Original Article

Combining quantitative trait loci analysis with physiological models to predict genotype-specific transpiration rates

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ABSTRACT

Transpiration is controlled by evaporative demand and stomatal conductance ($g_s$), and there can be substantial genetic variation in $g_s$. A key parameter in empirical models of transpiration is minimum stomatal conductance ($g_0$), a trait that can be measured and has a large effect on $g_s$ and transpiration. In Arabidopsis thaliana, $g_0$ exhibits both environmental and genetic variation, and quantitative trait loci (QTL) have been mapped. We used this information to create a genetically parameterized empirical model to predict transpiration of genotypes. For the parental lines, this worked well. However, in a recombinant inbred population, the predictions proved less accurate. When based only upon their genotype at a single $g_0$ QTL, genotypes were less distinct than our model predicted. Follow-up experiments indicated that both genotype by environment interaction and a polygenic inheritance complicate the application of genetic effects into physiological models. The use of ecophysiological or ‘crop’ models for predicting transpiration of novel genetic lines will benefit from incorporating further knowledge of the genetic control and degree of independence of core traits/parameters underlying $g_s$ variation.

Key-words: Arabidopsis thaliana; Ball–Berry; drought; stomatal conductance; transpiration; water-use efficiency.

INTRODUCTION

The distribution, survival and fecundity of plant species depend upon the timing and availability of rhizospheric water (Lu et al. 1998; Heschel et al. 2002; Donovan et al. 2007). The frequency and severity of drought stress and extreme weather patterns, however, are predicted to increase in many locations worldwide (Sangakkara et al. 2001; Dai et al. 2004). Consequently, rhizospheric water deficits will intensify, potentially reducing crop yields and plant fitness (Araus et al. 2002; Kumar et al. 2008; Chenu et al. 2009). Concurrently, added demands will be placed on irrigation water as food crop production increases in an attempt to match world population growth (Howell 2001). These factors combine to make enhancing the drought adaptation of crops a vital component of contending with future limited water resources (Araus et al. 2002; Tardieu 2003; Campos et al. 2004).

Detailed studies in plant physiology have revealed that maintaining plant productivity in the face of drought involves both constitutive and inducible characteristics (Chaves et al. 2003) and is strongly associated with stomatal regulation of gas exchange and water-use efficiency (WUE) (e.g. Buckley & Mott 2002; Comstock 2002; Franks & Farquhar 2007). CO₂ uptake (photosynthesis, $A$) and water loss (transpiration, $E$) both occur through stomata, resulting in a trade-off between acquiring CO₂ for growth versus losing water; this is a fundamental constraint on land-plant form and physiology. Stomatal closure minimizes water loss and can be a rapid and effective strategy; however, it results in reduced $A$ and growth (Schultze 1986; Geber & Dawson 1997; Katul et al. 2009). Stomata are highly dynamic. They open to a maximum amount under high light and water availability, and close in response to external signals such as elevated vapour pressure deficit as well as internal signals of drying like increased concentrations of abscisic acid in xylem sap (Taiz & Zeiger 2010).

Generally, plants in more drought-prone environments exhibit lower minimum stomatal conductance ($g_0$), as $g_0$ is negatively correlated with WUE (Christman et al. 2008; Galmes et al. 2011). Additionally, $g_0$ is positively correlated with daytime stomatal conductance ($g_{aw}$) and $A$ (Christman et al. 2008). Arabidopsis thaliana has been shown to exhibit both genetic and environmental variation in $g_0$ (Christman et al. 2008; Fletcher et al. 2013). Given the importance of $g_0$, commonly used models of plant $E$ include $g_0$ as an independent parameter (e.g. Ball et al. 1987; Barnard & Bauerle 2013; Leuning 1995; Medlyn et al. 2011).

