Changes in the vertical distribution of leaf area enhanced light interception efficiency in maize over generations of selection

Raphael Perez, Christian Fournier, Llorenç Cabrera-Bosquet, Simon Artzet, Christophe Pradal, Nicolas Brichet, Tsu-Wei Chen, Romain Chapuis, Claude Welcker, Francois Tardieu

Changes in the vertical distribution of leaf area enhanced light interception efficiency in maize over generations of maize selection

Raphaël P.A. Perez[1,2], Christian Fournier[1], Llorenç Cabrera-Bosquet[1], Simon Artzet[1], Christophe Pradal[2], Nicolas Brichet[1], Tsu-Wei Chen[1,3], Romain Chapuis[4], Claude Welcker[1], François Tardieu[1]

[1] Université de Montpellier, INRA, Montpellier SupAgro, UMR LEPSE, Montpellier, France

[2] Université de Montpellier, CIRAD, INRA Montpellier SupAgro, UMR AGAP, Montpellier, France

[3] Institute of Horticultural Production Systems, Leibniz Universität Hannover, Hannover, Germany

[4] Université de Montpellier, INRA, Montpellier SupAgro, UE DIASCOPE, Montpellier 34060, France;

[5] Université de Montpellier INRIA, , Montpellier, France

Author for correspondence:

François Tardieu

Tél: +33 499 612 632

Email: francois.tardieu@inra.fr

Total word count (excluding summary, references and legends):	5874	No . of figures:	7
Summary:	199	No. of Tables:	3
Introduction:	805	No. of Supporting information files:	1
Materials and Methods:	2499		
Results:	1834		
Discussion:	675		
Acknowledgements:	61		

Summary

Breeders select for yield, thereby indirectly selecting for traits that contribute to it. We tested if breeding has affected a range of traits involved in plant architecture and light interception, via the analysis of a panel of 60 maize hybrids released from 1950-2015. This was based on nvel traits calculated from reconstructions derived from a phenotyping platform. The contribution of these traits to light interception was assessed in virtual field canopies composed of 3D plant reconstructions, with a model tested in a real field. Two categories of traits had different contributions to genetic progress. (i) The vertical distribution of leaf area had a high heritability and showed a marked trend over generations of selection. Leaf area tended to be located at lower positions in the canopy, thereby improving light penetration and distribution in the canopy. This potentially increased the carbon availability to ears, via the amount of light absorbed by the intermediate canopy layer, (ii) Neither the horizontal distribution of leaves in the relation to plant rows nor the response of light interception to plant density showed appreciable trends with generations. Hence, among many architectural traits, the vertical distribution of leaf area was the main indirect target of selection.

1

Introduction

Plant architecture, i.e. the arrangement of plant leaf area in space and time, affects light interception and the way in which plants make use of the intercepted light for photosynthesis. For instance, a rapid coverage of the inter-row increases the amount of light intercepted by the canopy and depends on the horizontal distribution of leaf area, e.g. via a change with time of leaf orientation in relation to the plant row (Girardin & Tollenaar 1994). Increased leaf erectness tends to decrease the proportion of incident light intercepted by the canopy, butt improves light use efficiency by the canopy (Niinemets 2010). This is because the relationship between incident light and photosynthesis is non-linear. Erect architectures avoid upper leaves receiving saturating light and allow lower leaves in the canopy to receive more light (Long et al, 2006). Overall, traits involved in plant 3D architecture tend to have smaller effects on light interception than leaf area but their effects increase with phenological stages and becomes appreciable at canopy closure, when self and/or mutual shading occur (Perez *et al.* 2018; Chen *et al.* 2018a). Furthermore, the metabolic cost of changes in architecture is negligible compared with that of increasing leaf area in terms of nitrogen and carbon resources, so even small increases in the use of incident light obtained by optimizing plant architecture may have a beneficial effect on biomass accumulation.

Four conditions are necessary for any trait to have an interest in breeding (Sadras & Richards 2014). First, the considered architectural trait needs to result in an appreciable effect on total light interception and/or on the distribution of light interception in the canopy. Secondly, it needs a high genetic variability and heritability in the current genetic diversity. Thirdly, alleles of interest should not have been fixed so a genetic variability remains in current breeding populations after generations of selection. Finally, breeding is highly facilitated if the considered trait has a minimum trade off with other traits, so its improvement correlates with yield in a simple way ('scalability', Sadras & Richards, 2014). The multiple criteria for sorting out architectural traits make it difficult to establish a hierarchy of their interests for breeding, which inevitably depends on the weight that one places on each criterion. An alternative is to consider *a posteriori* the trends of each of these traits over generations of selection (Duvick & Cassman 1999).

Breeders essentially select based on yield but, doing so, they can indirectly select for traits that contribute to it, often termed secondary traits (Reynolds & Langridge 2016). This can happen if the effect of the considered trait is positive regardless of environmental scenarios. We have

argued that most traits involved in adaptation to abiotic stresses do not belong to this category because positive alleles change from year to year so they cannot be selected based on yield (Tardieu et al., 2018). Conversely, if the four above-mentioned criteria were met for architectural traits, one would expect a trend in these traits over generations of selection.

The emergence of phenotyping platforms now allows measuring complex traits on thousands of plants, in particular leaf area and biomass (Furbank & Tester 2011; Tardieu *et al.* 2017), but also light interception (Cabrera-Bosquet *et al.* 2016), plant competitiveness (Chen *et al.* 2018a) or stomatal conductance (Alvarez Prado *et al.* 2018). In particular, it is now possible to dissect biomass accumulation of hundreds of genotypes into components of the Monteith equation, thereby giving the possibility of detection of genomic regions associated with these traits (Chen *et al.* 2018a). Images can be taken from different directions, so 3D plant reconstructions can be built for mimicking each plant in the greenhouse every day of the plant cycle. This potentially allows calculating architectural traits, but also their consequences on light interception either in conditions in which plants were grown, or in field virtual canopies composed of 3D virtual plants.

