

Independent evolution of ab- and adaxial stomatal density enables adaptation

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1 Summary

- 2 • Are organisms free to reach their adaptive optima or constrained by hard-
3 wired developmental programs? Recent evidence suggests that the arrangement
4 of stomata on abaxial (lower) and adaxial (upper) leaf surfaces may be an
5 important adaptation in plants, but stomatal traits on each surface likely share
6 developmental pathways that could hamper evolution.

- 7 • We reviewed the quantitative genetics of stomatal density to look for loci that
8 (1) affected ab- or adaxial density independently or (2) pleiotropically affected
9 stomatal density on both surfaces. We also used phylogenetic comparative
10 methods to test for independent versus correlated evolution of stomatal traits
11 (density, size, and pore index) on each surface from 14 amphistomatous wild
12 tomato taxa (*Solanum*; Solanaceae).

- 13 • Naturally occurring and laboratory-induced genetic variation alters stomatal
14 density on one surface without affecting the other, indicating that develop-
15 ment does not strongly constrain the spectrum of available mutations. Among
16 wild tomato taxa, traits most closely related to function (stomatal pore index
17 and density) evolved independently on each surface, whereas stomatal size was
18 constrained by correlated evolution.

- 19 • Genetics and phylogenetics demonstrate mostly independent evolution of stom-
20 atal function on each leaf surface, facilitating largely unfettered access to fitness
21 optima.

22 **Keywords**

23 Adaptation, correlated evolution, developmental constraint, phylogenetic compara-
24 tive methods, quantitative genetics, *Solanum*, stomata, stomatal ratio

25 **Introduction**

26 Are traits able to evolve independently of one another or they constrained by de-
27 velopment, genetic, or functional connections? Here, we examine whether stomata
28 on the abaxial ('lower') surface of the leaf evolve independently of adaxial ('upper')
29 stomata. Stomata are microscopic pores on the leaf surface formed by a pair of guard
30 cells. The density, size, and arrangement of stomata on a leaf set the maximum stom-
31 atal conductance to CO₂ diffusing into a leaf and the amount of water that transpires
32 from it (Parkhurst, 1978; Sack et al., 2003; Franks and Farquhar, 2001; Galmés et al.,
33 1975). Hence, stomatal traits like density, size, and ratio of upper to lower stomata
34 have strong effects on carbon assimilation and water-use efficiency.

35 An unresolved question is whether stomatal size and density on each leaf surface
36 can evolve independently or are tethered together by shared development. Stomata
37 are most often found only on the lower leaf surface (hypostomy), but occur on both
38 surfaces (amphistomy) in some species (Metcalf and Chalk, 1950; Parkhurst, 1978;
39 Mott et al., 1984), especially herbs (Salisbury, 1927; Muir, 2015) and plants from
40 open habitats (Mott et al., 1984; Gibson, 1996; Jordan et al., 2014). The proportion
41 of stomata found on the upper surface also tends to increase during domestication,
42 even as the total stomatal density stays constant (Milla et al., 2013). Amphistomy
43 increases CO₂ diffusion within the leaf by opening up a second parallel pathway in

44 the intercellular airspace for diffusion from substomatal cavities to mesophyll cell
45 walls. However, stomata on the upper surface in particular may be costly. For
46 example, upper stomata increase the susceptibility to rust pathogens in *Populus*
47 (McKown et al., 2014). Amphistomy may also cause the palisade mesophyll to dry
48 out under strong vapor pressure deficits (Buckley et al., 2015). Muir (2015) reviewed
49 the literature on other possible fitness costs.

50 It is tempting to explain the striking diversity in stomatal ratio as the result of
51 natural selection optimally balancing the fitness costs and benefits. For this to be
52 true, stomatal traits on both surfaces must be free to evolve independently. There
53 are two reasons why independent evolution may be difficult. First, upper and lower
54 stomata share developmental pathways, so mutations that alter the size or pattern-
55 ing on one surface could pleiotropically affect stomata on the other surface. Second,
56 epidermal patterning may be tightly linked to, and therefore constrained by, overall
57 ab-adaxial patterning in the leaf. In bifacial leaves with well differentiated spongy
58 and palisade mesophyll layers ab-adaxial polarity is established very early in leaf de-
59 velopment and required for blade outgrowth (Waites and Hudson, 1995; McConnell
60 and Barton, 1998). If stomatal development is integrated into overall adaxial/abaxial
61 patterning through shared regulatory pathways, then mutations that alter stomatal
62 ratio could pleiotropically disrupt normal leaf development. Since spongy and pal-
63 isade mesophyll layers specialize in CO₂ diffusion and light harvesting, respectively,
64 to optimize carbon gain, such disruption could be deleterious. Hence, populations
65 may be unable to respond to selection on stomatal ratio because of antagonistic
66 pleiotropy, preventing them from reaching their adaptive optima.

