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Journal

Plant, cell & environment, 39(1)

ISSN

0140-7791

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Publication Date

2016

DOI

10.1111/pce.12598

Peer reviewed

Plant, Cell & Environment

Plant, Cell and Environment (2015) doi: 10.1111/pce.12598

Original Article

Genetic variation in circadian regulation of nocturnal stomatal conductance enhances carbon assimilation and growth

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ABSTRACT

Circadian resonance, whereby a plant's endogenous rhythms are tuned to match environmental cues, has been repeatedly shown to be adaptive, although the underlying mechanisms remain elusive. Concomitantly, the adaptive value of nocturnal transpiration in C_3 plants remains unknown because it occurs without carbon assimilation. These seemingly unrelated processes are interconnected because circadian regulation drives temporal patterns in nocturnal stomatal conductance, with maximum values occurring immediately before dawn for many species. We grew individuals of six *Eucalyptus camaldulensis* genotypes in naturally lit glasshouses and measured sunset, predawn and midday leaf gas exchange and whole-plant biomass production. We tested whether sunrise anticipation by the circadian clock and subsequent increases in genotype predawn stomatal conductance led to rapid stomatal opening upon illumination, ultimately affecting genotype differences in carbon assimilation and growth. We observed faster stomatal responses to light inputs at sunrise in genotypes with higher predawn stomatal conductance. Moreover, early morning and midday stomatal conductance and carbon assimilation, leaf area and total plant biomass were all positively correlated with predawn stomatal conductance across genotypes. Our results lead to the novel hypothesis that genotypic variation in the circadian-regulated capacity to anticipate sunrise could be an important factor underlying intraspecific variation in tree growth.

Key-words: adaptation; anticipation hypothesis; biomass enhancement; circadian clock; CO_2; gas exchange; genotype; memory; nocturnal transpiration; stomata.

INTRODUCTION

The circadian clock is an endogenous timer of metabolic processes that regulates transcriptional activity over time in the cells of plants and other organisms. There is ample evidence indicating that such circadian regulation is adaptive (Dodd *et al.* 2005; Johnson & Kyriacou 2006; Resco *et al.* 2009b; Yerushalmi & Green 2009; Vaze & Sharma 2013). Circadian resonance, whereby a plant's endogenous rhythmicity is finely tuned to match environmental cues, leads to increased fitness, and, conversely, fitness suffers from increasing circadian dissonance (Went 1960; Green *et al.* 2002). However, understanding the processes by which circadian regulation confers a fitness advantage has proven more challenging. Earlier hypotheses discussed the role of circadian regulation in shifting photophobic processes, such as DNA replication or cell division, to the night-time period ('escape from light' hypothesis; Pittendrigh 1993) or the separation of mutually incompatible metabolic processes, such as photosynthesis and nitrogen fixation in cyanobacteria (Mitsui *et al.* 1986). Alternatively, the 'anticipation' hypothesis, whereby circadian regulation anticipates highly predictable environmental cues and prepares cellular metabolism accordingly, remains the most common explanation for the growth and fitness advantage provided by circadian clocks. However, direct tests of this hypothesis are rare (Goodspeed *et al.* 2012) and, as previously noted by Johnson & Kyriacou (2006), 'like many other evolutionary ideas that are eminently reasonable – remains just a good idea'.

Circadian regulation, in conjunction with direct physiological responses to vapour pressure deficit and other environmental drivers, is involved in the exchange of water vapour and carbon dioxide between leaves and the atmosphere, through the regulation of stomatal conductance and photosynthesis (Hennessey *et al.* 1993; Resco de Dios *et al.* 2012). An unresolved question is whether circadian regulation, which enhances water loss throughout the night, could be adaptive because there is no carbon assimilation in the dark (Bucci *et al.* 2004; Dawson *et al.* 2007; Resco de Dios *et al.* 2013b). Indeed, the temporal pattern of nocturnal stomatal conductance, largely driven by circadian rhythms, is often characterized by decreased stomatal conductance in the first hours of darkness, followed by significant increases later in the night, and reaching a peak immediately before dawn (Hennessey *et al.* 1993; Resco de Dios *et al.* 2013a, 2015).

One of the mechanisms hypothesized to be involved in the endogenous rise of nocturnal conductance is the availability of starch because starch deficient *Arabidopsis* mutants did not show enhanced predawn stomatal conductance (Lasceve *et al.* 1997). Indeed, nocturnal stomatal conductance has been

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reported to increase in response to elevated CO_2 (Zeppel *et al.* 2012), which is contrary to the daytime trend, and increases in starch availability under elevated CO_2 could be the mechanism underlying such positive response of nocturnal stomatal conductance to CO_2 (Easlon & Richards 2009). However, the generality of this observation is still under examination (Resco de Dios *et al.* 2013b), and recent studies question the relevance of carbohydrates for regulating the endogenous rise in stomatal conductance (Resco de Dios *et al.* 2015).

There is ample evidence of genetic variation in nocturnal stomatal conductance and transpiration within species (Christman *et al.* 2008; Phillips *et al.* 2010; Schoppach *et al.* 2014). The cause and fitness implications of intraspecific variation in nocturnal water use remain uncertain, but associations between circadian-regulated night-time water use, daytime gas exchange rates and plant fitness proxies across genotypes may provide insight into the mechanisms and evolutionary/ecological significance of variable nocturnal leaf gas exchange within species.

Here, we tested whether circadian increases in predawn stomatal conductance promote increased carbon assimilation and enhanced growth across six different *Eucalyptus camaldulensis* genotypes. We first tested whether the different genotypes varied in predawn stomatal conductance. We then hypothesized that genotype predawn conductance would be positively correlated with daytime stomatal conductance (Christman *et al.* 2008; Drake *et al.* 2013) and that the time to respond to early morning radiation inputs would be shorter when stomata are 'primed' prior to sunrise. Finally, we tested whether genotypic variation in stomatal priming was related to genotype fitness by examining the relationship between genotype predawn conductance and three fitness proxies: carbon assimilation, leaf area and total biomass. Experiments were conducted under two CO_2 concentrations (ambient and elevated), to test whether higher predawn conductance under elevated CO_2 is correlated with changes in the availability of carbohydrates (Easlon & Richards 2009; Zeppel *et al.* 2012). Although environmental conditions of CO_2 or other environmental drivers may alter the magnitude of nocturnal conductance (Barbour & Buckley 2007, Rosado *et al.* 2012), its temporal pattern and predawn values are driven by circadian regulation (Hennessey *et al.* 1993; Resco de Dios *et al.* 2013a, 2015).

