

Competitive ability not kinship affects growth of *Arabidopsis thaliana* accessions

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Summary

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- In many organisms, individuals behave more altruistically towards relatives than towards unrelated individuals. Here, we conducted a study to determine if the performance of *Arabidopsis thaliana* is influenced by whether individuals are in competition with kin or non-kin.
- We selected seven pairs of genetically distinct accessions that originated from local populations throughout Europe. We measured the biomass of one focal plant surrounded by six kin or non-kin neighbours in *in vitro* growth experiments and counted the number of siliques produced per pot by one focal plant surrounded by four kin or non-kin neighbours.
- The biomass and number of siliques of a focal plant were not affected by the relatedness of the neighbour. Depending on the accession, a plant performed better or worse in a pure stand than when surrounded by non-kin plants. In addition, whole-genome microarray analyses revealed that there were no genes differentially expressed between kin and non-kin conditions.
- In conclusion, our study does not provide any evidence for a differential response to kin vs non-kin in *A. thaliana*. Rather, the outcome of the interaction between kin and non-kin seems to depend on the strength of the competitive abilities of the accessions.

Introduction

The theory of kin selection (Hamilton, 1964) proposes that behaviour among individuals should depend upon their genetic relatedness. This is because individuals can transmit copies of their own genes not only through their own reproduction, but also by favouring the reproduction of kin, such as siblings or cousins. Importantly, co-operation among kin is much more likely to evolve by kin selection if relatives and nonrelatives can be distinguished and behaviour altered in response to that information (Lehmann & Perrin, 2002). Studies in species as diverse as bacteria, amoeba, insects, birds, mammals and humans have indeed revealed that individuals modulate their behaviour depending on the social context and the relatedness of interacting individuals (Sherman, 1977; Pfennig & Collins, 1993; Komdeur, 1994; Sundström *et al.*, 1996; Buchan *et al.*, 2003; Langer

et al., 2004; Sherborne *et al.*, 2007; Gibbs *et al.*, 2008; Mehdiabadi *et al.*, 2008).

Because plants are often aggregated and seed dispersal skewed towards a limited distance away from the maternal plant, neighbours are likely to comprise kin and non-kin, a situation that may favour kin recognition and weaker competitive reactions towards kin than towards unrelated individuals. Several studies have investigated whether plants respond differently when in the presence of kin and non-kin, and they have yielded contrasting results. While some studies have reported that plants perform better when they grow in competition with siblings (e.g. Willson *et al.*, 1987; Donohue, 2003; Dudley & File, 2007) others have reported the opposite effect (Allard & Adams, 1969; Antonovics & Ellstrand, 1984; Milla *et al.*, 2009) or no significant differences (McCall *et al.*, 1989; Argyres & Schmitt, 1992). A recent study found that sagebrush (*Artemisia tridentata*) plants responded differently to volatiles emitted by clipped branches of self and different neighbours

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(Karban & Shiojiri, 2009). Some studies also found contrasting results depending on the traits studied (Tonsor, 1989), or the growth conditions (Andalo *et al.*, 2001).

There are several possible explanations for these discrepancies. First, they may be attributable to differences across species in the relative effects of kin selection and resource partitioning (Cheplick & Kane, 2004 and references therein; Monzeglio & Stoll, 2008). Thus, a strong effect of kin selection in some species may lead to higher fitness in kin groups while a strong effect of resource partitioning may lead to the reverse effect with greater fitness in mixed groups. Secondly, the differences among studies may be attributable to differences in experimental design and uncontrolled genetic variations affecting growth and reproductive success (see Donohue, 2003). Several studies have reported intrinsic differences in competitive abilities between genotypes (Cheplick, 1997; Gustafson *et al.*, 2004; Latta *et al.*, 2004; Cahill *et al.*, 2005). Thus, the choice of genotypes used in the experiments may affect the outcome of competition, with some genotypic combinations resulting in higher growth in kin groups and others in higher growth in non-kin groups (Donohue, 2003). Because most studies investigating the effect of kin group structure have not controlled for family effects, it is difficult to determine the relative contributions of resource partitioning, differences in competitive abilities and kin effects to growth and competition. Moreover, many studies have been performed with seeds collected from the field, so in such studies uncontrolled maternal effects may also contribute to differences in growth and reproductive success (Donohue, 2003).

In this study we tested for the occurrence of kinship effects in *Arabidopsis thaliana* by using clearly identified accessions and by exploring reciprocal interactions between pairs of accessions. In addition, microarrays were used to look for genes differentially expressed in kin vs non-kin conditions. We chose *A. thaliana* because: it produces a high number of seeds with low dispersal, favouring interactions between individuals; a high number of accessions with known genetic relatedness are available from seed stock centres; and genomic tools such as DNA microarrays can be used to assess intraspecific changes in gene expression. Seven pairs of accessions representing a wide geographical area were selected and their interactions were analysed in two performance assays (early growth and silique production).

Kin selection theory predicts that individuals living with kin should invest less in competition, leading to a better reproductive performance compared with individuals living in non-kin conditions (Hamilton, 1964). By contrast, resource partitioning, or niche partitioning, predicts that nonrelated individuals competing within a group will partition resources more efficiently than related individuals and therefore have greater fitness (Young, 1981; Price & Waser, 1982). Finally, a difference in competitive ability between individuals predicts no consistent effect of relatedness on

the outcome of competition. We define competitive ability as the ability of a plant to acquire resources from the environment to the detriment of competitors or the ability to inhibit the growth and reproduction of competitors (Goldberg, 1996; Aarssen & Keogh, 2002). In the latter case, the relative fitness of a genotype in competition with itself or with another genotype should depend only on the relative competitive abilities of the genotypes and not on kin effects. Our goal was to test whether *A. thaliana* accessions always grow better in the presence of kin, suggesting the occurrence of kin selection, or always grow better in the presence of non-kin, suggesting resource partitioning, or grow differently depending on the accession, suggesting varying competitive abilities.

