

# Genetic variation in *Arabidopsis thaliana* for night-time leaf conductance

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## ABSTRACT

Night-time leaf conductance ( $g_{\text{night}}$ ) and transpiration may have several adaptive benefits related to plant water, nutrient and carbon relations. Little is known, however, about genetic variation in  $g_{\text{night}}$  and whether this variation correlates with other gas exchange traits related to water use and/or native habitat climate. We investigated  $g_{\text{night}}$  in 12 natural accessions and three near isogenic lines (NILs) of *Arabidopsis thaliana*. Genetic variation in  $g_{\text{night}}$  was found for the natural accessions, and  $g_{\text{night}}$  was negatively correlated with native habitat atmospheric vapour pressure deficit ( $\text{VPD}_{\text{air}}$ ), suggesting lower  $g_{\text{night}}$  may be favoured by natural selection in drier habitats. However, there were also significant genetic correlations of  $g_{\text{night}}$  with daytime gas exchange traits expected to affect plant fitness [i.e. daytime leaf conductance, photosynthesis and intrinsic water-use efficiency ( $\text{WUE}_i$ )], indicating that selection on daytime gas exchange traits may result in indirect selection on  $g_{\text{night}}$ . The comparison of three NILs to their parental genotypes identified one quantitative trait locus (QTL) contributing to variation in  $g_{\text{night}}$ . Further characterization of genetic variation in  $g_{\text{night}}$  within and among populations and species, and of associations with other traits and native habitats will be needed to understand  $g_{\text{night}}$  as a putatively adaptive trait.

**Key-words:** adaptation; *FRIGIDA*; intraspecific variation; night-time transpiration; nocturnal; stomatal conductance.

## INTRODUCTION

A diverse range of  $C_3$  and  $C_4$  plant species exhibit significant night-time stomatal opening and transpirational water loss (Musselman & Minnick 2000; Caird, Richards & Donovan 2007a; Dawson *et al.* 2007; Marks & Lechowicz 2007). Although this night-time water loss is not accompanied by carbon gain, it can be a substantial portion of a plant's total daily water loss, with reports ranging from

5–30% depending on species and ambient conditions (Benyon 1999; Snyder, Richards & Donovan 2003; Bucci *et al.* 2004, 2005; Daley & Phillips 2006; Caird *et al.* 2007a; Caird, Richards & Hsiao 2007b; Fisher *et al.* 2007; Kavanagh, Pangle & Schotzko 2007; Scholz *et al.* 2007). A number of hypotheses have been suggested for the potential adaptive value, or lack thereof, of night-time stomatal conductance ( $g_{\text{night}}$ ) and transpiration ( $E_{\text{night}}$ ) (Donovan, Linton & Richards 2001; Snyder *et al.* 2003, 2008; Barbour *et al.* 2005; Daley & Phillips 2006; Caird *et al.* 2007a; Dawson *et al.* 2007; Fisher *et al.* 2007; Marks & Lechowicz 2007; Scholz *et al.* 2007). From an evolutionary perspective, these hypotheses can be grouped into three general categories that are not mutually exclusive: (1) that night-time water loss is not a substantial cost, and thus, under weak selection or selectively neutral; (2) that substantial  $g_{\text{night}}$  and  $E_{\text{night}}$  are an indirect effect of genetic and mechanistic links to daytime gas exchange rates, which are the more important traits and under stronger selection; and (3) that substantial  $g_{\text{night}}$  and/or  $E_{\text{night}}$  confer a direct benefit on plant growth and fitness in some habitats by enhancing various aspects of plant nutrient, water and carbon relations. All of these categories make the assumption that there is a genetic basis for variation in  $g_{\text{night}}$ , but little is known about the genetic variation for  $g_{\text{night}}$  within species and thus the potential for direct or indirect selection on this trait.

There is growing evidence that there is considerable environmentally induced variation in  $g_{\text{night}}$  and  $E_{\text{night}}$ , and that regulation of these traits can be attributed to some of the same factors well known to regulate daytime conductance ( $g_{\text{day}}$ ) and transpiration ( $E_{\text{day}}$ ) (Caird *et al.* 2007a). For example, decreasing water availability (drought and salinity) and higher atmospheric vapour pressure deficit ( $\text{VPD}_{\text{air}}$ ) are associated with decreasing  $g_{\text{night}}$  (Rawson & Clarke 1988; Donovan *et al.* 1999; Daley & Phillips 2006; Caird *et al.* 2007a,b; Cavender-Bares, Sack & Savage 2007; Dawson *et al.* 2007; Kavanagh *et al.* 2007). Whereas these studies have focused on correlations of environmental variation and  $g_{\text{night}}$ , comparisons of species and populations in 'common garden' experiments that minimize environmental variation provide a means of identifying underlying genetic differentiation for a trait (Arntz & Delph 2001). For

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example, patterns of population genetic differentiation for daytime gas exchange traits [e.g.  $g_{\text{day}}$ , photosynthesis ( $A$ ), water-use efficiency (WUE)] and their associations with native habitat support the hypothesis that genetic differentiations in these traits are adaptive (reviewed in Arntz & Delph 2001). For daytime gas exchange traits, the further use of phenotypic selection analyses has gone on to test adaptive hypotheses for gas exchange traits (e.g. Dudley 1996; Sherrard & Maherali 2006; Donovan *et al.* 2007).

