

Photoreceptor-mediated kin recognition in plants

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Summary

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- Although cooperative interactions among kin have been established in a variety of biological systems, their occurrence in plants remains controversial.
- Plants of *Arabidopsis thaliana* were grown in rows of either a single or multiple accessions.
- Plants recognized kin neighbours and horizontally reoriented leaf growth, a response not observed when plants were grown with nonkin. Plant kin recognition involved the perception of the vertical red/far-red light and blue light profiles. Disruption of the light profiles, mutations at the *PHYTOCHROME B*, *CRYPTOCROME 1* or *2*, or *PHOTOTROPIN 1* or *2* photoreceptor genes or mutations at the *TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS1* gene required for auxin (growth hormone) synthesis impaired the response. The leaf-position response increases plant self-shading, decreases mutual shading between neighbours and increases fitness.
- Light signals from neighbours are known to shape a more competitive plant body. Here we show that photosensory receptors mediate cooperative rather than competitive interactions among kin neighbours by reducing the competition for local pools of resources.

Introduction

Preferential helping of relatives has been observed for a wide range of taxa. For instance, in vertebrate (bird, mammal) species, helpers preferentially aid closer relatives during breeding (Griffin & West, 2003). In the social amoebae *Dictyostelium discoideum*, cells cooperate preferentially with relatives and aggregate to form multicellular fruiting bodies (Hirose *et al.*, 2011). In humans, as the cost of helping increases, the share of help given to kin increases, whereas that given to nonkin decreases (Stewart-Williams, 2007). These differential responses require mechanisms to discriminate between kin and nonkin, but these mechanisms are often difficult to establish (Mehlis *et al.*, 2008) and some have only recently been elucidated. In *Dictyostelium discoideum*, kin recognition depends on the presence of matching pair of alleles of two genes involved in cell–cell adhesion (Hirose *et al.*, 2011). In humans, sibling detection involves monitoring subsystems specialised for registering maternal perinatal association with the individual's biological mother, and duration of sibling coresidence (Lieberman *et al.*, 2007).

The occurrence of preferential cooperation among plant kin has become controversial, particularly after the pioneer studies of Dudley and collaborators. The reduced root growth of plants of *Cakile edentula* grown with sibling compared to strangers (Dudley & File, 2007) has been interpreted as the result either of chemical signals leading to cooperative interactions or of enhanced competition (Dudley & File, 2008; Klemens, 2008; Murphy & Dudley, 2009; Biedrzycki & Bais, 2010; Biedrzycki *et al.*, 2010; Masclaux *et al.*, 2010; Willis *et al.*, 2010; Bhatt

et al., 2011; Fang *et al.*, 2013). It is difficult to investigate the occurrence of cooperative interactions among kin by analysing quantitative changes in growth, because kinship can also reinforce the competition for local pools of resources available for growth (Milla *et al.*, 2009, 2012; Masclaux *et al.*, 2010; Willis *et al.*, 2010; File *et al.*, 2012b; Lepik *et al.*, 2012; Dudley *et al.*, 2013). *Artemisia tridentata* plants exposed to volatile cues from clipped close relatives experience less leaf herbivory over the growing season than those that received cues from clipped neighbours that were more distantly related (Karban *et al.*, 2013). Mycorrhizal partners become more abundant when *Ambrosia artemisiifolia* is grown with siblings compared to strangers (File *et al.*, 2012a).

Due to selective light absorption by photosynthetic pigments, plant canopy shade-light exhibits low red to far-red ratios (R:FR) and low blue-light irradiances (Smith, 2000; Casal, 2013). In sparse canopies, the R:FR of the radiation propagating horizontally can be reduced by selective far-red light reflection on neighbour foliage even before mutual shading among plants is established (Ballaré *et al.*, 1987; Smith *et al.*, 1990). As the canopy closes, horizontally propagating blue light is also reduced before the horizontally placed leaves are severely shaded (Ballaré *et al.*, 1991). The responses to low R:FR and low blue-light signals include increased growth of stems and/or petioles, and a redirection of leaf growth vertically and/or horizontally (Casal, 2013). These responses, called 'shade-avoidance reactions' increase the competitive ability of plants (Schmitt, 1997; Schmitt *et al.*, 1999, 2003; Smith, 2000; Weinig, 2000), which place their leaves at better light strata within the canopy at the expense of

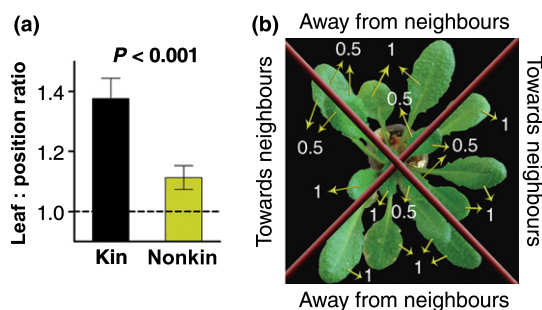


Fig. 1 Arabidopsis leaf position responds differentially to kin and nonkin neighbours. (a) Leaf-position ratio (number of leaves away from neighbours: number of leaves towards neighbours) in rows formed either by a single accession (kin, average of multiple accessions) or by random mixtures of multiple accessions (nonkin). Ratio = 1, corresponds to the null hypothesis of random leaf display. Accessions: Columbia, Landsberg *erecta*, Nossen, Cvi, SHA, BAY and RLD. Data are means $\pm$ SEM of 29 (Nonkin) or 21 rows (Kin, 3 rows per accession). The significance of *t*-test is indicated. (b) Representative seedling grown with kin neighbours. The lines help to dissect the leaves growing either away from neighbours or towards them in the row (in the example, leaf-position ratio = 9 : 6 = 1.5).

overtopped neighbours that become shaded. The changes in R : FR and blue light caused by neighbours are (respectively) perceived mainly by phytochrome B (phyB) and cryptochrome 1 (cry1) (Sellaro *et al.*, 2010; Keller *et al.*, 2011). Blue light can also be perceived by phototropin 1 (phot1) and phot2 (Christie, 2007) but their role in shade-avoidance responses is not unequivocally documented.