Recently, the parameterization of quantitative genetics via quantitative trait loci (QTL) in ecophysiological models has emerged as a way to predict and understand the causal basis of trait variation across multiple environments (Reymond et al. 2003; Yin et al. 2004; Hammer et al. 2006; Collins et al. 2008). This technique offers the ability to in silico predict the phenotypic outcome from breeding with known QTL that describe trait variation (e.g. Tardieu 2003), providing insight into how a genotype will respond to the environment. Genetically based descriptions of stomatal responses to environmental drivers are needed to advance...
leaf water flux estimates because at best, current stomatal conductance ($g_s$) models use a combination of physiological and empirical parameters to predict how $g_s$ will respond to climate constraints (Damour et al. 2010). QTL for $g_s$ have been discovered in *A. thaliana* (Fletcher et al. 2013) and understanding how these loci influence $g_s$ is important for parameterizing $g_s$ models. Hence, the phenotypic effect of these *A. thaliana* $g_s$ QTL can represent one or more causal polymorphisms at each identified genomic region—an important first step for replacing empirical approximations with functional genomics. Ultimately, incorporating genetic parameters into $g_s$ models may allow for improved predictions of $A_s$, biomass, $E$, yield and *in silico* simulation of diverse genotypes (Blanco et al. 2002; Hammer et al. 2006; Bertin et al. 2010).

The primary objective of this study was to measure and model $E$ for Kas-1 and Tsu-1, two *A. thaliana* parental lines with divergent WUE (McKay et al. 2008), as well as individuals from a recombinant inbred line (RIL) population created from a reciprocal cross of the parental accessions. We simulated $E$ with a three-dimensional spatially explicit plant model, MAESTRA (multi-array evaporation stand tree radiation assay), originally developed by Wang & Jarvis (1990) and described in detail by Medlyn (2004). In this work, we aim to predict genotype specific $E$ values by incorporating information on $g_s$ QTL.

**MATERIALS AND METHODS**

**Plant material**

We examined two accessions of *A. thaliana*, Kas-1 (CS903) and Tsu-1 (CS1640) (hereafter referred to as Kas and Tsu), known to be divergent in WUE (McKay et al. 2003; Juenger et al. 2010). Kas is native to Kashmir, India (34.5°N, 76°E). Tsu is from Tsushima, Japan (34.41°N, 129.33°E), a much warmer and wetter climate than Kas (McKay et al. 2003, 2008; Christman et al. 2008). In addition to the two parental lines, we investigated a population of 341 RILs created from a reciprocal cross between Tsu and Kas (McKay et al. 2008).

**Experiment 1: Minimum stomatal conductance and leaf area in parental lines**

Prior to planting, 56 6.35 cm × 8.89 cm black form pots were lined with polyester batting to prevent soil loss from the bottom of the pots. Pots were filled with Profile Porous Ceramic (PPC) Greens Grade dry soil (Profile Products LLC, Buffalo Grove, IL, USA) to 1 cm below the lip of the pot. All pots were placed in non-slatted flats and bottom-filled with water, left to soak overnight and siphoned off twice to leach any salts from the soil. Four Kas or Tsu seeds were randomly assigned and sown at the centre of each pot and placed in flats.

Immediately after sowing, flats were filled with half-strength Hoagland’s solution, covered with clear plastic domes to prevent excess evaporation, and stratified in a dark refrigerator at approximately 4 °C for 5 d. Soil surfaces were misted to saturation twice daily until germination. After cold stratification, the flats were transferred to a growth chamber and grown under 8:16 h (light : dark) photoperiod, with approximately 330 μmol m−2 s−1 PPFD (photosynthetic photon flux density) at crown height. Temperatures were set to 23 °C and 40% relative humidity (RH) during the light period, and 18 °C and 60% RH in the dark. Temperature and RH gradually ascended to daytime conditions over the course of half an hour (mimicking sunrise), and likewise in the transition to dark conditions (sunset). Germination occurred 2 d after transfer to the growth chamber with clear plastic domes remaining on the flats for 3 d post-germination. Approximately 1 week after germination, plants were thinned to one per pot.

The pots were flood-irrigated every 3–4 d by filling the flats with water and allowing the pots to become saturated for 5–10 min before siphoning off the water. This allowed the plants to experience wet conditions without the risk of root hypoxia/anoxia. Once a week, half-strength Hoagland’s solution was used in place of water. During the second portion of the experiment, a gradual dry-down was imposed on the plants, decreasing gravimetric water content by up to 10% each day. Mean container maximum water capacity was approximately 93% at the beginning of the dry-down and ended near 40% gravimetric water content.