We tested if, and which architectural traits were indirectly selected by breeders in a panel involving 60 maize hybrids released in the last sixty years (1950 to 2015), which displays an increasing yield over generations of selection of about 100 kg ha^{-1} year^{-1} (Welcker et al unpublished data), similar to that in another panel (Hammer *et al.* 2009). This panel was analysed in a phenotyping platform, while the interest of architectural traits for light interception was analysed in virtual canopies made of 3D plants, after assessing the validity of such canopies in comparison to a field experiment. We also tested if the observed trends also apply to plants subjected to water deficit. Besides, light interception by hybrids belonging to each generation of selection was also considered at different plant densities, because it has been argued that a major cause of genetic progress of maize yield is the ability of recent hybrids to grow at high densities (Duvick 2005; Assefa *et al.* 2018).

1

Materials and Methods

Plant material and experiments

The panel consisted of 60 maize (*Zea mays*. L.) hybrids released in Europe from 1950 and 2015, which were the most successful in their generation according to the Arvalis database (https://www.arvalisinstitutduvegetal.fr) and experts in the field. These hybrids belonged to mid-early/mid-late maturity groups, with leaf number from 14 to 18, and originated from the programmes of selection of different breeding companies based in Europe.

The panel was tested in two experiments performed in controlled and field conditions. The platform experiment was conducted at the PhenoArch installation (https://www6.Montpellier.inra.fr/lepse_eng/M3P/PHENOARCH-platform; (Cabrera-Bosquet *et al.* 2016) hosted at the Montpellier Plant Phenotyping Platforms, M3P (https://www6.montpellier.inra.fr/lepse/M3P) in spring 2017 (from April 6th to May 31th). Plants were subjected to two levels of soil water content, either well-watered (WW) or water deficit (WD) (for details see Brichet *et al.* 2017). Seeds were sown in 9-l pots (0.19 m diameter, 0.4 m high) filled with a 30:70 (v/v) mixture of a clay and organic compost, and thinned to one plant per pot at the 3 appeared leaf stage. Pots were arranged in 28 rows of 60 plants (with an inter-row distance of 40cm and an inter-plant distance of 20 cm within the row). One replicate of each hybrid was randomly positioned within each line, leading to a total of 14 replicates per hybrid and per irrigation treatment. Air temperature and relative humidity were measured at eight positions in the greenhouse every 15 min (Rotronic, HC2A, Bassersdorf, Switzerland). Temperature was maintained at 26 ± 3°C during the day and 18 ± 1 °C during the night. The amount of incident light at each plant position was estimated using hemispherical images and the outside incident photosynthetic photon flux density (PPFD) measured every 15 min with a sensor on the top of the glasshouse (for details see Cabrera-Bosquet *et al.* 2016). Additional incident light was provided by lamps when external solar radiation was below 300 W m^{-2} or to extend the photoperiod with 400 W HPS Plantastar lamps (OSRAM, Munich, Germany) with 0.4 lamps m^{-2}. All experimental, environmental and 3D collected data were stored in the PHIS information system (Neveu *et al.* 2019).

A field experiment was hosted at DIAPHEN (https://www.phenome-fppn.fr/phenome_eng/Facilities/Montpellier-Field) at INRA Mauguio (southeast of France, 43°36' N, 03°58'E) between May and September 2017. Eleven hybrids of the panel, selected according

to contrasting phenotypes (erectness, total leaf area) and year of release, were sown on 19 May 2017 in plots of 16.7 m² with four 5.2 m long rows 0.8 m apart and grown under irrigation, with two replications for each hybrid.

3D reconstruction of plants in the greenhouse

The estimation of architectural phenotypic traits was based on 3D plant reconstructed from images analysis (Cabrera-Bosquet *et al.* 2016; Brichet *et al.* 2017). Virtual 3D reconstructions were generated every day for each of the 1680 plants of the platform by analysing RGB (2056 x 2454) plant images taken from 13 views (12 lateral views from 30° rotational difference and one top view) (for details see Cabrera-Bosquet et al., 2016 and Brichet et al., 2017). Plant pixels were distinguished from the background via threshold algorithms and morphological operators using OpenCV libraries (Bradski & Kaehler 2008 http://opencv.org) and converted into mm² by calibrating camera positions using reference objects. Total plant leaf area was obtained every day via calibration curves constructed using multiple linear regression models based on processed images taken in <="" span="">13 directions against measurements of leaf area at different stages (Brichet *et al.* 2017). Virtual 3D representations were computed using a space carving algorithm (Kutulakos & Seitz 2000) and represented by a set of 3D voxels of 0.512 cm³ each. This voxel set was then transformed into a triangular mesh using the marching cube algorithm (Lorensen & Cline 1987) and a 90% decimation (Schroeder *et al.* 1992) implemented in a VTK library (Schroeder *et al.* 1995). This transformation into triangular meshes was achieved in order to obtain a 3D surface reconstruction that conserved the plant leaf area estimated by regressions with pixel number. The open-source software pipeline *Phenomenal* (https://github.com/openalea/phenomenal; Fournier *et al.* 2015) processed the images, stored intermediate results and distributed the computation on the grid via the cyber-infrastructure *InfraPhenoGrid* (Pradal *et al.* 2017) embedded in the scientific workflow system *OpenAlea* (Pradal *et al.* 2008).

Vertical distribution of plant area in the greenhouse

Plant height (h_{plant}) was estimated as the highest pixel of a plant. The whorl height (h_{whorl}) was estimated as the highest point of the vertical axis including the plant central axis (Table 1), detrmined using the 3D coordinates of the centres of gravity of each voxel structuring the considered 3D plant. First, 3D coordinates of these centres of gravity were projected on the horizontal plan (x,y) and the number of voxels located (vertically) at each (x,y) coordinate was counted (Fig 1A). The central axis of the plant was defined as the (x_c,y_c) coordinate with the

maximal voxel count. Whorl height was estimated as the z coordinate of the topmost voxel x_c-y_c located on the central axis (Fig. 1B and Fig. SI 1A).