Because photosensory receptors are able to perceive subtle signals generated by neighbours (Ballaré *et al.*, 1987; Smith *et al.*, 1990) and reorient growth of aerial organs (Maddonni *et al.*, 2003; Christie, 2007), we investigated whether they are involved in kin recognition and cooperative interactions. Single plants of *Arabidopsis thaliana* were grown in pots (to minimise belowground interference) forming rows of kin or nonkin neighbours.

Materials and Methods

Growth conditions

Plants of *Arabidopsis thaliana* (L.) Heynh were grown in single rows of seven cylindrical pots (10-mm diameter, 74-mm height), containing one seedling each and secured by means of Styrofoam supports, in a glasshouse (Supporting Information Fig. S1).

Experiments and plant material

In a first experiment (Fig. 1), leaf-position ratio was measured in seedlings of the accessions Columbia, Landsberg *erecta*, Nossen, Cvi, SHA, BAY and RLD grown in rows of a single genotype (pure rows of kin) or rows of mixed genotypes (where different accessions were included at random without repetitions within each row).

In a second experiment (Fig. 2), leaf-position ratio was measured in seedlings of the accession Columbia at different times

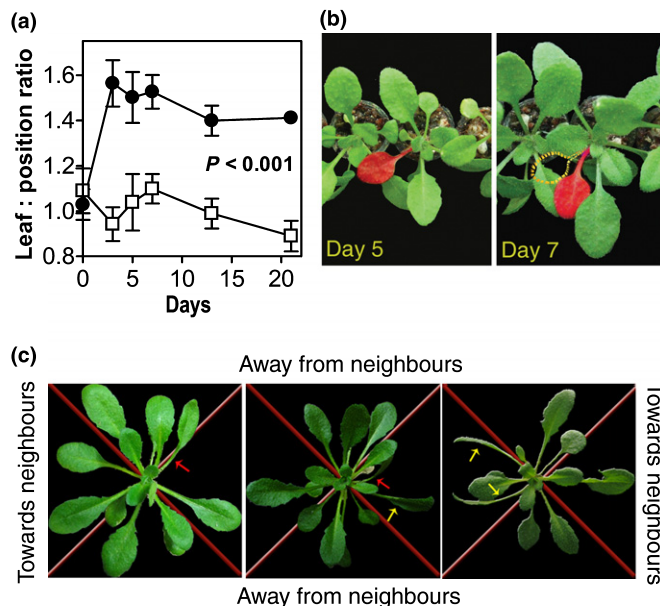


Fig. 2 Arabidopsis plants reorient leaf growth in response to kin neighbours. (a) Time course of leaf-position ratio in rows formed by kin seedlings. At time 0 (T0), the seedlings were arranged in rows. Controls were left isolated throughout the experiment. With kin, circles; isolated, squares. Data are means $\pm$ SEM of 18 replicate rows or 36 isolated control plants. The significance of *t*-test for day 3 (the earliest time point after the beginning of treatments) is indicated. (b) Representative leaf (false coloured) changing its growth direction in a row formed by kin neighbours: originally oriented towards its neighbour (day 5), the leaf then grows towards the empty space out of the row of neighbours (day 7). (c) While changing their horizontal position the leaves showed bent petioles (red arrows) and/or torsion of the petioles leading to vertical orientation of the leaf blades (yellow arrows).

after their arrangement in rows. Leaf-position ratio was simultaneously measured in controls grown in similar pots but isolated from neighbours.

In a third experiment (Fig. 3) seedlings of the accessions Columbia, Landsberg *erecta*, Nossen, Cvi, SHA, BAY and RLD were grown in rows of a single genotype (pure rows of kin) and used for the measurements of light profiles.

In a fourth experiment (Fig. 4), leaf-position ratio was measured in seedlings of the Columbia wild-type and of the *phyB-9* (Reed *et al.*, 1993), *cry1* (*hy4-B104*) (Bruggeman *et al.*, 1996), *cry2-1* (Guo *et al.*, 1998), *phot1-5* (Liscum & Briggs, 1995; Huala *et al.*, 1997), *phot2-1* (Kagawa *et al.*, 2001), *cry1* (*hy4-b104*) *cry2-1* (Buchovsky *et al.*, 2008), *phot1-5 phot2-1* (Liscum & Briggs, 1995; Kagawa *et al.*, 2001), *ft-10* or *ft-10 phyB-9* (Strasser *et al.*, 2010) mutants (all in the Columbia background) grown in pure rows of kin. Leaf-position ratio was also measured in seedlings of the Columbia wild-type grown in pure rows placed under either green (Lee filters number 089) or yellow (Lee filters number 101) plastic filters with lateral ventilation to distort either the normal R : FR signals (sunlight, 1.09; under filter, 0.24) and/or the blue-light signals.

In a fifth experiment (Fig. 5), seedlings of the Columbia wild-type were grown isolated (i.e. without nearby neighbours) and a green plastic filter (1.5-cm tall, 2-cm wide) was placed vertically,