We simultaneously used three Decagon SC-1 Leaf Porometers (Decagon Devices, Inc., Pullman, WA, USA) to determine $g_{soy}$ and $g_s$. The porometers were cross-calibrated and allowed to equilibrate to ambient temperature and RH for at least 30 min prior to measurement. *A. thaliana* $g_s$ has been shown to remain consistent throughout the night (Christman et al. 2008); but nevertheless, minimum and daytime $g_s$ values were recorded for all replicates between 4 and 2 h pre-dawn and at solar noon. $g_s$ measurements were taken with the aid of photosynthetically inactive light-emitting diode headlamps [four layers of green Clearphane® Film (Item CL2405-GN; Highland Supply Corp., Highland, IL, USA) emitting <1 μmol m−2 s−1 PAR (photosynthetically active radiation) at 0.2 m from the light source (Eveready Battery Co., Inc., US Patent D592,782)] to avoid PAR-driven stomatal opening. $g_{soy}$ values were recorded inside the environmentally controlled growth chamber and all $g_s$ values were obtained in ~30 s using the SC-1’s automatic mode. This allowed consistent measurements between plants and days, and ensured that stomatal environmental reaction times were not reached (e.g. Zeiger & Field 1982). All $g_s$ measurements were collected from similar-aged, non-damaged leaves ($n = 23$) over 2 d during both wet and dry conditions.

A destructive harvest immediately followed the final day of $g_s$ measurements. All replicates were harvested by removing the rosette from the roots with a razor blade at the base of the stem. We dissected leaves from the stems and laid them flat on a white sheet of paper for overhead photographing. Leaf areas were calculated from the photographs with ImageJ (Schneider et al. 2012).
Experiment 2: Leaf-level gas exchange in a RIL population

Tsu, Kas and individuals from the RIL population were sown in 7.6 cm pots containing Fafard 4P mix (Conrad Fafard Inc., Agawam, MA, USA) in 2 replicates per genotype, and stratified in the dark at 4 °C for 5 d. The plants were transferred to a Conviron ATC60 growth chamber (Controlled Environments, Winnipeg, MB, Canada) set for 8:16 h (light : dark) days. Temperature and RH were 23 °C and 40% during the day, and 20 °C and 50% at night. Plants were grown for approximately 6 weeks before gas exchange measurements. Leaf-level gas exchange data were collected with a CIRAS-2 portable gas exchange system fitted with a PLC(6) cuvette (PP Systems, Amesbury, MA, USA). Mean cuvette conditions were as follows for the light measurements: 397 ppm CO₂, 299 μmol m⁻² s⁻¹ PPFD, 32% RH and 23 °C. Each plant’s measurements were averaged over 10 readings taken approximately every 10 s, post-equilibration. Prior to dark gas exchange measurements, plants were dark-adapted in the growth chamber for 20–28 h. Dark gas exchange data were collected in a dark room (0 μmol m⁻² s⁻¹ PPFD) at 23 °C. Cuvette environmental conditions for dark measurements were as follows for the light measurements: 397 ppm CO₂, 299 μmol m⁻² s⁻¹ PPFD, 32% RH and 23 °C. Each plant’s measurements were averaged over 10 readings taken approximately every 10 s, post-equilibration. Prior to dark gas exchange measurements, plants were dark-adapted in the growth chamber for 20–28 h. Dark gas exchange data were collected in a dark room (0 μmol m⁻² s⁻¹ PPFD) at 23 °C. Cuvette environmental conditions for dark measurements were set to mimic those recorded in the light, with the exception of PPFD.