67 Multiple reviews of stomatal development conclude that stomatal traits are inde-
68 pendently controlled on each surface (Lake et al., 2002; Bergmann and Sack, 2007),

69 but there is little evidence for this claim. Nor is there strong evidence from the
70 developmental literature for tight linkage between ab-adaxial polarity and stomatal
71 development (Kidner and Timmermans, 2010; Pillitteri and Torii, 2012). To fill this
72 gap, we use two complementary methods to directly test whether upper and lower
73 stomatal traits can evolve independently. First, we reviewed the genetic literature
74 for loci that effect stomatal density. If upper and lower stomatal densities can evolve
75 independently, then we expected to find loci that specifically alter density on the
76 upper or lower surface, but not both. Second, we took a phylogenetic comparative
77 approach to ask whether upper and lower stomata evolve independently among a
78 closely related group of wild tomato species (*Solanum* sect. *Lycopersicon* (Miller)
79 Wettstein in Engler & Prantl, sect. *Lycopersicoides* (A. Child) Peralta, and sect.
80 *Juglandifolia* (Rydberg) A. Child; Solanaceae) grown in a common garden. Both ge-
81 netic and phylogenetic comparisons indicate that stomatal density on one leaf surface
82 can evolve independently of density on the other surface. This implies that natural
83 or artificial selection should be able to optimize the ratio of stomata on the upper
84 and lower surface.

85 **Methods and Results**

86 **Genetics reveals partially independent control of ab- and adax-** 87 **ial stomatal density**

88 We reviewed the literature on quantitative trait locus (QTL) mapping and genome-
89 wide association studies (GWAS) of stomatal traits within and between species. We
90 searched broadly using Google Scholar and ISI Web of Knowledge, as well as by

91 looking through citations of and literature cited within studies we found. Seven
92 studies of four genera (*Brassica*, *Populus*, *Solanum*, *Oryza*) measured separate ab-
93 and adaxial stomatal trait loci (Table 1). Six used QTL mapping; one used GWAS.
94 We restricted our analysis to stomatal density because not all studies measured
95 stomatal size. We counted the number of loci that altered ab- or adaxial density,
96 but not both ('independent loci') and loci that altered ab- and adaxial density in the
97 same direction ('shared loci'). For example, if two loci increased abaxial density and
98 two loci increased adaxial density, and one locus for each surface colocalized, then
99 we counted this as two independent loci (one abaxial, one adaxial) and one shared
100 locus. If reported, we also indicated whether the authors found a significant genetic
101 correlation between ab- and adaxial stomatal density across all genotypes. One study
102 measured stomatal QTL at both ambient and elevated [CO₂] (Rae et al., 2006; Ferris
103 et al., 2002); we used only data from the ambient [CO₂] treatment. In another study,
104 QTL were determined at two life stages (Laza et al., 2010); we counted QTL if they
105 affected density at one life stage or both. Finally, some studies measured QTL in
106 the same species (Ishimaru et al., 2001; Laza et al., 2010) or even the same lines
107 (Chitwood et al., 2013; Muir et al., 2014b), albeit under different conditions, and are
108 clearly not independent data points.

109 Genetic studies reveal some correlation between stomatal densities on each surface,
110 but in all cases there are loci which alter stomatal density on one surface indepen-
111 dently of the other (Table 1). In some cases, there was no detectable genetic corre-
112 lation between stomatal densities on each surface, which would optimally facilitate
113 adaptive evolution. However, with few studies it is difficult to generalize about how
114 strongly genetic covariation between stomatal traits on each surface would constrain
115 responses to selection on microevolutionary timescales. It is also difficult to predict

116 macroevolutionary constraints from genetic correlations within species, as genetic
117 correlations themselves may evolve. Therefore, we next looked at macroevolutionary
118 patterns of correlated evolution using a phylogenetic approach.