Materials and Methods

Plant material

The small annual plant *Arabidopsis thaliana* (L.) Heynh. (Brassicaceae) was chosen as the model system for this study. The patterns of genetic polymorphism of many accessions collected in the Northern Hemisphere have been analysed for numerous DNA fragments representing 0.48 Mbp of the genome (Nordborg *et al.*, 2005). From these data, we chose seven pairs of accessions (Nordborg *et al.*, 2005) that cover most of the range of pairwise genetic diversity described in the Nordborg *et al.* study. The most closely related pair was Ren-1/Ren-11, which contained 0.0027 single nucleotide polymorphisms (SNPs) per site, whereas Bay-0/Mrk-0 was the most distantly related pair with 0.0047 SNPs per site (Supporting Information Table S1). Because of the high rate of selfing and because all accessions were initiated from a single individual, the within accession relatedness was very high (in particular because Nordborg *et al.* (2005) only kept accessions where they did not find any level of heterozygosity). We selected five pairs of accessions (NFA-8/NFA-10, Zdr-1/Zdr-6, Lp2-2/Lp2-6, Kz-1/Kz-9 and Ren-1/Ren-11) with pair members collected within a few hundred metres of each other, thus probably sharing the same habitat, and two pairs (Bay-0/Mrk-0 and Ts-1/LL-0) where the distance between pair members was 160 and 20 km, respectively. Moreover, this group of seven pairs encompassed a large geographical area, allowing any effects attributable to local environment or latitude to be minimized (Fig. 1). Seeds were obtained from the Nottingham Arabidopsis Stock Centre (NASc, Nottingham, UK). All accessions were collected in 2003 except Bay-0, Mrk-0 and Ts-1, which were collected in 1995. Seeds were donated to the seed stock centre (<http://www.arabidopsis.org>) and went through two or three generations by selfing before they were distributed (Luz Rivero, ABRC Germplasm curator, pers. comm.). Starting from *c.* 10 parent plants, we generated two more generations by

Accession	Country	Population	Latitude	Longitude	Stock nr.
NFA-8	England	NFA	51.25	0.41	CS22598
NFA-10			51.25	0.41	CS22599
Kz-1	Kazakhstan	KZ	49.83	73.01	CS22606
Kz-9			49.84	73.02	CS22607
Ren-1	France	Rennes	48.50	-1.41	CS22610
Ren-11			48.50	-1.41	CS22611
Bay-0	Germany	Bayreuth	49.00	11.00	CS22633
Mrk-0		Markt/Baden	49.00	9.30	CS22635
Zdr-1	Czech Rep.	Zdr	49.20	16.62	CS22588
Zdr-6			49.20	16.62	CS22589
Lp2-2	Czech Rep.	LP2	49.22	16.39	CS22594
Lp2-6			49.22	16.39	CS22595
Ts-1	Spain	Tossa de Mar	41.72	2.93	CS22647
LL-0		Llagostera	41.59	2.49	CS22650

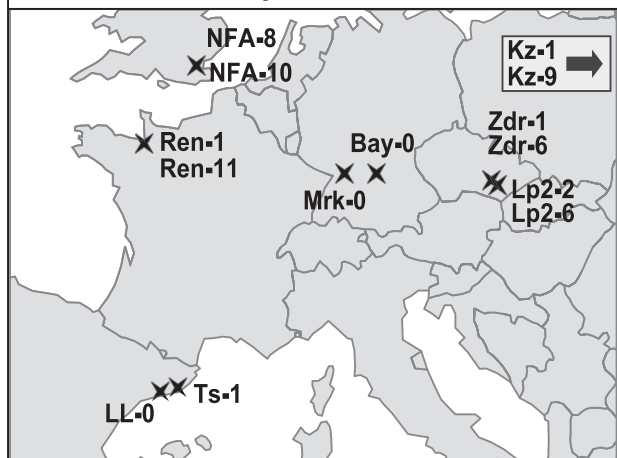


Fig. 1 Geographical origin and stock centre reference of the seven pairs of *Arabidopsis thaliana* accessions used in this study. All accessions but Kz-1 and Kz-9 are placed on the map. Data are based on Nordborg *et al.* (2005) and The Arabidopsis Information Resource (TAIR; <http://www.tair.org>).

selfing before conducting the experiments. Fresh batches of seeds were produced at the same time for all accessions in a growth chamber under standard conditions (22°C, photoperiod 16 h/8 h, relative humidity 65% and photosynthetic photon fluence rate 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$).

Measure of kinship effects

For competition analyses *in vitro*, we measured the biomass of a focal plant surrounded by six neighbours after 12 d of growth on sterile agar medium. For each pair of *A. thaliana* accessions, a seed was placed in the centre of a hexagon formed by six neighbouring seeds of the same accession (kin) or by six seeds of the other accession (non-kin). The distance between the focal plant and a neighbour was 0.5 cm and each agar plate (9 cm diameter) contained five groups of seedlings. The plates with kin and non-kin treatments were randomly placed in the growth chamber to eliminate any position effect. Agar plates were composed of half Murashige and Skoog (MS) medium (M5524; Sigma) supplemented with 0.25% sucrose and 0.8% agar (A1296;

Sigma). Seeds were surface-sterilized using a vapour-phase bleach sterilization method. Plates were stored at 4°C for stratification for 6 d and were then transferred to a growth room (22°C, photoperiod 10 h/14 h, relative humidity 65% and photosynthetic photon fluence rate 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$). After a growth period of 12 d, the aerial part of each focal seedling was cut and weighed on a high-precision balance. Focal plants not surrounded by six neighbours because of poor germination were not considered. The number of plants analysed for each replicate experiment is listed in Supporting Information Table S2. For the accessions Ren-1, Ren-11, Zdr-1, Zdr-6, Lp2-2, Lp2-6, and Kz-1, we performed a noncompetitive control experiment where each focal plant was grown alone in a plate. This treatment was randomized together with the competitive treatments.