We used the Easlon et al. (2014) data set, which contained identical A. thaliana accessions, to calculate the maximum Rubisco-limited rate of photosynthesis (V_cmax) and the maximum rate of electron transport (J_max) (Supporting Information Table S1). To obtain these values, we used whole-crown gas exchange A-C i responses [where A is photosynthesis (μmol m⁻² s⁻¹) and C_i is internal CO₂ concentration (mole fraction of CO₂)] with the Farquhar and von Caemmerer models (von Caemmerer & Farquhar 1982; Medlyn et al. (2007) (Supporting Information Table S1)). Using meteorological data, genotype-specific leaf-level physiological information and leaf and crown morphological parameters, MAESTRA computes whole-crown estimates of E and A by scaling up leaf-level calculations (e.g. Bauerle & Bowden 2011). Photosynthesis is calculated from the Farquhar–von Caemmerer biochemical sub-model (Farquhar & von Caemmerer 1982; Reynolds et al. 2009) coupled to the Ball–Berry–Leuning (BBL) g_s sub-model (Leuning 1995) (Eqn 1):

\[ g_{sw} = g_s + g_i \frac{A_i}{c_i - \Gamma}(1 + D_s/D_h) \]

where \( g_{sw} \) is to water, \( g_i \) is an empirically fitted parameter, \( A \) is the net carbon assimilation rate, \( c_i \) is the CO₂ mole fraction at the leaf surface, \( \Gamma \) is the CO₂ compensation point, \( D_s \) is the vapour pressure deficit and \( D_h \) is the empirical coefficient.

MAESTRA inserts \( g_{sw} \) into the isothermal form of the Penman–Monteith equation (Eqn 2) to spatially calculate E on a crown sub-volume basis, resulting in a whole-crown E estimate (Medlyn et al. 2007):

\[ \lambda E = \frac{m R_n + D_s g_s c_i M_h}{m + g_{sw} M_h} \]

where \( \lambda \) is the latent heat of water vapour (J mol⁻¹), \( E \) is transpiration per unit leaf area (mol m⁻² s⁻¹), \( m \) is the slope of the curve relating saturation water vapour pressure to temperature (Pa K⁻¹), \( R_n \) is the isothermal net radiation (W m⁻²), \( D_s \) is the vapour pressure deficit (kPa), \( g_s \) is the total leaf conductance to heat (mol m⁻² s⁻¹), \( c_i \) is the specific heat of air (1010 J kg⁻¹ K⁻¹), \( M_h \) is the molecular mass of air (29 × 10⁻³ kg mol⁻¹). \( \gamma \) is the psychometric constant (Pa K⁻¹) and \( g_{sw} \) is to water (mol m⁻² s⁻¹).

Model parameterization and validation

We parameterized Kas and Tsu accessions with measured values for the following important physiology parameters from the aforementioned independent experiments: \( g_s, g_i, V_{cmax}, J_{max} \), quantum yield of electron transport (\( \alpha \)) and dark respiration (\( R_d \)) (Table 1 and Supporting Information Table S1). Parameter effects were calculated with the method described in Bauerle et al. (2014). Following this method, we

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition</th>
<th>Kas value</th>
<th>Tsu value</th>
<th>Units</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>( g_0 )</td>
<td>Minimum value of ( g_s )</td>
<td>36.6</td>
<td>69.1</td>
<td>mmol m⁻² s⁻¹</td>
<td>This study</td>
</tr>
<tr>
<td>( V_{cmax} )</td>
<td>Maximum Rubisco-limited rate of photosynthesis</td>
<td>61.3</td>
<td>73.03</td>
<td>μmol m⁻² s⁻¹</td>
<td>Easlon et al. (2014)</td>
</tr>
<tr>
<td>( J_{max} )</td>
<td>Maximum rate of electron transport</td>
<td>96.43</td>
<td>122.31</td>
<td>μmol m⁻² s⁻¹</td>
<td>Easlon et al. (2014)</td>
</tr>
<tr>
<td>( R_d )</td>
<td>Dark respiration</td>
<td>1.47</td>
<td>1.28</td>
<td>μmol m⁻² s⁻¹</td>
<td>This study</td>
</tr>
<tr>
<td>( \Gamma )</td>
<td>CO₂ compensation point</td>
<td>40.6</td>
<td>30.9</td>
<td>μmol mol⁻¹</td>
<td>This study</td>
</tr>
<tr>
<td>LAREA</td>
<td>Leaf area of crown</td>
<td>0.0035</td>
<td>0.00416</td>
<td>mm²</td>
<td>This study</td>
</tr>
</tbody>
</table>
individually modelled $E$ with Kas and Tsu physiology, and systematically varied the three parameters with the largest effect ($g_0, J_{\text{max}}$ and $R_a$). We used the pooled Kas and Tsu means for the ‘base case’ values. Kas and Tsu measured values were used as the minimum and maximum, respectively. Complete lists of all parameter values, including Tsu and Kas morphology, are reported in Supporting Information Tables S1–S3.