119 **Stomatal pore area and density, but not size, evolve indepen-** 120 **dently on each surface**

121 **Stomatal trait measurements**

122 We measured stomatal density (SD) and guard cell length (GCL) from ab- and adax-
123 ial surfaces of 14 wild tomato species. Supporting Information Table S2 lists species
124 names and Tomato Genetic Resource Center accession numbers of seed sources.
125 There were 3-5 biological replicates per species, except the glabrous *S. chilense*, for
126 which we could only get an accurate count from one replicate. Species were grown
127 in a common garden at the experimental field at the University of the Balearic Is-
128 lands, as described in Muir et al. (2015). We made polyvinylsiloxane (Kerr Extrude
129 Medium, Orange, California, USA) casts of leaf surfaces from fully expanded adult
130 leaves. We painted casts with a thin coat of nail polish and mounted this on a glass
131 slide to count the number of stomata from three (proximal, medial, and distal) 0.571
132 mm^2 portions of the leaf area unobstructed by major veins. We measured average
133 GCL on 20 stomata per portion, 60 stomata per leaf surface examined. For each leaf
134 surface, we calculated Stomatal Pore Index (SPI) as $SD \times GCL^2$, where SD and GCL
135 are in units of stomata per mm^2 and mm, respectively. SPI indicates what proportion
136 of the leaf surface is occupied by stomatal pore and is closely related to maximum
137 stomatal conductance (Sack et al., 2003). Total SD and SPI were calculated as the
138 sum of ab- and adaxial values:

$$SD_{\text{tot}} = SD_{\text{ab}} + SD_{\text{ad}}$$

$$SPI_{\text{tot}} = SPI_{\text{ab}} + SPI_{\text{ad}}$$

139 The _{ab} and _{ad} subscripts denote stomatal traits values on the ab- and adaxial surface,
140 respectively. We measured total leaf stomatal conductance to CO₂ (g_s) under ambient
141 CO₂ concentrations (400 ppm) using an open-path infrared gas exchange analyzer
142 (LI-6400 or LI-6400XT, LI-COR Inc., Lincoln, NE, USA) as described in Muir et al.
143 (2015). Stomatal conductance was measured under optimal conditions to approach
144 maximum g_s . Steady-state measurements were taken at midday with saturating
145 irradiance (photosynthetically active radiation set to 1500 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$),
146 moderate relative humidity (40–60%), and 25°C leaf temperature.

147 **Phylogenetic methods**

148 Stomatal traits on each surface clearly differ from one another (Figure 1). The abax-
149 ial (lower) surface of tomato leaves usually have higher stomatal density and stomatal
150 pore index, but smaller guard cells. Although stomata from each surface clearly dif-
151 fer overall, shared developmental pathways may nevertheless constrain how stomatal
152 traits on each surface evolve. We tested whether ab- and adaxial stomatal traits
153 evolve independently using phylogenetic comparative methods. If ab- and adaxial
154 traits can evolve independently, then phylogenetic models assuming zero covariance
155 between traits should outperform models with covariance. Conversely, if ab- and
156 adaxial stomatal traits share common developmental pathways that constrain their
157 evolution, then models with positive covariance should outperform models without

158 covariance. To test this, we compared six models using the R package **mvMORPH**
159 (Clavel et al., 2015). We used a maximum likelihood phylogenetic tree inferred from
160 18 genes (Haak et al., 2014) using RAxML version 8.1.24 (Stamatakis, 2014). All
161 models allow separate average values for ab- and adaxial traits, but differ in two
162 respects. First, we compared Brownian motion (BM) to Ornstein-Uhlenbeck (OU)
163 models. In both BM and OU models, trait values evolve at rate σ . The only differ-
164 ence between BM and OU models is that the OU model includes an extra parameter
165 (denoted α) that pulls species back faster toward the long-run average (denoted θ).
166 Note that BM versus OU comparison only tests how tightly stomatal traits are con-
167 strained to evolve around the long-run average, not whether ab- and adaxial stomata
168 evolve independently. We tested for independent evolution by comparing BM and
169 OU models with and without evolutionary covariance between leaf surfaces. We
170 compared two BM models for each trait, one in which $\text{Cov}(\sigma_{\text{ab}}, \sigma_{\text{ad}})$ is estimated
171 (‘covary’ model) and another with the constraint $\text{Cov}(\sigma_{\text{ab}}, \sigma_{\text{ad}}) = 0$ (‘independent’
172 model). Similarly, we compared four OU models for each trait, three that allowed
173 covariance between ab- and adaxial evolution and one in which they evolved inde-
174 pendently. Specifically, we tested for covariance between diffusion rates ($\text{Cov}(\sigma_{\text{ab}},$
175 $\sigma_{\text{ad}})$ estimated, $\text{Cov}(\alpha_{\text{ab}}, \alpha_{\text{ad}}) = 0$), covariance between return rates ($\text{Cov}(\alpha_{\text{ab}}, \alpha_{\text{ad}}$
176 estimated, $\text{Cov}(\sigma_{\text{ab}}, \sigma_{\text{ad}}) = 0$), or covariance between both diffusion and return rates
177 ($\text{Cov}(\sigma_{\text{ab}}, \sigma_{\text{ad}})$ and $\text{Cov}(\alpha_{\text{ab}}, \alpha_{\text{ad}})$ estimated). The ‘independent’ model constrained
178 both $\text{Cov}(\sigma_{\text{ab}}, \sigma_{\text{ad}}) = 0$ and $\text{Cov}(\alpha_{\text{ab}}, \alpha_{\text{ad}}) = 0$. We incorporated measurement er-
179 ror using the standard error across biological replicates within species (Pennell et al.,
180 2015). Because we could not estimate measurement error for *S. chilense*, we used the
181 average measurement from the other species instead. All traits were log-transformed
182 for normality. We compared model fit using Akaike Information Criterion corrected
183 for small sample size (AICc).