For competition analyses in soil, we measured the silique production of a focal plant surrounded by four neighbours grown in the same pot. For each pair of *A. thaliana* accessions, a seedling was planted in the centre of a pot (7 cm diameter) containing 230 cm^3 of soil and was surrounded by four seedlings of the same accession (kin) or by four seedlings of the other accession (non-kin). Seedlings were germinated on agar plates similarly to the *in vitro* culture. After 4 d of growth, seedlings were transplanted to pots with a distance of 2.5 cm between the focal plant and the neighbours. The soil contained 65% humus, 10% sand, 15% perlite and 10% silt and was not complemented with fertilizer. Trays containing randomized pots were transferred to a glasshouse at 22°C under natural light with 16 h of supplemental lighting per day. Plants were watered twice a week to maintain a constant moisture level throughout the growth period which lasted *c.* 2 months. When leaves started to turn yellow, the aerial part of each focal plant was collected and the number of siliques was recorded. Only pots containing five plants were considered. The number of plants analysed for each experiment is listed in Table S2. A noncompetitive control experiment was performed with the accessions Ren-1 and Ren-11 where a single plant was grown per pot. This treatment was randomized together with the competitive treatments.

Statistical analyses

To test whether biomass and silique production of focal plants were influenced by the type of neighbour, we used two-way analysis of variance (ANOVA), where the type of neighbour (kin or non-kin) and the geographical origin of accessions were used as fixed factors. In addition, we tested whether biomass and silique production might be influenced by the different competitive abilities of the accessions. To this end, we separately analysed each pair of accession using two-way ANOVAs, where the accessions used as focal plant and as neighbour were considered as fixed factors.

Interactions between factors were tested in all analyses. As biomass was measured on one to three biological replicates, we categorized the replicates as random factors in the corresponding analyses (mixed models). Finally, to achieve residual normality, variables were normalized by square-root transformation. Variables were back-transformed for figures. All tests were two-tailed. *P*-values were estimated with restricted maximum likelihood (REML) methods using the software JMP 7.0 (SAS Institute Inc., Cary, NC, USA). The significance level $\alpha = 0.05$ was adjusted for multiple testing to $\alpha = 0.0375$ using the false discovery rate (FDR) method of Benjamini & Hochberg (1995).

Gene expression analyses

Seedlings were grown for 12 d on MS agar plates similarly to the *in vitro* growth assay. Approximately 75 focal seedlings (shoots and roots) per condition (kin or non-kin) were collected and stored at -80°C . Plant tissue was then ground in liquid nitrogen and RNA was extracted, reverse-transcribed and labelled with Cy3- or Cy5-dCTP as previously described (Reymond *et al.*, 2004). Labelled cDNA was hybridized to Complete Arabidopsis Transcriptome Micro-Array (CATMA) microarrays containing 22 473 gene-specific tags (Hilson *et al.*, 2004; Allemeersch *et al.*, 2005). Microarray hybridizations were performed in triplicate with a dye-swap design. Scanning, normalization and data analyses have been described previously (Reymond *et al.*, 2004). To control for multiple testing, we applied the FDR method.

Results

Competition influences the growth and reproductive performance of focal plants irrespective of the neighbours' genetic relatedness

In order to identify conditions conducive to competitive interactions between neighbours, we performed a preliminary experiment with the extensively studied accession Columbia (Col-0). We planted varying densities of seeds in a pot and measured the silique production at maturity and the total aerial biomass before bolting. As the number increased from one plant per pot to 20 plants per pot, silique production and leaf biomass per plant declined sharply, indicating that plants were experiencing competition (Supporting Information Fig. S1). We thus selected a design with five plants per pot, one focal plant and four neighbours, corresponding to an intermediate level of competition, for further studies.

To verify that the members of the selected accession pairs were also competing with each other in our experimental design, we compared the *in vitro* growth and silique number of a focal plant growing alone with those of a focal plant

surrounded by kin or non-kin neighbours. For Ren-1 and Ren-11, single culture provided a higher biomass and silique number than competition with either kin or non-kin plants (Fig. 2). Similar results were obtained for biomass with five other accessions tested for *in vitro* competition (Supporting Information Fig. S2).

Having shown that competition occurred with our experimental design, we then tested the effect of neighbours' genetic relatedness on the *in vitro* growth of seedlings and on the production of siliques. The *in vitro* performance assay had the advantage of examining plants interacting in sterile and well-controlled conditions, preventing the possible interference of other living organisms with the interaction, whereas growth in pots assessed the reproductive performance of the plants under more natural conditions. Overall, the biomass and silique production of the focal plants were significantly different between accessions within pairs (Table 1). However, there was no significant effect of neighbours' relatedness, indicating that interaction with kin was not systematically associated with higher growth and reproduction of focal plants. Furthermore, there was no

