Shifts in stomatal traits following the domestication of plant species

Rubén Milla*, Natalia de Diego-Vico and Nieves Martín-Robles

Departamento de Biología y Geología, Área de Biodiversidad y Conservación, Escuela Superior de Ciencias Experimentales y Tecnología, Universidad Rey Juan Carlos, c/Tulipán s/n, Móstoles 28933, Spain

* To whom correspondence should be addressed. Email: ruben.milla@gmail.com

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Abstract

Stomata are the major gates regulating substrate availability for photosynthesis and water loss. Although both processes are critical to yield and to resource-use efficiency, we lack a comprehensive picture on how domestication and further breeding have impacted on leaf stomata. To fill this gap, stomatal sizes and densities were screened in cultivated and wild ancestor representatives of a uniquely large group of 24 herbaceous crops. Anatomical data and gas-exchange models were combined to compute maximum potential conductance to water, separately for upper and lower leaf sides. The evolution of maximum conductance under domestication was diverse. Several crops increased, others decreased (noticeably high-conductance species), and others kept a similar potential conductance following domestication. It was found that the contribution of upper leaf sides to maximum conductance was statistically higher in cultivated than in wild ancestors. For crops showing this response, reduced stomatal density in the lower side of domesticated leaves was responsible for the observed ‘adaxialization’ of conductance. Increases in the size of stomata at the upper epidermis played a comparatively minor role. Nevertheless, this overall response was varied in magnitude and direction, signalling crop-wise specificities. Observed patterns reflect only potential conductances based on anatomical traits and should be used with care until actual physiological outcomes are measured. Together with advancements in the developmental genetics of stomata, our findings might hint at new breeding avenues, focused on stomata distribution. Provided urgent needs for increasing yields, the opportunities of enhancing traits of the physiological relevance of stomata should not be ignored.

Key words: Abaxial, adaxial, artificial selection, conductance, domestication, plant breeding, stomata, yield.

Introduction

The gradual domestication and improvement of certain wild plants were a keystone series of historical events (Zohary and Hopf, 2000). Driven by artificial selection, plants steadily increased yield and productivity during the course of this process (Evans, 1993). Worryingly, yield improvements are currently stagnant (Miflin et al., 2000). It is therefore critical to reflect on which the mechanisms were that promoted yield increase during domestication, and which of these might respond to further breeding. Here, we extensively explored how stomatal traits, as key drivers of mechanisms affecting photosynthetic productivity, water use, and plant body temperature, have changed from wild ancestors¹ to current domesticates.

¹ We use here the shortened term ‘wild ancestor’ to refer to the closest wild relative of an existent crop. This shortened is employed for simplicity, although we are aware that, for many crop species, domestication was a complex evolutionary process where the assignment of a unique ancestral gene pool is only a convenience.
habitats showing either low maximum stomatal conductance or finely tuned pore aperture responses (Hetherington and Woodward, 2003). However, domestication took wild plants from habitats where water availability was more unpredictable and scarcer, to agricultural environments with regular, predictable, and more abundant water supplies (Mckey et al., 2012). Selection for using water efficiently under such circumstances was probably relaxed, while maximizing CO₂ uptake and evaporative cooling were most likely favoured. Stomatal traits should have been under selection in this scenario. Some scant and disperse literature hints that domestication might have increased stomatal densities in crops such as pea, tomato, or cotton (Cornish et al., 1991; Kebede et al., 1994; Zayed 2010). Nevertheless, a comprehensive analysis of the evolution of stomatal traits under domestication is still missing.

There are additional reasons to expect domestication to produce genotypes with different stomatal traits from those of their wild ancestors. Stomata are functionally linked with other leaf traits that were subjects of either directional or unintended artificial selection. Dimensions like leaf lamina surface or thickness have increased during the domestication of several species (Navea et al., 2002). Thickening of leaves is known to augment mesophyll conductance and to modify vein distribution and density (Blonder et al., 2011). As the distribution of minor veins is in close coordination with that of stomata (Brodribb et al., 2010), changes in thickness should also influence the placement and density of stomata (Lawson et al., 2002). Similarly, leaf size may also modify relevant traits, including stomatal sizes, densities, and conductances, provided that leaf size–function relationships are allometric (Niklas et al., 2007). Some early evidence of decreasing stomatal density with leaf size is available (Salisbury, 1928; Jones, 1977). Moreover, cell size, or the size of nuclear genomes, are quantitative traits known to have increased in several domestication processes (Bull, 1965; Dunstone and Evans, 1974; Kingsbury, 2009). Provided that epidermal cell and genome sizes tend to scale positively with the size of guard cells (Salisbury, 1928; Beaulieu et al., 2008; Kumar and Yadav, 2010; Franks et al., 2012), stomata might have enlarged as a developmental correlate of increases in cell and genome dimensions.

