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Growing with siblings: a common ground for cooperation or for fiercer competition among plants?

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Recent work has shown that certain plants can identify their kin in competitive settings through root recognition, and react by decreasing root growth when competing with relatives. Although this may be a necessary step in kin selection, no clear associated improvement in individual or group fitness has been reported to qualify as such. We designed an experiment to address whether genetic relatedness between neighbouring plants affects individual or group fitness in artificial populations. Seeds of *Lupinus angustifolius* were sown in groups of siblings, groups of different genotypes from the same population and groups of genotypes from different populations. Both plants surrounded by siblings and by genotypes from the same population had lower individual fitness and produced fewer flowers and less vegetative biomass as a group. We conclude that genetic relatedness entails decreased individual and group fitness in *L. angustifolius*. This, together with earlier work, precludes the generalization that kin recognition may act as a widespread, major microevolutionary mechanism in plants.

Keywords: kin selection; kin recognition; *Lupinus angustifolius* L.; intraspecific competition; fitness; group selection

1. INTRODUCTION

Plant–plant competition is probably the only ubiquitous interaction in plant communities (Connell 1983). Patterns, proposals of mechanisms and theoretical developments in this field have been described in the ecological literature for decades (Tilman 1982; Casper & Jackson 1997; Craine 2006; Brooker & Kikvidze 2008). Yet progress in areas such as root foraging mechanisms, or the interplay between facilitation and competition, shows that the processes involved in plant–plant competition are more complex than previously thought (Rajaniemi 2007; Chu *et al.* 2008). An example of this is the renewed interest in the genetic determinants of intraspecific competition (for example, Falik *et al.* 2006; Boyden *et al.* 2008; Crutsinger *et al.* 2008; Hughes *et al.* 2008). In this context, a recent paper reported that plants of the annual *Cakile edentula* are able to identify kin in competitive settings and, more importantly, react by competing less aggressively against a close relative (Dudley & File 2007). When individuals were grown in pots with close relatives, root biomass, a surrogate of below-ground competitive ability, was lower than in individuals grown in the vicinity of strangers (Dudley & File 2007).

Dudley and File's findings challenge current thinking on plant evolution. A corollary of their experiment is that kin recognition and the subsequent reduction in root growth facilitate the development of relatives living in the neighbourhood. In other words, kin selection (a kind of group selection; Bell 1997) takes place at the expense of not maximizing root foraging potential of each

individual (Dudley & File 2007). Previous fieldwork with this species also suggests that kin selection may be a major selective force in the most genetically structured groups of *C. edentula* plants (Donohue 2003).

Recognition of kin and kin selection are long-held topics in animal evolution, where behavioural and chemical mechanisms for individual recognition are well known (Griffin & West 2002; Tibbetts & Dale 2007). However, the mechanisms of individual recognition are less than intuitive in plants and have received comparatively little attention (Callaway & Mahall 2007).

Results in *C. edentula* prompted immediate reactions in the literature (Callaway & Mahall 2007; de Kroon 2007; Dudley & File 2008; Klemens 2008). Callaway & Mahall (2007) emphasized that if kin recognition is shown to exert a strong effect on fitness, the pool of mechanisms that drive plant evolution will be increased. They considered that Dudley and File's study provides the core of evidence, while unravelling the effects of kin recognition on fitness should be relatively routine. Yet the kin groups of *C. edentula* did not show increased reproductive fitness, which makes root recognition questionably transferable to kin selection in that experiment. However, mechanisms that induce change in the developmental program of a plant in response to a neighbour's performance are described in the literature, providing ground for a direct link between kin recognition and kin selection. For instance, expression of certain fitness-related genes in *Arabidopsis thaliana* has indirect, pleiotropic effects on trait expression in neighbouring plants, but how pleiotropic effects are modulated by genetic relatedness among neighbours is unknown (Mutic & Wolf 2007).

Renewed attention on kin selection in plants after the report of root recognition should be welcomed with

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interest but, given its relevance, should be subject to rigorous criticism and testing (Klemens 2008). Since trait variation is negligible in terms of evolutionary importance unless it is subject to selection, understanding whether and how kin recognition may improve individual and/or group fitness should be a priority for evolutionary ecology research (see also de Kroon 2007; Klemens 2008). In animals, the positive effects of kin recognition mechanisms on inclusive fitness of relatives have been documented before (for example, Tsutsui *et al.* 2003), but this is not the general case for plants. Therefore, unless strong, comprehensive evidence shows that the skill of root recognition does indeed benefit groups of relatives, kin recognition should be solely viewed as an exciting ability of certain plant species with unknown ecological and evolutionary significance.

Although this topic is not completely new and some data are available, evidence from earlier literature in plant biology is inconclusive. Goodnight (1985) reported probably the first experience claiming an effect of group selection on a trait (leaf area) of *A. thaliana*, though implications in individual and group performance were not assessed. Another experiment with *A. thaliana* yielded non-conclusive results regarding the performance of groups of relatives versus groups of strangers: groups of siblings performed better under ambient CO_2, but performed worse under elevated CO_2 levels (Andalo *et al.* 2001). Other studies have shown kin selection in plants (for example, Tonsor 1989; Donohue 2003), or have found groups of strangers performing better (for example, Allard & Adams 1969; Schmitt & Ehrhardt 1987; Kelley 1989; Cheplick & Kane 2004). Thus, the overall consensus is far from established.