For night-time gas exchange traits, however, only a few common garden studies have identified species differences (Jordan, Brodribb & Loney 2004; Howard & Donovan 2007; Marks & Lechowicz 2007) and intraspecific variation among cultivars of agricultural and horticultural plants (see Supplementary Table S1 in Caird *et al.* 2007a). In a common garden study with woody saplings of 21 species, Marks & Lechowicz (2007) found that high nocturnal sap flux was characteristic of species that are generally associated with lower shade tolerance, but not with differences in soil water availability of native habitats. Associations among population level genetic differences in  $g_{\text{night}}$  and habitat have not been tested.

If habitat associations are found with variation among populations for a putatively adaptive trait, then this suggests that the population differentiation may be the evolutionary outcome of direct selection on that trait (Arntz & Delph 2001). However, population differentiation may also be caused by other processes including genetic drift, historical artefact or indirect selection because of underlying genetic correlations. Phenotypic correlations (including both environmentally and genetically based variation) have been found between  $g_{\text{night}}$  and  $g_{\text{day}}$  for desert plant species and Tasmanian conifers (Snyder *et al.* 2003; Jordan *et al.* 2004), and between nocturnal sap flux and leaf N and stem growth for saplings of 21 deciduous tree species (Marks & Lechowicz 2007). These correlations can be used to suggest how plant traits are functionally coordinated, and thus, may be providing a benefit or cost for plant growth and fitness. An understanding of the underlying genetic correlations can further suggest whether traits are independently affected by selection or jointly affected. For example, daytime gas exchange traits related to water use and WUE are thought to be under selection in water-limited habitats (Dudley 1996; Arntz & Delph 2001; McKay, Richards & Mitchell-Olds 2003; Donovan *et al.* 2007, but see Sherrard & Maherali 2006). If there is a strong genetic correlation between  $g_{\text{night}}$  and daytime gas exchange traits, then patterns of  $g_{\text{night}}$  may be greatly influenced by selection on the daytime rates, regardless of whether variation in  $g_{\text{night}}$  has any effect on plant fitness.

We investigated within-species genetic variation in maximum  $g_{\text{night}}$  using natural accessions of the model  $C_3$  annual plant *Arabidopsis thaliana* (L.) Heynh. Substantial  $g_{\text{night}}$  has been observed for a few accessions of *Arabidopsis*, and there is evidence for specific mutants having altered the magnitude of  $g_{\text{night}}$  (Lasceve, Leymarie & Vavasseur 1997; Leymarie, Lasceve & Vavasseur 1998, 1999; Dodd *et al.* 2005). The values of  $g_{\text{night}}$  are well above the functionally

defined cuticular conductances generally reported [minimum  $g_{\text{night}}$  when stomata are forced closed with desiccation or abscisic acid (ABA)] (Caird *et al.* 2007a,b; Cavender-Bares *et al.* 2007). Additionally, *Arabidopsis* shows substantial within-species genetic variation in integrated WUE estimated from leaf  $\delta^{13}\text{C}$ , and quantitative trait loci (QTL) have been identified that contribute to variation in leaf  $\delta^{13}\text{C}$  (McKay *et al.* 2003; Hausmann *et al.* 2005; Masle, Gilmore & Farquhar 2005).

In this growth chamber experiment, we explored within-species genetic variation by comparing 12 naturally occurring accessions of *Arabidopsis* representing populations from a variety of habitats around the world (Redei 1970) that differ in water relations traits (McKay *et al.* 2003; Juenger *et al.* 2005). We grew the plants and measured night-time and daytime gas exchange under well-watered conditions that would maximize the potential for high  $g_{\text{night}}$ . We asked three questions: (1) Is there genetic variation in maximum  $g_{\text{night}}$  of *Arabidopsis*? (2) Is  $g_{\text{night}}$  correlated to climate characteristics of the region where the accessions were collected (precipitation and VPD<sub>air</sub>)? (3) Are there genetic correlations between  $g_{\text{night}}$  and daytime gas exchange rates and  $\delta^{13}\text{C}$ ? Additionally, we used near isogenic lines (NILs) known to have divergent  $\delta^{13}\text{C}$  and/or  $g_{\text{day}}$  (McKay *et al.* 2003; Juenger *et al.* 2005) to investigate whether  $g_{\text{night}}$  and  $g_{\text{day}}$  are under similar genetic control.

## MATERIALS AND METHODS

### Plant material

We studied 12 accessions of *Arabidopsis thaliana* selected to represent populations from locations across the globe with contrasting temperature and precipitation (Table 1; McKay *et al.* 2003). Seeds were obtained from the *Arabidopsis* Biological Resource Center (ABRC, <http://www.arabidopsis.org>), and seed for each accession was increased using single seed descent. Of these accessions, nine are spring annuals, shorter living and capable of flowering without vernalization, and three are winter annuals, longer living and requiring vernalization to flower. Using latitude and longitude data provided by the Nottingham *Arabidopsis* Stock Center, the following climate data were obtained for each accession from a long-term (40 years) data set (New, Hulme & Jones 1998): mean annual VPD<sub>air</sub>, mean monthly VPD<sub>air</sub>, mean annual precipitation and mean monthly precipitation.