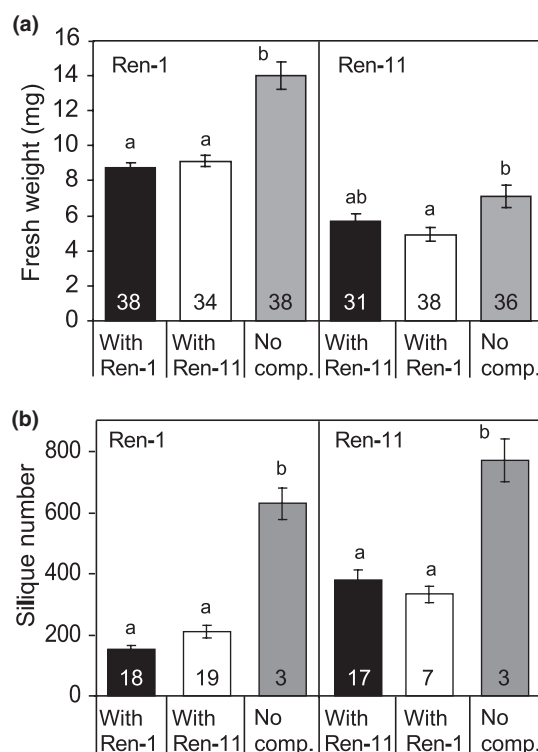


Fig. 2 Effect of neighbours on *in vitro* growth and reproductive output of the accessions Ren-1 and Ren-11. Mean (\pm SE) fresh weight (a) and silique number (b) of focal *Arabidopsis thaliana* plants grown alone ('No comp.'; grey bars), with kin neighbours (black bars) or with non-kin neighbours (white bars) are presented. Fresh weight was measured for plants grown *in vitro* for 12 d and silique number was measured for plants grown in pots until maturity (c. 2 months). The number of plants measured is reported in each bar. Bars with different letters differed at $P < 0.05$ (Tukey's HSD test).

Table 1 Effect of competition on *in vitro* growth and silique number of a focal *Arabidopsis thaliana* plant

Source	df (1)	df (2)		F	P
<i>In vitro</i> growth					
Accession	6	11.47		4.827	0.0109
Neighbour's genotype	1	3589		0.003	0.9561
Neighbour's genotype × accession	6	3589		1.087	0.3673
Source	df	SS	MS	F	P
Silique number					
Accession	5	10 494	2098	159.075	< 0.0001
Neighbour's genotype	1	15	15	2.272	0.192
Neighbour's genotype × accession	5	32.8	6.6	0.497	0.778
Residual	776	10 239	13.2		

Global two-way ANOVA analysis. For *in vitro* growth, mixed-model ANOVA was used. SS, sum of squares; MS, mean square. Significant values are in bold in Tables 1 and 2.

significant interaction between the genotype of neighbours and the origin of the accession, suggesting that the growth and silique production of a given accession were not affected by specific interactions between accessions within the pairs (Table 1).

A further analysis of each of the seven accession pairs revealed different competitive abilities between accessions (Table 2). For six out of the seven accession pairs tested, one accession always grew better when surrounded by kin than when surrounded by non-kin, while the other grew better when surrounded by non-kin than when surrounded

by kin. Similarly, one accession always produced more siliques when surrounded by kin for five out of six pairs of accessions, and vice versa for the other accession of the pair (Table 2). For instance, with the pair of accessions Ts-1/LL-0, we observed that the genetic identity of the neighbour had an influence on the weight and the number of siliques produced by the focal plant. The focal plant Ts-1 produced significantly less biomass when surrounded by Ts-1 than when surrounded by LL-0. In contrast, LL-0 produced significantly more biomass and siliques when surrounded by LL-0 than when surrounded by Ts-1 (Fig. 3a).

Table 2 Effect of accession and neighbour's genotype (kin or non-kin) on *in vitro* growth and silique number of a focal *Arabidopsis thaliana* plant

Accessions	Source	<i>In vitro</i> growth assay		Silique number assay	
		F	P	F	P
Kz-1/Kz-9	Accession	$F_{1,773} = 61.127$	< 0.0001	$F_{1,136} = 0.214$	0.64
	Neighbour's genotype	$F_{1,773} = 4.903$	0.0271	$F_{1,136} = 7.365$	0.008
	Neighbour's genotype × accession	$F_{1,773} = 0.075$	0.784	$F_{1,136} = 0.193$	0.66
Lp2-2/Lp2-6	Accession	$F_{1,410} = 13.391$	< 0.001	ND	ND
	Neighbour's genotype	$F_{1,410} = 21.708$	< 0.0001	ND	ND
	Neighbour's genotype × accession	$F_{1,410} = 0.053$	0.819	ND	ND
Ts-1/LL-0	Accession	$F_{1,289} = 191.442$	< 0.0001	$F_{1,111} = 239.586$	< 0.0001
	Neighbour's genotype	$F_{1,289} = 84.772$	< 0.0001	$F_{1,111} = 137.359$	< 0.0001
	Neighbour's genotype × accession	$F_{1,289} = 0.052$	0.82	$F_{1,111} = 0.773$	0.38
Ren-1/Ren-11	Accession	$F_{1,549} = 67.656$	< 0.0001	$F_{1,69} = 0.815$	0.37
	Neighbour's genotype	$F_{1,549} = 8.177$	0.0044	$F_{1,69} = 17.962$	< 0.0001
	Neighbour's genotype × accession	$F_{1,549} = 0.002$	0.965	$F_{1,69} = 0.153$	0.70
Mrk-0/Bay-0	Accession	$F_{1,497} = 1258.820$	< 0.0001	$F_{1,73} = 24.382$	< 0.0001
	Neighbour's genotype	$F_{1,497} = 227.724$	< 0.0001	$F_{1,73} = 14.110$	< 0.001
	Neighbour's genotype × accession	$F_{1,497} = 0.035$	0.852	$F_{1,73} = 4.718$	0.033
NFA-8/NFA-10	Accession	$F_{1,529} = 453.958$	< 0.0001	$F_{1,187} = 567.148$	< 0.0001
	Neighbour's genotype	$F_{1,529} = 152.827$	< 0.0001	$F_{1,187} = 1.875$	0.17
	Neighbour's genotype × accession	$F_{1,529} = 3.228$	0.073	$F_{1,187} = 0.021$	0.89
Zdr-1/Zdr-6	Accession	$F_{1,528} = 132.274$	< 0.0001	$F_{1,188} = 16.960$	< 0.0001
	Neighbour's genotype	$F_{1,528} = 40.122$	< 0.0001	$F_{1,188} = 21.782$	< 0.0001
	Neighbour's genotype × accession	$F_{1,528} = 1.268$	0.261	$F_{1,188} = 7.955$	0.005

Each pair of accession was analysed separately by two-way ANOVA; ND, not determined. Significant values are in bold in Tables 1 and 2.