184 SD and SPI evolution are constrained but ab- and adaxial traits are uncorrelated.
185 For both traits, OU models fit better than BM models, and models with covariance
186 between leaf surfaces performed worse than those without covariance (Table S1). We
187 found the opposite pattern for GCL. Evolution of this trait was best described by
188 a model without constraint but including covariance between ab- and adaxial GCL.
189 Since SPI_{tot} is closely related to stomatal conductance in these species (Figure 2), in-
190 dependent evolution of SPI_{tot} suggests little evolutionary constraint on how stomatal
191 conductance is partitioned across surfaces in different species.

192 Discussion

193 Adaptive evolution may be constrained if traits cannot evolve independently. In
194 particular, if traits share developmental pathways, then they may be unable to re-
195 spond differentially to selection. In this study, we examined whether stomata on the
196 abaxial (lower) and adaxial (upper) surfaces can evolve independently. We adduce
197 two new lines of evidence which suggest that stomatal function on each surface can
198 readily respond to selection. First, species possess heritable variation that allows
199 partially independent evolution of stomatal densities in response to selection; every
200 study reviewed found loci which alter stomatal density on one surface but not the
201 other. Second, the anatomical trait most closely connected to stomatal conductance,
202 stomatal pore index (Sack et al., 2003), evolves independently on ab- and adaxial
203 surfaces among wild tomato species. Together, these new lines of evidence demon-
204 strate that natural selection on stomatal arrangement is not strongly constrained by
205 development, although we lacked statistical power to detect weak constraint. It is
206 therefore likely that variation in how stomatal conductance is partitioned between

207 leaf surfaces is due to adaptive rather than nonadaptive forces.

208 Indeed, much recent evidence indicates that selection finely tunes the ratio of stomata
209 on the upper and lower leaf surface, although the adaptive significance of variation in
210 stomatal ratio is unresolved. Stomatal ratio affects leaf function, increasing CO₂ dif-
211 fusion (Parkhurst, 1978; Parkhurst and Mott, 1990; Gutschick, 1984; Parkhurst, 1994)
212 and hydraulic conductance outside the xylem (Buckley et al., 2015). As predicted,
213 amphistomy seems to be more common in circumstances when efficient CO₂ supply
214 is important, such as high irradiance (Mott et al., 1984; Gibson, 1996; Smith et al.,
215 1997; Jordan et al., 2014), thick leaves (Parkhurst, 1978; Muir et al., 2014a), herba-
216 ceous growth form (Salisbury, 1927; Muir, 2015), and domestication (Milla et al.,
217 2013). Despite potential benefits of amphistomy, most plant species are hypostoma-
218 tous, implying a fitness cost of upper stomata, such as increased infection by foliar
219 pathogens (Gutschick, 1984; McKown et al., 2014). For example, ‘upside-down’ (re-
220 supinate) leaves with the abaxial surface facing upward have re-evolved hypostomy
221 (Lyshede, 2002), strongly implying a cost of upward facing stomata.