For each plant, the vertical distribution of plant area was described by two variables (rh_{PAD} and b_{PAD}, Table 1) computed based on the number of plant voxels per horizontal layer of the canopy (1 voxel/8 mm thickness) extracted from 3D plant reconstructions (Munier-Jolain *et al.* 2013) (Fig 1C):

(eq1)

Where $f(z_{rel})$ is the cumulated leaf area (expressed relatively to total plant leaf area) from plant base to the height z_{rel} in the canopy. z_{rel} is the ratio between the absolute altitude of the considered voxel and the plant height. rh_{PAD} is the relative height in the canopy where the cumulated leaf area reached half of the total leaf area ($f(rh_{PAD})=0.5$). High values of rh_{PAD} indicate a concentration of plant area towards the top of the plant (Fig. SI 1B). b_{PAD} is an index of concentration of leaf area around $z_{rel} = rh_{PAD}$, with high values of b_{PAD} indicating a high concentration of plant area around rh_{pad}, whereas low values of b_{PAD} indicate an evenly distributed area in horizontal layers of the canopy. rh_{PAD} and b_{PAD} were estimated using the 'nls' function of R software (R Core Team 2017).

Leaf erectness (θ) was calculated as the mean of elevation angles (θ_p) of the triangular meshes composing the 3D reconstructions (Fig 1D). High values of θ indicate more erect leaves (Fig. SI 1C).

Horizontal distribution of plant area in the greenhouse

The projection of 3D plants on the horizontal plane, combined with the detection of the position of the plant central axis on this plane, allowed expressing the position of each voxel in polar coordinates, with a specific azimuth (az) and radius (r). Plant radius (Table 1) was estimated as the 95th quantile of the distribution of voxels radiuses (Fig. SI 2A). The estimation of traits related to plant azimuthal dispersion (σ_{az}) and orientation(Δ_{row}) were performed by i) detecting leaf tips by analysing peripheral voxel and isolating radial peaks (see supplementary material appendix A and Fig. SI 3 for the detailed procedure). Voxels with r < 25% of the maximum radius were removed as they were considered as artefacts, not distant enough from the stem to be considered as leaf tips (Fig. SI 2 B); ii) grouping leaves into azimuthal directions by applying a cluster analysis based on matrix distance of voxel azimuths, and considering the azimuth of the detected radial peaks as cluster medoids (medoids with difference in azimuth below 15° were merged to form a unique cluster) (Fig. SI 2C). Each group was characterized by the number of

voxels forming it, an azimuth (median azimuth of the grouped voxel) and a radius (median radius of the grouped voxels); iii) clustering the groups into two main azimuthal directions using a hierarchical cluster analysis (*hclust* procedure of the R software; R Core Team, 2017) applied on group azimuths weighted by the number of voxels per group (Fig. SI 2D). This clustering analysis allowed estimation of σ_{az}, which was computed as the mean difference between group azimuths with the associated main direction. As a result, σ_{az} can be viewed as the average deviation from a distichous phyllotaxy, i.e the higher σ_{az} the more a plant is dispersed in azimuth (Fig. SI 4A). Finally, Δ_{row} was estimated as the mean difference between the azimuth and the direction of plant rows; the higher $\Delta_{row,}$ the more perpendicular were the leaves in relation to plant rows (Fig. SI 4B).

In addition to comparing hybrids at given days after sowing (DAS), we also performed analyses at comparable phenological stages. For that, the number of visible and ligulated leaves were counted twice a week, and the date of tassel appearance was recorded for each plant. In particular, comparisons were performed at the date on which the ligules were visible on half of leaves that eventually emerged, i.e. when no tassel was visible in any of the studied hybrids (see Fig. SI 5). This was done to ensure that the architectural differences observed among hybrids at a given day after sowing were not the result of difference in phenological stages .

Imaging in the field experiment

In the field experiment, vertical upward-looking hemispherical images (2640 × 1760) were taken using a Sigma SD-14 camera equipped with a fisheye lens of 8 mm focal. For each micro-plot and date, 10 images were taken early in the morning (before 8 am). At each sampling date (June 15th, June 21st, June 29th, and July 19th), the camera was oriented North with the lens kept horizontally towards the sky and images were distributed over two diagonal segments placed between the two central rows of each micro-plot as detailed in Baret *et al.* (2018). Images are available at http://www.phis.inra.fr/ under an Open Source license (CC-BY-NC-SA). Images were analysed using the Ilastik (Ilastik software, version 1.21.7) (Sommer *et al.* 2011) built-in pixel classification machine learning procedure based on a training set of contrasting images involving micro-plots of different hybrids at different dates. For each image, total pixels corresponding to vegetation were extracted from background (sky) using a random forest classification method based on colour (Gaussian smoothing of 5 px) and texture (structure tensor eigenvalues of 1.6 px)

The ground cover (ratio of vegetation pixel to total pixel numbers, i.e. reciprocal of gap fraction, Fig. SI 6) was calculated in every micro-plot corresponding to each of the 11 studied hybrids. Average values of ground cover corresponding to every micro-plot were obtained using images taken at the central positions of each micro-plot. Ground cover (i.e. reciprocal of gap fraction) measured in the field at the four sampling dates was then compared to the RIE of the same hybrids in the greenhouse experiments, with comparisons performed for plants at the same phenological stage in both conditions.

