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Original Article

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

Gretchen A. Reuning[1], William L. Bauerle[1], Jack L. Mullen[2] & John K. McKay[2]

[1]*Department of Horticulture and Landscape Architecture and* [2]*Department of Bioagricultural Sciences and Pest Management, Colorado State University, Fort Collins, CO 80523-1173, USA*

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). CO_2 uptake (photosynthesis, A) and water loss (transpiration, E) both occur through stomata, resulting in a trade-off between acquiring CO_2 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_{day}) 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

Correspondence: W. L. Bauerle. Fax: 00 + 1 + 970 + 491 7745 970 491 7745; e-mail: bauerle@colostate.edu

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_0 have been discovered in *A. thaliana* (Fletcher *et al*. 2013) and understanding how these loci influence g_0 is important for parameterizing g_s models. Hence, the phenotypic effect of these *A. thaliana* g_0 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, 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_0 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_{day} and g_0. 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_0 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_0 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_{day} 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 ($n \approx 23$) 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 CO_2, 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 CO_2 concentration (mole fraction of CO_2)] with the Farquhar and von Caemmerer models (von Caemmerer & Farquhar 1981) using the PC software Photosyn Assistant (Dundee Scientific, Dundee, Scotland). To estimate leaf reflectance, absorbance and transmittance, we used a SPAD meter (SPAD-502; Minolta Camera Co. Ltd., Osaka, Japan). Conversion of SPAD readings to leaf reflectance, absorbance and transmittance followed Bauerle *et al.* (2004) (Supporting Information Table S1).

Transpiration model description

We used a three-dimensional spatially explicit plant E model, MAESTRA (previously named MAESTRO), to estimate daily E of the *A. thaliana* parental accessions and RILs (Wang & Jarvis 1990; Bauerle & Bowden 2011). MAESTRA has been validated and applied to estimate E in numerous studies, most of which are documented in a bibliography at the website http://maespa.github.io/. 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):

$$g_{sw} = g_o + g_1 A/(c_s - \Gamma)(1 + D_a/D_0), \quad (1)$$

where g_{sw} is g_s to water, g_1 is an empirically fitted parameter, A is the net carbon assimilation rate, c_s is the CO_2 mole fraction at the leaf surface, Γ is the CO_2 compensation point, D_a is the vapour pressure deficit and D_0 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):

$$\lambda E = \frac{mR_n + D_a g_h c_p M_a}{m + \gamma g_h / g_{sw}}, \quad (2)$$

where λ 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_a is the vapour pressure deficit (kPa), g_h is the total leaf conductance to heat (mol m^{-2} s^{-1}), c_p is the specific heat of air (1010 J kg^{-1} K^{-1}), M_a is the molecular mass of air (29×10^{-3} kg mol^{-1}), γ is the psychometric constant (Pa K^{-1}) and g_{sw} is g_s 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_0, g_1, V_{cmax}, J_{max}, quantum yield of electron transport (α) 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 1. Values for the physiological parameters for transpiration prediction that varied between Kas-1 and Tsu-1 accessions

Parameter	Definition	Kas value	Tsu value	Units	Source
g_0	Minimum value of g_s	36.6	69.1	mmol m^{-2} s^{-1}	This study
V_{cmax}	Maximum Rubisco-limited rate of photosynthesis	61.3	73.03	μmol m^{-2} s^{-1}	Easlon *et al.* (2014)
J_{max}	Maximum rate of electron transport	96.43	122.31	μmol m^{-2} s^{-1}	Easlon *et al.* (2014)
R_d	Dark respiration	1.47	1.28	μmol m^{-2} s^{-1}	This study
Γ	CO_2 compensation point	40.6	30.9	μmol mol^{-1}	This study
LAREA	Leaf area of crown	0.004416	0.005311	m^2	This study

individually modelled E with Kas and Tsu physiology, and systematically varied the three parameters with the largest effect (g_0, J_{max} and R_d). 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 μmol m^{-2} s^{-1}), RH (30:60%), T_{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 ± 1 mmol m^{-2} s^{-1} and RILs with a Tsu allele were assigned a g_0 of 48 ± 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_n) as fixed effects, plus a $G \times E_n$ 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_s 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_{max} and R_d.

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 Tsu 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).

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 ± 1 mmol m^{-2} s^{-1} for RILs containing the Kas allele and 48 ± 2 mmol m^{-2} s^{-1} for RILs with the Tsu allele. The mean measured E for the Kas-allele and Tsu-allele RILs were 3.07 mmol m^{-2} s^{-1} ($\pm$ 0.036 SEM) and 3.18 mmol m^{-2} s^{-1} ($\pm$ 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

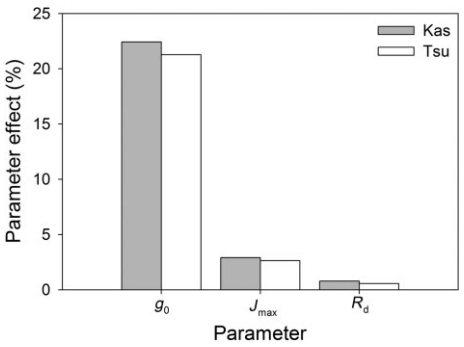

Figure 1. Transpiration estimate sensitivity analysis results for the three most influential physiology parameters for Kas-1 (Kas) and Tsu-1 (Tsu) *Arabidopsis thaliana* accessions. To obtain these results, we used both Kas and Tsu measured default physiology parameters and systematically varied minimum stomatal conductance (g_0), maximum rate of electron transport rate (J_{max}) and dark respiration (R_d) 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).