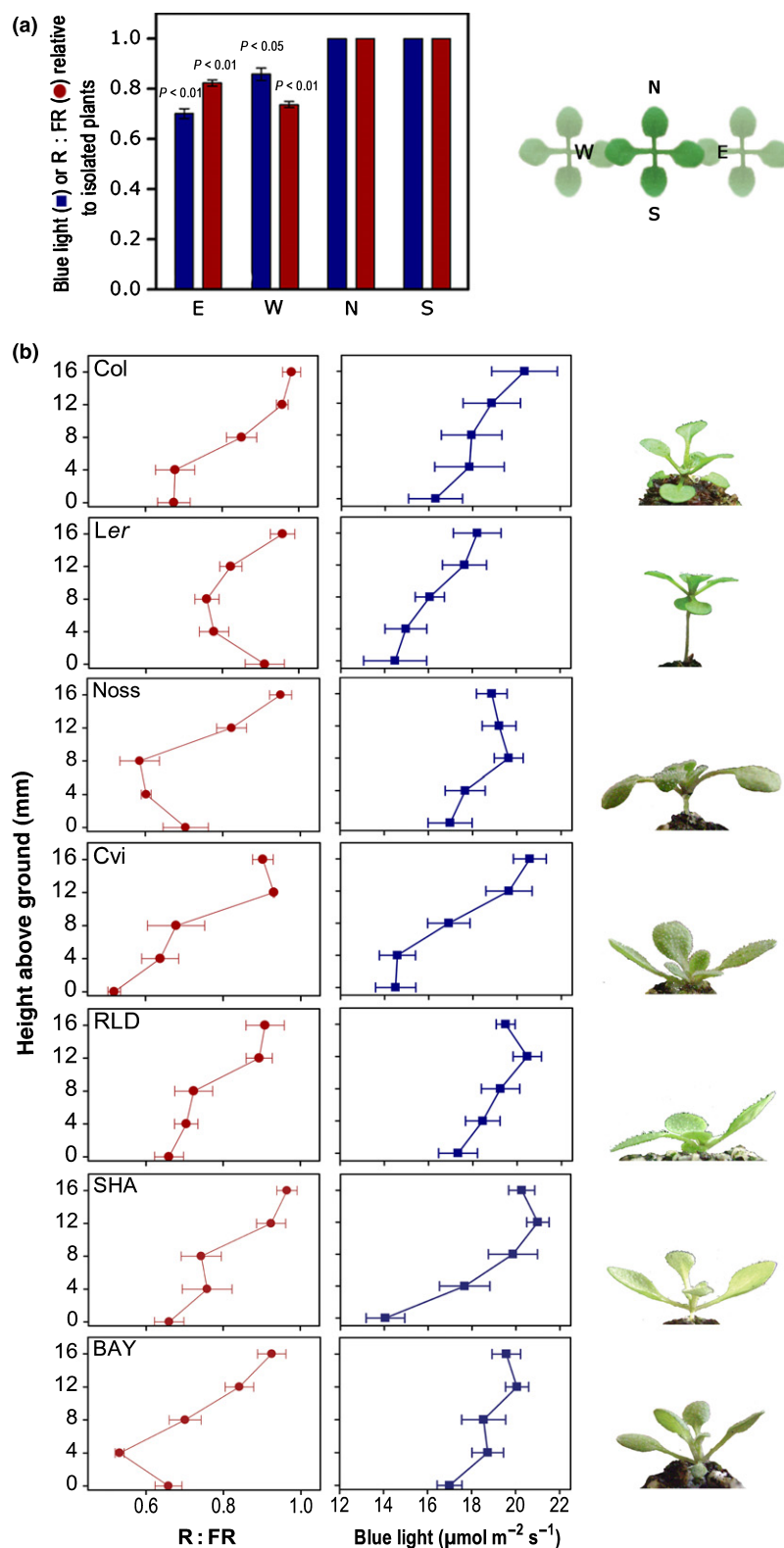


Fig. 3 Light signals produced by neighbours. (a) Selective reduction of blue light and R:FR within the row. The remote probe of the sensor was placed vertically (to characterise horizontally-propagating light), facing East, West, North or South and the plant rows were East–West oriented (see diagram). Data are means $\pm$ SEM of three *Arabidopsis* plants from different rows. The significance of *t*-test between row and isolated conditions is indicated for blue and R:FR facing East or West. (b) Different accessions generate different vertical patterns of the R:FR and blue light. Data are means $\pm$ SEM of three plants from different rows. Factorial ANOVA indicates significant (< 0.0001) main effects (accession, height) for R:FR and blue light and significant (< 0.001) interaction for R:FR. Representative seedlings of each accession (Columbia (Col), Landsberg *erecta* (Ler), Nossen (Noss), Cvi, RLD, SHA and BAY) are shown.

North–South-oriented, 1 cm from the centre of the plant either towards the East or the West side of the plant, to simulate the signals of the horizontally propagating light generated by a neighbour. A clear plastic filter was used as control. The number of

leaves growing towards the East, West, North or South was recorded 10 d later.

In a sixth experiment (Fig. 6), leaf-position ratio was measured in seedlings of the Landsberg *erecta* wild-type grown in rows