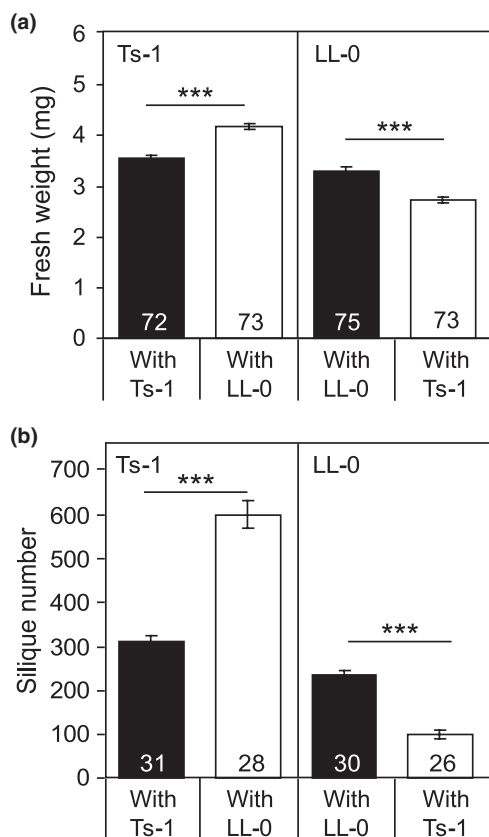


Fig. 3 *In vitro* growth and reproductive output of the accessions Ts-1 and LL-0 growing in competition. Mean (\pm SE) fresh weight (a) and silique number (b) of focal *Arabidopsis thaliana* plants grown with kin neighbours (black bars) or non-kin neighbours (white bars) are presented. Fresh weight was measured for plants grown *in vitro* for 12 d and silique number was measured for plants grown in pots until maturity (c. 2 months). The number of plants measured is reported in each bar. *** $P < 0.001$ (Student's *t*-test).

Thus, the presence of Ts-1 as a neighbour resulted in decreased growth of the focal plant, independent of its genotype, and the opposite effect was found for the accession LL-0. Similar to the *in vitro* assay, Ts-1 appeared to be a stronger competitor than LL-0: the accession LL-0 produced more siliques when growing in a pure stand than when growing with Ts-1, whereas Ts-1 produced fewer siliques in the pure stand (Fig. 3b). Growth in kin conditions thus resulted in two opposite effects, depending on the accession considered.

Data for all accession pairs are shown in a difference plot. The occurrence of kin selection predicts that the fresh weight or silique number difference between kin and non-kin conditions will always be positive (i.e. larger fresh weight or silique number of the focal plant when growing with kin) whereas resource partitioning predicts that the difference will always be negative (i.e. smaller fresh weight or silique number of the focal plant when growing with kin). Instead, we found that for all accession pairs the values were

positive for one accession and negative for the other, clearly indicating differences in competitive ability (Fig. 4). We found that Lp2-2 was a stronger competitor than Lp2-6, Ren-1 than Ren-11, Mrk-0 than Bay-0, NFA-8 than NFA-10, and Zdr-1 than Zdr-6, whereas Kz-9 was a stronger competitor in terms of growth, and Kz-1 was a stronger competitor in terms of silique production, but the effects were small (Fig. 4; Supporting Information Figs S3,S4,S5). Competitive ability and tolerance to competitors varied between accessions, as the effect of the neighbour on the focal plant differed in magnitude and was significant for some pairs and nonsignificant for others (Table 2). For instance, in the pair Zdr-1/Zdr-6, the number of siliques produced by the weaker competitor (Zdr-6) decreased by only c. 13% in the presence of the stronger competitor (Zdr-1), whereas it decreased by 58% for Zdr-1. This result suggests that Zdr-6 was less competitive than Zdr-1. By contrast, in the pair Ren-1/Ren-11, the number of siliques produced by both the weaker and stronger competitors decreased by c. 53% in presence of the stronger competitor (Ren-1), suggesting no differences in their tolerance to

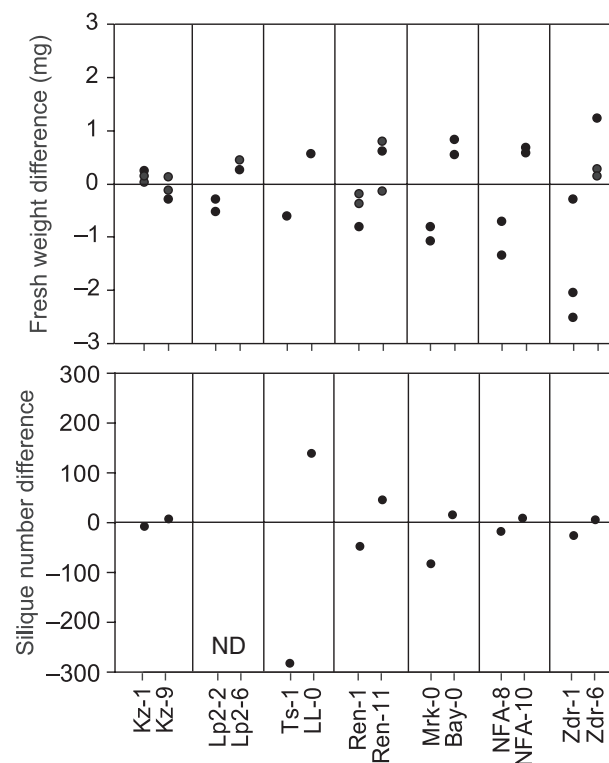


Fig. 4 Difference in fresh weight and silique number of focal *Arabidopsis thaliana* plants growing in kin and non-kin conditions. Each black dot corresponds to the difference found in a single experiment. A difference is calculated by subtracting the value measured in kin conditions from the value measured in non-kin conditions. Results obtained in one to three replicate experiments for each accession are presented. Fresh weight was measured for plants grown *in vitro* for 12 d and silique number was measured for plants grown in pots until maturity (c. 2 months). ND, not determined.

competitors. Interestingly, the stronger competitor of a pair was the accession that grew to a larger size when grown in single culture, suggesting that the competitive effect might be attributable to greater efficiency in exploiting the available resources (Fig. 2 and Fig. S2).