222 To optimally balance fitness costs and benefits, natural selection must be able to
223 change stomatal traits on one surface independently of the other. The present study
224 shows that this is likely true and strikingly consistent on micro- and macroevolu-
225 tionary timescales. Among *Populus trichocarpa* populations and *Solanum* species,
226 the ratio of adaxial to abaxial SPI (SPI ratio) evolves mostly by changes in stom-
227 atal density rather than guard cell size. Within *Populus*, populations are more am-
228 phistomatous at Northern latitudes with shorter growing seasons that may select for
229 faster carbon assimilation (McKown et al., 2014; Kaluthota et al., 2015). Latitudinal
230 variation *Populus trichocarpa* is due mostly to adaptive variation in adaxial stomatal
231 density (McKown et al., 2014; Porth et al., 2015). Stomatal density rather than size

232 may have responded more readily to selection because there is no genetic covariance
233 between ab- and adaxial stomatal density, permitting independent evolution (Porth
234 et al., 2015). In contrast ab- and adaxial guard cell length positively covary, likely
235 constraining evolution. Similarly, we found that over macroevolutionary timescales
236 most of the variation in SPI among wild tomato species is due to changes in adaxial
237 stomatal density rather than size. Indeed, stomatal density on each surface evolved
238 independently, whereas guard cell lengths positively covaried (Table S1). Adaptive
239 evolution will likely take advantage of traits that evolve independently because this
240 minimizes antagonistic pleiotropy. In a previous study, we found that loci affecting
241 adaxial stomatal density were likely fixed by selection, but we did not measure stom-
242 atal size (Muir et al., 2014b). Overall, patterns within and between species indicate
243 that selection on SPI ratio leads to greater change in stomatal densities rather sizes
244 on each surface. Based on the analysis here, we conclude that changing stomatal
245 density on one surface incurs less cost than changing size because the former is less
246 constrained by shared developmental pathways.

247 We caution that there are limitations of our analysis. First, although some loci alter
248 stomatal traits on one surface independently of the other, there are also loci that
249 affect both surfaces, leading to significant genetic correlations in some species (Ta-
250 ble 1). Such genetic correlations will slow adaptation even if they do not prevent
251 populations from eventually reaching an adaptive optimum in the long run. For
252 example, the relatively high genetic correlation between ab- and adaxial stomatal
253 density in *Oryza* may contribute to low variation in stomatal ratio between species
254 of this genus (Giuliani et al., 2013). Second, the sample size of the phylogenetic
255 comparisons is small and thus not statistically powerful. However, simulations show
256 that model identification (e.g. Brownian motion versus Ornstein-Uhlenbeck) is usu-

257 ally correct, even when sample sizes are moderate (Cressler et al., 2015; Ho and Ané,
258 2014). The dataset was also powerful enough to find significant correlated evolution
259 of guard cell size on ab- and adaxial surfaces, which we interpret as evidence of shared
260 developmental pathways. However, we cannot rule out some level of correlated evo-
261 lution for stomatal density and pore index below our threshold to detect. Finally,
262 stomatal traits measured in a common garden may be different than what occurs
263 naturally. For example, the ratio of stomatal density and size changes in response
264 to light (Gay and Hurd, 1975) and water stress (Galmés et al., 1975). Despite *ad*
265 *libitum* watering and fertilizer, our common garden in a Mediterranean climate may
266 have been more stressful for some tomato species than others, depending on their
267 habitat of origin, perhaps inducing stress-response phenotypes.

268 We recommend that future genetic and comparative studies of stomatal traits report
269 separate ab- and adaxial values for stomatal density and size. We also need to
270 determine developmental connections between abaxial/adaxial pattern specification
271 and epidermal development. For example, SPCH SILENT, an *Arabidopsis* mutant
272 that relatively normal adaxial stomatal density but no abaxial stomata (Dow et al.,
273 2014), suggesting possible links between SPCH and abaxial/adaxial patterning. The
274 molecular mechanisms may explain how stomata often develop differently on each
275 surface and why asymmetry between surfaces readily evolves.

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282 **Author contribution statement**

283 CDM, MAC, and JG designed and carried out the experiment. CDM analyzed the
284 data and wrote the manuscript.

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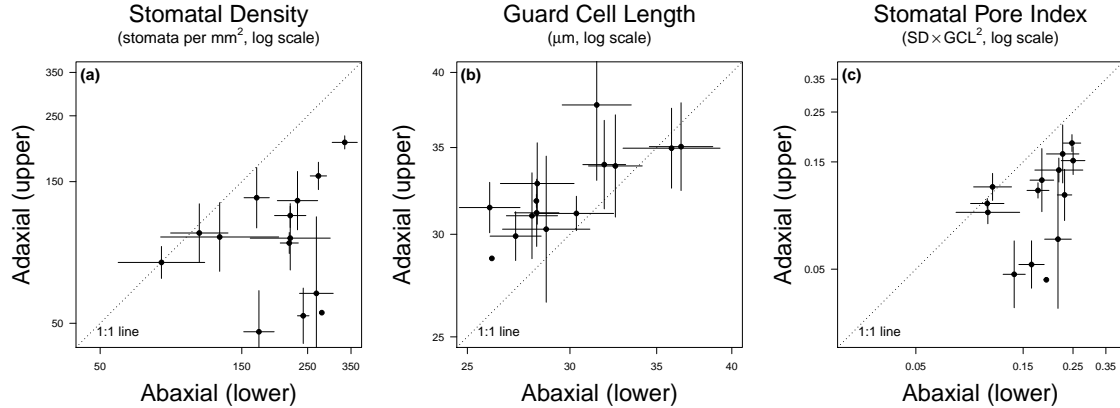