We set up a pot experiment to evaluate the fitness consequences of growing *Lupinus angustifolius* in the vicinity of close relatives (hereafter 'siblings'), unrelated individuals of the same population (hereafter 'moderate strangers'), or unrelated individuals from populations located far apart (hereafter 'full strangers'). Our null hypothesis is that genetic relatedness to neighbours should be irrelevant to the intensity of competition and thus would not affect individual or group fitness. On the basis of this null hypothesis, we tested the following explicit predictions. (i) Competition will be equally intense among full strangers, moderate strangers and siblings. Consequently, fitness will be similar for individual plants, regardless of their neighbours' identity. (ii) No evidence of kin selection will be found at the group level. Thus, cumulative fitness at the pot level will be independent of the genetic relatedness of individuals within the pot. (iii) Seeds sown close to each other will interact with neighbours at an earlier developmental stage than seeds sown at a greater distance. This will intensify competition and diminish individual and group fitness, regardless of whether individuals in a pot are relatives or strangers.

2. MATERIAL AND METHODS

(a) *Study species and seed origin*

Lupinus angustifolius L. is an annual legume, widespread as a weed across the Mediterranean Basin. It inhabits environments subjected to frequent disturbance, such as road or forest edges. It grows preferentially on acid sandy soils. This species is well suited for this experiment owing to its short life

cycle, which allows prompt assessment of fitness components. Also, three other biological features of this species make it especially suitable for evaluating kin selection effects in a pot experiment. (i) It is predominantly a passive selfer and, when outcrossing takes place, flowers are pollinated by an assembly of widely distributed generalist pollinators (Forbes *et al.* 1971). Consequently, reproduction is scarcely dependent on the local community of pollinators when grown artificially in a greenhouse. (ii) Seeds are heavy (0.1–0.9 g) and dispersal is limited, which provides an intense spatial genetic structure whereby individuals mainly interact with relatives. Restricted dispersal provides favourable ground to detect group selection effects (Cheplick 1992; Stevens & Wiley 1995). (iii) Its germination rate is almost 100 per cent after mechanical scarification. This allows the establishment of a planting design with little further alteration of the experimental plan.

In June 2007 seeds from *L. angustifolius* were obtained from three populations located over 100 km apart along a latitudinal gradient in western Spain (hereafter 'northern', 'central' and 'southern' populations, see table 1). Thus, individuals of a given population can be considered full strangers to those of any other population from an ancestry viewpoint. Three mother plants located at a minimum distance of 10 m from each other were randomly selected per population, and mature legumes were harvested from each mother plant. Progenies from a single mother plant are considered to be very close genetically because of the species's high selfing rate, and will be referred to hereafter as belonging to the same 'family line'.

(b) *Growing conditions and experimental design*

On 24 January 2008, a total number of 657 seeds were scarified by gently cracking the seed coat on the side opposite to the embryo with a pair of cutting pliers and sown in black round pots of 15 cm diameter per 20 cm in height (261 units) filled with 8 dm^3 of a substrate composed of 28 per cent sand, 15 per cent perlite and 56 per cent commercial peat. Pots were then placed on greenhouse shelves and subjected to regular automatic water sprinkling as needed to maintain plants under optimal common growing conditions. Pots were rotated fortnightly to avoid within-pot heterogeneity in light exposure and weeds were removed regularly.

Our experimental design aimed to test whether genetic relatedness among groups of plants sharing a pot affects fitness of focal individuals and groups of plants. Each pot was allocated a set of three seeds following one of three relatedness treatments: (a) siblings treatment (seeds belonging to the same family line); (b) moderate stranger treatment (seeds originating from the same population, but from different family lines); and (c) full stranger treatment (seeds originating from different populations). Seeds were sown in circles around the centre of the pot at two sowing distances: 2 or 10 cm apart from each other (near and far treatments, respectively, hereafter). The sowing distance treatments did not intend to mimic levels of population density in the field. Density was the same for all pots except controls, in which one seed was sown per pot (see below). Instead, sowing distance modulated the timing of when seedling roots come into contact with each other. This timing may modulate the responsiveness of root development and thus kin recognition and selection events. Sowing distance and relatedness treatments were factorized. Thus, for each of the two sowing distance treatments (near and far), 9 seeds per family line

Table 1. Climate data and location of seed origin populations and of the greenhouse site. (Data are long-term averages extracted from the 'Atlas Climatico Digital de la Peninsula Iberica', http://opengis.uab.es/wms/iberia/mms/index.htm. MAT: average annual temperature; T_{min}: average annual minimum temperature, T_{max}: average annual maximum temperature.)