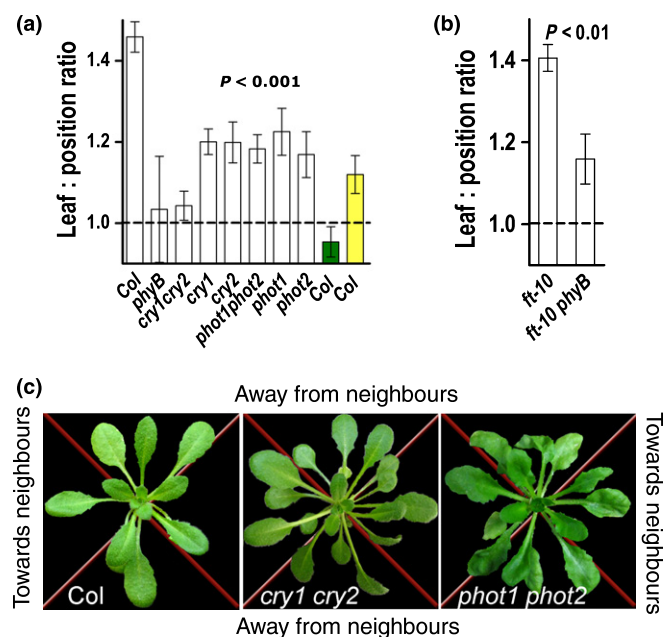


Fig. 4 Distortion of either the light signals or their perception impairs leaf-position responses to kin. (a) Leaf-position ratio in rows of kin seedlings of the following *Arabidopsis* genotypes: wild-type accession Columbia (Col), *phyB* mutant, *cry1 cry2* mutant, *cry1* mutant, *cry2* mutant, *phot1 phot2* mutant, *phot1* mutant or *phot2* mutant (all in the Columbia background). The seedlings were grown under sunlight (open bars) but wild-type seedlings grown under a filter that reduces the R : FR (green bar) or under a filter that reduces blue light (yellow bar) were also included. Data are means $\pm$ SEM of 24, 5, 10, 8, 8, 14, 10, 11, 5 and 10 replicate rows, respectively. The significance of Dunnett's tests (multiple comparisons with the Col control under sunlight) is indicated. (b) Leaf-position ratio in rows of kin seedlings of the *ft* and *ft phyB* mutants grown under sunlight (the effect of the *phyB* mutation is shown in the late-flowering *ft* background to avoid indirect consequences of early flowering caused by *phyB*). Data are means $\pm$ SEM of 9 and 8 replicate rows, respectively. The significance of *t*-tests is indicated. (c) Representative pictures of Col, *cry1 cry2* and *phot1 phot2* seedlings are included to illustrate the horizontal shade-avoidance phenotype of the blue-light photoreceptor mutants.

formed either by coetaneous or noncoetaneous (age difference of 7 d) seedlings for 3 wk. The youngest plants had two pairs of leaves, the same as the plants used for the second and third experiments.

In a seventh experiment (Fig. 7), leaf-position ratio, the degree of mutual and self-shading, and seed yield were measured in seedlings of the Columbia wild-type and of the *sav3* mutant (Tao *et al.*, 2008) grown in pure rows of kin. Isolated plants were also included to evaluate whether the mutation affected fitness independently of the presence of controls. In a variant of this experiment (conducted separately), leaf-position ratio and seed yield were measured in seedlings grown in alternate rows of *sav3* and Columbia seedlings (where each genotype was grown at alternate positions within the same row).

In an eighth experiment (Fig. 8), fitness was evaluated in seedlings of the accessions Columbia, Landsberg *erecta*, Nossen, Cvi, SHA, and RLD grown either in pure rows of kin or in alternate rows of two accessions (all possible combinations).

Leaf-position ratio

The number of leaves (> 3-mm length) within or outside the rows was recorded in 3-wk-old seedlings. Plants within the rows were observed from above (view perpendicular to the soil) through a cross drawn on a clear acetate sheet and centred on each seedling to define the sectors within or outside the rows (Figs 1, S1b).

Light profiles

The vertical profile of the R : FR and blue light was measured with a SpectroSense2 meter (Skye Instruments Ltd, Powys, UK) for six plants per accession. After removing the cosine corrector, the terminals of the red, far-red or blue channel sensors were placed facing the plant to be analysed (15 mm apart), at different heights. For Columbia, horizontal radiation coming from each cardinal point was also characterised with the sensor facing East, West, North or South.

Self-shading and mutual plant shading

The plants were photographed from above with a digital camera (A590 IS, 8MP, $\times 4$; Canon, Tokyo, Japan) first within the row and then isolated from neighbours. Finally, to measure plant leaf lamina area, all the leaves were separated from the plant and photographed. The petioles were erased by using Photoshop (Adobe Systems Inc., San José, CA, USA) software before measuring lamina area (Fig. S1d,e). The degree of self-shading was measured by erasing from the total leaf lamina area of a plant the area exposed by this plant photographed isolated from neighbours before dissection (Fig. S1d). The degree of shading by neighbours was measured by erasing from the total leaf lamina area of a plant the area exposed by this plant photographed within the row (i.e. the area not shaded by neighbours) (Fig. S1e). Leaf shading data are expressed as a proportion of the total leaf lamina area. Total area was unaffected by the *sav3* mutation (Columbia = 368 ± 30 mm², *sav3* = 416 ± 18 mm²). A plant was considered heavily shaded by neighbours when the latter covered > 20% of its lamina area because the distribution of frequency of mutual shading intensities was biphasic, with peaks separated by a minimum frequency at 20% mutual shading.

Fitness

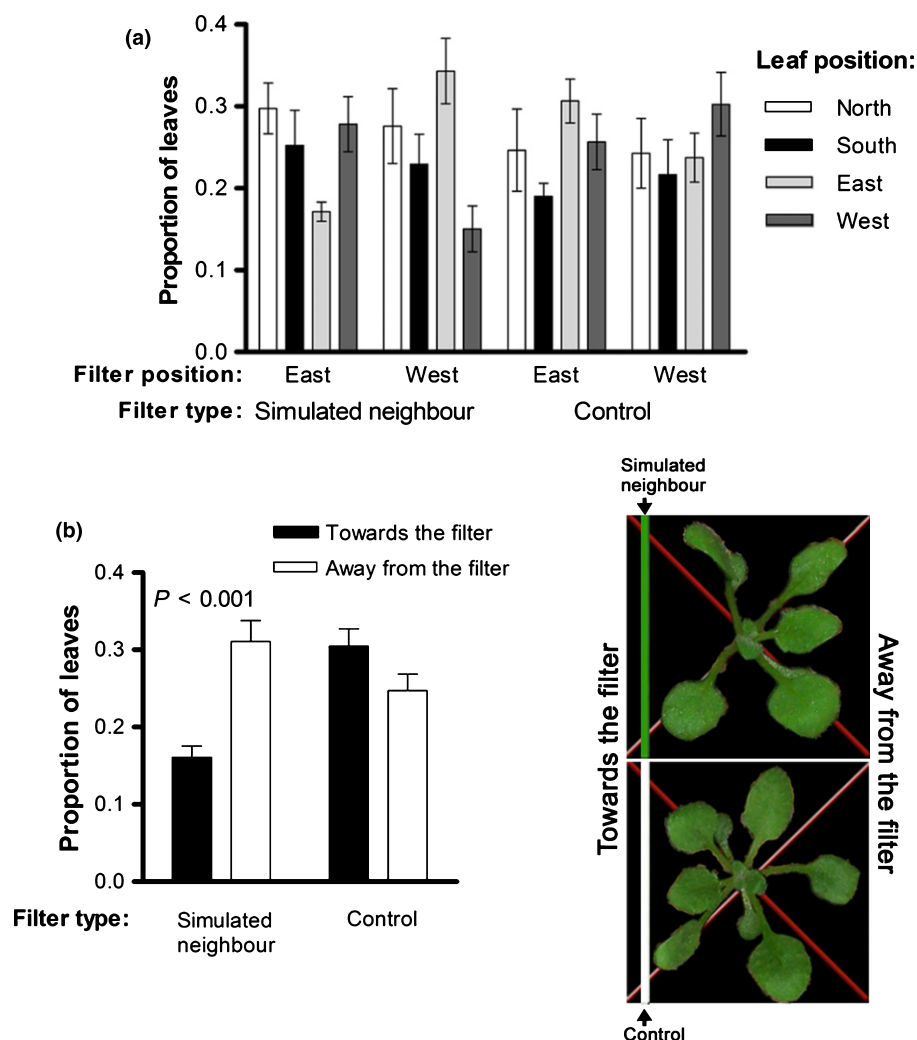
In order to evaluate fitness, seeds were harvested separately per plant and stored 7 d at room temperature before weighing.