MATERIALS AND METHODS

Plants and growing conditions

Seedlings from six different genotypes of *E. camaldulensis* ssp. *camaldulensis* were prepared from clonal hedges by the Commonwealth Scientific and Industrial Research Organization (see Supporting Information Table S1 for full details on provenances). The hedges were half-sib seedlings originating from provenances representing different geographic and climatic origins. After reaching an average height of 24.6 cm (±0.97; SE) and a basal diameter of 1.86 mm (±0.07), genotypes were transplanted into 6.9 L cylindrical pots and grown at the naturally lit (with 20% reduction of incident radiation) glasshouse facilities of the University of Western Sydney in Richmond, New South Wales, in south-eastern Australia. Each pot contained 7.5 kg of coarse-textured soil (supplied by the Australian Native Landscape, Richmond NSW, Australia), with a pH of 6.5. To ensure that no nutrient limitations occurred, the plants were fertilized every fortnight with a commercial liquid fertilizer (500 mL Aquasol, at 1.6 g L^{-1}; 23% N, 4% P, 18% K, 0.05% Zn, 0.06% Cu, 0.013% Mo, 0.15% Mn, 0.06% Fe and 0.011% B; Yates Australia, Padstow, NSW, Australia). Plants were grown in ambient (400 μmol mol^{-1}) or elevated (640 μmol mol^{-1}) CO_2 concentrations and were randomly assigned to one of two glasshouse bays per CO_2 treatment, within a randomized block design. Pots were rotated between (monthly) and within (weekly) glasshouse bays to further reduce potential glasshouse bay effects on plant performance. Air temperatures (25:17 °C, average day:night) and relative humidity (45:60%) were representative of average summer values in Richmond. The pots were daily watered to field capacity (until water drained from the bottom of the pot). Further details on glasshouse design and set-up are given by Ghannoum *et al.* (2010). No differences in height and diameter occurred between genotypes at experiment initiation [with $P > 0.05$, analysis of variance (ANOVA)].

Measurements and statistical analyses

Leaf gas exchange was measured with four cross-calibrated portable photosynthesis systems (LI-6400XT, Li-Cor Inc., Lincoln, NE, USA) 2 months after treatments were started in the glasshouse. Nocturnal gas exchange was measured during periods of 1 h centred at 2230 h (early night) and 0345 h (predawn). Natural dawn and dusk occurred at approximately 0600 and 1800 h, respectively. Conditions inside the LI-6400XT cuvettes were set to match the growth conditions previously described. Importantly, there were no significant differences in relative humidity [ANOVA, degrees of freedom (d.f.) = 1, 46, $F = 1.91$, $P = 0.17$], temperature (ANOVA, d.f. = 1, 46, $F = 1.91$, $P = 0.17$) or vapour pressure deficit (ANOVA, d.f. = 1, 46, $F = 1.25$, $P = 0.27$) in the glasshouses during periods when 'early night' and 'predawn' measurements were conducted. Genetic variation in nocturnal conductance was assessed by linear mixed models that included sampling time, CO_2 concentrations, genotype (with $n = 3$–6 in each combination) and their interactions as fixed variables, and with glasshouse bay nested within CO_2 concentration as random variables. These analyses were performed after examining whether the data conformed to assumptions of homoscedasticity and normality.

To test relationships between nocturnal and early morning stomatal conductance, we monitored gas exchange briefly after dawn by logging, every 15 s, the responses to 300 μmol m^{-2} s^{-1} of photosynthetically active radiation (PAR) during 5 min and the subsequent responses at 800 μmol m^{-2} s^{-1} PAR for 5 more minutes. While 5 min is generally not sufficient for steady-state stomatal responses, it is sufficient to test hypotheses regarding the time to react, and values should be correlated with those at steady state. We established whether stomatal priming led to higher early morning stomatal conductance by correlating predawn values with the maximum values at each PAR level, using mixed models that included CO_2 concentration as a random factor.

To test whether stomatal priming would also lead to faster stomatal responses, we calculated the time constant (τ, the time to reach 63% [$1 - e^{-1}$] of the maximum response at each PAR level; Woodward 1987) and determined its relationship with predawn stomatal values using mixed models that included CO_2 concentration as a random factor. PAR for gas exchange measurements was provided by the red–blue light-emitting diodes of the LI-6400XT (with a 7–13% blue fraction, according to the manufacturer).

To examine the relationships between genotype stomatal conductance and growth, we developed mixed-model regressions of genotype predawn conductance as a function of total plant carbon assimilation (also measured in the early morning) and total plant leaf area and biomass. These analyses included CO_2 concentration as a random factor.

Total plant leaf area was determined during harvest using a leaf area metre (LI-3100C, Li-Cor Inc.), and whole-plant gas exchange was determined by multiplying leaf carbon assimilation by total plant leaf area, assuming a 'big leaf' model structure. It is well known that big leaf scaling approaches are fairly limited, especially within complex canopies, given that leaf gas exchange is heterogeneous within canopies and varies as a function of factors such as leaf position or age (De Pury & Farquhar 1997), and this variation could also be under genetic control. These problems would have been minimized within our design, because the plants were less than 3 months old, and little time had elapsed for complexity in the canopy structure to be developed.

Total biomass (including above-ground and below-ground) was collected 2 weeks after gas exchange measurements and determined after oven drying until constant mass (48 h at 70 °C). Immediately after removing leaves for measurements of leaf area, the stem was cut and dried prior to assessing its biomass. The soil was then washed, and a 3 mm sieve was used to preserve the fine roots. Roots were subsequently dried and weighed. Although extreme care was taken to minimize root losses, we acknowledge some root biomass may have been lost using this technique. However, all plants were treated the same way, and any mass losses originating from this technique would have affected equally all plants, regardless of their genotype or CO_2 growth concentration.