site	coordinates	altitude (m a.s.l.)	MAT (°C)	T_{min} (°C)	T_{max} (°C)	rainfall (mm year^{-1})
northern origin	42°02′51″ N	942	10.0	4.0	16.0	897
Asturianos (Zamora province)	6°28′39″ W					
central origin	41°04′33″ N	700	13.0	7.0	19.0	671
Berruecopardo (Salamanca province)	6°39′27″ W					
southern origin	40°05′50″ N	516	15.0	9.0	21.0	1232
Jaraiz de la Vera (Cáceres province)	5°44′06″ W					
common garden site	40°18′48″ N	632	14.0	8.0	21.0	481
Móstoles (Madrid province)	3°52′57″ W					

were sown in full strangers pots, 12 in moderate strangers pots, and 12 in siblings pots. Thus, the experimental layout was unbalanced by design (see statistics below). In addition, seven extra seeds per family line were singly sown as controls of the experiment in the same pot size (single culture, hereafter).

Pots were arranged in four blocks (shelves) in the greenhouse. Each shelf contained at least two pots representing each experimental treatment for each family line. Location of pots within each shelf was randomized. An additional set of 20 seeds per family line was sown and placed on a nearby shelf to obtain extra seedlings to replace potential casualties at the early stage of the experiment, and to develop size-biomass equations for above-ground biomass estimation (see below).

Seeds that did not germinate or seedlings that died at an early stage (35 in total, approx. 5% of seeds sown initially), were replaced by extra individuals from the same family line grown on an adjacent shelf. Ninety-six per cent of the plants of the experiment survived until flowering initiation. All pots where a plant died before flowering were discarded from any statistical analysis.

(c) *Plant measurements and fitness estimates*

Above-ground vegetative growth was measured twice through two different estimates. Seedling height was measured to the nearest 0.5 cm to characterize early growth 25 days after sowing. Then, at the time of flowering, maximum and minimum plant canopy diameter and height were measured to the nearest 0.5 cm. After these measurements were taken, 30 plants of contrasting sizes were selected from the set of extra pots and measured for dimensions, and the above-ground fraction was clipped, oven-dried and weighed. Linear models relating these plant dimensions and above-ground dry mass were obtained for the 30 individuals and used for inferring above-ground dry mass of all remaining plants of the experiment. Plant size at flowering onset (g dry mass) was used as a proxy for the pool of internal plant resources available for reproduction.

Flower, fruit and seed production, and seed and fruit set, were measured at the end of the reproductive period. The number of fruits containing fully developed seeds and the number of pedicel scars per plant were counted. Flower number was obtained by adding the number of fruits to the number of pedicel scars found in the infructescence. Fruit set was then estimated as the ratio of number of fruits to number of flowers. One pod per plant was then harvested at random, and the number of fully developed seeds inside the pod and the number of undeveloped ovules were counted.

Seed set was then computed as the ratio of seed number to total number of developed and undeveloped ovules in that pod. All fully mature seeds of each individual were collected and counted to obtain the total number of fully mature seeds produced per plant by the end of its life cycle. The latter was considered the net contribution of an individual to the next generation in terms of viable offspring.

We used several surrogates of fitness to account for the effects of relatedness and sowing distance at two integration levels: individuals and groups. Individual fitness was considered the performance of each individual plant of the experiment as compared to controls of its family line and block. Group performance was total yield (of biomass, flowers, etc.) of triads in a pot. For estimating fitness at the individual plant level, single cultures were used as the controls of the experiment, and were assumed to represent maximum growth and potential fitness of each family line under common growing conditions. Thus, for each individual plant a response ratio of focal plant fitness to single culture fitness was calculated according to the following expression: $\text{fitness}_{ratio} = \text{fitness}_{focal}/\text{fitness}_{control}$. $\text{fitness}_{control}$ was the average score of single cultures per each family line and block. Response ratios, rather than relative competition intensity indices (*sensu* Weiglet & Jolliffe 2003), are recommended for designs similar to ours, because they are symmetrical for both increases and decreases in fitness relative to controls and do not assume a maximum threshold for competition (Goldberg *et al.* 1999). For statistical analyses (see below), log link function was implemented for response ratios, which improves statistical properties of the index (Hedges *et al.* 1999). Fitness of groups was estimated as the pot-level sum of fitness metrics of cumulative nature: above-ground vegetative biomass, number of flowers, number of fruits and number of seeds.

(d) *Data analysis*

Generalized linear mixed models (hereafter GLMMs; McCullagh & Nelder 1989) were used to disentangle the effects of fixed and random factors on fitness estimates. The different models developed included a link function of fitness measures at the individual and at the pot (group) levels as response variables, and a linear combination of fixed and random effects as explanatory variables. Given our planting design, all plants were used as both focal individuals and neighbours.

Models at the individual level incorporated relatedness of neighbours (siblings, moderate strangers or full strangers), sowing distance (near or far) and their interaction, as fixed-effect factors. Block was not included as a covariate

because, as explained above, fitness was expressed as a ratio of potential fitness relative to that of single cultures of each family line in each block, which in itself accounts for putative differences among blocks. Pot effects were considered by including fitness estimates at the pot level as a covariate. This term accounted for differences in productivity among individual pots due to putative uncontrolled environmental heterogeneity within greenhouse shelves. Population of seed origin and family line, nested within population, were included as random-effect factors.