Fig. 1. Ab- and adaxial stomatal density (SD; panel (a)) and stomatal pore index (SPI; panel (c)) evolve independently, whereas the guard cell lengths (GCL; panel (b)), a measure of stomatal size, positively covaries over evolution. In horizontally-oriented tomato leaves, ab- and adaxial surfaces are the lower and upper surface, respectively. Adaxial SD and SPI values tend to be lower than abaxial ones (most points fall below 1:1 line), whereas adaxial stomata tend to be larger (higher GCL) than abaxial ones. Each point is mean trait value for one of 14 wild tomato species; lines are +/- one standard deviation. One species, *S. chilense*, was only sampled once and therefore the standard deviation could not be estimated.

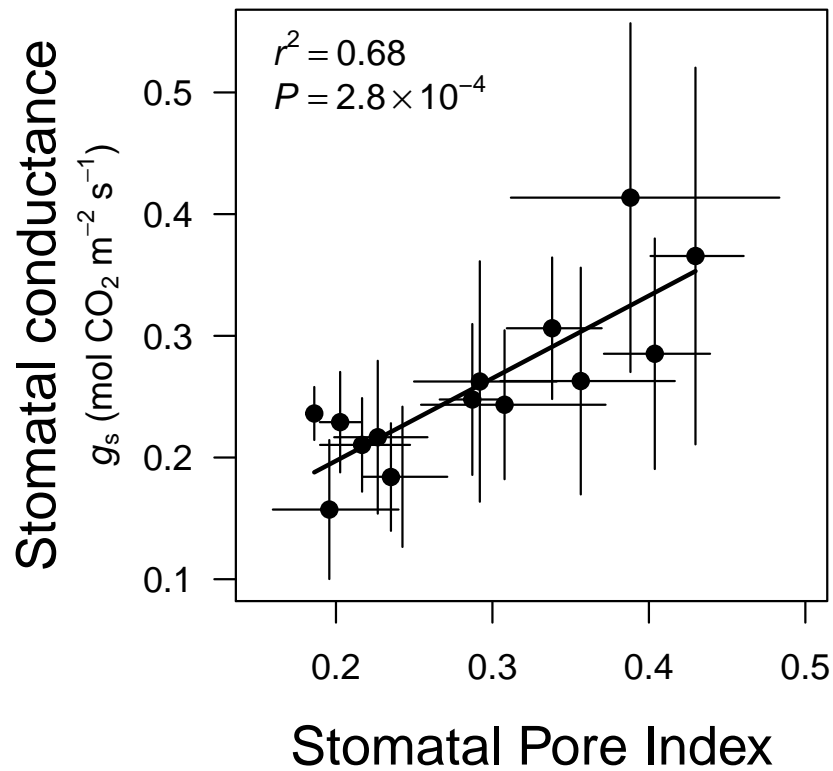


Fig. 2. Stomatal conductance to CO₂ (g_s) is directly proportional to stomatal pore index (SPI) in wild tomato species. g_s was measured at ambient CO₂ concentrations (400 μ mol CO₂ mol⁻¹ air), saturating irradiance (1500 μ mol quanta m⁻² s⁻¹), 25°C leaf temperature, and 40–60% relative humidity. Each point is the species mean; error bars are +/- one standard deviation.

Table 1. Many loci alter ab- or adaxial stomatal density independently, while others affect both surfaces. We reviewed seven studies (key to reference numbers below) in four genera. Loci were identified using quantitative trait locus mapping (QTL) or genome-wide association studies (GWAS). Shared loci altered both ab- and adaxial stomata density, whereas independent loci affected one or the other. The Data Source column refers to the table or figure in the reference where we found data. Muir et al. (2014b) did not report these analyses, but we calculated number of QTL using the same methods. We also indicate whether the study reported significant genetic correlation between ab- and adaxial stomatal density across all genotypes.