We included three NILs known to have divergent  $\delta^{13}\text{C}$  and/or  $g_{\text{day}}$  as compared to the natural accessions making up the background for these NILs (McKay *et al.* 2003; Juenger *et al.* 2005). The NILs delta 2.1 and delta 3.1, described by Juenger *et al.* (2005), contain a small portion of Cvi-1's chromosomes 2 and 3, respectively, in a *Ler-2* background. The NIL ColFRI has the genomic region containing a functional FRI introgressed from the Sf-2 accession into a Columbia background, which carries a null, recessive *FRIGIDA* allele (Lee & Amasino 1995; Johanson *et al.* 2000). FRI affects flowering time by its role in the vernalization response

**Table 1.** The origin locations (latitude, longitude and elevation), life history characteristics and number of days to flowering [least square (LS) mean  $\pm$  SE] of the 12 natural *Arabidopsis thaliana* accessions in the study

Accession	Abbreviation	Location	Latitude	Longitude	Elevation(m)	Winter/spring annual	Days to flowering (d)
6673	Col-0	Columbia, USA	N38.5	W92.5	50	Spring	24.4 $\pm$ 2.7
902	Cvi-0	Cape Verde Islands	N16	W24	1200	Spring	23.0 $\pm$ 1.8
1102	Db-1	Tenne, Germany	N50.5	E8.5	400–500	Spring	25.4 $\pm$ 2.7
1122	Edi-0	Edinburgh, UK	N56	E3	100–200	Winter	DNF
922	Hodja	Tajikistan	N43	E1		Spring	25.4 $\pm$ 2.7
903	Kas-1	Kashmir, India	N35	E77	1580	Winter	24.4 $\pm$ 2.7
20	Ler-0	Landsberg <i>erecta</i> ; Germany	N51	E12		Winter	25.9 $\pm$ 2.7
1643	Oy-1	Oystese, Norway	N60	E6	1–100	Spring	24.9 $\pm$ 3.7
1504	Sei-0	Seis am Schlern, Italy	N46.5	E11.5	1000–1500	Spring	25.8 $\pm$ 1.8
1640	Tsu-1	Tsushima, Japan	N33	E136	1–100	Spring	25.0 $\pm$ 2.5
1584	Van-0	Vancouver, Canada	N49.5	W123	1–100	Spring	26.9 $\pm$ 3.7
1638	Ws-3	Wassilewskija, Russia	N52	E30	100–200	Spring	21.3 $\pm$ 2.1

DNF, did not flower.

(Johanson *et al.* 2000), and introgression of a functional FRI has been found to increase both  $\delta^{13}\text{C}$  and flowering time (McKay *et al.* 2003).

The experimental design was a complete randomized block with three blocks and two replicates of each natural accession or NILs per block (90 plants total). Each replicate was an individual plant in a pot constructed from a 50 mL centrifuge tube with lid containing a small hole for plant growth. The bottom of the centrifuge tube was cut off, and the soil-filled tube was placed on top of a soil-filled Conetainer (Stuewe & Sons, Corvallis, OR, USA) to allow added volume for root growth (see McKay *et al.* 2001; Juenger *et al.* 2005). Each pot was initially planted with four seeds and thinned to one plant after germination. After planting, the pots were placed in a dark, cold room at 4 °C for 7 d and then transferred to a growth chamber with a 12 h photoperiod at the UC Davis Controlled Environment Facility. For growth and gas exchange measurements of the natural accessions and NILs, the daytime growth chamber conditions averaged approximately 28.1 °C and 34.9% relative humidity (RH), and night-time conditions averaged approximately 17.5 °C and 43.1% RH.

### Gas exchange measurements

Gas exchange measurements ( $g$ ,  $E$ ,  $A$ ) were obtained using a LI-6400 portable photosynthesis system (Li-Cor, Inc., Lincoln, NE, USA) with a custom-built cuvette that enclosed the whole canopy (rosette) of *Arabidopsis* (see McKay *et al.* 2001; Juenger *et al.* 2005). Preliminary measurements of several plants for gas exchange through 30 h intervals (logged every 10 s) indicated that *Arabidopsis*  $g_{\text{night}}$  remained high and steady through the night. Thus, daytime measurements were made during the interval 3–5 h after chamber lights came on and night-time measurements were made during the interval 2–4 h before chamber lights came on the following morning. During night-time measurements, a headlamp with a green safe light with intensity

not detectable by an LI-190 sensor [ $0 \mu\text{mol m}^{-2} \text{s}^{-1}$  photosynthetic photon flux density (PPFD); Li-Cor] was used to avoid promoting stomatal opening. There is no evidence from previous trials that the very low (non-detectable) intensity of green light used for these measurements caused any change in stomatal aperture. The cuvette enclosed the whole rosette of each plant, and measurements were logged after stability was attained in the chamber. For daytime and night-time measurements, cuvette PPFD was 350 and  $0 \mu\text{mol m}^{-2} \text{s}^{-1}$ , respectively, and  $\text{CO}_2$  was maintained at  $400 \mu\text{mol mol}^{-1} \text{CO}_2$ . Temperature and RH in the gas exchange cuvette were set to approximate ambient growth chamber conditions (approximately 28 °C and 35% RH during daytime measurements, and 18 °C and 43% RH during night-time measurements).

Each block was measured on a different day for comparisons of gas exchange rates among natural accessions and NILs. Plants of some accessions did not germinate or died prior to gas exchange measurements, resulting in some blocks having less than two plants of each accession available for sampling each day. After the gas exchange measurements were completed for an individual plant, leaf areas were obtained from a digital photograph of the canopy, and image analysis was performed using Scion Image Software program (Scion Corporation, Frederick, MD, USA). Intrinsic WUE ( $\text{WUE}_i$ ) was calculated as  $A/g_{\text{day}}$ .