Simulation of light interception and radiative variables in virtual canopies

Radiation interception efficiency (RIE) was estimated considering the 3D representation of each plant in a virtual toric field to represent a homogeneous canopy under three contrasting plant densities (5.5, 9 and 12.5 plants m^{-2}) with 0.8 m row spacing. The toricity allows one to remove border effects as if the canopy was infinite. Light interception was estimated using the Caribu light propagation model (Chelle & Andrieu 1998), combined with a sky radiance distribution model mixing SOC (Moon & Spencer 1942) and clear sky (Perez *et al.* 2002) distributins according the mixing ratio proposed by Mardaljevic (2000). These models were calibrated with daily meteorological data to reproduce appropriate sun path over a day (segmented into 46 directions).

The extinction coefficient (k) was calculated following the Beer-Lambert equation and was used to assess the penetration of light within the virtual canopy (Monsi & Saeki 2004):

(eq4)

k was estimated using the *'nls'* function of R software (R Core Team 2017) based on estimations of RIE and leaf area index (LAI) from 0 to 35 DAS for each of the 1680 simulated canopies.

The amount of light intercepted by each component (triangular mesh) of the reconstructed plants in the canopy allowed estimating three radiative variables: Z_{rell}, the depth above which half of the incident light was intercepted; $RIE_{0.4-0.7}$, the proportion of incident light intercepted by the canopy layer located between 40% and 70% of the canopy height (layer where ears are located; Brichet et al, 2017, Fig. SI 7) and σ_{Ei}, the standard deviation of the proportion of incident light intercepted per unit leaf area of plant components.

Assessing the contribution of architectural traits to light interception

The contribution of architectural traits to light interception was calculated for each virtual canopy via a regression model:

(eq5)

where ε is the residual effect. The absence of multi-collinearity between explanatory variables (traits) was checked using variance inflation factors (VIF<5). This procedure was performed between 9 and 35 DAS when all the plants were presented in the platform, and allowed the decomposition of model variance into its constitutive explanatory variables using the '*calc.relimp'* function of the '*relaimpo'* R package (Grömping 2006).

Genotypic means of RIE for each hybrid in virtual canopies

Fourteen virtual canopies were built for each hybrid and water treatment grown in the greenhouse. Each virtual canopy involved replicated 3D representations of one of the 14 plants observed in the greenhouse for the corresponding hybrid and water treatment, positioned in the virtual canopy with its exact architecture and azimuthal position as in the greenhouse. RIE was calculated for the 14 virtual canopies, and its mean value was considered as the genotypic mean for the considered hybrid.

We challenged the validity of this method, first by comparing it with another method based on the random distribution of the 14 plant representations of each hybrid in a single virtual canopy, second by comparing the outputs of both methods with the results obtained for the 11 hybrids in the field experiment. In the alternative method for building a virtual canopy, the fourteen 3D representations corresponding to the studied hybrid were randomly distributed in a single virtual canopy. This was replicaed seven times to account for the effect of randomness of permutations. The mean RIE for these replications was considered as the genotypic mean for the considered hybrid. Finally, the genotypic means obtained with both methods for the 11 hybrids were compared with the ground covers (reciprocal of gap fraction based on hemispherical images in the field) of the same hybrids in the field experiment.

Heritability

Heritabilities were estimated using the following mixed-effect model for each variable:

(eq2)

Where Y_{ijsd} is the phenotypic value of the considered variable for plant i for the water scenario s and the day d, μ_{sd} is the overall mean, G_{jsd} is the effect of genotype j and E_{ijsd} the residual effect. Models were fitted for each water scenario and every day after sowing. Broad sense heritabilities on plant leaf area (LA) and the seven architectural traits (h_{whorl}, θ, rh_{PAD}, b_{PAD}, radius, σ_{az} and Δ_{row}) were calculated as:

(eq3)

The variance components were estimated using the '*lmer*' procedure of the '*lme4*' R package (R Core Team, 2017).

Results

A high genetic variability and heritability for architectural traits describing the vertical distribution of leaf area.

The first category of architectural traits was associated to the vertical distribution of leaf area, in particular the relative altitude in the canopy at which half of leaf area was cumulated (rh_{PAD}), the concentration of leaf area at this altitude (b_{PAD}) and leaf erectness (θ). The relative altitude rh_{PAD} and erectness were correlated (r=-0.64, Table 2), meaning that the leaf area of most erect plants was concentrated at lower relative positions of the canopy than in less erect plants. This somewhat counter-intuitive result is linked to the fact that rh_{PAD} is calculated relative to plant height that increases with erectness (Table 2). The index rh_{PAD} tended to increase with time, whereas the concentration of leaf area at the altitude rh_{PAD} (b_{PAD}) increased during the plant cycle (Fig. 2). Leaf erectness tended to decrease with time for all hybrids, with a large genetic variability at each date (Fig. 2). The intra-genotypic variability was lower than inter-genotypic variability for these three variables, which presented a high heritability during the whole growth cycle with maximum of 0.83, 0.80 and 0.58, respectively, at 20-25 days for leaf erectness and rh_{PAD} and at 30 days for b_{PAD} (Fig. 3). They defined the second axis of a principal component analysis (PCA) and showed either weak or no correlation with other variables (Table 2 and Fig. SI 8).

The second category of architectural traits described plant vigour, in particular leaf area (LA), the height of the whorl (h_{whorl}) and plant radius that all increased with time and presented a high genetic variability (Fig 2 & Fig. SI 9). These variables defined the first axis of the PCA, (Fig. SI 8), were highly correlated at all phenological stages (Table 2) and had a medium to high heritability that increased with time (Fig. 3).

The third category described the orientation of the projections of leaves over a horizontal plane, namely the azimuthal dispersion (σ_{az}) and the orientation of plants in relation to the row along the plant cycle (Δ_{row}). These two variables increased with time (Fig. SI 9). The increase in σ_{az} meant that the vertical plane of plants, easy to identify at the beginning of the plant cycle, tended to be less and less obvious at later stages. The increase of Δ_{row} meant that the average vertical plane of plants tended to orient towards a direction perpendicular to plant rows. Indeed, at the beginning of the experiment (9 days after sowing), the proportion of plants with orientation toward the inter row (Δ_{row}>45°) was 50%, and progressively increased with canopy closure to reach up to 80% 35 days after sowing (Fig. SI 10). A high plant-to-plant variability was observed

for these variables (Fig. SI 9), with very low heritability (Fig. 3), thereby suggesting a low genetic control for plant orientation in relation to the plant row.