In sum, research from diverse fields jointly suggests that stomatal traits should have changed during domestication, but a broad-scale screening of stomata evolution under artificial selection is not available. We aimed to fill this gap by investigating maximum potential stomatal conductance, derived from stomatal density and size data, of an ample set of 24 taxonomically diverse herbaceous crops and their wild ancestors. We hypothesized that stomatal conductance has generally increased during domestication and further breeding, keeping pace with well-known increases in yield. This screening should provide relevant baseline information to refocus attention on stomatal traits as key contributors to future improvements in yield, particularly under steadily increasing atmospheric temperatures and CO₂ levels.

Materials and methods

Study system and leaf sampling

Our study target was a set of 24 taxonomically diverse herbaceous crops (Supplementary Table S1 at JXB online). For each crop we obtained seed lots of two accessions: one representative of a modern, domesticated stage of the species, and another of its most likely wild ancestor (see Supplementary Table S1 for accession identifiers, seed donors, and literature source for wild ancestor assignment). Part of this collection of seeds was grown under a common garden regime in 2010, and part during 2011. Approximately 20 seeds per accession were set to germinate in dark, cold growth chambers, and when radicle emergence was observable were taken to individual 5 × 5 × 10 cm containers set in a greenhouse (Universidad Rey Juan Carlos, Móstoles-Madrid, Spain: 40°18′48″N-3°52′57″W, mean annual temperature 14 °C, mean annual precipitation 481 mm; long-term data from http://opengis.uab.es/wms/iberia/mms/index.htm). Seedlings were kept in the greenhouse for 2–4 weeks, depending on the crop. Then, 15 individuals per accession were transplanted outdoors to free-rooting planting beds in an experimental field beside the greenhouse. Watering in the greenhouse and in the experimental fields was supplied at dawn and/or sunset through regular automatic water sprinkling and drip irrigation, respectively, and as needed to maintain plants under optimal growing conditions. All the above plant growth procedures were carried out sequentially throughout season, matching the most appropriate time of the year with the performance of each species, although always concurrently and at the same spatial location for the two accessions of each crop.

Measurement and counting of stomata

When leaves were mature and fully expanded, one sun-oriented leaf of each of ten individual plants per accession was harvested in the mid-morning and immediately stored at –18 °C until sample processing. Five of these leaves were randomly chosen and left at 4 °C thawing overnight in between soaked germination paper to recover turgidity. A variable-sized piece of each leaf lamina was then cut at an equal distance from the tip and base of the blade, and at the right side of the mid-vein, equidistant from the mid-vein and leaf margin. The adaxial and abaxial sides of each piece of the blade were permanently imprinted in a dentist’s impression mould (Gewie Berry 3MESPE rapid; Sultan Healthcare, Hannover, Germany). Once the mould had hardened, nail varnish imprints were obtained and mounted on microscope slides. The number of stomata in each varnish image was counted in three separate inter-vein regions of variable size (0.02–2.74 mm², depending on crop-specific leaf size and abundance of stomata). The length of guard cells (L, defined as distance between the joints of both guard cells farthest from the pore, μm) was measured in two stomata of each of the above regions. All together, 1437 epidermal regions were surveyed for stomatal density counts (48 accessions × two leaf sides × five leaves × three replicates per leaf side, minus three lots of missing data), and the length of 2874 guard cells was measured (two per replicate) (Supplementary Table S2 at JXB online).