MAESTRA $E$ estimates for Tsu and Kas were compared with the measured $E$ values obtained from a separate leaf-level gas exchange experiment (Experiment 2). The mean measured day:night values for environmental conditions were used to parameterize the MAESTRA simulation: PAR (302.5 $\mu$mol m$^{-2}$ s$^{-1}$), RH (30:60%), $T_{\text{air}}$ (28.24 °C) and wind speed (0.5 m s$^{-1}$). We used 12 randomly selected $g_0$ values (Experiment 1) for Kas and Tsu $E$ simulations to obtain an estimate of modelling error.

We previously identified a QTL for $g_0$ in this population, located at the top of chromosome 1 (Fletcher et al. 2013). We modelled $E$ for RILs based upon allelic class at this QTL, where RILs with a Kas allele were assigned a $g_0$ of $39 \pm 1$ mmol m$^{-2}$s$^{-1}$ and RILs with a Tsu allele were assigned a $g_0$ of $48 \pm 2$ mmol m$^{-2}$s$^{-1}$. For each of the $g_0$ QTL allele classes, the model was separately parameterized with both Kas and Tsu values at the other physiological parameters (Table 1 and Supporting Information Table S1), which had a minimal influence on the predicted $E$ values MAESTRA $E$ estimates were compared with the measured gas exchange $E$ values for the RILs.

**Statistical analyses**

All statistical analyses were completed with JMP (JMP Pro 10; SAS Institute Inc., Cary, NC, USA). To determine genotype and environmental effects on $g_0$, we performed an ANOVA using $g_0$ as the response variable, with genotype ($G$) and environment ($E_0$) as fixed effects, plus a $G \times E_0$ interaction in the model (Supporting Information Table S4). The difference between wet and dry $g_0$ values was analysed with a one-way ANOVA, and means were compared with the Tukey–Kramer honestly significant difference (HSD) method. Kruskal–Wallis rank sum tests with the Steel–Dwass method for comparisons of all pairs were performed on the non-parametric data, including validation of Tsu and Kas measured versus predicted $E$. Transpiration values for each RIL were calculated as least squares means from a model incorporating line, replicate and gas exchange chamber temperature.

For the QTL analysis of the slope of $g$, as a function of light intensity, we performed a genome scan with a single-QTL model using Haley–Knott regression in the R/qtl program (Broman et al. 2003; Broman & Sen 2009) of the R statistical package (R Development Core Team 2008) for the difference in conductance between darkness and light with cytoplasm (i.e. maternal parent) as an interacting covariate. A significance threshold was determined based upon 1000 permutations. ANOVA was performed on the QTL model to calculate effect size, percentage variance explained and the log10 likelihood ratio (LOD) score for each QTL (Supporting Information Table S5).

**RESULTS**

A sensitivity analysis of MAESTRA model parameter effects was performed to identify the three most influential parameters for $E$ prediction (Fig. 1). Minimum stomatal conductance had the largest effect on $E$ for both accessions, followed by $J_{\text{max}}$ and $R_a$.