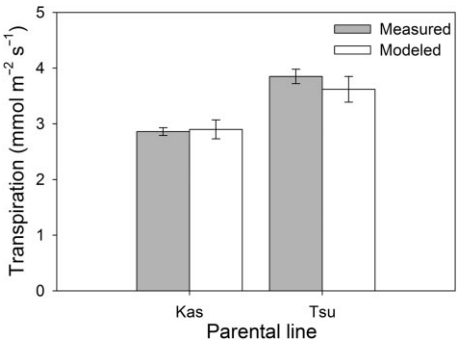

Figure 2. Measured versus MAESTRA-estimated transpiration (E) for Tsu and Kas accessions. Error bars represent standard error of mean (SEM). Measured Tsu E was 3.85 mmol m^{-2} s^{-1} (± 0.13 SEM) and modelled was 3.62 mmol m^{-2} s^{-1} (± 0.23 SEM). Likewise, Kas measured E was 2.86 mmol m^{-2} s^{-1} (± 0.07 SEM) and predicted was 2.90 mmol m^{-2} s^{-1} (± 0.17 SEM). 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}).

measured Tsu-allele RILs, and likewise for Kas-allele RILs. Measured Kas-allele and Tsu-allele RIL E are significantly different ($P < 0.05$; Fig. 3).

Our modelling approach inherently assumes that the effect of QTL alleles on g_0 is constant across environments; however, this assumption is unlikely to be true. To test the degree to which g_0 varies within a genotype across environments, we measured g_0 values for Kas and Tsu under

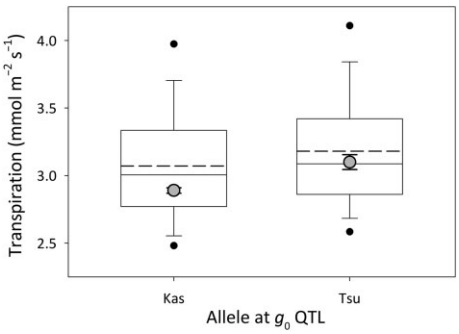

Figure 3. Measured versus simulated transpiration (E) values for Tsu-allele recombinant inbred lines (RILs) and Kas-allele RILs. Measured E is represented by boxes and whiskers, while simulated E is represented by the grey circles (mean) with error bars indicating modelled values of minimum stomatal 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 ($P = 0.049$).

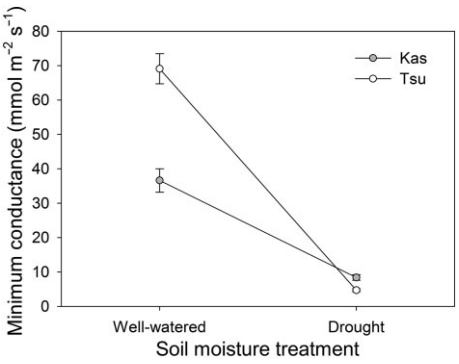

Figure 4. Mean minimum stomatal conductance (g_0) norm of reaction plot for Kas-1 and Tsu-1 genotypes under wet and dry soil moisture conditions. Tsu experienced a significantly sharper decline in g_0 between wet and dry conditions than Kas (mean ± SE, $P < 0.0001$).

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_s 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

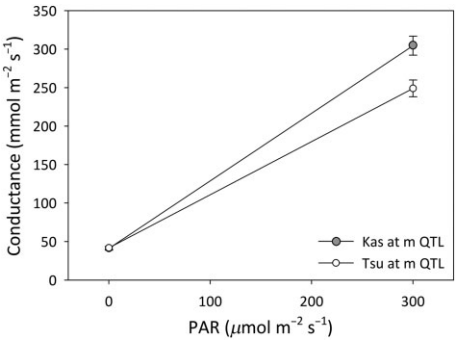

Figure 5. Dependence upon genotype of light response of stomatal conductance. A QTL (quantitative trait loci) analysis on the top of chromosome 3 shows differences among recombinant inbred lines (RILs) with Kas cytoplasm in the slope (*m*) of conductance as a function of photosynthetic active radiation (PAR) (mean ± SE).

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 C_3 species *E* estimates at the whole-crown 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_0 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_1 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_1 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_1. 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_1, Γ 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. Unravelling the current linkages, however, is impeded by the lack of high-throughput phenotyping methods for some key model variables (e.g. g_1). 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_1 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.