Computation of maximum stomatal conductance to water (Gwmax)

To permit a direct interpretation of the functional consequences of anatomical changes in stomata, we chose to model conductance instead of computing stomatal area indexes. To this end, we made use of the models of Franks and Farquhar (2001, 2007) and Franks and Beerling (2009) to work out maximum potential conductance from our anatomical variables. Stomatal size (S) was defined as guard cell length (L) multiplied by total width (W) of the closed guard cell pair. W was taken as L/2 for stomata of dicots and L/8 for monocots (Franks and Farquhar, 2001, 2007). Stomatal size and density were then used to calculate maximum stomatal conductance...
to water \((G_{\text{wmax}}, \text{ mol m}^{-2} \text{ s}^{-1})\) following Franks and Farquhar (2001, 2007) and Franks and Beerling (2009) as:

\[
G_{\text{wmax}} = \frac{d}{v} \times \frac{D}{d_{\text{max}}} / (l \times \pi)
\]

where \(d\) is the diffusivity of water vapour in air \((\text{m}^2 \text{ s}^{-1})\), \(v\) is the molar volume of air \((\text{m}^3 \text{ mol}^{-1})\) at 25 °C, \(D\) is stomatal density \((\text{mm}^{-2})\) and \(d_{\text{max}}\) is the maximum area of the open stomatal pore, while \(l\) is its depth. \(d_{\text{max}}\) was approximated as \(\pi d^2/2\), where \(d\) is stomatal pore length, assumed to be \(L/2\) (Franks and Farquhar, 2007). Stomatal pore depth \((l\) for fully open stomata was taken as equal to \(W/2\), assuming guard cells inflate to a circular cross-section. Taken together, \(d_{\text{max}}, l,\) and \(D\) determine maximum diffusive conductance to water vapour or \(\text{CO}_2\) (Franks and Farquhar, 2001). The equation makes increases in \(D\) impact positively over conductance, while the effect of stomatal size is counterbalanced between positive and negative. On the one hand, longer and wider stomata promote gas exchange, as signified by \(d_{\text{max}}\) taking part of the numerator. On the other hand, deep pores hinder diffusion (Franks and Farquhar, 2007), which is quantified by the inclusion of the \(l\) term in the denominator. From here onwards, results are presented only for conductance to water, but note that \(G_{\text{wmax}} = G_{\text{wmax}}/l\).

\(G_{\text{wmax}}\) was computed separately for each side of the leaf as \(G_{\text{wmax}}\) and \(G_{\text{wmax}}\), for adaxial and abaxial surfaces, respectively. Conductance of the two sides of the leaf \((G_{\text{wmax}+\text{ab}})\) was further calculated as \(G_{\text{wmax}+\text{ab}} = G_{\text{wmax}+\text{ab}}\). The ratio of \(G_{\text{wmax}+\text{ab}}\) to \(G_{\text{wmax}+\text{ab}}\) \((G_{\text{wmax}+\text{ab}})\) was used as an index of the distribution of stomatal conductance on each side of the leaf. Average changes in any given variable during each crop domestication process were calculated as \(\frac{\text{Variable}_{\text{domesticated}} - \text{Variable}_{\text{wild}}}{\text{Variable}_{\text{wild}}}\) (e.g. \(G_{\text{wmax}+\text{ab}} - G_{\text{wmax}}\)). This change is denoted as \(\Delta\text{Variable}\) (e.g. \(\Delta G_{\text{wmax}+\text{ab}}\)) throughout the paper.