To validate the MAESTRA model on *A. thaliana*, we compared measured and predicted $E$ in Kas and Tsu. We observed $E$ in Kas to be 0.99 mmol m$^{-2}$ s$^{-1}$ lower than in Tsu (Fig. 2). Modelled versus measured $E$ estimates were not statistically different from one another (Kas: $P = 0.68$; Tsu: $P = 0.69$). Measured $T_{\text{su}}$ $E$ was 3.85 mmol m$^{-2}$ s$^{-1}$ (±0.13 SEM) and modelled was 3.62 mmol m$^{-2}$ s$^{-1}$ (±0.23 SEM). Likewise, Kas measured $E$ was 2.86 mmol m$^{-2}$ s$^{-1}$ (±0.07 SEM) and predicted was 2.90 mmol m$^{-2}$ s$^{-1}$ (±0.17 SEM).

Next, we compared measured and simulated $E$ for Tsu- and Kas-allele RILs to test the accuracy of measured RIL $g_0$ values for predicting $E$ with all other parameters remaining constant at Kas or Tsu physiology. RILs were selected based upon known genotypes at the $g_0$ QTL of interest: containing either a Kas or Tsu allele at the locus. The RIL $g_0$ parameter values used in the model were as follows: $39 \pm 1$ mmol m$^{-2}$s$^{-1}$ for RILs containing the Kas allele and $48 \pm 2$ mmol m$^{-2}$s$^{-1}$ for RILs with the Tsu allele. The mean measured $E$ for the Kas- and Tsu-allele RILs were 3.07 mmol m$^{-2}$ s$^{-1}$ (±0.036 SEM) and 3.18 mmol m$^{-2}$ s$^{-1}$ (±0.048 SEM), respectively. Comparatively, MAESTRA-simulated $E$, using measured RIL $g_0$ values with Kas physiology for Kas-allele RILs and Tsu physiology for Tsu-allele RILs, yielded the following predictions: 2.89 mmol m$^{-2}$ s$^{-1}$ for Kas-allele RILs and 3.10 mmol m$^{-2}$ s$^{-1}$ for Tsu-allele RILs. Simulated $E$ values for Tsu-allele RILs predicted higher $E$, which is in line with the

**Figure 1.** Transpiration estimate sensitivity analysis results for the three most influential physiology parameters for Kas-1 (Kas) and Tsu-1 (Tsu) *Arabidopsis thaliana* accessions. To obtain these results, we used both Kas and Tsu measured default physiology parameters and systematically varied minimum stomatal conductance ($g_0$), maximum rate of electron transport rate ($J_{\text{max}}$) and dark respiration ($R_a$) one at a time during MAESTRA model runs. We used a pooled Kas and Tsu mean as the ‘base case’ parameter. Tsu parameters were used as the maximum values, and Kas parameters were used as the minimum parameter values. Parameter effect values were calculated with the method described in Bauerle et al. (2014).
Figure 2. Measured versus MAESTRA-estimated transpiration ($E$) for Tsu and Kas accessions. Error bars represent standard error of mean (SEM). Measured $E$ was 3.85 mmol m$^{-2}$ s$^{-1}$ ($\pm 0.13$ SEM) and modelled was 3.62 mmol m$^{-2}$ s$^{-1}$ ($\pm 0.23$ SEM). Likewise, Kas measured $E$ was 2.86 mmol m$^{-2}$ s$^{-1}$ ($\pm 0.07$ SEM) and predicted was 2.90 mmol m$^{-2}$ s$^{-1}$ ($\pm 0.17$ SEM). Kas measured and simulated $E$, as well as Tsu measured and simulated $E$, are significantly different ($P < 0.05$). Tsu versus Kas measured and estimated $E$ are significantly different from each other ($P < 0.05$). The mean measured day : night values for environmental conditions were used to parameterize the MAESTRA simulation: photosynthetically active radiation (302:5 μmol m$^{-2}$ s$^{-1}$), relative humidity (30:60%), air temperature (28:24 °C) and wind speed (0.5 m s$^{-1}$).