Reference	Taxa	Method	Shared loci	Independent loci	Data source	Significant genetic correlation?
(1)	<i>Brassica oleracea</i> L.	QTL	2	1	Table 1	not reported
(2, 3)	<i>Populus trichocarpa</i> Torr. & A. Gray ex Hook.	GWAS	0	9	Table 3 ²	no
(4)	<i>Solanum</i> species	QTL	7	17	unpub. result	no
(5)	<i>Solanum</i> species	QTL	7	14	Supplemental Figure 10	yes
(6, 7)	<i>Populus</i> species	QTL	0	6	Figure 4 ⁷	not reported
(8)	<i>Oryza sativa</i> L.	QTL	1	2	Table 1	yes
(9)	<i>Oryza sativa</i> L.	QTL	1	8	Table 3	yes

¹ Hall et al. (2005); ² McKown et al. (2014); ³ Porth et al. (2015); ⁴ Muir et al. (2014b); ⁵ Chitwood et al. (2013); ⁶ Ferris et al. (2002); ⁷ Rae et al. (2006); ⁸ Ishimaru et al. (2001); ⁹ Laza et al. (2010)

Table 2. Phylogenetic comparisons reveal independent evolution of ab- and adaxial stomatal density (SD) and stomatal pore index (SPI), but shared developmental pathways for ab- and adaxial guard cell length (GCL). We compared Brownian motion (BM) and Ornstein-Uhlenbeck (OU) models. Under the BM model, average trait values (θ) evolve without bounds at rate σ , whereas under the OU model, trait values are bounded. α is the return rate toward θ in the OU model. For both OU and BM models, we compared models with (‘covary’) and without (‘independent’) covariance between ab- and adaxial traits. We compared models using Akaike Information Criteria corrected for small sample size (AICc). Δ AICc for a model is the difference its AICc and that of the model with lowest AICc. Hence, for the best-supported model Δ AICc = 0. k is the number of parameters estimated for a particular model.

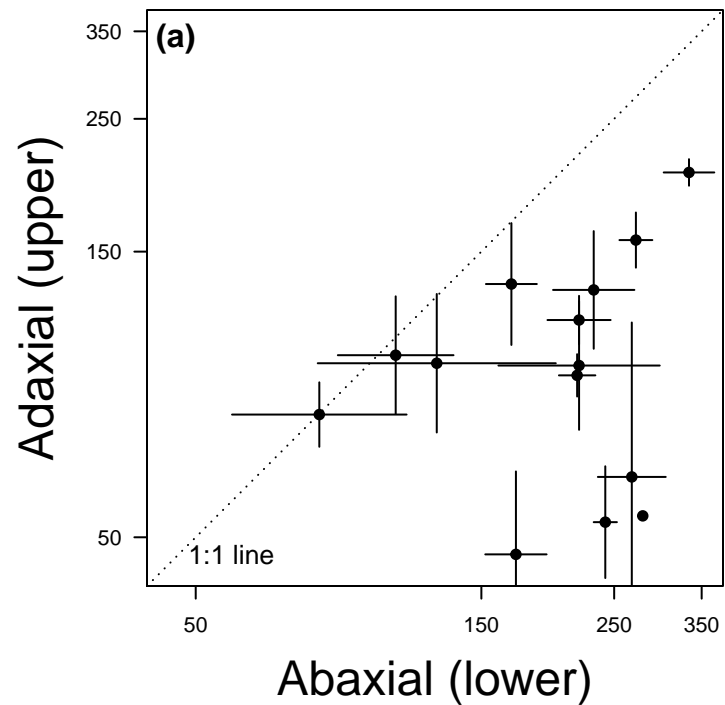
Model	Model parameters				Δ AICc		
	θ	σ	α	k	GCL	SD	SPI
BM1		covary	–	5	0	6.5	7
BM2		independent	–	4	10.1	11.8	12.4
OU1	separate	covary	covary	8	19.9	13.4	14.9
OU2		covary	independent	7	7.8	7.5	5.5
OU3		independent	covary	7	7.8	3.3	4.6
OU4		independent	independent	6	13.1	0	0