### Statistical analyses

Either raw or log-transformed data complied with assumptions of normality and homoscedasticity of general linear models. Also, the design was balanced and full factorial. We thus used two-way analysis of variance (ANOVA) to evaluate the effects of taxonomic crop identity, domestication status (domestic vs wild ancestor), and their interaction, on \(G_{\text{wmax}+\text{ab}}\) and \(G_{\text{wmax}+\text{ab}}\). Crop identity was introduced in the models as a random-effect factor, while domestication status was a fixed-effect factor. Stomatal size \((S)\) and density \((D)\), and \(G_{\text{wmax}}\) were analysed using three-way ANOVA analogous to the models described above, but adding leaf side (adaxial vs abaxial) as an additional fixed-effect predictor. Bonferroni-corrected multiple comparisons were carried out if domestication status, leaf side, and their interaction were significant predictors. \(G_{\text{wmax}+\text{ab}}\) was log transformed prior to analysis to make the index symmetric to changes in numerator and denominator. \(D\) was log transformed to comply with general linear model assumptions. Relationships between \(S\) and \(D\), dependence of \(G_{\text{wmax}}\) on variations in \(S\) and \(D\), of \(\Delta G_{\text{wmax}+\text{ab}}\) on variations in \(\Delta S\) and \(\Delta D\), and of \(\Delta G_{\text{wmax}+\text{ab}}\) on variations in \(\Delta S_{\text{ab}}\) and \(\Delta D_{\text{ab}}\) were all assessed fitting type I regressions. To get a more precise quantification of the relative importance of \(S\) and \(D\) variation in accounting for total variance in \(G_{\text{wmax}}\), we ran hierarchical partitioning of variance analyses (Chevan and Sutherland, 1991). This procedure applies a hierarchical partitioning algorithm to assign an independent and joint contribution of each explanatory variable to account for variance in the response. As joint contributions were negligible, we used the independent component, scaled as a percentage of total explained variance, to compare the relative contributions of \(S\) and \(D\) over \(G_{\text{wmax}}\) variation. Analyses of hierarchical partitioning of variance were implemented using the hier.part package available for the R platform (R Core Development Team, 2008). All other analyses were performed using SPSS 15.0.

### Results

#### Domestication effects on \(G_{\text{wmax}+\text{ab}}\) and \(G_{\text{wmax}+\text{ab}}\)

Overall, maximum stomatal conductance to water vapour \((G_{\text{wmax}+\text{ab}})\) was statistically similar between domesticated varieties and their wild ancestor counterparts. Several crops, such as \(\text{Vicia}, \text{Cichorium, and Avena}\), showed a modest decrease in \(G_{\text{wmax}+\text{ab}}\) with domestication, while others like \(\text{Arachis, Gossypium, and Phaseolus}\) slightly increased conductance (Fig. 1A and Supplementary Fig. S1 at JXB online).

It was interesting to note that eight of the 11 wild ancestors with the highest conductance \((3.9 \text{ mol m}^{-2} \text{ s}^{-1} \text{ or higher})\) had their conductance decreased with domestication (Fig. 1A). However, a generalized pattern of either augmented or diminished \(G_{\text{wmax}+\text{ab}}\) following domestication was statistically absent (Fig. 1A and Supplementary Table S3 at JXB online).

In contrast, the distribution of stomatal conductance among adaxial and abaxial leaf surfaces \((G_{\text{wmax}+\text{ab}})\) was statistically dissimilar among domestication statuses (Figs. 1B and 2C). \(G_{\text{wmax}+\text{ab}}\) was generally higher for domesticated lines, although the magnitude and direction of the response was diverse among crop species (Fig. 1B).

#### Stomata size and density and their relationship to \(G_{\text{wmax}+\text{ab}}\) and \(G_{\text{wmax}+\text{ab}}\)

We found no trade-off of size versus density of stomata in our dataset, in either the adaxial or the abaxial sides of our leaves, and regardless of whether all accessions were considered together or split between domesticated and wilds (Fig. 3). \(G_{\text{wmax}+\text{ab}}\) was strongly and positively related to stomatal density, while variation in the size of stomata was statistically irrelevant to variation in \(G_{\text{wmax}+\text{ab}}\) (Fig. 4A, B). This resulted in changes in \(G_{\text{wmax}+\text{ab}}\) during domestication, if present, being strongly driven by changes in stomatal density (Fig. 4C, D). Moreover, the domestication response of the distribution of stomatal conductance among adaxial and abaxial leaf sides, i.e. \(\Delta G_{\text{wmax}+\text{ab}}\) was caused chiefly by changes in stomatal numbers, with sizes playing a less relevant role \((r=0.86 \text{ vs } r=0.39, \text{ Fig. 4E, F})\). All the above were supported by the results of hierarchical partitioning analyses, which showed that the variance in \(G_{\text{wmax}}\) accounted for by \(D\) was much larger than that accounted for by \(S\) (see insets in Fig. 4A, C, and E). Therefore, even if stomatal size tended to increase at the adaxial side of domesticated leaves, the domestication effect over \(G_{\text{wmax}+\text{ab}}\) was triggered chiefly by the trend of domesticates to show lower stomatal densities at the abaxial side of their leaves (Supplementary Figs S2 and S3).