Results

Kin recognition

In order to investigate separately the occurrence of kin recognition and its fitness consequences, we searched for outputs of kin recognition involving no immediate reductions in growth

Fig. 5 *Arabidopsis* plants reorientate leaf growth away from simulated neighbours. Plants were grown isolated from neighbours in the presence of a green filter that simulates the presence of a neighbour or a clear filter used as control. The filters were placed either towards the East or the West side of the plant. The proportion of leaves growing towards each cardinal point was recorded 10 d later. (a) Proportion of leaves in each position as affected by filter type and position. Data are means $\pm$ SEM of five plants. Three-way ANOVA indicates significant interaction ($P = 0.0012$) among quadrant, filter type and filter position (main effects and interactions between two factors are not significant, $P > 0.1$). (b) Proportion of leaves growing towards the filter (i.e. towards the West if the filter is placed to the West side of the plant, and towards the East if the filter is placed to the East side of the plant) or away from the filter (i.e. towards the East if the filter is placed to the West side of the plant, and towards the West if the filter is placed to the East side of the plant) as affected by filter type. The same data shown in (a) were grouped according to the indicated pattern. Two-way ANOVA showed significant interaction ($P < 0.0001$), the significance of Bonferroni post-tests is indicated.



(which can be interpreted as the result of exacerbated competition for local pools of resources). We cultivated seedlings of *Arabidopsis thaliana* forming a single-row canopy and recorded leaf position. Plants grown with kin showed a high ratio between the number of leaves positioned away from the neighbours and the number of leaves placed towards the neighbours in the row (Fig. 1a,b). In plants grown with nonkin neighbours (random mixture of accessions within the row) leaf position did not significantly deviate from random (leaf-position ratio = 1, Fig. 1a).

The leaf-position ratio changed rapidly in response to kin neighbours (Fig. 2a). To investigate the nature of the response we recorded the position of target leaves at different time points. The leaf-position ratio of the cohort present at time 0 changed from the original value of 1.1 (random disposition) to 1.4 (SE = 0.1, $P < 0.01$) 5 d later. Therefore, the change in leaf position was caused by a reorientation of leaf growth towards the empty space out of the row of plants (Fig. 2b) and not by changes in phylotaxis. During leaf transition to its new position, the petiole often showed bending and/or torsion leading to a vertical position of the lamina (Fig. 2c).

Mechanism of kin recognition

Because one seedling was grown in each pot, in current experiments kin recognition was likely based on features of the aerial environment. The presence of neighbours within the row generated a radially asymmetric light environment, where both R : FR and blue light were reduced towards the neighbours compared to open places (Fig. 3a). The vertical profile of R : FR and of blue light was recorded for the radiation propagating horizontally within the rows formed by different accessions. The minimum value of R : FR and of blue light correlated with the height of more dense foliage of the neighbour and therefore differed between individuals with different body shape (Fig. 3b).

In order to test the role of R : FR signals in kin recognition, we used the *phyB* mutant because *phyB* is the main photoreceptor of R : FR. *phyB* failed to rearrange its leaves in response to kin neighbours (Fig. 4a). Because the *phyB* mutant flowers early and this could affect the leaf position ratio, we also analysed the effect of the *phyB* mutation in the late-flowering *ft* background, which overrides the flowering phenotype of *phyB*. The results confirmed the failure of the *phyB* mutant to accommodate its leaves in

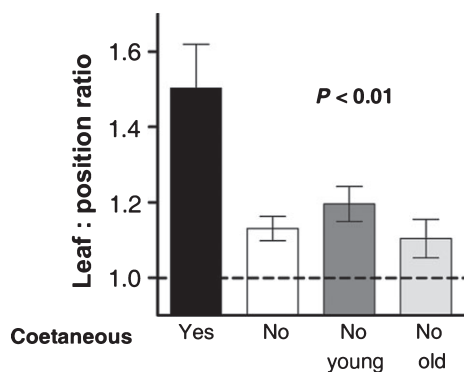


Fig. 6 Noncoetaneous neighbours are not recognized as kin. Leaf-position ratio in rows formed either by coetaneous or noncoetaneous (age difference of 7 d) kin seedlings *Arabidopsis* accession Landsberg *erecta*. For noncoetaneous seedlings, the average and the values corresponding to each age are presented. Data are means $\pm$ SEM of five (coetaneous) or seven (no coetaneous) replicate rows. The significance of Dunnett's test is indicated.