Interestingly, the pairwise genetic differences between accessions from the same population were only slightly smaller than the differences between accessions that were separated by *c.* 1500 km (Table S1), suggesting that the within-population differentiation between accessions should have been sufficient to observe kin recognition effects if they occurred. Consistent with this view, the *in vitro* experiments on pairs in which the accessions were separated by *c.* 1500 km (Bay-0/Ts-1 and Ren-1/Lp2-2) gave very similar results to those on pairs in which the accessions were from the same population (Supporting Information Fig. S6).

In summary, growth in kin conditions did not result in systematic reduced competition or a better reproductive performance. Instead, we found that the existence of a strong and a weak competitor in most pairs tested in this study was the key factor that controlled the outcome of the interaction between accessions. Although the effect on growth and reproduction appeared consistent with the kin selection theory when one weak competitor was surrounded by its kin, the reciprocal comparison did not confirm this result, arguing against a kin selection phenomenon.

Comparison of gene expression between kin and non-kin conditions

The second aim of our study was to investigate whether there was a difference in gene expression between focal plants grown with kin and those grown with non-kin. Such a difference might be interpreted as recognition, or a differential investment in competition dependent on the kin/non-kin environment. Whole-genome DNA microarrays analyses were performed on two pairs of accessions (Mrk-0/Bay-0 and NFA-10/NFA-8) exhibiting strong differential growth in our *in vitro* test. We compared gene expression between seedlings growing *in vitro* surrounded by their kin and seedlings that were surrounded by non-kin. For each accession, the experiment was repeated three times independently, giving a total of 12 microarrays. Using an FDR threshold of 10%, we could not find genes that were significantly up- and down-regulated in kin vs non-kin conditions in a single accession. Similarly, in analysis of all accessions together (12 replicates in total), we were again unable to identify any differentially expressed gene that fitted the selection criteria. Overall, these data indicate that, under our experimental set-up, growth in kin and non-kin conditions did not produce differences in transcript signature (Supporting Information Table S3).

Discussion

Our results clearly show that the relatedness (kin vs non-kin) of neighbours had no significant effect on the growth of focal plants and the number of siliques they produced. However, the biomass and fitness of plants were strongly influenced by the genotype (accession type) of the competing plants. These results thus show a large difference in competitive abilities of the accessions tested, but a lack of specific responses of accessions to whether they are in kin or non-kin conditions.

This conclusion contrasts with that of an earlier study on *A. thaliana*, which concluded that kin selection effects were present on the basis of genotypes performing better in a pure stand than in a mixture (Andalo *et al.*, 2001). However, in this study the association with relatedness was reversed by elevated CO₂ concentrations, suggesting that factors other than just relatedness are operating. Andalo *et al.* pooled data over five experiments where focal plants were grown with five different treatment genotypes. A re-analysis of the data (kindly supplied by Christophe Andalo) shows that in each set of four non-kin experiments there was at least one genotype against which the focal plant grew poorly and this pairing had a large impact on the mean productivity of the focal plant in non-kin conditions when data were pooled. Unpooled data show that, for each focal genotype (there were five in all), focal plant productivity was actually better at ambient CO₂ when surrounded by non-kin than when surrounded by kin in approximately half the 20 non-kin experiments. Thus, Andalo *et al.*'s results may reflect differences in competitive ability between genotypes (as we also have found in our study) rather than kinship effects.

Similarly, the association found between within-group relatedness and productivity and fitness in many other studies may be attributable to differences among genotypes in competitive ability. In the sea rocket *Cakile edentula*, plants surrounded by siblings had, on average, higher reproductive success. However, there was a family effect, with some families having a higher fitness when competing with siblings and other families having a higher fitness when competing with unrelated families (Donohue, 2003), showing that effect direction varied. A more recent study of kin effects in *C. edentula* showed that biomass allocation to fine roots was higher when plants competed with unrelated individuals than when they competed with kin, which has been suggested to be a result of kin recognition (Dudley & File, 2007). However, this conclusion has been a subject of debate because of statistical issues with the data analyses (Klemens, 2007; though see Dudley & File, 2008). Moreover, kin groups did not show increased reproductive fitness, raising the question of the significance of the results of this study (Milla *et al.*, 2009), although the design of the experiment might have precluded a good measure of fitness consequence (Dudley & File, 2008). Tonsor (1989) showed

that relatedness affected performance in *Plantago lanceolata*, with a higher number of flowering plants per pot in high-relatedness treatments. However, Tonsor suggested that these patterns were more likely to be a result of growth-rate variation being lower in high-relatedness treatments than an outcome of altruism (i.e. in low-relatedness treatments one plant often dominated). He further suggested that the direction of the effect might well have been reversed under different resource conditions, as found by Andalo *et al.* (2001). Family effects were also found in *Triplasis purpurea* (Cheplick & Kane, 2004), where only one out of five pairs of families showed better growth and reproduction when grown with relatives, leading the authors to conclude that overall better growth with non-kin resulted from resource partitioning. Similarly, Milla *et al.* (2009) found no evidence overall for kin-selected co-operation in kin relative to non-kin treatments in *Lupinus angustifolius*, but once again family lines varied in their response. In general, it appears that studies in plants provide strong evidence for growth and fitness being influenced by the genotype of neighbours, but the effect of the relatedness between neighbours is unpredictable and there is as yet no conclusive evidence of kin recognition affecting the outcome of competition and the fitness of interacting individuals.