The genetic progress affected the vertical distribution of leaf area

Modern hybrids tended to show a markedly different vertical distribution compared with older hybrids, together with a slightly lower leaf area. The variables describing the vertical distribution of leaf area were the most correlated with the year of release of considered hybrids (r = -0.72, -0.44 and 0.44 for rh_{PAD}, b_{PAD} and leaf erectness respectively in both WW and water deficient plants of the panel, Table 3). Leaf area correlated negatively with year of release in both water treatments, meaning that leaf area tended to slightly decrease with genetic progress (Table 3). However, this tendency disappeared if plants were compared at a given leaf stage rather than at a given date (Fig. SI 11). The plant radius and whorl height were either independent or weakly correlated with year of release. As expected because of its low heritability, the reorientation of the vertical plane of leaves in relation to plant rows (Δ_{row}) was not associated with genetic progress (Table 3).

Hence, leaves tended to be more concentrated at low relative altitudes of the canopy (decreasing values of rh_{PAD}), and distributed more evenly with height (decreasing values of b_{PAD}) over generations of selection (Fig. 4 and Table 3). These changes were observed in both well-watered and water deficit plants, with parallel positive relationships without significant difference in slopes.

Modern hybrids presented improved light penetration and distribution in the canopy, thereby increasing the amount of light absorbed in the canopy layer where ears are located.

Virtual canopies, simulating plants in the field, were built to calculate the genotypic means of light interception efficiency for each hybrid. They involved 3D representations of the 14 plants observed in the greenhouse for the corresponding hybrid. Genotypic means of RIE were calculated either by averaging the RIE of fourteen virtual canopies involving every plant replication of the considered hybrid, or calculating the RIE of a virtual canopy involving the same 14 virtual plants placed at random. The genotypic means of RIE obtained by both methods were closely correlated (Fig. 5, inset), suggesting that neither method appreciably biases the tendencies for genetic progress. The correlations between RIE and genotypic means of ground cover observed in the field were slightly better with the first than with the second method (Fig. SI 12), with satisfactory correlations at the four studied dates (r=0.53, 0.57, 0.62 and 0.75 for June

15th, June 21st, June 29th, and July 19th, respectively Fig 5). Correlation coefficients increased with phenological stage, probably because the gap fraction method has a precision that increases with leaf area. As a consequence, further paragraphs only refer to the first method that was considered as adequately representing the genotypic means of RIE translated to the field.

The total amount of light intercepted by virtual canopies was essentially maintained throughout generations of selection with the usual density of 9 plants m^{-2}, with a slight decrease over generations due to the decrease in leaf area (Fig. SI 13). Variations in light interception efficiency were largely explained by the traits proposed in this study, the latter contributing to 82 - 95% of the total variance depending on days after sowing (Fig. 6). Leaf area was the main determinant of light interception during the whole plant cycle (50% contribution) whereas architectural variables, considered jointly, accounted for 40% of light interception. The cumulated contributions to light interception of leaf erectness, rh_{PAD} and b_{PAD} ranged from 5% at the beginning of the plant cycle (9 DAS) to 22% at the 12-leaf stage (35 DAS). Regarding the contribution of architectural traits to the proportion of light intercepted in the layer between 40 and 70% of plant height (RIE 0.4-0.7; Fig 6), it sarply increased over time to reach 80%, rh_{PAD} and leaf erectness accounting for respectively around 60% and 20% of the associated explained variability.

Because leaf area was located lower in the canopy of recent hybrids, one observed a decrease over generations of the relative altitude in the canopy at which 50% of incident light is absorbed (Z_{rell}, Fig. 7B). Consistently, the extinction coefficient (k) was lowest in recent hybrids, suggesting a better penetration of light within the canopy (Fig. 7A). In old hybrids, the upper 10% of the canopy intercepted 50% of the incident light (Z_{rell}= 0.9) for the usual plant density of 9 plants m^{-2}. In modern hybrids, this upper canopy layer allowed better light penetration, so 50% of incident light was still available in the canopy layers located below 80% of the canopy height. Furthermore, light was intercepted more evenly in the canopy, with a significant decrease in variance of the amount of light intercepted per unit leaf area by the different triangular meshes of the canopy (σ_{Ei}, Fig. 7C). The increased light penetration in the canopy of recent hybrids was linked to the lower proportion of leaves in the upper canopy layer (Fig. 4A and Fig. 6b), but also to more erect leaves that intercept less light per unit leaf area (Fig. 4B). Similar tendencies were observed if plants were compared at a given leaf stage rather than at a given date (Fig. SI 14). The increased light penetration in the canopy may have two advantages.

- First, because the relationship between light and photosynthesis is markedly non-linear, a better distribution of light in the canopy allows better radiation-use efficiency by avoiding that the

upper 10% of the canopy presents light-saturated photosynthesis whereas the lower layers receive low light.

- Second, because the carbon supply to the ear essentially comes from the leaves located around it (Palmer et al, 1973). The proportion of incident light intercepted in this layer was calculated here as that between the altitudes in the canopy ranging from 40 to 70% of plant height, regardless of generations (Fig. SI 7). It largely increased over generations of selection, from 5% up to 20% in oldest and most recent hybrids, respectively (Fig. 7D). In addition, the leaf area in the same layer increased over generations of selection (Fig. SI 14). Hence, the amount of photosynthates accumulated by the canopy was probably more available to the ear over generations of selection. This was attributable to a change in the vertical distribution of leaf area rather than to a change in ear height (Figure SI 7).