Based on the results from this correlative approach, and as a further complement to those results, we employed a path model to directly test for the hypothesized mechanisms underlying relationships between predawn stomatal conductance and biomass (Wright 1934). While correlative approaches allow us to test for the significance of relationships between two variables, with path models, we were able to directly assess the significance of the hypothesized mechanisms across more complex routes. The potential routes to be tested included direct effects of predawn stomatal conductance on the values of early morning stomatal conductance and on the time for stomata to respond, which, in turn, would affect early morning carbon assimilation that would affect whole-plant biomass.

We additionally measured the concentration of soluble carbohydrates and starch to understand their relationship with potential CO_2 effects on stomatal conductance. A 1 cm^2 round piece of leaf was collected at dusk and flash frozen in liquid nitrogen and analysed following previously described methods (Mitchell *et al*. 2013).

All analyses were performed in the R software environment using base packages, lme4 (Bates *et al.,* 2014), car (Fox & Weisberg 2011), influence.ME (Nieuwenhuis *et al*. 2012) and lavaan (Rosseel 2012). R^2 in mixed models was computed following Nakagawa & Schielzeth (2014).

RESULTS

Night-time stomatal conductance was higher at predawn than at early night (Fig. 1 and Table 1), and there was significant variation in nocturnal stomatal conductance across genotypes. While there was no CO_2 effect and no $CO_2 \times$ genotype interaction, there was a significant time $\times$ CO_2 interaction, such that conductance was higher under elevated CO_2 but only at predawn (Fig. 1a and Table 1). The effect of CO_2 on nocturnal stomatal conductance was not driven by carbohydrate availability, as we observed no CO_2 effect on starch, soluble sugars or total non-structural concentrations (only total non-structural carbohydrates shown, Table 1).

Genotypic variation in predawn (but not in early night) stomatal conductance was associated with subsequent daytime processes. For instance, genotype predawn stomatal conductance was strongly associated with early morning stomatal conductance and assimilation at both PAR levels and across CO_2 treatments (linear mixed model, $P < 0.01$, $0.35 \leq R^2 \geq 0.37$, Fig. 2). However, there was no relationship between genotype stomatal conductance in the early night and stomatal conductance and assimilation in the early morning (linear mixed model, $P \geq 0.13$, Supporting Information Fig. S1).

τ showed a negative correlation with predawn stomatal conductance (linear mixed model, $P = 0.04$, $R^2 = 0.3$, Fig. 3a), indicating that the response time to morning light decreased as predawn conductance increased. Moreover, genotypes with more rapidly opening stomata (smaller τ) had higher leaf carbon assimilation (linear mixed model, $P < 0.0001$, $R^2 = 0.60$, Fig. 3b).

Concomitantly, genotypes with higher predawn stomatal conductance showed significantly higher plant carbon assimilation (linear mixed model, $P < 0.0001$, $R^2 = 0.67$), as well as significantly higher leaf area (linear mixed model, $P = 0.033$, $R^2 = 0.29$) and marginally significant higher plant biomass (linear mixed model, $P = 0.098$, $R^2 = 0.19$, Fig. 4). However, there was no significant relationship between genotype early night stomatal conductance and morning carbon assimilation, or leaf area (linear mixed model, $P > 0.4$).

Finally, the path model (Fig. 5) indicated that the mechanism explaining the higher biomass for genotypes with higher predawn stomatal conductance was associated with the effect of predawn stomatal conductance on early morning conductance (at $P < 0.10$). Additionally, we also observed that the relationship between predawn stomatal conductance and early morning assimilation was mediated by τ ($P < 0.05$). In turn, this early morning increase in carbon assimilation led to higher biomass ($P < 0.05$).

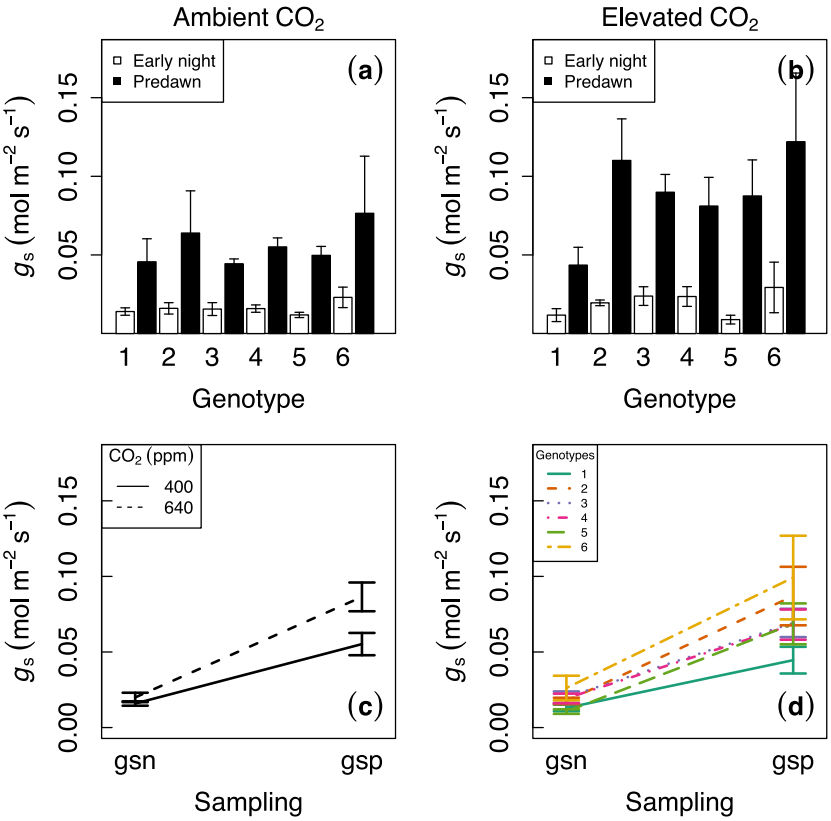

Figure 1. Mean (±SE) stomatal conductance (g_s) for each sampling time (gsn and gsp indicate g_s at early night and at predawn, respectively) and genotype under ambient (a) and elevated (b) CO_2 and effect of the interactions between sampling time and growth CO_2 (c) or genotype (d) on g_s.