Our expression data are also consistent with a lack of kinship effect on growth and fitness. Overall, no gene was differentially expressed when focal plants were grown with kin and with non-kin. The differential expression of genes in the context of kin vs non-kin is a prerequisite for kin recognition and kin selection to operate. To date there have been no studies determining how patterns of gene expression are affected by the kin structure of the group. In both ants (Wang *et al.*, 2008) and *Drosophila melanogaster* (Kent *et al.*, 2008), the level of expression of individuals was affected by the genotypic composition of other group members. However, these studies did not investigate whether within-group relatedness affected patterns of gene expression. Such studies would be of great interest.

The biochemical or molecular mechanisms underlying the effects of neighbours on the growth and reproduction of focal plants are unknown. Given our experimental design, an effect of shade avoidance is unlikely. Seedlings growing *in vitro* were small and the leaves did not overlap each other frequently. In pots the number of plants was relatively small and the growth of focal plants was affected by neighbours before the leaves started to overlap each other (data not shown). It is therefore likely that the observed differences in competitive ability are attributable to a different capacity of each accession to acquire resources from the soil or the inhibitory effect of one accession on the other. The differential expression or activity of ion transporters, the ability to transfer solutes from roots to shoots, the release of growth-inhibiting substances, the sequestration of resources, the release of aboveground volatiles and light-capture efficiency

are some non-mutually exclusive parameters that could vary between competing plants and explain the results. Clearly, more biochemical or genetic studies will be necessary to resolve the question of why some accessions had higher competitive abilities than others.

In conclusion, our study does not provide any evidence for a differential response to kin vs non-kin in *A. thaliana*. Rather, the outcome of competition experiments between kin and non-kin depends on the strength of the competitive abilities of the families/accessions used. Therefore, a global analysis of multiple interactions may give the impression that kin selection is operating if there is an overrepresentation of weak competitors in the non-kin treatment. Thus, systematic reciprocal analyses of pair-wise competition experiments need to be undertaken before drawing general conclusions on the nature of these interactions. There is also the possibility that kinship effects are only manifested in specific biotic or abiotic environments (e.g. Goodnight, 1985). In a study carried out in the field, seedlings of the pasture grass *Anthoxanthum odoratum* had a higher fitness when grown with siblings than when grown with unrelated individuals (Antonovics & Ellstrand, 1984). In a related experiment, *A. odoratum* plants attacked by aphids showed a better survivorship when growing with unrelated neighbours than when growing with siblings (Schmitt & Antonovics, 1986), illustrating the potential role of biotic factors in these interactions. However, with the exception of these two experiments conducted at a natural site and that of Donohue (2003) with *C. edentula* transplanted into its native environment, most studies so far have tested the response of plants to neighbours in glasshouses or growth cabinets in carefully controlled conditions, where nutrients were not limiting and in the absence of diseases. It would be interesting to repeat these experiments in a natural environment where abiotic and biotic factors might influence the outcome of the interactions. Finally, the choice of the model plant can be an important factor. Like the self-incompatibility system, which is only present in some plant species (Sanabria *et al.*, 2008), kin recognition mechanisms might not be universal. Further study of kin recognition in other plants with different life histories and reproductive modes is thus necessary before more definite and general conclusions can be drawn on whether kin recognition occurs in plants.