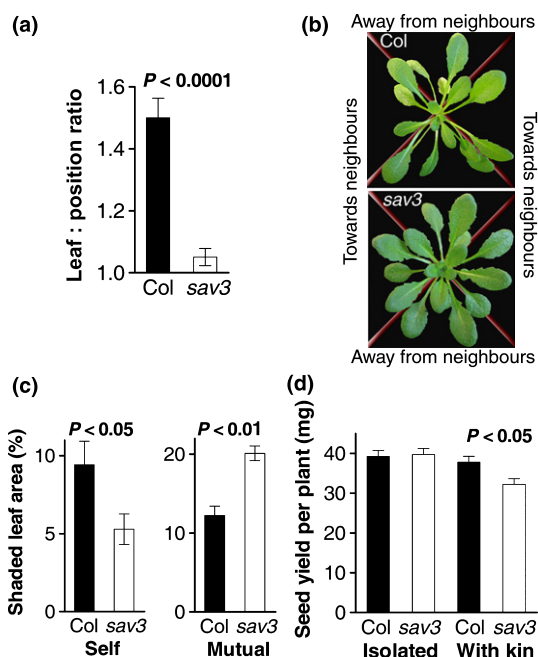


Fig. 7 Leaf-position responses increase self-shading, reduce mutual shading and enhance fitness when plants have kin neighbours. (a) Leaf-position ratio in rows formed by kin seedlings *Arabidopsis* accession Columbia either of the wild-type (Col) or of the *sav3* mutant (mutant of an auxin-synthesis gene in the Columbia background). Data are means $\pm$ SEM of 9 (Col) or 7 (*sav3*) replicate rows. (b) Representative seedlings from Col and *sav3* rows. (c) Compared to Col rows, *sav3* rows have seedlings showing reduced self-shading and increased mutual shading. Data are means $\pm$ SEM of 15 plants from different rows. (d) Compared to Col rows, *sav3* rows show reduced seed yield (note normal yield in isolated *sav3* controls). Data are means $\pm$ SEM of 13 (Col) or 15 (*sav3*) replicate rows or 28 isolated plants. The significance of *t*-tests is indicated. Multiple regression analysis indicates that the terms representing canopy condition and the interaction between genotype and canopy condition are significant at $P < 0.005$.

response to kin neighbours (Fig. 4b). This response was also impaired when wild-type kin were grown under selective filters that distort the R : FR (Fig. 4a).

In order to test the role of blue-light signals in kin recognition, we used *cry1*, *cry2*, *cry1 cry2*, *phot1*, *phot2*, and *phot1 phot2* mutants. All of these mutants showed reduced leaf-position responses to kin neighbours, with the strongest phenotype corresponding to *cry1 cry2* (Fig. 4a,c). This response was also impaired when wild-type kin were grown under selective filters that distort the blue-light environment (Fig. 4a).

The aforementioned results suggest that the leaf-position response is caused by neighbour signals of the horizontally propagating radiation, and signals of insufficient magnitude reach the leaves when the neighbour is not a kin. To test this possibility, plants were grown isolated from nearby neighbours with a green plastic filter placed vertically either towards the East or towards the West side of the plant, simulating the presence of a neighbour. This filter produced strong neighbour signals by reducing the R : FR (from 0.94 ± 0.03 in the clear control filter to 0.40 ± 0.01) and blue irradiance ($54 \pm 9\%$ of the clear filter control) of the horizontally propagating irradiation (measured at 4 mm height) coming from the side where it was placed. Leaves grew away from the plastic filter that simulated the presence of a neighbour (Fig. 5). A clear plastic filter, used as control, did not affect the direction of leaf growth (Fig. 5).

We compared the leaf position responses in rows formed by coetaneous or noncoetaneous neighbours of the same genotype. The rows formed by alternate plants with an age difference of 7 d failed to show the leaf-position response (Fig. 6). Neither the eldest nor the youngest plants rearranged their leaves away from the row of neighbours (Fig. 6). Because plants of different age have different stature, these results reinforce the idea that kin recognition requires matching body shapes. Furthermore, they underscore the importance of horizontally propagating radiation because the smaller (younger) plants were shaded by their taller neighbours from above and yet did not rearrange their leaves.

The promotion of petiole growth and the vertical reorientation of leaf growth in response to low R : FR require the growth hormone auxin (Tao *et al.*, 2008; Kozuka *et al.*, 2010). The horizontal leaf-growth response reported here could also require auxin. To test this idea we used the *sav3* mutant, affected in the *TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS1* (*TAA1*) gene involved in the synthesis of auxin in response to low R : FR (Tao *et al.*, 2008). *sav3* failed to reorient leaf growth in the presence of kin neighbours (pure *sav3* rows, Fig. 7a,b).

Fitness consequences: kin recognition provides mutual benefit

We investigated whether recognizing a kin provides any advantage when the plant grows with kin neighbours. We took advantage of the fact that *sav3* mutant seedlings are morphologically indistinguishable from their wild-type, with the exception of its impaired leaf-position response. We compared pure rows of either wild-type plants (that respond to their kin) or *sav3* plants (that do not respond to their kin). The occurrence of leaf-position responses towards kin neighbours showed fitness