Table 1. Results of linear mixed models on the effects of CO_2, genotype and their interactions on nocturnal stomatal conductance (g_s) and on TNC, with sampling time as an additional fixed factor affecting g_s and with glasshouse bay nested within CO_2 concentration as random variables

	g_s			TNC		
Factor	χ^2	d.f.	*P*-value	χ^2	d.f.	*P*-value
CO_2	1.56	1	0.211	1.68	1	0.194
Genotype	12.34	5	0.030	28.59	5	<0.001
Sampling	78.09	1	<0.001			
CO_2 × genotype	3.63	5	0.604	2.02	5	0.846
CO_2 × sampling	5.70	1	0.017			
Genotype × sampling	5.39	5	0.370			
CO_2 × genotype × sampling	1.76	5	0.893			

TNC, total non-structural carbohydrates; d.f., degrees of freedom.

DISCUSSION

We observed genetic correlations between nocturnal stomatal conductance and genotype fitness, quantified as morning carbon assimilation, leaf area and biomass, in *E. camaldulensis* (Figs 4 and 5). These relationships between nocturnal stomatal responses and carbon metabolism were only apparent for conductance at predawn (Figs 2 and 3), but not at early night (Supporting Information Fig. S1). The stronger relationships between predawn and daytime processes (rather than early night and daytime processes) indicate that it is not nocturnal conductance *per se* that affects daytime responses, but rather the magnitude of nocturnal conductance immediately prior to dawn. The temporal pattern of nocturnal stomatal conductance is predominantly driven by circadian regulation, which leads to gradual increases in stomatal conductance through the night period (Hennessey *et al.* 1993; Resco de Dios *et al.* 2013a, 2015). It thus follows that strong circadian regulation, with increasing stomatal conductance until predawn, fostered higher daytime carbon assimilation and plant growth in *E. camaldulensis*, consistent with the anticipation hypothesis.

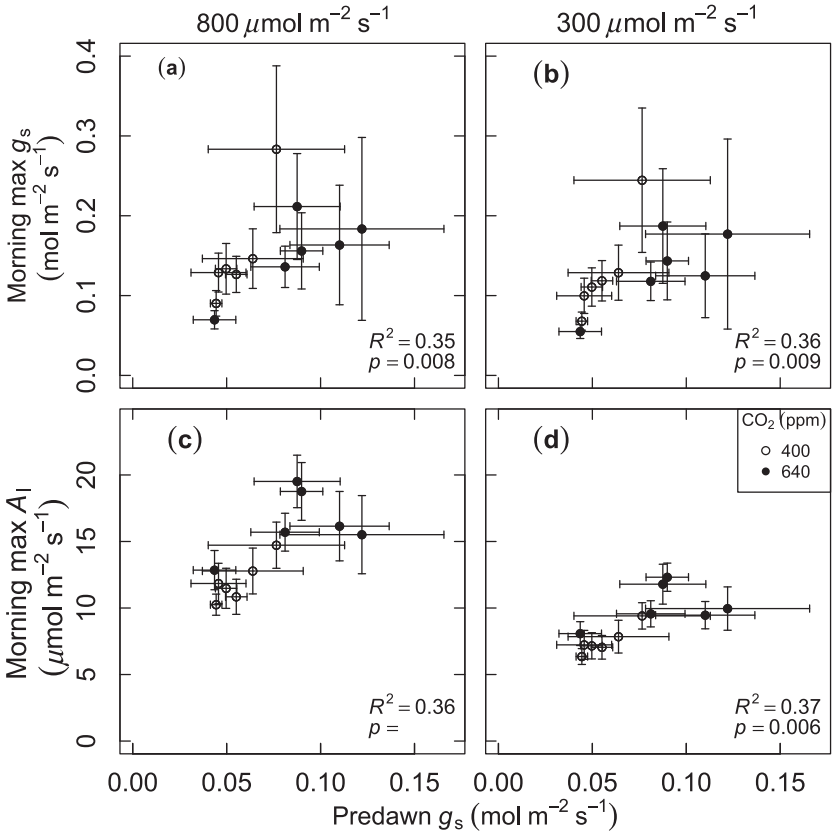

Figure 2. Predawn stomatal conductance (g_s) is positively correlated with the maximum g_s (a, b) and carbon assimilation (A_l, c, d) measured in response to 300 (b, d) and 800 μmol m^{-2} s^{-1} (a, c) of photosynthetically active radiation in the early morning. Values indicate the mean ($n = 4$ and ±SE) for each genotype under each CO_2 concentration. *P*-values reflect the results of Wald tests on linear mixed model that included CO_2 concentration as a random factor.

Circadian regulation of nocturnal conductance and plant fitness

The circadian clock is known to 'orchestrate' the temporal pattern of transcription (Michael *et al.* 2008). Indeed, important differences in the transcriptome of wild-type *Arabidopsis*, relative to those in arrhythmic mutants where circadian regulation has been impaired, were observed 1 h before dawn, and those included differences in the activation state of genes related to abscissic acid and stomatal behaviour (Legnaioli *et al.* 2009). Additionally, circadian rhythms in root hydraulic conductivity could also provide the mechanistic basis for the endogenous rise in predawn stomatal opening (Caldeira *et al.* 2014). Here, we show that the capacity of the stomata to anticipate sunrise then fostered stomatal responses to morning light, decreased diffusion limitations to photosynthesis and, ultimately, was genetically correlated with enhanced growth.