Figure 3. Measured versus simulated transpiration ($E$) values for Tsu-allele recombinant inbred lines (RILs) and Kas-allele RILs. Measured $E$ is represented by boxes and whiskers, while simulated $E$ is represented by the grey circles (mean) with error bars indicating modelled values of minimum stomatal conductance ($g_0$) ±1 SE from the mean. The mean measured Kas-allele RIL $g_0$ values and Tsu-allele RIL $g_0$ values were used as the modelled $g_0$ values (39 ±1 mmol m$^{-2}$ s$^{-1}$ for Kas-allele and 48 ±2 mmol m$^{-2}$ s$^{-1}$ for Tsu-allele RILs), with all other parameter values held constant at either Kas or Tsu physiology (Table 1 and Supporting Information Tables S1–S3). Measured Kas allele and Tsu allele $E$ are significantly different at $\alpha = 0.05$ level ($P = 0.049$).

Figure 4. Mean minimum stomatal conductance ($g_0$) norm of reaction plot for Kas-1 and Tsu-1 genotypes under wet and dry soil moisture conditions. Tsu experienced a significantly sharper decline in $g_0$ between wet and dry conditions than Kas (mean ± SE, $P < 0.0001$).

well-watered and dry soil conditions. For each genotype, we plotted a reaction norm of their phenotypes across the two environments (Fig. 4). Our results show that Kas has a narrower range of $g_0$ values than Tsu, with Kas’ mean range of $g_0$ values starting at 36.6 mmol m$^{-2}$ s$^{-1}$ under wet conditions and ending at 6.4 mmol m$^{-2}$ s$^{-1}$ under dry conditions. Tsu’s range of $g_0$ values between wet and dry conditions was 69.1–4.7 mmol m$^{-2}$ s$^{-1}$. Tsu experienced a steeper decline in $g_0$ throughout the course of the drought, with a mean wet to dry condition $g_0$ difference of 64.4 mmol m$^{-2}$ s$^{-1}$. Kas had a significantly lower difference between wet and dry condition $g_0$ of 30.2 mmol m$^{-2}$ s$^{-1}$ (Fig. 4), with a pairwise comparison of Kas to Tsu being significantly different ($P < 0.0001$). In other words, Kas maintained a more static $g_0$ value throughout the course of the dry down, relative to Tsu. Although Kas had a lower mean $g_0$ in the wet environment, it maintained a higher mean $g_0$ than Tsu during drought (Fig. 4). ANOVA showed $G$, $E_n$ and the $G \times E_n$ interaction terms to be highly significant (Supporting Information Table S4, $P < 0.0001$) for predicting $g_0$.

Our effort to predict genotype-specific $E$ values using a single QTL for $g_0$ showed promise, but the predictions were not as accurate as we had hoped. One explanation is that traits other than $g_0$ are genetically variable. To test this idea, we examined genetic variation in the difference in $g_0$ between darkness and light conditions by scanning for QTL. We identified a QTL on the top of chromosome 3 for RILs with Kas cytoplasm (Fig. 5). This QTL explained 5.8% of the variance in this change in conductance (Supporting Information Table S5). RILs with the Kas allele at this QTL had a higher slope of conductance as a function of light intensity than those with the Tsu allele, despite having similar values of $g_0$.

Although this QTL only explained approximately 60% as much variance as the $g_0$ QTL did (Fletcher et al. 2013), the higher conductance in the higher light environment allows it to confound the differences based upon the $g_0$ QTL.

DISCUSSION

The motivation of the current study was to develop a modelling approach for predicting $E$ responses of diverse plant
genotypes. Models that predict $E$ as a function of both genetics and environmental factors should explain empirical data better than current models that do not include genetic attributes. Our analysis and empirical results support the concept proposed by Reymond et al. (2003) that combining QTL information and ecophysiological models aids in prediction at the whole-plant scale. QTL data for $g_0$ also show promise for improving $E$ estimates among A. thaliana RILs, which could account for the variation in stomatal behaviour among genotypes. We focus upon a single QTL associated with $g_0$ because of the substantial influence of leaf-level $g_0$ traits on leaf to global scale $E$ estimates (e.g. Bauerle et al. 2014). The parameterization of quantitative genetics via QTL in ecophysiological models has emerged as a way to predict and understand the genetic basis of trait variation across multiple environments, an important first step for adding functional genomics to leaf water flux estimates (e.g. Hammer et al. 2006; Collins et al. 2008; Chenu et al. 2009).