### Plant biomass and flowering time

Plants in two of the three blocks were harvested following gas exchange measurements for above-ground biomass determination, and leaf N and carbon isotope ratio ( $\delta^{13}\text{C}$ ). Plants were dried at 60 °C and weighed for dry biomass. Leaf tissue was ground and analysed for leaf N and  $\delta^{13}\text{C}$  at the UC Davis Stable Isotope Facility (<http://stableisotopefacility.ucdavis.edu>). Within a species where leaf size and morphology are similar, leaf  $\delta^{13}\text{C}$  reflects WUE integrated over the lifetime of the leaf (Farquhar,

**Table 2.** Correlation coefficients (*r*) for genotypic correlations (above the diagonal, *n* = 12) and phenotypic correlations (below the diagonal, *n* = 47 for gas exchange traits, 28–38 for above-ground biomass, leaf N and δ<sup>13</sup>C and 13–14 for flowering time) for natural accessions of *Arabidopsis thaliana*

	<i>g</i> <sub>night</sub>	<i>g</i> <sub>day</sub>	A	WUE <sub>i</sub>	δ <sup>13</sup> C	Leaf N	Biomass (above-ground)	Flowering time
<i>g</i> <sub>night</sub>	–	0.95***	0.75**	–0.81***	–0.57	–0.15	0.01	0.09
<i>g</i> <sub>day</sub>	0.84***	–	0.87***	–0.84***	–0.53	–0.31	0.06	0.03
A	0.60***	0.73***	–	–0.61*	–0.38	–0.38	–0.01	–0.34
WUE <sub>i</sub>	–0.51***	–0.59***	–0.01	–	0.62*	0.10	0.13	–0.26
δ <sup>13</sup> C	–0.13	–0.04	0.22	0.49**	–	–0.27	0.49	–0.07
Leaf N	–0.28	–0.54**	–0.66***	–0.15	–0.37*	–	–0.75**	0.05
Biomass (above-ground)	0.33	0.46*	0.72***	0.43*	0.59***	–0.63***	–	0.13
Flowering time	0.75**	0.66*	0.28	–0.64*	N/A	N/A	N/A	–

Asterisks indicate significance, \**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001. N/A, traits only collected on non-overlapping blocks.

Ehleringer & Hubick 1989; Ehleringer, Phillips & Comstock 1992). A higher (less negative) leaf δ<sup>13</sup>C reflects greater WUE. Plants in the third block were allowed to continue growing so that the number of days to flowering could be calculated as the number of days from germination to bolting.

**Statistics**

Natural accessions were compared for gas exchange characters (*g*<sub>night</sub>, *E*<sub>night</sub>, *g*<sub>day</sub>, A) with two-way analysis of variance (ANOVA) (PROC GLM, SAS Institute, Inc., Cary, NC, USA) with accession and block as fixed effects. *Ler*-0, delta 2.1 and delta 3.1 were also compared for gas exchange characters (*g*<sub>night</sub>, *E*<sub>night</sub>, *g*<sub>day</sub>, A) with two-way ANOVA. *Col*-0 and *ColFRI* were compared using a two-way ANOVA. Data were log transformed and weighted by the inverse of the variance as necessary to meet assumptions of normality and homoscedasticity. Reported results are back-transformed least square (LS) means and SEs. For the 12 accessions, we calculated the correlations between characteristics of their native habitat (mean annual VPD<sub>air</sub>, mean monthly VPD<sub>air</sub>, mean annual precipitation, mean monthly precipitation) and plant traits (using accession means). Additionally, we calculated genetic correlations (using accession means) and phenotypic correlations (using individual plants) among the follow traits: *g*<sub>night</sub>, *g*<sub>day</sub>, A, WUE<sub>i</sub>, δ<sup>13</sup>C, leaf N, pre-flowering above-ground biomass and number of days to flowering, except for phenotypic correlations where the need for destructive harvests resulted in traits collected on separate blocks (see Table 2).

**RESULTS**

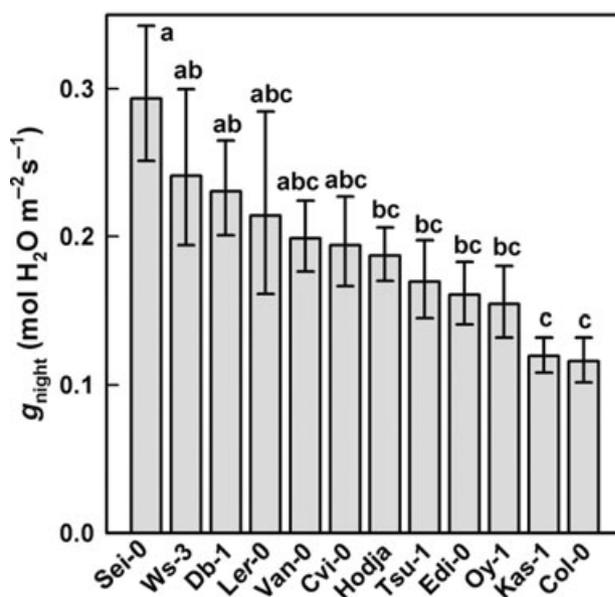
**Genetic variation in *g*<sub>night</sub>**

Each of the *Arabidopsis* natural accessions exhibited substantial *g*<sub>night</sub>. The 12 accessions differed significantly for *g*<sub>night</sub> (Fig. 1), with *g*<sub>night</sub> varying 2.5-fold. Under our experimental conditions, with daytime and night-time VPD<sub>leaf</sub> averaging 1.6 and 0.6 kPa, respectively, *E*<sub>night</sub> was 23–33% of