Models at the pot (group) level were built as follows. Relatedness to neighbours, sowing distance and their interaction were included as fixed-effect factors, and block (shelf) was included as a random-effect factor. Only vegetative biomass, early height, flower number, fruit number and seed number were considered cumulative fitness estimates and thus included as response variables in group-level analyses.

Model specifications were as follows. Most response variables were either counts or ratios, or were significantly right-skewed: thus, Poisson error and log link function distribution were specified. This approach also minimizes potential biases due to skewed plant size distributions (see Klemens 2008). Parameter estimation was performed through restricted maximum likelihood, which is recommended when dealing with unbalanced designs (Patterson & Thompson 1971). As our design was unbalanced, we used Satterthwaite's method to determine the approximate denominator degrees of freedom for tests (Verbeke & Molenberghs 1997). Effects of random factors were tested using Wald Z-statistic tests, and those of fixed factors were tested with F-tests (Littell *et al.* 1996). All GLMM computations were performed using SAS Macro program GLIMMIX, which iteratively calls SAS Procedure MIXED until convergence (SAS v. 9.0).

Our design for fixed-effect factors was factorial. Thus, multiple *post hoc* comparisons among levels of relatedness were performed as follows. If sowing distance was significant in GLMMs, or if the interaction term for sowing distance and relatedness to neighbours was significant, multiple comparisons among levels of relatedness to neighbours were performed using the SLICE option of the LSMEANS statement of SAS v. 9.0, corresponding to the effect of a given factor tested at the different levels of the other factors (Schabenberger *et al.* 2000). If not, multiple comparisons of simple main effects for relatedness to neighbours were carried out only when this factor was significant in GLMMs (see Herrera *et al.* 2001).

To estimate whether initial differences in plant height (surrogate of competitive ability for light) among plants sharing a pot increase or decrease during plant growth, we proceeded as follows. We expressed the height of a given plant as a percentage of the summed height of the three individuals in its pot, using the equation:

$$\text{Height index} = (\text{Height}_{\text{focal}}/(\text{Height}_{\text{focal}} + \text{Height}_{\text{neighbour 1}}$$
$$+ \text{Height}_{\text{neighbour 2}})) \times 100.$$

Height index was calculated for each plant at the seedling and adult (flowering) stages. Height index accounts for average plant size differences between seedlings and adults, and also scores each individual relative to its neighbours in the pot. Then, we fitted a Type I least-squares linear regression between Height index$_{\text{seedling}}$ and Height index$_{\text{adult}}$. Type I

regression calculates regression parameters so that error in the dependent (y) variable is minimized. Type I regression is more appropriate when there is an *a priori* functional relation of dependency between y and x variables (Sokal & Rohlf 1995). If initial height differences in a pot exacerbate through time, then the Height index$_{\text{seedling}}$ versus Height index$_{\text{adult}}$ slope will be greater than 1, whereas if initial differences are buffered throughout ontogeny, the slope will be lower than 1. We fitted regressions separately for each relatedness treatment, and then carried out slope heterogeneity tests among treatments to determine whether individuals being planted in contrasting neighbourhoods affect growth trajectories and putative asymmetry in competition for light. Least square regression fitting, slopes heterogeneity tests and *post hoc* multiple comparison of slopes among the three relatedness treatments were carried out using (S)MATR v. II (D. S. Falster, D. I. Warton & I. J. Wright, http://www.bio.mq.edu.au/ecology/SMATR).

3. RESULTS

Plant survival until flowering initiation reached 96 per cent. As a rule, controls grown in single culture showed better performance for all cumulative fitness components, except for plant height at the seedling stage ($p < 0.01$ for all tests, on the basis of multiple comparisons after GLMMs with metrics of individual fitness as response variables, but expressed as absolute measures, instead of relative to controls; results not shown). This indicates that individuals seeded in triads did indeed experience competitive interactions (see fig. (a) in the electronic supplementary material).

(a) *Effects of family line and population of origin*

Family line was found to have either a consistent trend (flower number, $p = 0.067$; fruit number, $p = 0.075$; seed number, $p = 0.075$; fruit set, $p = 0.075$) or a significant effect (above-ground vegetative biomass, $p < 0.05$; early height, $p < 0.05$) on all fitness metrics, except seed set. Population of origin, however, did not affect any of the fitness components ($p > 0.1$ for all fitness metrics).

Several of the family lines tended to perform better for most reproductive fitness components (e.g. family lines 1, 3 or 6), while others performed generally worse (e.g. family lines 2, 5 or 7; figure 1; see also fig. (b) in the electronic supplementary material). It is remarkable that the relative ranking of family lines for reproductive estimates of fitness was mostly independent of that for vegetative estimates. Also, in spite of the general effect of family line in GLMMs, the relative ranking of genotypes showed noticeable variation among experimental situations (data are shown for flower production, but similar interactions occur for all other fitness estimates). Relative ranking of family lines coming from different populations was not consistent among different fitness estimates or among experimental treatments (figure 1). This further reflects the absence of population of origin effects.