The improved distribution of absorbed light applied to low and high densities

We performed simulations with three plant densities because it has been stated that part of the genetic progress originates from the plant ability to grow at high densities. Studied densities were 9 plants m^{-2}, common in Europe, plus a high density, 12.5 plants m^{-2}, increasingly used for silage, and a low density of 5.5 plants m^{-2} that was used at the beginning of XXth century in southern regions. These simulations were performed with the 3D representations of plants in the greenhouse regardless of plant density, thereby neglecting the adaptation of plant architecture in the considered range of plant densities. Indeed, calculations performed on datasets collected on previous studies suggested a low interaction between these adaptations and generations of selection (Sangoi *et al.* 2002; Ma *et al.* 2014) or genotypes derived from distinct maize germplasm groups (Ku *et al.* 2016) (Fig. SI 15). Overall, the relationships between studied variables and generations were essentially parallel for the three studied densities (Fig. 7). In view of the absence of appreciable interaction between generations and adaptation of architecture, this parallelism probably holds in spite of our simplifying hypothesis of neglecting adaptation of architecture. The high density allowed interception of a large amount of light in the canopy layer that includes ears, so modern hybrids could make the best use of incident light even at high densities. Besides, a steeper slope of progress can be visualized at 9 plants m^{-2} for the extinction coefficient (k) and the variance of irradiance within the canopy (σ_{Ei}) (Fig. 7 A & C). Our analysis therefore suggests that changes in canopy architecture were favourable at all densities, thereby allowing the use of high densities without penalty, with a specific advantage of genetic progress at the common plant density rather than higher or lower densities.

Discussion

The recent development of methods allowing *in situ* characterization of plant architecture, in combination with light models, enables one to explore the capacity of plant to intercept light (Burgess *et al.* 2017). Here we demonstrate that such approach applied to a phenotyping platform (Cabrera-Bosquet *et al.* 2016), helps to overcome the technical bottleneck of conducting genetic analysis on architectural traits (Tardieu *et al.* 2017). Comparatively to modelling studies based on architectural models, our approach is based on accurate and actual 3D reconstructions avoiding any change in the phenotypic correlation among architectural traits that may emerge from uncontrolled combinations of allometries used in these models.

The dissection of 3D reconstruction allowed identification of novel traits related to either vertical or horizontal distribution of leaf area. The trait that underwent the clearest indirect selection by breeders was the vertical distribution of leaf area (rh_{PAD}) that appreciably contributed to light penetration in the canopy. The change in vertical distribution over generations of selection allowed optimizing the utilization of light in the different canopy layers, and a better interception by the layer containing the leaves that mainly supply carbon to the ear (Palmer *et al.* 1973) . This is in agreement with the plausible enhancement of yield trough improved photosynthesis at ear height, as suggested in previous studies (Long *et al.* 2006; Hammer *et al.* 2009). Another advantage of more even leaf area and light distribution along the canopy is to reduce the stomatal limitation to photosynthesis and save water, especially under stress condition (Chen *et al.* 2018b). In addition, a deeper penetration of light within the canopy may optimize nitrogen supply, thus enhancing phtotosynthetic capacity (Mantilla-Perez & Salas Fernandez 2017). Interestingly, the changes in vertical distribution of either leaf area or light interception were essentially continuous over generations of selection, without clear breaking point or step, suggesting that they were obtained via rearrangement of a large number of allelic values rather than via the introduction of key novel alleles.

The correlation between vertical distribution of leaf area and leaf erectness suggests that the latter has appreciably contributed to the former. Leaf erectness has been identified as a heritable trait and a key component of high-yielding varieties in cereals species (Mantilla-Perez & Salas Fernandez 2017). We confirm here that erectness largely contributes to light interception and its penetration within the canopy (Truong *et al.* 2015; Burgess *et al.* 2017), and that it has been indirectly selected by breeders. Erectness was empirically selected over generations of selection, which corroborate with morphological changes observed in maize hybrids selected in American (Duvick & Cassman 1999) and Chinese programs (Ma *et al.* 2014).

We also estimate a new indicator related to the horizontal arrangement of leaf area (σ_{az} and Δ_{row}). As previously indicated by Drouet & Moulia (1997), we observed a non-random distribution of azimuths, with maize leaves turning toward inter-row spaces over time and thus ensuring a better capture of the available incident light (Maddonni *et al.* 2001). Traits related to leaf horizontal orientations were not heritable, suggesting either the absence of genetic variability of such traits or that the alleles related to leaf re-orientation were already fixed before selection programs. This outcome contradicts the genetic variability of phytochrome-mediated responses to low red to far-red ratio (R/FR) as suggested by Maddonni (2002).

Finally, the enhancement of light penetration over generations of selection occurred for all planting densities, with a slightly better progression at 9 plants m^{-2}, density considered as the current agronomic optimum plant density (Assefa *et al.* 2018). This suggests that selected changes in plant architecture were driven to fulfil the physiological trade-off imposed by a specific agronomic management. However, light simulations were performed under virtual canopy that did not integrate plant-plant interactions and thus overlooked the potential architectural plasticity under contrasting densities. Because of the absence of appreciable interaction between this plasticity and generation of selection, the relative differences in y-intercepts of each regression lines were likely to be underestimated in this study, but the main result, namely common response to density regardless of generations of selection probably holds.