Bauerle & Bowden (2011) identified $g_0$ as a key parameter for accurate $E$ estimates in the Ball–Berry family of equations (e.g., Ball et al. 1987; Leuning 1995). Subsequently, Barnard & Bauerle (2013) found $g_0$ to have the greatest influence on C3 species $E$ estimates at the whole-plant level and Bauerle et al. (2014) showed that $g_0$ has a substantial influence on $E$ estimates across an array of environmental conditions at the regional and continental scale. Genetically based descriptions of stomatal responses to environmental drivers are needed to advance $g_0$ for $g_s$ models because they currently only use a combination of physiological and empirical parameters to predict how $g_s$ will respond to climate constraints (Damour et al. 2010). Hence, we focused upon the genetic characterization of $g_0$, in the widely used Ball–Berry family of equations.

We observed that Tsu, with relatively high well-watered $g_s$ values, transpired water faster and also reduced $g_s$ more rapidly than Kas accessions in response to a gradual dry-down (Fig. 4). Tsu experienced the sharpest decline in $g_0$ while simultaneously using the most water. On average, Kas individuals lost 73 g (combined $E$ and evaporative water loss) of water compared to Tsu’s 100 g over the course of 11 days. Figure 4 illustrates Tsu and Kas norms of reaction over the course of the dry-down, and shows the $G \times E_n$ interaction that occurs as soil moisture is depleted and Kas/Tsu responses differ. Such genotype by environment interaction, where the effect size of a single mutation varies as a function of environments, will prove challenging to standard ecophysiology models that assume all plants are equally sensitive to environmental variables.

Even if we identify all $g_0$ QTL and perfectly account for all $G \times E_n$ at these QTL, other parameters in the Ball–Berry models may also have heritable variation. For example, $g_s$ has recently been identified as the second most influential parameter for transpiration estimates (Barnard & Bauerle 2013; Bauerle et al. 2014); however, it is unclear if and how $g_s$ varies among species (summarized in Xu & Baldocchi 2003). Our follow-up experiment identified a QTL for the slope of $g_s$ as a function of light intensity (Fig. 5). Even with the minimal power in our RIL experiment, we have identified genetic variation in both traits and a single major QTL for $g_0$ and another QTL related to $g_s$. Together, these results show that the genetics of these traits are somewhat independent and thus adding genetic parameters for both traits should improve genotype-specific predictions of $E$.

The present generation of ecophysiology models treats genetic variation simplistically with species-specific physiology parameter values. The value of $g_0$ in the Ball–Berry family of equations functions independently of other parameters; however, the remaining physiology parameters (e.g. $g_s$, $\Gamma_1$ and $A$) are linked, reacting in parallel to environmental factors. A new genetic interpretation is needed to separate the parameters that may not have a constant proportionality. Unraveling the current linkages, however, is impeded by the lack of high-throughput phenotyping methods for some key model variables (e.g. $g_s$). In addition, it may be that current ecophysiological models are not the ideal medium for genetic attribute additions. On the contrary, Chenu et al. (2009) was successful at scaling up genetic variability in leaf growth responses to water deficit. Our results with regard to $E$ models suggest future research to investigate whether the linkage among parameters (e.g. $g_s$ and $A$) persists or should be constructed to act independently (e.g. $g_0$). Such an analysis would be complicated by the need to control for each parameter. Future investigations should attempt to incorporate genetic information that accounts for the variation in plant traits and provide alternative $g_s$ estimates that separate their linkage.

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**SUPPORTING INFORMATION**

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

**Table S1.** List of MAESTRA physiological model parameters that did not vary amongst Tsu-1 and Kas-1 accessions. If the parameter abbreviation is different in the MAESTRA model input file, our abbreviation is followed in parentheses by the abbreviation specifically used in the MAESTRA input file.

**Table S2.** MAESTRA canopy structure model parameters.

**Table S3.** MAESTRA site-specific model parameters.

**Table S4.** ANOVA table for model of $g_0$ in dry down experiment.

**Table S5.** ANOVA table for $g_1$ related QTL.