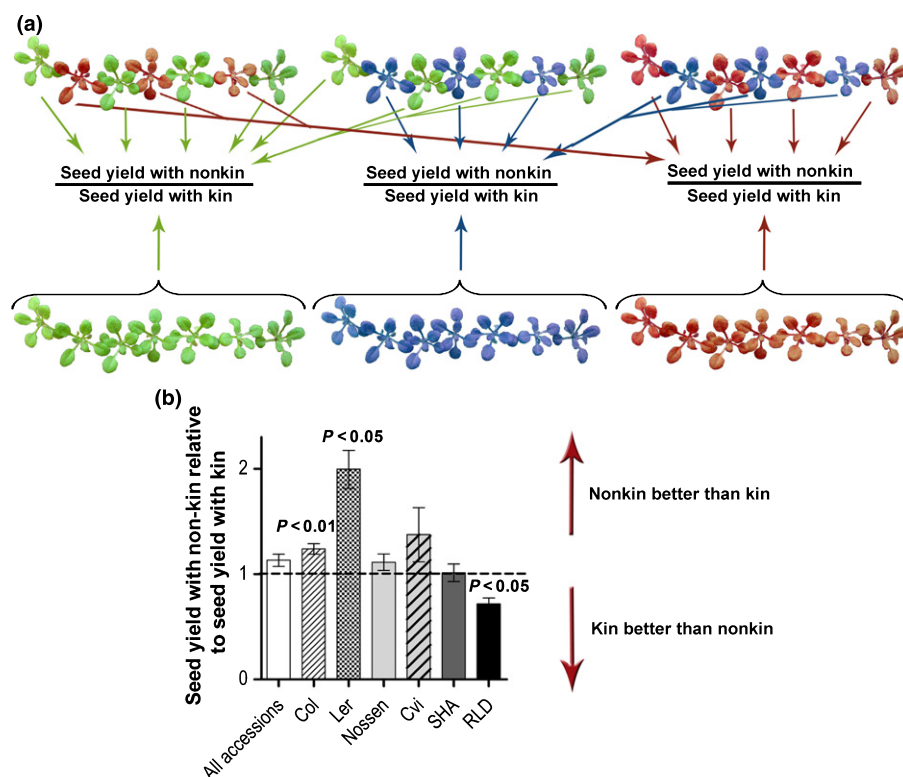


Fig. 8 Fitness consequences of growing with kin or nonkin. (a) Arabidopsis seedlings were grown either in pure rows of one genotype or in rows of two alternate genotypes (different false colours represent different accessions) and seed yield was recorded separately to calculate the nonkin/kin seed-yield ratio. (b) Ratio between seed yield with nonkin and seed yield with kin. The first column is the average of all accessions and the subsequent columns show the ratio for each accession. In each case, the ratio was compared to the kin/kin ratio (1, indicated by the dashed line) and the results of *t*-tests showing significant differences are indicated. Note that nonkin/kin ratios larger than 1 indicate that for that accession it is better to grow with nonkin, whereas ratios < 1 indicate the opposite. Data are means $\pm$ SEM of three rows for each accession.

consequences that can be disaggregated into positive and negative components. First, the re-orientation of leaf-growth direction increased the degree of leaf self-shading (Fig. 7c). The leaf illustrated in Fig. 2(b) departed from its original position facing the neighbour but ended up partially shaded by a leaf of the same plant. Therefore, the leaf-position response involves a cost (Pignocchi *et al.*, 2006; West *et al.*, 2007a,b). Second, the leaf-position response also involves a benefit because it reduced the degree of mutual shading among plants (Fig. 7c). Actually, the leaf-position response strongly reduced the probability of becoming severely shaded by neighbours (proportion of plants with $> 20\%$ of the leaf area under shade: Wild-type = 0.05, *sav3* = 0.55, $P < 0.01$). In the present system, cost (increased self-shading) and benefit (reduced mutual shading) can be measured in the same currency. The decrease in mutual shading outweighed the increase in self-shading (Fig. 7c) and therefore the leaf-position response involves a net benefit for the actor within its lifespan.

Shading can compromise fitness by reducing the radiation available for photosynthesis and to test this possibility, plants were allowed to grow to maturity and produce seeds. When grown with kin neighbours, seed yield was significantly higher in the wild-type than in the *sav3* mutant (Fig. 7d). This provides unequivocal evidence of adaptive kin discrimination (Biernaskie, 2011). Plants of the *sav3* mutant showed wild-type seed yield when grown isolated from neighbours (Fig. 7d), indicating that the fitness consequences of the *sav3* mutation are expressed only when grown with kin. Therefore, the cooperation among Arabidopsis kin plants is a case of mutual benefit (Pignocchi *et al.*, 2006; West *et al.*, 2007a,b).

Fitness consequences of cheating kin neighbours

We also investigated whether when mixed with wild-type seedlings, the *sav3* mutant behaves as a 'selfish cheater' (West *et al.*, 2007a,b). For this purpose we evaluated the behaviour of the wild-type and the *sav3* mutant in mixed rows formed by alternate seedlings of each one of the two genotypes. The wild-type placed the leaves out of the position of *sav3* neighbours, while *sav3* did not show the leaf-position response (Leaf-position ratio: wild-type = 1.30 ± 0.08 ; *sav3* = 1.09 ± 0.05 , $P < 0.05$). By the end of the experiments with mixed rows, *sav3* showed higher seed yield per plant than the wild-type (seed weight, mg, mean $\pm$ SE, wild-type = 20 ± 1 , *sav3* = 28 ± 2 , $P < 0.05$).

Fitness consequences: Is it better to grow with a kin?

We also investigated whether kin recognition results in kin selection; that is, whether for a plant it is better to grow with a kin or a nonkin. We cultivated plants of different accessions either in pure rows (i.e. with kin neighbours) or as alternate mixtures of two accessions within the row (i.e. nonkin neighbours) in all possible pair combinations (Fig. 8a). We recorded seed yield per plant and to eliminate the intrinsic differences among accessions, for each accession we normalised seed yield to the values observed in pure rows (i.e. average normalised seed yield in kin rows was 1 for each accession) (Fig. 8a). Considering the average seed-yield ratio for all the accessions there was no significant difference between growing with kin or non kin (Fig. 8b). The latter average resulted from accessions with a ratio not significantly different from 1, and some accessions with a ratio significantly higher or

lower than 1 (Fig. 8b). We do not regard these differences among accessions as general; rather, we consider that the hierarchy is valid only for the tested conditions.

