_m, mitochondrial and stomatal effects could be accounted for, the principal problem still remaining is that Rubisco-limited photosynthesis under such conditions is driven by combined effects of Rubisco activase and Rubisco. In the case of heat and cold stress conditions, the situation is further complicated because of time-dependent reductions in enzymatic capacities (Niinemets and Keenan, 2014). On the other hand, the problems associated with *in vitro* assays at low and high temperatures can originate from low reaction rates and changes in the substrate concentration in the assay media due

to evaporation, respectively, which may increase the error associated with these measurements. It may well be that the lower Q_{10} values for K_c at higher temperatures observed for *in vivo* data compared with *in vitro* data (Fig. 5B) reflect the outlined problems with measurements at higher temperature. We suggest that both *in vivo* and *in vitro* measurements provide informative insight into the potential and actual *in vivo* kinetics of Rubisco and we call for more comparative measurements of *in vivo* and *in vitro* Rubisco kinetics in economically and ecologically relevant species.

Modeling Rubisco-dependent photosynthesis: how much do all the observed differences in temperature kinetics matter?

Although statistically important differences were observed in a number of Rubisco kinetic characteristics determined in *in vitro* studies (Table 3), the differences were relatively small, and the key question is how much do these differences matter when considered together? A comparison of simulated Rubisco-dependent photosynthesis among C_3 cool and warm species demonstrates that the temperature adaptation indeed improves modeled photosynthesis of C_3 cool species under lower temperatures, especially at lower chloroplastic CO_2 concentration, while the temperature adaptation increases modeled photosynthesis of C_3 warm species under higher temperatures, especially at higher chloroplastic CO_2 concentration (Fig. 7). This is an important outcome, suggesting that in addition to higher-level cellular, whole leaf and whole plant adaptation responses (e.g. Björkman *et al.*, 1980; Silim *et al.*, 2010; Muhl *et al.*, 2011), enzyme-level adaptation can lead to significant modifications in realized leaf photosynthesis in plants adapted to different climates. Of course, it is only a simulation for two groups of species that have relatively similar Rubisco characteristics and only considers the potential Rubisco-limited photosynthesis. Nevertheless, across all species, the activation energy for $S_{c/o}$ (Fig. 3A) and the deactivation energy for k_{cat} scaled with the growth temperature (Galmés *et al.*, 2015), indicating that the simulations shown for C_3 cool and warm species constitute a part of a broad trend of Rubisco temperature acclimation. Combining temperature responses of Rubisco-limited and RuBP-regeneration-limited photosynthesis further indicates that Rubisco-limited photosynthesis is a key driver of realized photosynthesis over much of the ambient temperature response for a large fraction of physiologically relevant quantum flux densities and chloroplastic CO_2 concentrations (Galmés *et al.* 2014*a*).

The difference among C_3 cool and warm plants was predicted to increase with increasing CO_2 concentration (Fig. 7). This difference in Rubisco temperature characteristics is expected to enhance the competitive potential of C_3 warm species relative to C_3 cool species in future climates with higher atmospheric CO_2 concentration and air temperature (Kirtman *et al.*, 2013). We believe that for realistic simulation of carbon gain in future climates, models of C_3 photosynthesis need to be modified to incorporate different Rubisco temperature kinetics of broad species groups to reflect modifications in Rubisco temperature kinetics to species' growth environment. Even by doing this, we still need to recognize the inherent variability in species' Rubisco temperature responses within species groups that we cannot explain by species' growth environment or by phylogenetic relationships (Fig. 3). Such a variability is of particular significance when species group averages need to be used for simulation of photosynthesis for any given species lacking measured Rubisco kinetic traits (or for derivation of FvCB photosynthesis model parameters). However, it is likely of less concern for simulating photosynthesis of multi-species canopies where species-specific effects average out and average kinetics for species groups are more appropriate.