(b) *Effects of genetic relatedness to neighbours and sowing distance*

We found higher reproductive fitness in individual plants sharing a pot with full strangers. Response ratios for flower, fruit and seed production were highest for individuals growing in the vicinity of full strangers (table 2;
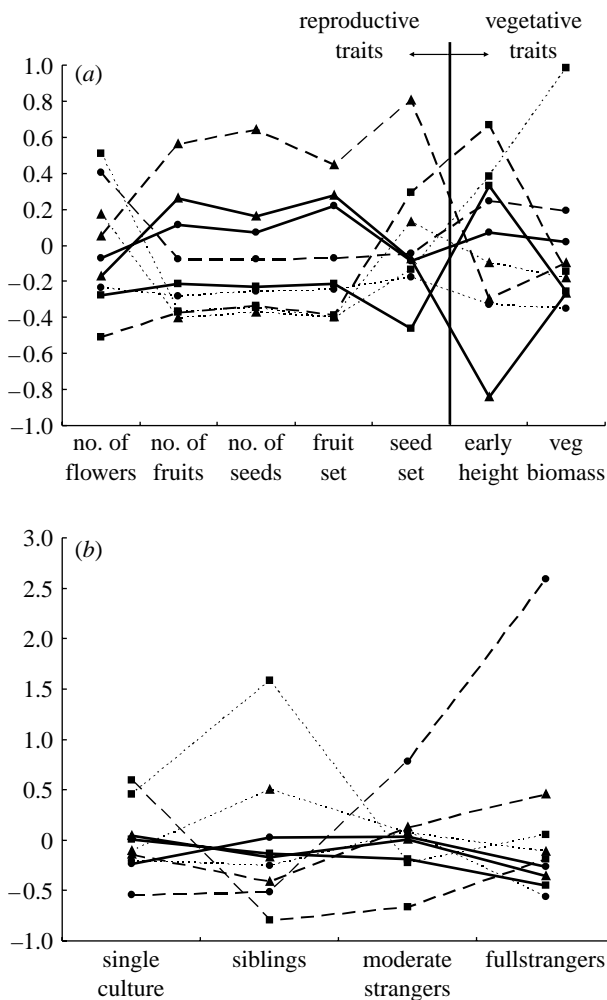
Figure 1. Two examples of representative norm of reaction graphs for the family lines used in this experiment. (*a*) Ranking of family lines as a function of fitness components. Solid lines: circles, family 1 south; squares, family 2 south; and triangles, family 3 south. Dashed lines: circles, family 4 central; squares, family 5 central; and triangles, family 6 central. Dotted lines: circles, family 7 north; squares, family 8 north; and triangles, family 9 north. (*b*) Relative ranking of genotypes as a function of flower production, for each of the three levels of genetic relatedness to pot neighbours, and of single cultures. Solid lines: circles, family 1 south; squares, family 2 south; and triangles, family 3 south. Dashed lines: circles, family 4 central; squares, family 5 central; and triangles, family 6 central. Dotted lines: circles, family 7 north; squares, family 8 north; and triangles, family 9 north. All data were *Z*-standardized within (*a*) each fitness metric or (*b*) each treatment level to make rank ordering comparable among fitness metrics or treatments, respectively. Data in (*b*) were averaged across all treatment levels of sowing distance. Line patterns denote different populations of origin, while different family lines within population are denoted by dot patterns.

figure 2). Fruit set scores for plants surrounded by full strangers were even higher than those attained by control plants of their respective blocks grown in single culture. However, early growth was highest in plants living in the vicinity of siblings (table 2; figure 2; see also fig. (a) in the electronic supplementary material for patterns on an absolute measure scale).

Analysis at the group level revealed that groups of plants consisting of individuals from different populations (full strangers) produced more flowers than groups of

siblings or moderate strangers (table 3; figure 3). Above-ground plant biomass was also highest for groups of full strangers. However, early growth was highest for groups of siblings. Other fitness components analysed at the group level did not show any particular trend (table 3; figure 3). Sowing distance was not significant for any of the fitness metrics in the group or individual-based analyses (tables 2 and 3).

(c) *Plant height growth trajectories as a function of relatedness to neighbours*

Individuals that were taller at the seedling stage also tended to be taller at the adult stage (figure 4). However, there was considerable deviation from this statistically significant tendency, deviation being of different extent depending on relatedness treatment (figure 4). Initial plant height differences within a given pot generally decreased as plants grew until flowering, since slopes were significantly smaller than 1 (figure 4). The slope of this relationship differed among relatedness treatments, on the basis of slope heterogeneity tests: the slope of full strangers was significantly higher than that of moderate strangers and siblings ($p < 0.05$, result not shown). Thus, regression fits and parameters are shown separately for each treatment in figure 4.