1

Table 1: Overview of the phenotypic traits estimated from 3D reconstructions

Symbols	Units	Traits	area arrangement
LA	m^2	Plant leaf area	-
h_{whorl}	cm	Whorl height	vertical
θ	degrees	Leaf erectness	vertical & horizontal
rh_{PAD}	-	Plant relative height where half plant leaf area is reached	vertical
b_{PAD}	-	Distribution of leaf area along plant height	vertical
radius	cm	Plant radius	horizontal & vertical
σ_{az}	degrees	Azimuthal dispersion	horizontal
Δ_{row}	degrees	Azimuthal deviation from row	horizontal

Table 2: Pearson correlation coefficients between architectural traits 35 days after sowing. Data originate from well-watered plants. Correlation is based on individual plant values (Correlation when the ligules were visible on half of leaves that eventually emerged is presented Table S2)

	LA	h_{whorl}	h_{plant}	radius	Θ	σ_{az}	Δ_{row}	rh_{PAD}	b_{PAD}
LA	1								
h_{whorl}	0.65 ***	1							
h_{plant}	0.60***	0.78***	1						
radius	0.51***	0.44***	0.68***	1					
θ	-0.17***	0.05 n.s	0.34***	0.09**	1				
σ_{az}	0.11***	0.00 n.s	-0.09**	-0.15***	-0.16***	1			
Δ_{row}	-0.15***	-0.17***	-0.22***	-0.05*	-0.06 n.s	-0.21***	1		
rh_{PAD}	0.30***	0.25***	-0.14***	-0.03 n.s	-0.64***	0.21***	-0.01 n.s	1	
b_{PAD}	0.11***	-0.19***	-0.25***	-0.07*	-0.22***	0.14***	0.07*	0.09**	1

Table 3: Pearson correlation coefficients between the studied variables and year of release among water treatments (35 days after sowing). Correlation is based on average values of all replicates per hybrid.

Architectural traits	WW	WD
LA	-0.48 ***	-0.49 ***
h_{whorl}	-0.33 **	-0.28 *
radius	0.15 n.s	0.21 n.s
θ	0.44 ***	0.43 ***
σ_{az}	-0.42 ***	-0.48 ***
Δ_{row}	-0.02 n.s	0.04 n.s
rh_{PAD}	-0.72 ***	-0.76 ***
b_{PAD}	-0.44 ***	-0.58 ***

References

Alvarez Prado S., Cabrera-Bosquet L., Grau A., Coupel-Ledru A., Millet E.J., Welcker C. & Tardieu F. (2018) Phenomics allows identification of genomic regions affecting maize stomatal conductance with conditional effects of water deficit and evaporative demand. *Plant, Cell & Environment* **41**, 314–326.

Assefa Y., Carter P., Hinds M., Bhalla G., Schon R., Jeschke M., … Ciampitti I.A. (2018) Analysis of Long Term Study Indicates Both Agronomic Optimal Plant Density and Increase Maize Yield per Plant Contributed to Yield Gain. *Scientific Reports* **8**, 1–11.

Baret F., Madec S., Irfan K., Lopez J., Comar A., Hemmerlé M., … Tixier M.H. (2018) Leaf-rolling in maize crops: From leaf scoring to canopy-level measurements for phenotyping. *Journal of Experimental Botany* **69**, 2705–2716.

Bradski G. & Kaehler A. (2008) *Learning OpenCV: Computer vision with the OpenCV library*. " O'Reilly Media, Inc.," Cambridge, MA, USA.

Brichet N., Fournier C., Turc O., Strauss O., Artzet S., Pradal C., … Cabrera-Bosquet L. (2017) A robot-assisted imaging pipeline for tracking the growths of maize ear and silks in a high-throughput phenotyping platform. *Plant Methods* **13**, 96.

Burgess A.J., Retkute R., Herman T. & Murchie E.H. (2017) Exploring Relationships between Canopy Architecture, Light Distribution, and Photosynthesis in Contrasting Rice Genotypes Using 3D Canopy Reconstruction. *Frontiers in Plant Science* **8**, 1–15.

Cabrera-Bosquet L., Fournier C., Brichet N., Welcker C., Suard B. & Tardieu F. (2016) High-throughput estimation of incident light, light interception and radiation-use efficiency of thousands of plants in a phenotyping platform. *New Phytologist* **212**, 269–281.

Chelle M. & Andrieu B. (1998) The nested radiosity model for the distribution of light within plant canopies. *Ecological Modelling* **111**, 75–91.

Chen T.-W., Cabrera-Bosquet L., Alvarez Prado S., Perez R., Artzet S., Pradal C., … Tardieu F. (2018a) Genetic and environmental dissection of biomass accumulation in multi-genotype maize canopies. *Journal of Experimental Botany*, 1–12.

Chen T.W., Stützel H. & Kahlen K. (2018b) High light aggravates functional limitations of cucumber canopy photosynthesis under salinity. *Annals of Botany* **121**, 797–807.

Drouet J.L. & Moulia B. (1997) Spatial re-orientation of maize leaves affected by initial plant orientation and density. *Agricultural and Forest Meteorology* **88**, 85–100.

Duvick D.N. & Cassman K.. (1999) Post – Green Revolution Trends in Yield Potential of Temperate Maize in the United States. *Crop Science* **39**, 1622–1630.

Duvick D.N. (2005) The Contribution of Breeding to Yield Advances in maize (Zea mays L.). *Advances in Agronomy* **86**, 83–145.

Fournier C., Artzet S., Mielewczik M., Brichet N., Sirault X., Cohen-boulakia S., … Artzet S. (2015) Phenomenal : a software framework for model-assisted analysis of high throughput plant phenotyping data. In *IAMPS 2015 (International Workshop on Image Analysis Methods for the Plant Sciences)*. .

Furbank R.T. & Tester M. (2011) Phenomics - technologies to relieve the phenotyping bottleneck. *Trends in Plant Science* **16**, 635–644.

Girardin P. & Tollenaar M. (1994) Effects of intraspecific interference on maize leaf azimuth. *Crop Science* **34**, 151–155.

Grömping U. (2006) R package relaimpo: relative importance for linear regression. *Journal Of Statistical Software* **17**, 139–147.

Hammer G.L., Dong Z., McLean G., Doherty A., Messina C., Schussler J., … Cooper M. (2009) Can Changes in Canopy and/or Root System Architecture Explain Historical Maize Yield Trends in the U.S. Corn Belt? *Crop Science* **49**, 299.