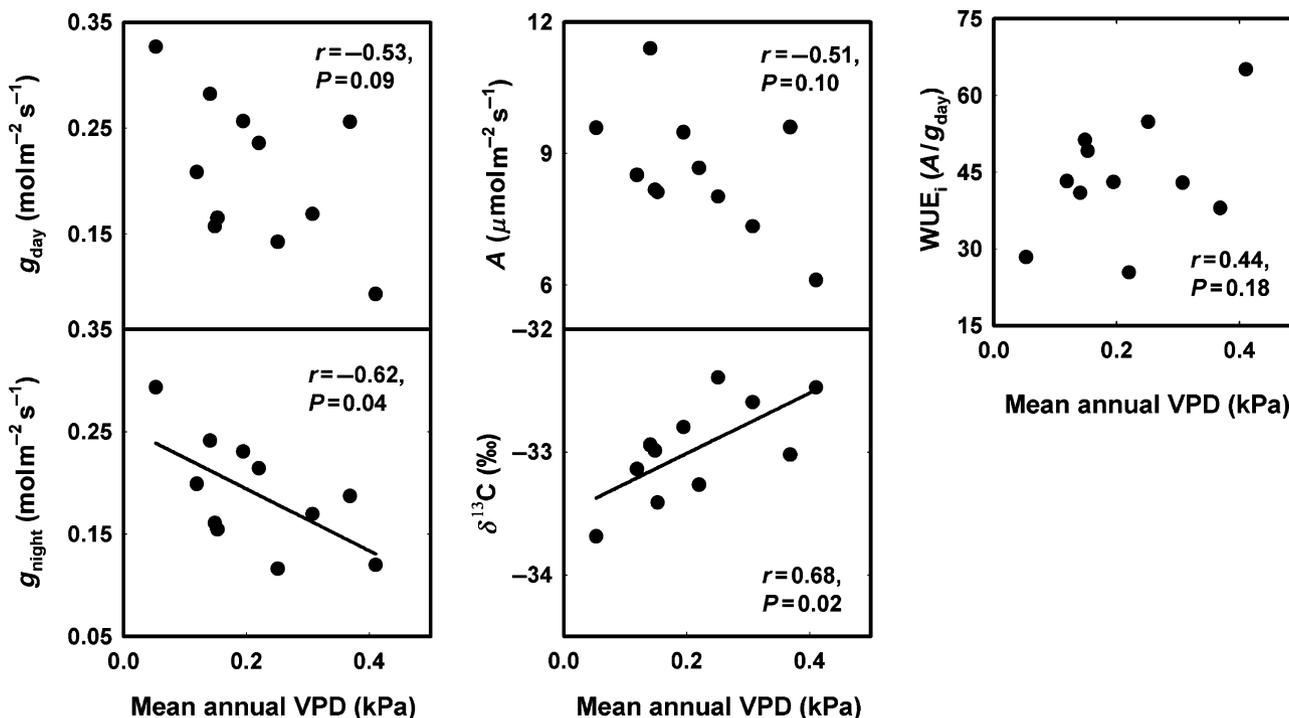
daytime rates among accessions, representing a substantial fraction of total daily water loss occurring during dark, non-photosynthetic times. The accessions also differed significantly for *g*<sub>day</sub> (*P* < 0.001), A (*P* < 0.001), WUE<sub>i</sub> (*P* < 0.001), δ<sup>13</sup>C (*P* < 0.001), leaf N (*P* = 0.01), above-ground biomass (*P* < 0.001) and flowering time (*P* < 0.001).

**Correlations of *g*<sub>night</sub> with characteristics of accession native habitats**

Among accessions, *g*<sub>night</sub> was negatively correlated with the native habitat mean annual VPD<sub>air</sub> (Fig. 2). When the relationship between *g*<sub>night</sub> and monthly mean VPD<sub>air</sub> was examined, the maximum *r* values occurred during the spring



**Figure 1.** Night-time total leaf conductance (*g*<sub>night</sub>) in 12 natural accessions of *Arabidopsis thaliana*. Different letters indicate significantly different *g*<sub>night</sub> values among the accessions (*P* < 0.05). Conductance data are back-transformed least square (LS) means and SE (*n* = 2–6 per accession).



**Figure 2.** Correlations between native habitat mean annual atmospheric vapour pressure difference ( $\text{VPD}_{\text{air}}$ ) and accession traits in the common garden experiments: night-time leaf conductance ( $g_{\text{night}}$ ), daytime leaf conductance ( $g_{\text{day}}$ ), photosynthesis ( $A$ ), carbon isotope discrimination ( $\delta^{13}\text{C}$ ) and intrinsic water-use efficiency ( $\text{WUE}_i$ ) for the *Arabidopsis thaliana* accessions. The 'Cvi' accession is excluded here because of unavailability of  $\text{VPD}_{\text{air}}$  data. Data are back-transformed least square (LS) means.

(February–March) and fall (September–November) months. There was no correlation between  $g_{\text{night}}$  and native habitat mean annual or mean monthly precipitation ( $r = 0.11$ ,  $P = 0.76$  and  $r < 0.26$ ,  $P > 0.44$  for all months, respectively). For daytime traits, the relationships of  $g_{\text{day}}$  and  $A$  to mean annual  $\text{VPD}_{\text{air}}$  trended in the same negative direction, but were not significant (Fig. 2). Mean annual  $\text{VPD}_{\text{air}}$  was positively correlated with  $\delta^{13}\text{C}$ , but not with  $\text{WUE}_i$  (Fig. 2).