4. DISCUSSION

Kin selection takes place when phenotypes that reduce fitness repression on their related neighbours are selected (Kelly 1996; Griffin & West 2002). Overall, the results of this study rule out any evidence of kin selection in *L. angustifolius* in our current experimental setting: plants surrounded by siblings did not outperform plants surrounded by full or moderate strangers, and group performance for any fitness metric did not rank siblings, moderate strangers and full strangers in descending order. The only recurrently significant effect of the genetic assembly of pots was that individuals benefit from growing in the vicinity of full strangers. This is in disagreement with our initial hypothesis that the genetic composition of the neighbourhood should be irrelevant to individual fitness. However, genetic effects pointed towards the opposite direction of kin selection. Our results also supported the suitability of the experimental design. Controls with single cultures provided the best performance, indicating that the groups of plants sharing a pot did have competitive interactions with their neighbours, irrespective of related-ness or sowing distance (see fig. (a) in the electronic supplementary material). Furthermore, the different family lines for seed source represented contrasting genotypes, as contrasting phenotypes were obtained when sown under common growing conditions (see fig. (b) in the electronic supplementary material). This supports the use of seeds from the same family line as a surrogate of close genetic relatedness.

Despite its putative ecological and evolutionary rel-evance, the significance of root recognition is largely unknown and has been examined in a limited number of studies (Hess & de Kroon 2007). Dudley and File's experiment found signs of kin recognition through root interactions, but did not detect any effect on individual or group fitness (Dudley & File 2007). The few previous experiments that have directly dealt with this issue have

Table 2. Fixed-factor effects of GLMMs for fitness metrics at the individual plant level of analysis. (See text for details on model construction.)

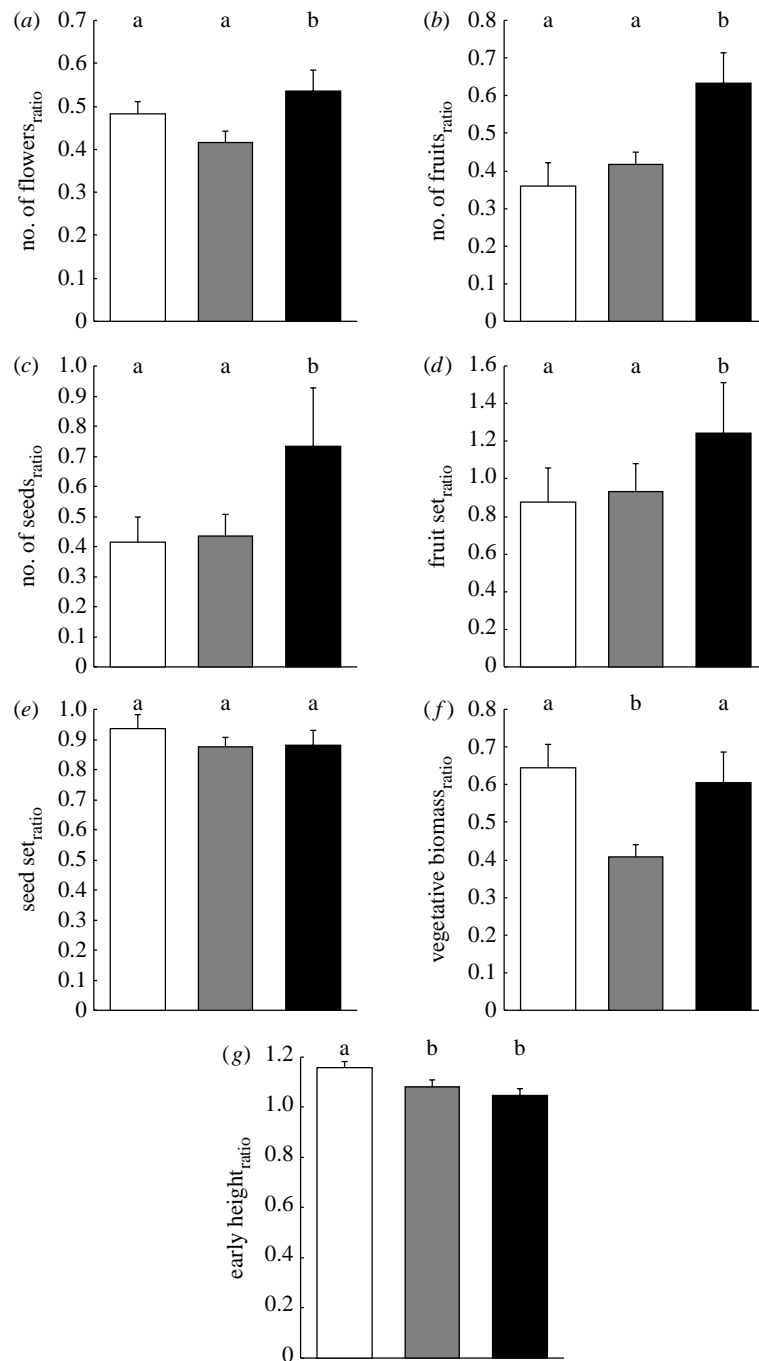|	relatedness		sowing distance		relatedness × distance	
	F-ratio	*p*-value	*F*-ratio	*p*-value	*F*-ratio	*p*-value
number of flowers$_{ratio}$	4.56	0.011	2.19	0.140	1.31	0.271
number of fruits$_{ratio}$	5.77	0.004	9.04	0.003	0.57	0.565
number of seeds$_{ratio}$	6.27	0.002	0.36	0.546	1.48	0.230
fruit set$_{ratio}$	32.49	<0.001	28.25	<0.001	7.09	0.001
seed set$_{ratio}$	0.01	0.995	1.26	0.265	3.10	0.048
vegetative biomass$_{ratio}$	3.32	0.037	1.85	0.175	0.09	0.770
early height$_{ratio}$	11.11	<0.001	0.61	0.435	0.16	0.854