Many previous studies have documented that circadian resonance increases fitness (Dodd *et al.* 2005; Yerushalmi & Green 2009; Yerushalmi *et al.* 2011), and these studies had attributed, but not directly tested, that such increases in fitness were dominated by the clock-driven capacity for anticipating environmental cues. As far as we are aware, our genetic correlations may provide some of the first direct evidence linking this circadian-driven stomatal 'anticipation' (i.e. opening before dawn) with fitness (as indicated by C assimilation, leaf area and biomass). Circadian regulation is demonstrated by self-sustained oscillations with a 24 h period, a step we did not take here because our previous work demonstrated that circadian regulation was the main driver of predawn values of stomatal conductance (Resco de Dios *et al.* 2013b, 2013a, 2015).

We move beyond previous studies that showed that the level of predawn conductance was related to early morning conductance (Drake *et al.* 2013), by additionally showing that the time for stomata to respond to light inputs is also related to predawn conductance, and that this process increases carbon assimilation across genotypes. This observation could provide a mechanistic explanation for previous studies where relationships between nocturnal water loss and traits that confer fast growth had been observed across multiple species (Daley & Phillips 2006; Marks & Lechowicz 2007; Rohula *et al.* 2014) or across multiple genotypes within a given species (Christman *et al.* 2008).

More detailed studies will be needed to describe the mechanisms underlying the observed genetic correlations between predawn stomatal conductance, early morning photosynthesis and total plant leaf area and biomass. In particular, the link between early morning photosynthesis and biomass is based on correlations and should be considered as a hypothesis requiring further testing (Fig. 5). For example, early morning photosynthesis may have a limited impact on whole-day C gain,

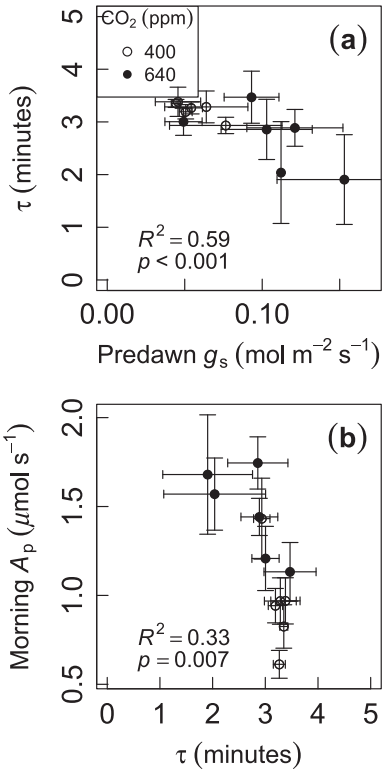

Figure 3. Time to reach 63% of the final stomatal conductance (g_s) in response to 5 min at 800 μmol m^{-2} s^{-1} of photosynthetically active radiation in the early morning (τ) is significantly correlated with predawn g_s (a). In turn, τ is significantly correlated with early morning plant carbon assimilation (A_p, b). These results indicate that carbon input is related to stomatal responsiveness (τ), which, in turn, is related to predawn g_s. Values indicate the mean ($n = 4$, ±SE) for each genotype under each CO_2 concentration. *P*-values reflect the results of Wald tests on linear mixed models that included CO_2 concentration as a random factor. Small error bars may be hidden.

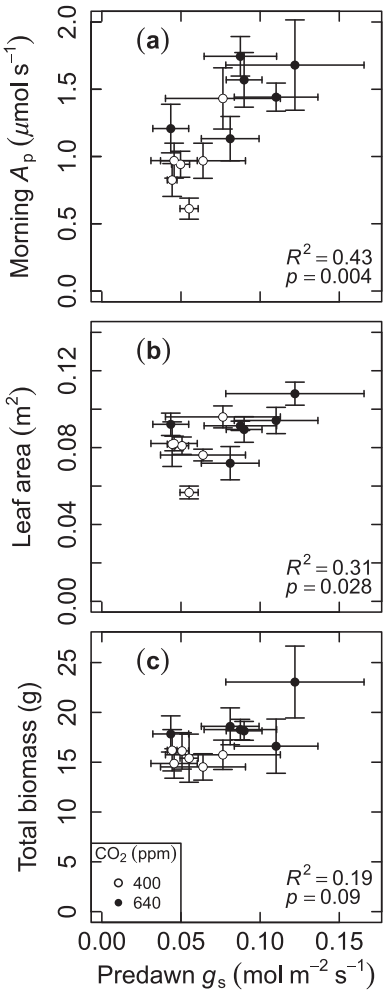

Figure 4. Values of early morning plant carbon assimilation (A_p), leaf area and total biomass are significantly correlated with predawn stomatal conductance (g_s). Values indicate the mean (n=4, ± SE) for each genotype under each CO_2 concentration. Small error bars may be hidden. *p*-values reflect the results of Wald tests on linear mixed models that included CO_2 concentration as a random factor.

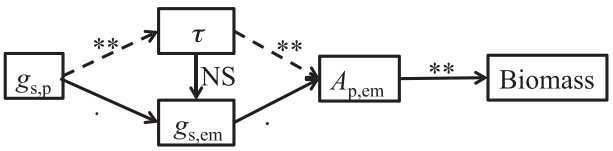

Figure 5. Results and significance levels for the path model on hypothesized relationships between relating predawn stomatal conductance ($g_{s,p}$), the time constant of stomatal responses (τ, with high τ indicating slow-responsive stomata), early morning stomatal conductance ($g_{s,em}$) and carbon assimilation ($A_{p,em}$) and final biomass. Straight and dashed lines indicate hypothesized positive and negative relationships, respectively. Significance levels are indicated by NS, * and ** that indicate $P > 0.10$, $P < 0.10$ and $P < 0.001$, respectively.

and early morning assimilation may be correlated with assimilation at other times. In addition to 'stomatal priming' mechanistically leading to more responsive (after sunset) stomata and to an enhancement of C gain, stomatal priming could also be part of a trait syndrome that confers enhanced growth and indirectly increases plant fitness. At any rate, we have shown that variation in stomatal priming is an important factor underlying intraspecific variation in growth.