In fact, the problem of selecting 'the appropriate' set of kinetic parameters for modeling C_3 photosynthesis has a long history starting with the publication of the FvCB photosynthesis model (Farquhar *et al.*, 1980). Early model applications used *in vitro* measurements for *Atriplex glabriuscula* (Badger and Collatz, 1977) or *Spinacia oleracea* (Jordan and Ogren, 1984), while today researchers increasingly use the *in vivo* dataset based on *N. tabacum* (Bernacchi *et al.*, 2001). While further *in vivo* datasets are becoming available (Walker *et al.*, 2013), *in vitro* parameters from either Badger and Collatz (1977) or Jordan and Ogren (1984) remain widely used today. In fact, according to Thomson Reuters Web of Science (accessed 3 May 2016), Farquhar *et al.* (1980), the paper with the FvCB model, has been cited 3563 times (232 times in 2015), while Bernacchi *et al.* (2001) has been cited 442 times (42 times in 2015) (see also Niinemets *et al.* (2015) for an analysis of the frequency of use of different Rubisco kinetics for a subset of studies using the FvCB model in simulating canopy photosynthesis). The comparison of the three datasets, *in vitro* Jordan and Ogren (1984), *in vivo* Bernacchi *et al.* (2001) and *in vivo* Walker *et al.* (2013), with the *in vitro* C_3 average dataset derived in our study demonstrated significant differences in predicted Rubisco-limited photosynthesis (Fig. 8). Further, the variability among the photosynthesis estimates derived by the three kinetics datasets is much greater than the variability among the estimated photosynthesis derived by the *in vitro* average kinetics for C_3 cool and warm datasets developed in the current study.

A detailed examination of differences among model estimates indicated that both *in vivo* datasets provided lower estimates of Rubisco-limited photosynthesis than the C_3-average and Jordan and Ogren (1984) *in vitro* datasets (Fig. 8). This is an important difference for interpretation of the FvCB model's parameters, in particular V_{cmax}, the maximum carboxylase activity of Rubisco, derived from gas-exchange data (Rogers, 2014). Use of the *in vivo* kinetics of Bernacchi *et al.* (2001) to derive V_{cmax} from net assimilation *vs.* CO_2 response curves leads to much higher, even more than 50% higher, estimates of V_{cmax} at 25 °C than use of the *in vitro* Jordan and Ogren (1984) kinetics. This difference is not fully appreciated by the modeling community, especially by modelers working at canopy, landscape, regional and global levels. Obviously, part of the large difference of the *in vitro* Jordan and Ogren (1984) and *in vivo* Bernacchi *et al.* (2001) kinetics could reflect lack of mesophyll diffusion conductance in parameter estimation in the study of Bernacchi *et al.* (2001), especially given

the important temperature effects on mesophyll conductance (Bernacchi *et al.*, 2002). This is partly confirmed by the smaller differences in *in vitro* and *in vivo* estimates from the dataset of Walker *et al.* (2013) that is derived considering mesophyll conductance. Nevertheless, as our study demonstrates, there are species-specific differences in Rubisco temperature kinetics, and this comparison again emphasizes the principal problem of using a single Rubisco temperature kinetics to simulate photosynthesis of all C_3 species. Researchers should be fully aware about the limitations and associated uncertainties of using a single Rubisco temperature dataset.

Concluding remarks

The purpose of our study was two-fold: to summarize all the existing information on Rubisco temperature kinetics and analyse the relationships between Rubisco temperature kinetics within and among the phylogenetic and functional groups. We have standardized all the available *in vitro* Rubisco temperature data and constructed an extensive database that allows for direct comparison of Rubisco temperature kinetics without possible study-to-study differences due to assay buffer composition. We believe that in addition to identification of what is available, a key strength of this analysis is recognition of the gaps in data coverage. Too often relevant research is not conducted because there is a feeling in the community that 'all this has already been measured'. As our analysis demonstrates, at least concerning Rubisco temperature kinetics, this opinion is an illusion and the coverage of several species groups is poor, suggesting that there clearly is room for high quality Rubisco temperature kinetic measurements over the years to come. This new research could potentially challenge some of the relationships developed here.

The other widespread opinion is that *in vitro* data coming from different labs and obtained using somewhat different methodologies cannot be used for broad analyses. We believe that the standardization functions developed by us largely solve this issue. Furthermore, we observed that activation energies and Q_{10} values that describe the magnitude of temperature-dependent change of given Rubisco kinetics are not very sensitive to the corrections developed, suggesting that our analysis of Rubisco temperature trait covariation is robust. However, we strongly advise against uncritical use of data by simply pooling the information without paying due attention to the experimental details.

From an immediate practical perspective, we provide separate average Rubisco temperature kinetics for C_3 species from cool and warm habitats and argue that use of separate kinetics is warranted in models of carbon gain, especially for simulation of future conditions. Comparison of Rubisco temperature kinetics widely used so far further suggests that the modeling community needs to rethink the concept of 'single species Rubisco fits all'. In fact, the variability in photosynthesis predictions from widely used single species datasets was much bigger than the variability between photosynthesis predictions by C_3 cool and warm datasets developed here. Although we acknowledge the inertia in the modeling community, at least uncertainties of using single species models need consideration in simulating photosynthesis from leaf to globe.

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