Discussion

We propose a mechanism of kin recognition involving: (1) the generation of different vertical patterns of R:FR and blue light of the horizontally propagating radiation (Fig. 3); (2) the perception of these R:FR and blue-light patterns by the photosensory receptors phyB, cry1, cry2 and phot1, phot2 (Fig. 4); (3) the action of the auxin (growth hormone)-synthesis gene *TAA1* downstream of the photoreceptors (Fig. 7a,b); and (4) the redirection of leaf growth away from the position of kin neighbours (Figs 1, 2). The evidence in favour of this mechanism is based on: (1) the selective reorientation of leaf growth away from the direction of neighbours observed in rows formed by kin and not in rows formed by nonkin (Fig. 1); (2), the measurement of selective light signals generated by kin neighbours (Fig. 3); (3), the impaired leaf response observed in mutants of photosensory receptor or auxin synthesis genes and when the light signals are distorted (Figs 4, 7); and (4), the reorientation of leaf growth in response to simulations of the light environment produced by kin neighbours (Fig. 5). This mechanism of kin recognition involves phenotype matching because the height where the light signal produced by the 'sender' is more intense (particularly the strongest reduction in R:FR) and coincides with the height where the foliage of the 'receiver' is denser when 'sender' and 'receiver' are kin neighbours (Fig. 3b). Nonkin produce comparatively weak light signals at the position where the 'receiver' has its leaves. Accordingly, plants did not show the leaf-position response when grown with noncoetaneous kin (Fig. 6).

The alternative hypothesis that in our experiments another sensory mechanism is involved in the perception of kinship and photosensory receptors are required only for the expression of the leaf-position response appears unlikely. The occurrence of belowground signals was minimised by the used of one pot per seedling. Volatile signals could be produced, but we observed comparable leaf-position responses to the presence of plastic filters that simulate the light signals of neighbours without producing volatiles (Fig. 5). To the best of our knowledge, neither belowground nor volatile signals have been shown to change the growth direction of leaves. Clearly, our data do not argue against the contribution of belowground signals to the control of root growth (Dudley & File, 2007; Biedrzycki *et al.*, 2010; File *et al.*, 2012a; Fang *et al.*, 2013) and the contribution of volatile signals to control plant defences (Karban *et al.*, 2013) under natural conditions.

The occurrence of preferential cooperation among plant kin is controversial (Dudley & File, 2008; Klemens, 2008; Milla *et al.*, 2009, 2012; Murphy & Dudley, 2009; Biedrzycki & Bais, 2010; Biedrzycki *et al.*, 2010; Masclaux *et al.*, 2010; Willis *et al.*, 2010; Bhatt *et al.*, 2011; File *et al.*, 2012b; Lepik *et al.*, 2012). The reason for this is that fitness consequences of the interaction with neighbours depend on the competitive ability of the neighbour, niche partitioning and cooperative interactions. Therefore, the

comparison between kin and nonkin neighbours shows the balance between these components and not a clean evaluation of cooperative interactions. Conversely, by comparing kin with or without the ability to exhibit leaf-position responses to kin we were able to show that if a plant has kin neighbours a mutually beneficial rearrangement of the leaves alleviates the competition for local pools of light (mutual shading) (Fig. 7c) and increases fitness (Fig. 7d). This does not mean that for a plant it is necessarily better to grow with a kin than with a nonkin neighbour (Masclaux *et al.*, 2010; Willis *et al.*, 2010; Biernaskie, 2011; Milla *et al.*, 2012). Actually, under our conditions, the average of multiple accessions showed similar fitness with kin and nonkin (Fig. 8b). Because the differences in competitive ability are expected to cancel out when multiple accessions are averaged, the similar average yield with kin or nonkin indicates that the other two components that define the consequences of the interaction with neighbours have similar magnitude; that is, the mutual benefit observed between kin neighbours approximately compensates the enhanced competition for local pools of resources between them. In other words, a fitness benefit from the leaf behaviour indicates kin selection, but the potential of niche partitioning and other processes (File *et al.*, 2012b) would preclude seeing higher fitness in kin groups.

Our genetic analysis has revealed several mutants that fail to rearrange their leaves in response to kin. When mixed with the wild-type, the *sav3* mutant did not rearrange its leaves and this conferred a fitness advantage over the wild-type. The latter result does not indicate that the population frequency of *sav3* mutants should be favoured over evolutionary time at the expense of the wild-type that shows 'honest' behaviour. First, 'honest' behaviour implies direct benefit to the wild-type actor when grown next to wild-type neighbours (Fig. 7d) and indirect benefits through wild-type relatives (West *et al.*, 2007a). Second, the *sav3* mutation also impairs competitive interactions with taller neighbours, which would reinforce the direct benefit of the wild-type gene to the actor (West *et al.*, 2007a).

Shade-avoidance responses involve the perception of low R:FR mainly by phyB and of low blue light mainly by cry1 (Sellaro *et al.*, 2010; Keller *et al.*, 2011). Although phototropins provide adaptive value with respect to establishment and growth of plants, the physiological processes that generate this advantage have not been defined (Pedmale *et al.*, 2010). Both phot1 and phot2 could be important for acclimation to shade (Casal, 2013). Whilst a role in vertical shade-avoidance (enhanced stem and petiole growth, leaf hyponasty) has not been documented, here we show that phot1 and phot2 are important for horizontal leaf shade-avoidance responses (Fig. 4a,c).

Because plants are sessile organisms, the genotype of the interacting neighbour is not a choice for them. Due to limited dispersal, plants often have coetaneous kin neighbours that tend to place the leaves at similar vertical strata, which would enhance mutual shading and competition for light, but this competition is alleviated by the horizontal rearrangement of the leaves towards empty areas with higher R:FR and blue-light signals. Classical shade-avoidance reactions increase plant stature placing the leaves at better light strata within the canopy at the expense of the light

received by the overtopped neighbours; that is, shade signals were known to increase the competitive ability of plants (Schmitt, 1997; Schmitt *et al.*, 1999, 2003; Smith, 2000; Weinig, 2000). Conversely, here we show that phyB-mediated perception of R:FR and cry1, cry2, phot1 and phot2-mediated perception of blue light generate cooperative rather than competitive interactions when the neighbour is a coetaneous kin. These cooperative interactions would be particularly important in agricultural crops involving genetically similar individuals grown at high densities with different distances among plants within and between rows.

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Fig. S1 Experimental procedures.

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