In response to a decrease in predawn relative humidity, Auchincloss *et al.* (2014) observed a decline in predawn stomatal conductance, but no change in subsequent daytime carbon assimilation in sunflower. The authors interpreted this result as proof that predawn stomatal regulation does not affect early morning photosynthesis; however, our results provide an alternative explanation to this finding. The circadian clock regulates the temporal pattern of transcription based on the conditions experienced in the previous days (Graf *et al.* 2010). Therefore, the application of a 'pulse' of low relative humidity, which leads to immediate reductions in predawn stomatal conductance, would not have altered circadian regulation. The stomata exposed to low humidity would have been pre-conditioned to respond to early morning radiation inputs and would have done so at the same rate as stomata exposed to high humidity, following the removal of the environmental constraint of high vapour pressure deficit.

CO_2 effects on nocturnal stomatal conductance

The positive effect of CO_2 growth concentration on stomatal conductance was only significant at predawn (Table 1 and Fig. 1). Earlier work hypothesized that the positive effect of CO_2 on stomatal conductance, when occurring, could be attributed to increases in carbohydrate concentrations, as carbohydrates provide some of the osmoticant and C skeletons for ATP production necessary to regulate stomatal opening (Easlon & Richards 2009). However, we are not aware of previous studies directly testing this hypothesis by measuring carbohydrate concentrations and nocturnal conductance under elevated CO_2. Our data did not support this hypothesis because we did not observe a change in carbohydrate concentrations under elevated CO_2. Alternative mechanisms could be driving this response, including changing stomatal sensitivities to abscissic acid under elevated CO_2 (Levine *et al.* 2009).

Implications and outlook

Eucalyptus camaldulensis is a riverine species, with relatively low values of nocturnal conductance, compared with species from drier environments. We did not have enough data to meaningfully test for associations between water availability at the genotype's origin and nocturnal conductance. However, it is tempting to hypothesize that our findings provide an explanation for a major conundrum in nocturnal water research, which is why the rates of nocturnal conductance and water loss are typically highest (up to 25% of daytime water loss or more) in species growing in deserts (Ogle *et al.* 2012), savannas (Ogle *et al.* 2012) and Mediterranean ecosystems (Barbeta *et al.* 2012), which are some of the driest environments on Earth. The period for carbon assimilation in C_3 or C_4 plants in water-limited ecosystems is very narrow, typically only a few hours in the early morning, before the daily onset of strongly desiccating atmospheric conditions (Huxman *et al.* 2004; Resco *et al.* 2009a). Therefore, it would be adaptive to have high predawn stomatal conductance in water-limited ecosystems, which would lead to rapid stomatal response to early morning light coincident with lower vapour pressure deficit; this strategy would maximize carbon assimilation before environmental conditions cause stomatal closure. Although *E. camaldulensis* grows in riparian environments, seedlings lacking groundwater access will still be exposed to intermittency of water availability.

Perhaps the next major challenge will be to understand why predawn stomatal conductance varies amongst genotypes and species, instead of being always high, and whether this is due to differences in the strength of clock regulation, the degree of clock resonance or local site factors. Differences in circadian period and intensity have often been related to differences in photoperiod. Genotypes of *E. camaldulensis* came from different provenances (Supporting Information Table S1), and the distance between provenances may have been large enough to drive major clinal variation in photoperiod (Hut *et al.* 2013). Moreover, variation in the perception of blue light across genotypes may have contributed to the predawn stomatal response as well (Taiz & Zeiger 2006).

It is important to note that high nocturnal conductance may conflict with other demands for water during the night. Decreasing nocturnal conductance and water loss are necessary to reduce xylem tension and thus favour leaf expansion and growth (Müller *et al.* 2014), stem refilling and cavitation repair (Daley & Phillips 2006) and hydraulic lift (Neumann *et al.* 2014). Additionally, increasing nocturnal water use may decrease daytime water availability. Future work could address how these multiple trade-offs for water allocation overnight interact across genotypes and species, ultimately affecting fitness across a wide range of environmental stress intensities and types.

More broadly, the finding that genotypes with higher predawn conductance show higher daytime gas exchange and productivity may represent an important mechanism by which intraspecific variation in plant growth occurs, where genetic variation in the capacity for anticipating sunrise would be an important trait underlying differences in C uptake and growth. This novel hypothesis on the mechanisms driving intraspecific variation deserves further testing. Our results thus contribute to the up and coming field of ecological memory (Ogle *et al.* 2015), by demonstrating the selective advantage of plants using information from the recent past to prepare plant metabolism to respond to predictable changes in the environment.

ACKNOWLEDGMENTS

We gratefully acknowledge funding from the Australian Science Industry and Endowment Fund (SIEF grant RP04-122) to D.T., a Ramón y Cajal Fellowship to V.R.D. (RYC-2012-10970) and a Research Exchange Program Grant from the Hawkesbury Institute for the Environment at the University of Western Sydney to M.L. We remain indebted to comments from T. O'Grady, J. Voltas, J.G. Alday, R. Norby, K. Mott and an anonymous reviewer on earlier versions of this manuscript and to G.D. Farquhar, S.G. Southerton, M. Battaglia, L. Pinkard and the full team behind the SIEF grant.

REFERENCES

Auchincloss L., Easlon H.M., Levine D., Donovan L. & Richards J.H. (2014) Pre-dawn stomatal opening does not substantially enhance early-morning photosynthesis in *Helianthus annuus*. *Plant Cell Environment*, **37**, 1364–1370.

Barbeta A., Ogaya R. & Peñuelas J. (2012) Comparative study of diurnal and nocturnal sap flow of *Quercus ilex* and *Phillyrea latifolia* in a Mediterranean holm oak forest in Prades (Catalonia, NE Spain). *Trees*, **26**, 1651–1659.

Barbour M.M. & Buckley T.N. (2007) The stomatal response to evaporative demand persists at night in *Ricinus communis* plants with high nocturnal conductance. *Plant, Cell & Environment*, **30**, 711–721.

Bates D., Maechler M., Bolker B. & Walker S. (2014) lme4: linear mixed-effects models using Eigen and S4. R package version 1.1-7, URL: http://CRAN.R-project.org/package=lme4%3E.