### Genetic correlations of $g_{\text{night}}$ with other traits

We found highly significant genetic correlations among the gas exchange traits (Table 2);  $g_{\text{night}}$  was positively correlated with  $g_{\text{day}}$  and  $A$ , and negatively correlated with  $\text{WUE}_i$  (Fig. 3);  $g_{\text{day}}$  was positively correlated with  $A$  and negatively correlated with  $\text{WUE}_i$  (Table 2). There was no genetic correlation of  $g_{\text{night}}$  or  $g_{\text{day}}$  with leaf N, above-ground biomass or flowering time. Phenotypic correlations generally paralleled the genotypic correlations in direction and significance for the gas exchange traits, but not for leaf N, above-ground biomass and flowering time (Table 2).

### NIL comparisons to natural accessions

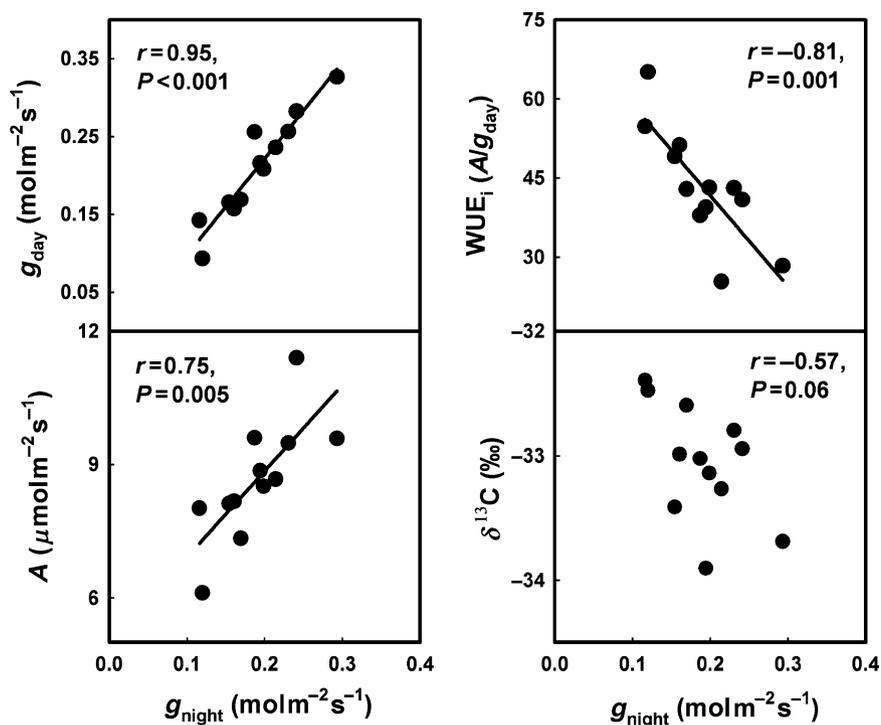
NILs delta 2.1 and delta 3.1 did not differ in  $g_{\text{night}}$  when compared to the *Ler-0* natural accession from which they were derived ( $P = 0.18$ ; Fig. 4), but they did have a higher

$g_{\text{day}}$  than *Ler-0* ( $P = 0.01$ ). In contrast, *ColFRI* plants had a lower  $g_{\text{night}}$  but a similar  $g_{\text{day}}$  when compared to the *Col-0* natural accession from which it was derived ( $P = 0.05$  and  $P = 0.74$ , respectively).

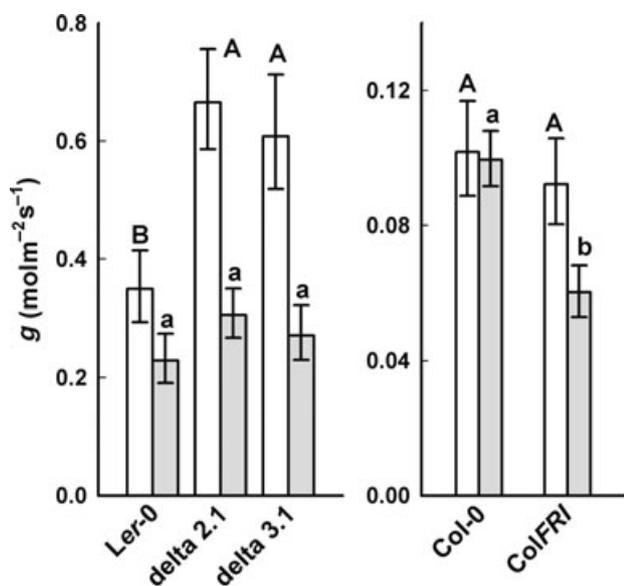
### DISCUSSION

The stomata of *Arabidopsis* did close partially during the dark, but  $g_{\text{night}}$  remained substantially greater than cuticular conductance throughout the night, similar to previous reports for the species (Leymarie *et al.* 1998, 1999; Dodd, Parkinson & Webb 2004; Dodd *et al.* 2005). We found within-species genetic variation for  $g_{\text{night}}$  among the 12 accessions, as well as for the other traits:  $g_{\text{day}}$ ,  $A$ ,  $\text{WUE}_i$ ,  $\delta^{13}\text{C}$ , leaf N, above-ground biomass and flowering time. The variation in  $g_{\text{night}}$  is consistent with that previously reported for  $\delta^{13}\text{C}$  and flowering time for a larger set of natural accessions of *Arabidopsis* (McKay *et al.* 2003), and consistent with genetic variation for daytime gas exchange characters for other plant species (reviewed in Arntz & Delph 2001). Within-species genetic variation for  $g_{\text{night}}$  suggests there is potential for selection to operate on this trait if it is directly or indirectly related to plant growth and fitness. However, variation alone does not necessarily mean that selection for or against the trait has occurred.