407 **Supporting Information**

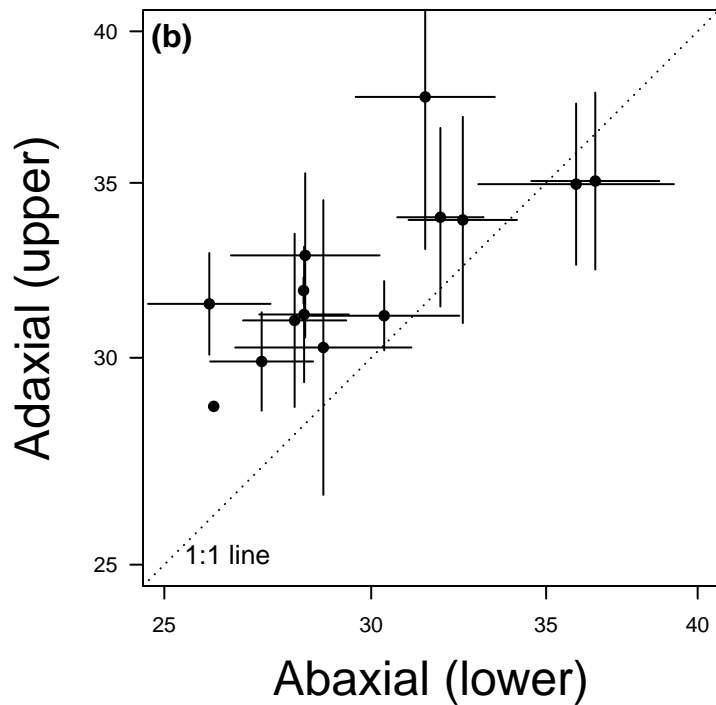
Table S1. Wild tomato species and Tomato Genetic Resource Center (TGRC) accession numbers.

Species	TGRC Number
<i>S. arcanum</i> Peralta	LA2153
<i>S. cheesmaniae</i> (Riley) Fosberg	LA1035
<i>S. chilense</i> Dunal	LA1782
<i>S. chmielewskii</i> D.M.Spooner, G.J.Anderson & R.K.Jansen	LA1327
<i>S. galapagense</i> S.C. Darwin & Peralta	LA0930
<i>S. habrochaites</i> S. Knapp & D.M. Spooner	LA2196
<i>S. lycopersicoides</i> Dunal	LA2951
<i>S. lycopersicum</i> var. <i>cerasiforme</i> (Dunal) D.M. Spooner, G.J. Anderson & R.K. Jansen	LA1320
<i>S. neorickii</i> D.M. Spooner, G.J. Anderson & R.K. Jansen	LA1322
<i>S. pennellii</i> Correll	LA1272
<i>S. pennellii</i> var. <i>puberulum</i> Correll	LA1926
<i>S. peruvianum</i> L.	LA2964
<i>S. pimpinellifolium</i> L.	LA0114
<i>S. sitiens</i> I.M. Johnst.	LA4115

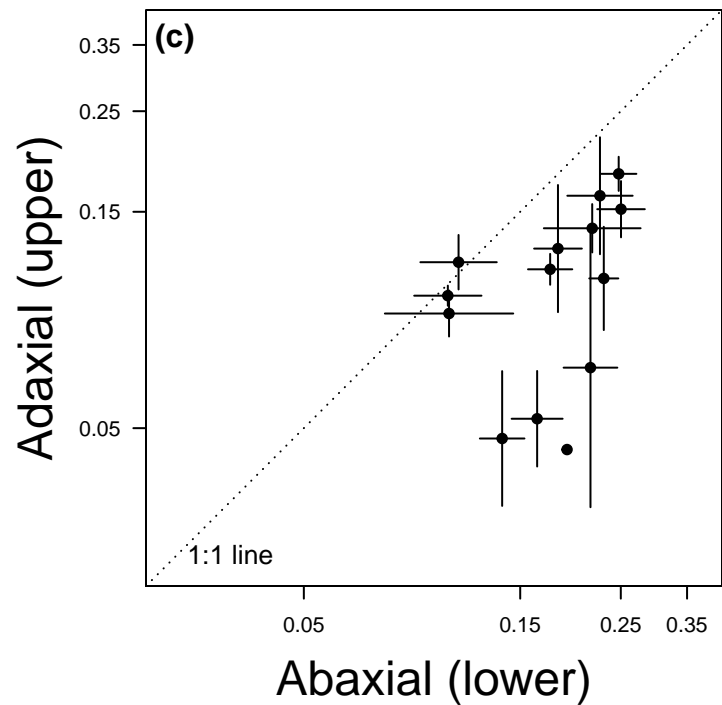
Stomatal Density
(stomata per mm², log scale)



Guard Cell Length
(μm , log scale)



Stomatal Pore Index
($\text{SD} \times \text{GCL}^2$, log scale)



Stomatal conductance

g_s (mol CO₂ m⁻² s⁻¹)

0.5

0.4

0.3

0.2

0.1

$r^2 = 0.68$

$P = 2.8 \times 10^{-4}$

Stomatal Pore Index

0.5