Bucci S.J., Scholz F.G., Goldstein G., Meinzer F.C., Hinojosa J.A., Hoffmann W. A. & Franco A.C. (2004) Processes preventing nocturnal equilibration between leaf and soil water potential in tropical savanna woody species. *Tree Physiol*, **24**, 1119–1127.

Caldeira C.F., Jeanguenin L., Chaumont F. & Tardieu F. (2014) Circadian rhythms of hydraulic conductance and growth are enhanced by drought and improve plant performance. *Nat Commun*, **5**, 5365.

Christman M.A., Richards J.H., McKay J.K., Stahl E.A., Juenger T.E. & Donovan L.A. (2008) Genetic variation in *Arabidopsis thaliana* for night-time leaf conductance. *Plant Cell Environ*, **31**, 1170–1178.

Daley M.J. & Phillips N.G. (2006) Interspecific variation in nighttime transpiration and stomatal conductance in a mixed New England deciduous forest. *Tree Physiol*, **26**, 411-419.

Dawson T.E., Burgess S., Tu K.P., Oliveira R.S., Santiago L.S., Fisher J.B., . . . Ambrose A.R. (2007) Nighttime transpiration in woody plants from contrasting ecosystems. *Tree Physiol*, **27**, 561–575.

De Pury D.G.G. & Farquhar G.D. (1997) Simple scaling of photosynthesis from leaves to canopies without the errors of big-leaf models. *Plant, Cell and Environment*, **20**, 537–557.

Dodd A.N., Salathia N., Hall A., Kevei E., Toth R., Nagy F., . . . Webb A.A.R. (2005) Plant circadian clocks increase photosynthesis, growth, survival, and competitive advantage. *Science*, **309**, 630–633.

Drake P.L., Froend R.H. & Franks P.J. (2013) Smaller, faster stomata: scaling of stomatal size, rate of response, and stomatal conductance. *J Exp Bot*, **64**, 495–505.

Easlon H.M. & Richards J.H. (2009) Photosynthesis affects following night leaf conductance in *Vicia faba*. *Plant Cell Environ*, **32**, 58–63.

Fox J. & Weisberg S. (2011) *An R Companion to Applied Regression* (2nd ed.). Sage Publications, Thousand Oaks, CA, USA.

Ghannoum O., Phillips N.G., Sears M.A., Logan B.A., Lewis J.D., Conroy J.P. & Tissue D.T. (2010) Photosynthetic responses of two eucalypts to industrial-age changes in atmospheric [CO_2] and temperature. *Plant, Cell & Environment*, **33**, 1671–1681.

Goodspeed D., Chehab E.W., Min-Venditti A., Braam J. & Covington M.F. (2012) *Arabidopsis* synchronizes jasmonate-mediated defense with insect circadian behavior. *Proc Natl Acad Sci U S A*, **109**, 4674–4677.

Graf A., Schlereth A., Stitt M. & Smith A.M. (2010) Circadian control of carbohydrate availability for growth in *Arabidopsis* plants at night. *Proc Natl Acad Sci USA*, **107**, 9458–9463.

Green R.M., Tingay S., Wang Z.-Y. & Tobin E.M. (2002) Circadian rhythms confer a higher level of fitness to *Arabidopsis* plants. *Plant Physiol.*, **129**, 576–584.

Hennessey T., Freeden A. & Field C. (1993) Environmental effects on circadian rhythms in photosynthesis and stomatal opening. *Planta*, **189**, 369–376.

Hut R.A., Paolucci S., Dor R., Kyriacou C.P. & Daan S. (2013) Latitudinal clines: an evolutionary view on biological rhythms. *Proc Biol Sci*, **280**, 20130433.

Huxman T.E., Snyder K.A., Tissue D.T., Leffler A.J., Ogle K., Pockman W.T., . . . Schwinning S. (2004) Precipitation pulses and carbon fluxes in semiarid and arid ecosystems. *Oecologia*, **141**, 254–268.

Johnson C.H. & Kyriacou C.P. (2006) Clock evolution and adaptation: whence and whither? In: *Endogenous Plant Rhythms* (eds A. Hall & H. McWatters), pp. 237–260. Blackwell Publishing Ltd, Oxford, UK.

Lasceve G., Leymarie J. & Vavasseur A. (1997) Alterations in light-induced stomatal opening in a starch-deficient mutant of *Arabidopsis thaliana* L. deficient in chloroplast phosphoglucomutase activity. *Plant Cell Environ*, **20**, 350–358.

Legnaioli T., Cuevas J. & Mas P. (2009) TOC1 functions as a molecular switch connecting the circadian clock with plant responses to drought. *EMBO J*, **28**, 3745–3757.

Levine L., Richards J. & Wheeler R. (2009) Super-elevated CO_2 interferes with stomatal response to ABA and night closure in soybean (*Glycine max*). *J Plant Physiol*, **166**, 903–913.

Marks C.O. & Lechowicz M. (2007) The ecological and functional correlates of nocturnal transpiration. *Tree Physiol*, **27**, 577–584.

Michael T.P., Mockler T.C., Breton G., McEntee C., Byer A., Trout J.D., . . . Chory J. (2008) Network discovery pipeline elucidates conserved time-of-day-specific cis-regulatory modules. *PLoS Genet*, **4**, e14.

Mitchell P.J., O'Grady A.P., Tissue D.T., White D.A., Ottenschlaeger M.L. & Pinkard E.A. (2013) Drought response strategies define the relative contributions of hydraulic dysfunction and carbohydrate depletion during tree mortality. *New Phytol*, **197**, 862–872.

Mitsui A., Kumazawa S., Takahashi A., Ikemoto H. & Arai T. (1986) Strategy by which nitrogen-fixing unicellular cyanobacteria grow photoautotrophically. *Nature*, **323**, 720–722.

Müller L.M., von Korff M. & Davis S.J. (2014) Connections between circadian clocks and carbon metabolism reveal species-specific effects on growth control. *J. Exp. Bot.*, **65**, 2915–2923.