### Discussion

Here, we have shown that domestication of herbaceous agricultural crop species did not entail a generalized increase or decrease in the maximum potential stomatal conductance of leaves. This result was accompanied by a tendency of domesticates for displaying a larger fraction of conductance at the upper side of leaves, in comparison with their wild ancestors.
This study is the first comprehensive multi-crop assessment on the consequences of domestication and further breeding for stomatal anatomical traits. Together, the set of crops of this study accounts for 31% of global croplands (http://faostat.fao.org, 2010 data), and takes in a diversity of phylogenetic origins, domestication processes, geographies, and intensities (e.g. from anciently domesticated Mesoamerican Poaceae like maize, to incipient European Fabaceae crops such as lupins). The obtained patterns, therefore, are sufficiently general to depict a broad picture of domestication effects on stomata. Also, the range of stomatal densities and sizes within our study system encompasses a large portion of the known variation in these plant traits worldwide (71% for density and 59% for size; see Hetherington and Woodward, 2003). It is

Fig. 1. Bisector plots representing maximum stomatal conductance to water (Gwmax_ad+ab) (A), or its adaxial:abaxial ratio (Gwmax_ad:ab) (B) of domesticated versus wild ancestor accessions of each crop. Crops above the y=x line showed higher Gwmax_ad+ab or Gwmax_ad:ab in the domesticated than in the wild ancestor accessions, and vice versa for crops below y=x. Data are means ±SEM (n=5). Capsicum a=Capsicum annuum; Capsicum b= Capsicum baccatum. See Supplementary Table S3 for details on statistical analyses.
also fair to note that broad-scale multi-species approaches, like ours here, compromise detailed survey within each crop. Thus, drawing conclusions with regard to the behaviour of each specific crop should be done with care. The patterns shown here are of better use for appreciating generalities and suggesting future, more detailed, work. Moreover, only maximum potential conductances have been reported here. Actual functional relevance of our anatomical measures, and SEM. Details on the results of statistical analyses available in Supplementary Table S4 at JXB online.
Fig. 4. Relationship between maximum stomatal conductance ($G_{\text{wmax}}$) and its anatomical components: stomatal size ($S$) and density ($D$). (A, B) $G_{\text{wmax ad+ab}}$ versus $D_{\text{ad+ab}}$, and $G_{\text{wmax ad+ab}}$ versus $S$. The sizes of stomata ($S$) were averaged among adaxial and abaxial leaf surfaces. Density ($D_{\text{ad+ab}}$) and conductance ($G_{\text{wmax ad+ab}}$) are adaxial and abaxial scores summed together. (C, D)
physiological responses to suboptimal environments, remain to be evaluated. Below, we discuss the functional and breeding implications of the observed patterns, and how stomatal densities and sizes have contributed to them.

Domestication effects over potential conductance and its distribution among upper and lower leaf sides

Taking our multiple crops together, we found that potential conductance of domesticate leaves did not differ in a consistent manner from that of their wild ancestors. This is at odds with our initial expectation. However, it should be taken into account that most of the wild ancestors of our herbaceous crops are probably high-growth plant species, when put into the context of other wild plants (Craine, 2009). This type of productive species exhibits high gas-exchange rates (Wright et al., 2004), and artificial selection may have encountered a top threshold to conductance in these wild species, which is difficult to improve further. It is well known, for instance, that augmenting the surface of stomata when stomatal density is already high can saturate effective conductance (Mott et al., 1982). This might have been the case in our study system, because those wild ancestors exhibiting particularly high levels of conductance were those that tended to decrease conductance with domestication (see Fig. 1A). An alternative to circumvent the limits of already-high conductances is to distribute stomatal pores more evenly among leaf surfaces. This occurred in our data set for those crop species that showed ‘adaxialization’ of potential conductance. Changing the distribution of potential conductance among leaf sides can affect instantaneous gas-exchange rates, increasing actual conductance up to 25% (Mott et al., 1982). The mechanisms underlying putative increases in conductance when stomatal function is transferred to the adaxial side are several. First, more evenly distributed stomata widen their spacing, and thus increase diffusion pathway routes (Mott et al., 1982). Secondly, resistance to diffusion is lower in the boundary layer of the adaxial epidermis, which should promote higher conductance to water for single stomata placed at the upper side of leaves (Parkhurst, 1978). Finally, stomata at the upper side of leaves are more sensitive to water stress (Aston, 1978; Mott and O’Leary, 1984). Thus, an increase in upper-side stomata should only be affordable for species evolved under benign and predictable water supply regimes, like those raised under artificial selection.