We assessed relationships between climate characteristics of the native habitats and the accession plant traits in a common garden setting to determine if the accession



**Figure 3.** Scatterplots representing genetic correlations for night-time leaf conductance ( $g_{\text{night}}$ ) with daytime leaf conductance ( $g_{\text{day}}$ ), photosynthesis ( $A$ ), integrative water use efficiency ( $\delta^{13}\text{C}$ ) and intrinsic water-use efficiency ( $\text{WUE}_i$ ;  $A/g_{\text{day}}$ ) among natural accessions of *Arabidopsis thaliana*. Data are back-transformed least square (LS) means.



**Figure 4.** Total leaf conductance ( $g$ ) during the day (open bars) and night (shaded bars) in *Arabidopsis thaliana* accession Ler-0 and two near isogenic lines (NILs) derived from a cross between Ler-0 and Cvi (left) and accession Col-0 and ColFRI NIL (right). Plants were grown in a common garden growth chamber under identical conditions. Note the difference in scales between panels. Capital letters denote significant differences in  $g_{\text{day}}$ , and lower case letters denote significant differences in  $g_{\text{night}}$ . Data are back-transformed least square (LS) means and SE ( $n = 2-6$ ).

differentiation was consistent with a hypothesized evolutionary outcome of selection on these traits (Arntz & Delph 2001). Because  $g_{\text{night}}$  can lead to water loss at night with no concomitant carbon gain, the potential for a high  $g_{\text{night}}$  might be disadvantageous in water-limited habitats, but under weak selection or selectively neutral in wetter habitats.  $\text{VPD}_{\text{air}}$  can be a proxy for habitat water availability because it is the atmospheric end of the leaf-to-air vapour pressure gradient driving plant water loss: habitats with higher temperatures and lower precipitation have a higher  $\text{VPD}_{\text{air}}$ . Comstock & Ehleringer (1992) found that more of the among-population genetic variation in  $\text{WUE}$  for an arid shrub (*Hymenoclea salsola*) was explained by models including the source habitat  $\text{VPD}_{\text{air}}$  as compared to just including precipitation or temperature. The accession differences in  $g_{\text{night}}$  among *Arabidopsis* under well-watered conditions, and the negative correlation between mean annual  $\text{VPD}_{\text{air}}$  and  $g_{\text{night}}$  suggest that an inherently lower  $g_{\text{night}}$  and associated night-time water loss were favoured by selection in drier habitats. However, there was also a significant correlation between mean annual  $\text{VPD}_{\text{air}}$  and  $\delta^{13}\text{C}$ , and non-significant trends between mean annual  $\text{VPD}_{\text{air}}$  and  $g_{\text{day}}$ ,  $A$  and  $\text{WUE}_i$ , raising the issue of which of these traits is actually under direct selection. This common garden study cannot determine whether the observed among-accession variation in  $g_{\text{night}}$  is likely the result of direct selection on  $g_{\text{night}}$  or the result of indirect selection mediated through  $g_{\text{day}}$ ,  $A$ ,  $\text{WUE}$  or other unmeasured traits, but assessing genetic correlations can provide some insights into the potential for indirect selection.

There were significant genetic correlations between  $g_{\text{night}}$  and daytime gas exchange traits, such that the accessions

with the highest  $g_{\text{night}}$  also had the greatest  $g_{\text{day}}$  and  $A$ , and the lowest  $WUE_i$ . The genetic correlations could reflect pleiotropy, close linkage of loci individually affecting each trait and/or linkage disequilibrium among loci that are not physically linked, presumably by correlated selection on both traits. Given the expectation for selection in water-limited habitats to act on  $WUE_i$  and its components of  $g_{\text{day}}$  and  $A$ , it is then reasonable to suggest that the accession differentiation in  $g_{\text{night}}$  may be caused by direct selection on  $WUE_i$ ,  $g_{\text{day}}$  or  $A$ , and associated indirect selection on  $g_{\text{night}}$ . This could lead to accession differentiation in  $g_{\text{night}}$  that was correlated with native habitat aridity even if  $g_{\text{night}}$  was selectively neutral or slightly disadvantageous. Studies of these plants with phenotypic selection analysis would be necessary to actually test whether there is direct or indirect selection on  $g_{\text{night}}$ .

For the natural accessions, higher  $g_{\text{night}}$  was associated with lower  $WUE_i$ . Although there was also a negative trend for  $g_{\text{night}}$  with integrative WUE ( $\delta^{13}\text{C}$ ), the genetic correlation was not significant. The relationship between  $g_{\text{night}}$  and WUE is logical from the point of view that there are two mechanisms – carbon gain and water loss – that affect WUE and increased water loss as by  $E_{\text{night}}$  would increase water loss without carbon gain, thereby lowering whole-plant WUE. However, it is worth considering that the often-used measures of  $WUE_i$  and integrated WUE ( $\delta^{13}\text{C}$ ) are leaf-level surrogates for whole-plant WUE that are related to daytime carbon gain and water loss, and thus, do not account for water loss by  $E_{\text{night}}$ . Positive correlations between  $g_{\text{night}}$  and both  $A$  and  $g_{\text{day}}$  may be driving the relationship with  $WUE_i$  because it is calculated as the ratio of  $A$  to  $g_{\text{day}}$ . Similarly for  $\delta^{13}\text{C}$ ,  $g_{\text{night}}$  being highly correlated to  $g_{\text{day}}$  may be the indirect cause for the trend. Regardless, the implications for the lack of significant relationship between  $g_{\text{night}}$  and  $\delta^{13}\text{C}$  are significant in that night-time water loss is typically ignored or considered negligible in  $C_3$  and  $C_4$  plants. This may be particularly important in crop species, some of which are known to have substantial  $g_{\text{night}}$  (Rawson & Clarke 1988; Musselman & Minnick 2000; Caird *et al.* 2007b) and where  $\delta^{13}\text{C}$  plays a large role in selection criteria for new cultivars better suited to habitats with contrasting water availability.