Figure 2. Effect of growing in the vicinity of siblings (white bars), moderate strangers (grey bars) or full strangers (black bars) on several fitness components examined at the individual plant level (mean ± 1 SEM). Data of far and near sowing distances were combined, since no significant effects of this factor were found on fitness metrics. Different letters mean significant differences at $p = 0.05$ on the basis of multiple comparisons. See fig. (*a*) in the electronic supplementary material for patterns expressed in absolute measures, instead of ratios relative to each family line's single cultures.

Table 3. Fixed-factor effects of GLMMs for fitness metrics at the group (=pot) level of analysis. (See text for details on model construction.)

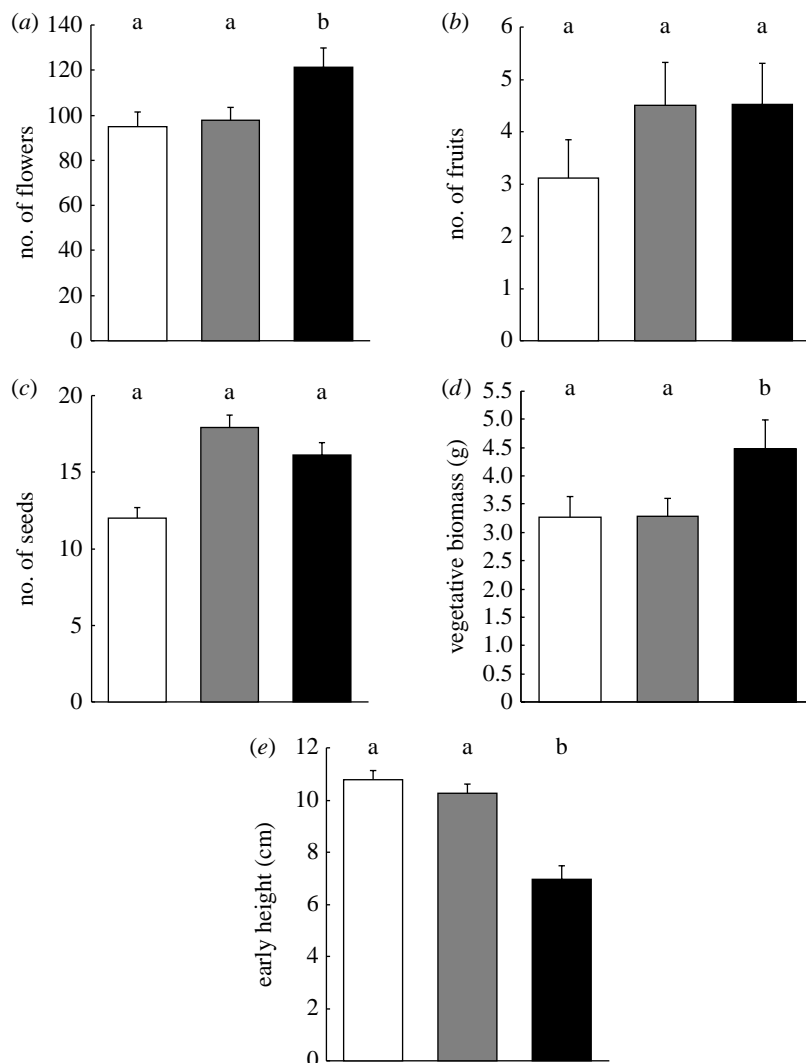|	relatedness		sowing distance		relatedness×distance	
	F-ratio	*p*-value	*F*-ratio	*p*-value	*F*-ratio	*p*-value
number of flowers	4.71	0.011	0.03	0.856	1.63	0.200
number of fruits	1.19	0.307	0.41	0.524	0.56	0.572
number of seeds	1.22	0.299	0.70	0.405	0.68	0.508
vegetative biomass	3.40	0.036	0.60	0.441	0.90	0.409
early height	18.40	<0.001	0.17	0.677	0.01	0.993

Figure 3. Performance of groups of three plants of siblings (white bars), moderate strangers (grey bars) or full strangers (black bars; mean ± 1 SEM). Data of far and near sowing distances were combined, since no significant effect of this factor was found. Different letters mean significant differences at $p=0.05$ on the basis of multiple comparisons.

obtained conflicting results (e.g. Cheplick & Kane 2004 against Tonsor 1989). The evidence found in our work, along with that in other experiments (Allard & Adams 1969; Schmitt & Ehrhardt 1987; Kelley 1989; Cheplick & Kane 2004), conforms to the view that individuals living in the vicinity of strangers, and microcosms composed of unrelated individuals, attain higher fertility rates.