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References

- Aarssen LW, Keogh T. 2002. Conundrums of competitive ability in plants: what to measure? *Oikos* 97: 531–542.
- Allard RW, Adams J. 1969. Populations studies in predominantly self-pollinating species. XIII. Intergenotypic competition and population structure in barley and wheat. *American Naturalist* 103: 621–645.
- Allemeersch J, Durinck S, Vanderhaeghen R, Alard P, Maes R, Seeuws K, Bogaert T, Coddens K, Deschouwer K, Van Hummelen P *et al.* 2005. Benchmarking the CATMA microarray. A novel tool for Arabidopsis transcriptome analysis. *Plant Physiology* 137: 588–601.
- Andalo C, Goldringer I, Godelle B. 2001. Inter- and intragenotypic competition under elevated carbon dioxide in *Arabidopsis thaliana*. *Ecology* 82: 157–164.
- Antonovics J, Ellstrand NC. 1984. Experimental studies of the evolutionary significance of sexual reproduction. I. A test of the frequency-dependent selection hypothesis. *Evolution* 38: 103–115.
- Argyres AZ, Schmitt J. 1992. Neighbor relatedness and competitive performance in *Impatiens capensis* (Balsaminaceae): a test of the resource partitioning hypothesis. *American Journal of Botany* 79: 181–185.
- Benjamini Y, Hochberg Y. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society B* 57: 289–300.
- Buchan JC, Alberts SC, Silk JB, Altmann J. 2003. True paternal care in a multi-male primate society. *Nature* 425: 179–181.
- Cahill JF Jr, Kembel SW, Gustafson DJ. 2005. Differential genetic influences on competitive effect and response in *Arabidopsis thaliana*. *Journal of Ecology* 93: 958–967.
- Cheplick GP. 1997. Responses to severe competitive stress in a clonal plant: differences between genotypes. *Oikos* 79: 581–591.
- Cheplick GP, Kane KH. 2004. Genetic relatedness and competition in *Triplaris purpurea* (Poaceae): resource partitioning or kin selection? *International Journal of Plant Sciences* 165: 623–630.
- Donohue K. 2003. The influence of neighbor relatedness on multilevel selection in the Great Lakes sea rocket. *American Naturalist* 162: 77–92.
- Dudley SA, File AL. 2007. Kin recognition in an annual plant. *Biology Letters* 3: 435–438.
- Dudley SA, File AL. 2008. Yes, kin recognition in plants! *Biology Letters* 4: 69–70.
- Gibbs KA, Urbanowski ML, Greenberg EP. 2008. Genetic determinants of self identity and social recognition in bacteria. *Science* 321: 256–259.
- Goldberg DE. 1996. Competitive ability: definitions, contingency and correlated traits. *Philosophical Transactions of the Royal Society of London B* 351: 1377–1385.
- Goodnight CJ. 1985. The influence of environmental variation on group and individual selection in a cress. *Evolution* 39: 545–558.
- Gustafson DJ, Gibson DJ, Nickrent DL. 2004. Competitive relationships of *Andropogon gerardii* (Big Bluestem) from remnant and restored native populations and select cultivated varieties. *Functional Ecology* 18: 451–457.
- Hamilton WD. 1964. The genetical evolution of social behaviour. I & II. *Journal of Theoretical Biology* 7: 1–52.
- Hilson P, Allemeersch J, Altmann T, Aubourg S, Avon A, Beynon J, Bhalerao RP, Bitton F, Caboche M, Cannoot B *et al.* 2004. Versatile gene-specific sequence tags for *Arabidopsis* functional genomics: transcript profiling and reverse genetics applications. *Genome Research* 14: 2176–2189.
- Karban R, Shiojiri K. 2009. Self-recognition affects plant communication and defense. *Ecology Letters* 12: 502–506.
- Kent C, Azanchi R, Smith B, Formosa A, Levine JD. 2008. Social context influences chemical communication in *Drosophila melanogaster* males. *Current Biology* 18: 1384–1389.
- Klemens JA. 2007. Kin recognition in plants? *Biology Letters* 4: 67–68.
- Komdeur J. 1994. The effect of kinship on helping in the cooperative breeding Seychelles warbler (*Acrocephalus sechellensis*). *Proceedings of the Royal Society B – Biological Sciences* 256: 47–52.
- Langer P, Hogendoorn K, Keller L. 2004. Tug-of-war over reproduction in a social bee. *Nature* 428: 844–847.
- Latta RG, Mackenzie JL, Vats A, Schoen DJ. 2004. Divergence and variation of quantitative traits between allozyme genotypes of *Avena barbata* from contrasting habitats. *Journal of Ecology* 92: 57–71.
- Lehmann L, Perrin N. 2002. Altruism, dispersal, and phenotype-matching kin recognition. *American Naturalist* 159: 451–468.
- McCall C, Mitchell-Olds T, Waller DM. 1989. Fitness consequences of outcrossing in *Impatiens capensis*: test of the frequency-dependent and sib-competition models. *Evolution* 43: 1075–1084.
- Mehdiabadi NJ, Kronforst MR, Queller DC, Strassmann JE. 2008. Phylogeny, reproductive isolation and kin recognition in the social amoeba *Dictyostelium purpureum*. *Evolution* 63: 542–548.
- Milla R, Forero DM, Escudero A, Iriondo JM. 2009. Growing with siblings: a common ground for cooperation or for fiercer competition among plants? *Proceedings of the Royal Society B – Biological Sciences* 276: 2531–2540.
- Monzeglio U, Stoll P. 2008. Effects of spatial pattern and relatedness in an experimental plant community. *Evolutionary Ecology* 22: 723–741.
- Nordborg M, Hu TT, Ishino Y, Jhaveri J, Toomajian C, Zheng H, Bakker E, Calabrese P, Gladstone J, Goyal R *et al.* 2005. The pattern of polymorphism in *Arabidopsis thaliana*. *PLoS Biology* 3: e196.
- Pfennig DW, Collins JP. 1993. Kinship affects morphogenesis in cannibalistic salamanders. *Nature* 362: 836–838.
- Price MV, Waser DM. 1982. Population structure, frequency-dependent selection, and the maintenance of sexual reproduction. *Evolution* 36: 35–43.
- Reymond P, Bodenhausen N, Van Poecke RM, Krishnamurthy V, Dicke M, Farmer EE. 2004. A conserved transcript pattern in response to a specialist and a generalist herbivore. *Plant Cell* 16: 3132–3147.
- Sanabria N, Goring D, Nurnberger T, Dubery I. 2008. Self/nonself perception and recognition mechanisms in plants: a comparison of self-incompatibility and innate immunity. *New Phytologist* 178: 503–514.
- Schmitt J, Antonovics J. 1986. Experimental studies of the evolutionary significance of sexual reproduction. IV. Effect of neighbor relatedness and aphid infestation on seedling performance. *Evolution* 40: 830–836.
- Sherborne AL, Thom MD, Paterson S, Jury F, Ollier WE, Stockley P, Beynon RJ, Hurst JL. 2007. The genetic basis of inbreeding avoidance in house mice. *Current Biology* 17: 2061–2066.
- Sherman PW. 1977. Nepotism and the evolution of alarm calls. *Science* 197: 1246–1253.
- Sundström L, Chapuisat M, Keller L. 1996. Conditional manipulation of sex ratios by ant workers - a test of kin selection theory. *Science* 274: 993–995.
- Tonsor SJ. 1989. Relatedness and intraspecific competition in *Plantago lanceolata*. *American Naturalist* 134: 897–906.
- Wang J, Ross KG, Keller L. 2008. Genome-wide expression patterns and the genetic architecture of a fundamental social trait. *PLoS Genetics* 4: e1000127.
- Willson MF, Hoppes WG, Goldman DA, Thomas PA, Katusic-Malmberg PL, Bothwell JL. 1987. Sibling competition in plants: an experimental study. *American Naturalist* 129: 304–311.
- Young JPW. 1981. Sib competition can favor sex in two ways. *Journal of Theoretical Biology* 88: 755–756.

Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Effect of density on growth and silique production of the Columbia (Col-0) accession.

Fig. S2 Effect of neighbours on *in vitro* growth of different accessions.

Fig. S3 Reproductive output of all accession pairs growing in competition.

Fig. S4 *In vitro* growth of different accession pairs growing in competition (part a).

Fig. S5 *In vitro* growth of different accession pairs growing in competition (part b).

Fig. S6 *In vitro* growth of accessions that are distant geographically (>1500 km) growing in competition.

Table S1 Pairwise genetic differences between the accessions used in this study

Table S2 Raw data for biomass and silique number measurements

Table S3 Microarray data for inter- and intragenotypic comparisons

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