The crop species that augmented $Gw_{max ad:ab}$ during domestication could prove suitable targets for plant breeding to pursue the promotion of productivity and/or water-use efficiency. This possibility is reinforced by findings of stomatal development being under different genetic or hormonal control in upper versus lower sides of leaves, which has been shown in Solanum and Arabidopsis (Hetherington and Woodward, 2003). Quantitative trait loci assignments for adaxial and abaxial stomata have also been reported in rice (Ishimaru et al., 2001). Breeding for stomatal sizes and numbers has followed aims as diverse as disease resistance, evaporative cooling, retention of pollutants, and water-use efficiency (Jones, 1987). Success has proved difficult to achieve because the coupling of stomatal conductance to yield and resource-use efficiency is elusive and is mediated by many factors (Jones, 1987; Condon et al., 2004). Thus, the patterns of adaxialization found here for a number of species, accompanied by current (Ishimaru et al., 2001; Hetherington and Woodward, 2003) and future genetic studies, may provide opportunities for the implementation of new breeding strategies worth exploring. However, as stated above, caution should be kept while interpreting our anatomical results, and additional physiological studies are required to expand potential conductances to actual gas-exchange responses occurring under diverse environmental scenarios.

Nevertheless, the response of $Gw_{max ad:ab}$ to domestication was diverse. Crops like Cicer, Gossypium, and Lactuca maintained either similar or even more abaxial distribution of stomata after domestication. No particular phylogenetic, historical, or functional group patterning was detected that could account for such diversity in the response. More detailed analyses would be needed in this regard to get a deeper insight on the reasons for among-crop variability found here. Also, subtler stomatal traits ignored in our broad screening study, such as shape and size of companion cells, species-specific pore length to guard cell width ratios, pore opening regulation, and the spatial patterning of stomatal distribution within the lamina, can all result in changes in stomatal conductance (Weyers and Lawson, 1997; Aasamaa et al., 2001; Franks and Farquhar, 2007; Haworth et al., 2013). Evolution of these traits during domestication, and their trade-offs with stomatal conductance, might have generated scatter and crop-specific variability in the degree of adaxialization of the stomatal function.

Size and density of stomata, and their influence on domestication effects over conductance

Maximum potential conductance per unit leaf area is a function of size and density of stomata (Franks and Beerling, 2009). Stomatal density has a direct and straightforward effect on conductance. Stomatal size, however, contributes in a more counterbalanced way. Whereas pore size directly increases conductance, it is associated with deeper pores, which increase resistance to gas diffusion (Franks and Farquhar, 2007). Diffusive conductance of a given stomatal pore is roughly proportional to the ratio of pore area to depth.
Two small stomata then have a higher joint conductance than a larger one of double pore area. Accordingly, we found that stomatal conductance, and its response to domestication, was chiefly driven by changes in stomatal density, rather than modifications in stomatal size. The domestication response of conductance ratio among adaxial and abaxial surfaces was also primarily governed by changes in stomatal numbers. However, increases in the size of adaxial stomata also contributed, although more modestly, to the transfer of conductance to the upper side of leaves (Fig. 4, and Supplementary Figs S2 and S3). Large stomata have less proficient closure and opening regulation, and respond poorly to drought (Aasamaa et al., 2001; Hetherington and Woodward, 2003; Drake et al., 2013). However, they are well suited under constantly humid conditions (Hetherington and Woodward, 2003). Therefore, under the more abundant and predictable water supplies experienced by plants in artificial environments (Mckey et al., 2012), the evolution of larger stomata could have been under a selective advantage. This was clearly the case for the adaxial stomata of most of the crops considered here (Supplementary Fig. S2C).