The delta 2.1 and delta 3.1 NILs had substantially lower WUE ( $\delta^{13}\text{C}$ ) and higher  $g_{\text{day}}$ , but similar  $g_{\text{night}}$ , compared with plants of the genetic background genotype *Ler-0*. *ColFRI* plants had opposite phenotypes with lower  $g_{\text{night}}$  in *ColFRI* plants than in *Col-0* plants, but no difference in  $g_{\text{day}}$ . These differences are the first we know of that show a differential effect for  $g_{\text{day}}$  and  $g_{\text{night}}$  because of specific genomic regions. This result also demonstrates a new physiological function of *FRIGIDA*, suggesting network models of flowering time may be missing a phenotypic dimension. Even with this start of knowledge of specific genetic effects on  $g_{\text{night}}$ , the next challenge is to elucidate the exact mechanism(s) underlying variation, because many genes function to affect gas exchange and WUE via multiple mechanisms. For example, the *Arabidopsis ERECTA* mutation in *Ler* has been shown to have a large effect on  $\delta^{13}\text{C}$  via mechanisms

affecting both transpiration, including stomatal density and conductance, and photosynthesis, including mesophyll cell distribution and reduced  $A_{\text{max}}$  (Masle *et al.* 2005). Thus, for the natural variation observed in this study, it is likely that multiple genes acting through multiple pathways ultimately affect  $g_{\text{night}}$ .

Although there is a highly significant genetic correlation between  $g_{\text{night}}$  and  $g_{\text{day}}$  among the natural accessions, it is not altogether surprising that the two processes are not under identical physiological or genetic control. There are several effectors of stomata which should affect  $g_{\text{day}}$  and  $g_{\text{night}}$  separately (i.e. factors affecting photosynthetic processes would affect  $g_{\text{day}}$  but not  $g_{\text{night}}$ ), and thus it is expected that some genomic regions will affect one process but not the other. For the delta 2.1 and delta 3.1 NILs in this study, it is probable that the genomic region that differs from the parental genotype is responsible for factor(s) which affect(s)  $g_{\text{day}}$  but not  $g_{\text{night}}$ ; the opposite would be true for *FRIGIDA*. However, the tight correlation between  $g_{\text{night}}$  and  $g_{\text{day}}$  may also be caused by factors which affect stomata in similar ways, such as morphological or anatomical differences among accessions that affect both  $g_{\text{night}}$  and  $g_{\text{day}}$  in the same manner (e.g. stomatal size and density). Differences in stomatal size and density could at least partially explain the observed variation in  $g_{\text{night}}$  among these *Arabidopsis* accessions as well as the tight  $g_{\text{night}}-g_{\text{day}}$  correlation. Furthermore, there is evidence that the magnitude of  $g_{\text{day}}$  affects the magnitude of  $g_{\text{night}}$  through effects on  $A$  and carbohydrate metabolism (Easlon, personal communication), which could be a physiological mechanism behind the observed correlation. Thus,  $g_{\text{night}}$  is likely affected by several genetic and physiological factors which may influence its magnitude separately or in tandem with  $g_{\text{day}}$ .

The emphasis on  $g_{\text{night}}$  and water availability in this common garden *Arabidopsis* study can be contrasted with the results from the common garden study with saplings of 21 tree species of Marks & Lechowicz (2007). Although that study used a different method (sap flux) to assess the integrated effects of  $g_{\text{night}}$  and  $E_{\text{night}}$  that were measured as whole-canopy instantaneous gas exchange in this study, the methods at different scales have generally been shown to be in good agreement (Caird *et al.* 2007a,b; Marks & Lechowicz 2007). For the tree saplings, high nocturnal sap flux was not associated with species differences in soil water availability of native habitats, but was associated with fast growing shade-intolerant species. The strong cross-species correlation between nocturnal sap flux and leaf N suggests that high nocturnal sap flux may sustain carbohydrate export and other processes driven by dark respiration needed to support fast growth (Marks & Lechowicz 2007). In *Arabidopsis*, there were no genetic or phenotypic correlations of  $g_{\text{night}}$  with leaf N or pre-flowering above-ground biomass which would suggest a similar mechanistic or fitness advantage. The differences between the two studies are perhaps not surprising given the differences in life history and sampling scales for the annual *Arabidopsis* and a set of deciduous woody species.

In summary, our finding of an association between *Arabidopsis* variation in  $g_{\text{night}}$  and aridity of the native habitats suggests that selection may have shaped this within-species variation, but the strong genetic correlations with daytime gas exchange traits raise the possibility that selection on  $g_{\text{night}}$  could have been indirect. Further characterization of genetic variation in  $g_{\text{night}}$  within and among populations and species, and of associations with other plant traits, plant fitness and native habitats will be needed to understand  $g_{\text{night}}$  as a putatively adaptive trait.

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