Ku L., Ren Z., Chen X., Shi Y., Qi J., Su H., … Chen Y. (2016) Genetic analysis of leaf morphology underlying the plant density response by QTL mapping in maize (Zea mays L.). *Molecular Breeding* **36**, 63.

Kutulakos K.N. & Seitz S.M. (2000) Theory of shape by space carving. *International Journal of Computer Vision* **38**, 199–218.

Long S.P., Zhu X.G., Naidu S.L. & Ort D.R. (2006) Can improvement in photosynthesis increase crop yields? *Plant, Cell and Environment* **29**, 315–330.

Lorensen W.E. & Cline H.E. (1987) Marching cubes: A high resolution 3D surface construction algorithm. In *ACM siggraph computer graphics*. pp. 163–169.

Ma D.L., Xie R.Z., Niu X.K., Li S.K., Long H.L. & Liu Y.E. (2014) Changes in the morphological traits of maize genotypes in China between the 1950s and 2000s. *European Journal of Agronomy* **58**, 1–10.

Maddonni G.A., Chelle M., Drouet J.L. & Andrieu B. (2001) Light interception of contrasting azimuth canopies under square and rectangular plant spatial distributions: Simulations and crop measurements. *Field Crops Research* **70**, 1–13.

Maddonni G.A. (2002) Maize Leaves Turn Away from Neighbors. *Plant Physiology* **130**, 1181–1189.

Mantilla-Perez M.B. & Salas Fernandez M.G. (2017) Differential manipulation of leaf angle throughout the canopy: Current status and prospects. *Journal of Experimental Botany* **68**, 5699–5717.

Mardaljevic J. (2000) Daylight simulation: validation, sky models and daylight coefficients.

Monsi M. & Saeki T. (2004) On the Factor Light in Plant Communities and its Importance for Matter Production. *Annals of Botany* **95**, 549–567.

Moon P. & Spencer D. (1942) Illumination from a non-uniform sky. *Transactions of the Illumination Engineering Society*, 707–726.

Munier-Jolain N.M., Guyot S.H.M. & Colbach N. (2013) A 3D model for light interception in heterogeneous crop: Weed canopies: Model structure and evaluation. *Ecological Modelling* **250**, 101–110.

Neveu P., Tireau A., Hilgert N., Nègre V., Mineau-Cesari J., Brichet N., … Cabrera-Bosquet L. (2019) Dealing with multi-source and multi-scale information in plant phenomics: the ontology-driven Phenotyping Hybrid Information System. *New Phytologist* **221**, 588–601.

Niinemets Ü. (2010) A review of light interception in plant stands from leaf to canopy in different plant functional types and in species with varying shade tolerance. *Ecological Research* **25**, 693–714.

Palmer a F.E., Heichel G.H. & Musgrave R.B. (1973) Patterns of translocation, respiratory loss, and redistribution of C in maize labelled after flowering. *Crop Science* **13**, 371–376.

Perez R.P.A., Dauzat J., Pallas B., Lamour J., Verley P., Caliman J.-P., … Faivre R. (2018) Designing oil palm architectural ideotypes for optimal light interception and carbon assimilation through a sensitivity analysis of leaf traits. *Annals of Botany* **121**, 909–926.

Perez R., Ineichen P., Moore K., Kmiecik M., Chain C., George R. & Vignola F. (2002) A new operational model for satellite-derived irradiances: description and validation. *Solar Energy* **73**, 307–317.

Pradal C., Artzet S., Chopard J., Dupuis D., Fournier C., Mielewczik M., … Cohen-Boulakia S. (2017) InfraPhenoGrid: A scientific workflow infrastructure for plant phenomics on the Grid. *Future Generation Computer Systems* **67**, 341–353.

Pradal C., Dufour-Kowalski S., Boudon F., Fournier C. & Godin C. (2008) OpenAlea: a visual programming and component-based software platform for plant modelling. *Functional Plant Biology* **35**, 751.

R Core Team (2017) R: A Language and Environment for Statistical Computing.

Reynolds M. & Langridge P. (2016) Physiological breeding. *Current Opinion in Plant Biology* **31**, 162–171.

Sadras V.O. & Richards R.A. (2014) Improvement of crop yield in dry environments: Benchmarks, levels of organisation and the role of nitrogen. *Journal of Experimental Botany* **65**, 1981–1995.

Sangoi L., Gracietti M.A., Rampazzo C. & Bianchetti P. (2002) Response of Brazilian maize hybrids from different eras to changes in plant density. *Field Crops Research* **79**, 39–51.

Schroeder W.J., Martin K. & Lorensen W. (1995) *The Visualization Toolkit: an Object Oriented approach to 3D graphics*. Kitware.

Schroeder W.J., Zarge J.A. & Lorensen W.E. (1992) Decimation of triangle meshes. In *ACM siggraph computer graphics*. pp. 65–70.

Sommer C., Straehle C., Kothe U. & Hamprecht F.A. (2011) Ilastik: Interactive learning and segmentation toolkit. In *Proceedings - International Symposium on Biomedical Imaging*. pp. 230–233.

Tardieu F., Simonneau T. & Muller B. (2018) The Physiological Basis of Drought Tolerance in Crop Plants : A Scenario-Dependent Probabilistic Approach. *Annual Review of Plant Biology* **69**, 733–759.

Tardieu F., Cabrera-Bosquet L., Pridmore T. & Bennett M. (2017) Plant Phenomics, From Sensors to Knowledge. *Current Biology* **27**, R770–R783.

Truong S.K., McCormick R.F., Rooney W.L. & Mullet J.E. (2015) Harnessing Genetic Variation in Leaf Angle to Increase Productivity of Sorghum bicolor. *Genetics* **201**, 1229–1238.

1