The size and number of stomata were unrelated across the species of our dataset. This occurred within wild accessions, within domesticated lines, and also when all accessions were considered together. Density and size of stomata correlate negatively when examined within species, or as a response of specific genotypes to environmental variation (e.g. Sapra et al., 1975). However, when this relationship is examined at the macroevolutionary level, and covering a high number of species and botanical lineages, a more diverse picture arises. Species with a high number of stomata per unit leaf area tend to bear small stomata, and vice versa (Hetherington and Woodward, 2003; Franks and Beerling, 2009). In contrast, at moderate to low values of size and density, all strategies are allowed by natural selection, with a very loose relationship between both parameters (Hetherington and Woodward, 2003; Franks and Beerling, 2009). In this sense, when a modest number of species are surveyed, as in the current study, it is very unlikely that the worldwide extremes of size and density are taken in, and thus the S versus D trade-off does not show up. Even so, the results in Fig. 3 are congruent overall with the macroevolutionary patterns mentioned above, with more variation in the S versus D relationship at the low ends of both variables, and less variation at the high range of both S and D (Fig. 3). This variation signals a relaxation of the physiological trade-off between S and D when plants evolve under domestication, parallel to the pattern found for macroevolution under natural selection (Franks and Beerling, 2009; Steinthorsdottir et al., 2013). Relaxation of trade-offs is common in evolution under artificial selection. For example, trophic and parasitic trade-offs implying metabolic or tissue-forming costs for the plant have been relaxed during the domestication of a number of species (Kiers et al., 2007; Archetti, 2009; Macfadyen and Bohan, 2010). Other cases include the relaxation of trade-offs between investing in growth or in chemical defence (Herms and Mattson, 1992), which has been reported for several crops (Rosenthal and Dirzo, 1997; Mondolot et al., 2008). Disruption of trade-offs might contribute to the lack of relationship among stomatal size and number within our domesticate accessions.

Improvements of photosynthetic rates and of water-use efficiency have proved elusive for traditional breeders and biotechnologists alike (Evans, 1993; Jones, 1987; Condon et al., 2004; Denison, 2012). Stomatal distribution may hint at a comparatively poorly explored avenue for increasing productivity and/or resource-use efficiency. The allocation of stomata to the upper or lower sides of leaves can modulate the amount of water transpired, the amount of CO₂ being taken up by the leaf, and, most importantly, the exchange ratios among both gases (i.e. water-use efficiency). Further physiological studies should delve further into this research line and provide actual gas-exchange data in response to domestication and under diverse ecological scenarios. The patterns described here provide a baseline of what has been unintentionally modified in terms of anatomical stomatal distribution during domestication as a guide for future directional breeding. Stomata are the key regulators of the water-per-carbon exchange of plants (Hetherington and Woodward, 2003). Clearly, with the current urgent needs for yield increases (Miflin et al., 2000), stomatal distribution merits increased attention.

**Supplementary data**

Supplementary data are available at *JXB* online.

**Supplementary Table S1.** Common name, taxonomic and domestication status, and seed origin information of each accession of the 24 domesticated-wild ancestor pairs used in this study.

**Supplementary Table S2.** Arithmetic mean and standard deviations of stomatal densities (mm⁻²) and guard cell lengths (μm) for the adaxial and abaxial leaf sides of domesticated (D) and wild ancestor (W) accessions of each crop.

**Supplementary Table S3.** Effects of domestication status (wild vs domesticated), of crop identity, and of leaf side (adaxial vs abaxial) on the several stomatal parameters used in this study.

**Supplementary Fig. S1.** Bisector plots of maximum stomatal conductance at the adaxial (A) (Gwmax_ad) and abaxial (B) (Gwmax_ab) side of leaves.

**Supplementary Fig. S2.** Bisector plots of stomatal sizes (S) and densities (D) of the wild and domesticated accessions of each crop.

**Supplementary Fig. S3.** Bisector plots of the adaxial:abaxial ratios of stomatal densities (A) (D_ad:ab) and sizes (B) (S_ad:ab) of the wild and domesticated accessions of each crop.

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