Nakagawa S. & Schielzeth H. (2014) A general and simple method for obtaining R2 from generalized linear mixed-effects models. *Methods Ecol Evol*, **4**, 133–142.

Neumann R.B., Cardon Z.G., Teshera-Levye J., Rockwell F.E., Zwieniecki M.A. & Holbrook N.M. (2014) Modelled hydraulic redistribution by sunflower (*Helianthus annuus* L.) matches observed data only after including night-time transpiration. *Plant Cell Environ*, **37**, 899–910.

Nieuwenhuis R., Te Grotenhuis M. & Pelzer B. (2012) Influence.ME: tools for detecting influential data in mixed effects models. *The R Journal*, **4**, 38–47.

Ogle K., Barber J.J., Barron-Gafford G.A., Bentley L.P., Young J.M., Huxman T. E., . . . Tissue D.T. (2015) Quantifying ecological memory in plant and ecosystem processes. *Ecol Lett*, **18**, 221–235.

Ogle K., Lucas R.W., Bentley L.P., Cable J.M., Barron-Gafford G.A., Griffith A., . . . Tissue D.T. (2012) Differential daytime and night-time stomatal behavior in plants from North American deserts. *New Phytol*, **194**, 464–476.

Phillips N.G., Lewis J.D., Logan B.A. & Tissue D.T. (2010) Inter- and intraspecific variation in nocturnal water transport in *Eucalyptus*. *Tree Physiol*, **30**, 586–596.

Pittendrigh C. (1993) Temporal organization: reflections of a Darwinian clock-watcher. *Annu Rev Physiol*, **55**, 17–54.

Resco de Dios V., Diaz-Sierra R., Goulden M.L., Barton C.V., Boer M.M., Gessler A., . . . Tissue D.T. (2013a) Woody clockworks: circadian regulation of night-time water use in *Eucalyptus globulus*. *New Phytol*, **200**, 743–752.

Resco de Dios V., Goulden M.L., Ogle K., Richardson A.D., Hollinger D.Y., Davidson E.A., . . . Moreno J.M. (2012) Endogenous circadian regulation of carbon dioxide exchange in terrestrial ecosystems. *Global Change Biol.*, **18**, 1956–1970.

Resco de Dios V., Roy J., Ferrio J.P., Alday J.G., Landais D., Milcu A. & Gessler A. (2015) Processes driving nocturnal transpiration and implications for estimating land evapotranspiration *Sci Rep*, **5**, 10975.

Resco de Dios V., Turnbull M.H., Barbour M.M., Ontedhu J., Ghannoum O. & Tissue D.T. (2013b) Soil phosphorous and endogenous rhythms exert a larger impact than CO_2 or temperature on nocturnal stomatal conductance in *Eucalyptus tereticornis*. *Tree Physiol.*, **33**, 1206–1215.

Resco V., Ewers B.E., Sun W., Huxman T.E., Weltzin J.F. & Williams D.G. (2009a) Drought-induced hydraulic limitations constrain leaf gas exchange recovery after precipitation pulses in the C_3 woody legume, *Prosopis velutina*. *New Phytologist*, **181**, 672–682.

Resco V., Hartwell J. & Hall A. (2009b) Ecological implications of plants' ability to tell the time. *Ecol Lett*, **12**, 583–592.

Rohula G., Kupper P., Räim O., Sellin A. & Sõber A. (2014) Patterns of night-time water use are interrelated with leaf nitrogen concentration in shoots of 16 deciduous woody species. *Environ Exp Bot*, **99**, 180–188.

Rosado B.H.P., Oliveira R.S., Joly C.A., Aidar M.P.M. & Burgess S.S.O. (2012) Diversity in nighttime transpiration behavior of woody species of the Atlantic Rain Forest, Brazil. *Agricultural and Forest Meteorology*, **158–159**, 13–20.

Rosseel Y. (2012) *lavaan*: an *R* package for structural equation modeling. *J Stat Soft*, **48**, 1–36.

Schoppach R., Claverie E. & Sadok W. (2014) *Genotype-dependent Influence of Night-time Vapour Pressure Deficit on Night-time Transpiration and Daytime Gas Exchange in Wheat.* Funct Plant Biol.

Taiz L. & Zeiger E. (2006) *Plant Physiology.* (4th ed.). Sinauer Associates, Inc, Sunderland, MA.

Vaze K.M. & Sharma V.K. (2013) On the adaptive significance of circadian clocks for their owners. *Chronobiol Int*, **30**, 413–433.

Went F.W. (1960) Photo- and thermoperiodic effects in plant growth. *Cold Spring Harb Symp. Quant. Biol.*, **25**, 221–230.

Woodward F.I. (1987) Scale. In: *Climate and Plant Distribution*, pp. 19–38. Cambridge University Press, Cambridge, UK.

Wright S.S. (1934) The method of path coefficients. *Ann. Math. Stat.*, **5**, 161–215.

Yerushalmi S. & Green R.M. (2009) Evidence for the adaptive significance of circadian rhythms. *Ecology Letters*, **12**, 970–981.

Yerushalmi S., Yakir E. & Green R.M. (2011) Circadian clocks and adaptation in *Arabidopsis*. *Molecular Ecology*, **20**, 1155–1165.

Zeppel M.J.B., Lewis J.D., Chaszar B., Smith R.A., Medlyn B.E., Huxman T.E. & Tissue D.T. (2012) Nocturnal stomatal conductance responses to rising [CO_2], temperature and drought. *New Phytol*, **193**, 929–938.

Received 31 March 2015; received in revised form 10 June 2015; accepted for publication 19 June 2015

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Fig. S1. Early night stomatal conductance (g_s) is not positively correlated with the maximum g_s (a, b) and carbon assimilation (A, c, d) measured in response to 300 (b, d) and 800 μmol m^{-2} s^{-1} (a, c) of photosynthetically active radiation in the early morning. Values indicate the mean ($n = 4$, ±SE) for each genotype under each CO_2 concentration. P-values reflect the results of Wald tests on linear mixed model that included CO_2 concentrations as a random factor.

Table S1: Origin for each of the six genotypes of Eucalyptus camaldulensis used in this study.