Multiple mechanisms may account for this response pattern. First, one may ask whether plants in our experiment competed mainly for light or for below-ground resources. We have indirect evidence that light competition was weak during the course of plant growth, since

early height differences within pots tended to be diluted as the experiment progressed. Individuals in full stranger pots maintained initial height differences more than those in sibling or moderate stranger pots. However, slope of Height index$_{adult}$ versus Height index$_{seedling}$ was always much lower than 1, indicating fading of early height hierarchies with time. Also, in our experiment, individuals growing in the vicinity of siblings showed significantly faster early growth (figure 2). This effect was also described during the early growth of sibling groups of *Phytolacca* (Willson *et al.* 1987). This suggests that emergence phenology, seedling architecture and leaf
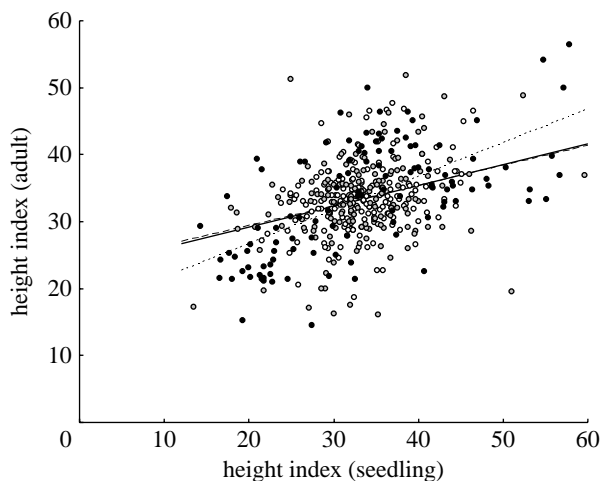
Figure 4. Scatter plot showing plant height growth trajectories during the experiment, from seedling to adult plants. Height indices are percentage contribution of each plant to the summed height of the three individuals in its pot (see §2). Heterogeneity of slopes tests detected significant differences at $p = 0.05$ among relatedness treatments, particularly between full strangers (black circles, dotted line; $y = 16.75 + 0.50x$; $R = 0.62$; $p < 0.001$) and moderate strangers (grey circles, dashed line; $y = 23.37 + 0.30x$; $R = 0.29$; $p < 0.001$), and between full strangers and siblings (white circles, solid line; $y = 23.04 + 0.31x$; $R = 0.32$; $p < 0.001$), after *post hoc* multiple comparison of slopes. Thus, regression fits and parameters are shown separately for each treatment.

display are more similar among sibs, forcing plant shoots to elongate more intensely to obtain greater exposure to sunlight and overshadow their neighbours, which evidences early light competition. Higher stature of *Lupinus* sibs did not result in comparatively better performance at later stages of development, in line with patterns found in other plant mixtures (Jensen & Meyer 2004). This result, together with the pattern that family lines that performed better in terms of vegetative growth were not the same ones that excelled in reproductive measures, suggests that fitness components are not always directly and positively correlated (see figure 1; see also fig. (b) in the electronic supplementary material). In annuals of indeterminate growth, similar to our study subject here, factors such as meristem limitation may generate compromises between vegetative growth and reproductive output, leading to different outcomes for each performance measure (Geber 1990).

Regarding below-ground processes, two contrasting explanations may have generated the obtained pattern. If root investment were traded off with reproductive allocation, as frequently argued in experiments in this field (Gersani *et al.* 2001), then plants in the vicinity of strangers may have invested less in roots, due to more efficient resource partitioning, and allocated more resources to reproduction. This view is unlikely to be in line with the pattern in Dudley & File (2007). However, pot size artefacts cast doubts on most previous evidence advocating this trade-off (Hess & de Kroon 2007). The other possibility is that root overproduction did not trade off with plant size or reproductive biomass; rather, it promoted greater resource acquisition, leading to larger plant biomass and reproduction in general.

Under this scenario, the costs of making more roots may be offset by the benefits of greater resource acquisition and plant growth (de Kroon 2007). If root production was greater in the presence of strangers, as suggested by Dudley & File (2007), this may lead to a pattern opposite to that expected under a kin selection scenario, which is precisely what we find here. This second possibility of root interaction pattern seems more plausible to us and is compatible with Dudley & File's (2007) findings. Clearly, more detailed studies are needed to shed light on the causal mechanisms in experiments on the genetics of intraspecific competition.

As highlighted above, kin recognition through roots is mechanistically compatible with the results shown here. This leads to the question of how and why individuals recognize each other through root interactions. Individual root recognition has been predominantly identified in experiments showing that the root tip growth of a plant responds differently when the plant runs into the roots of another plant than when it runs into its own roots, i.e. self/non-self (S/NS) root discrimination (Mahall & Callaway 1991; de Kroon *et al.* 2003). A plant's ability to distinguish its own roots should be an inherent skill, because root growth occurs in a remarkably heterogeneous space (Hodge 2004). This forces roots to explore alternative space and to respond differently to encounters with itself, another self, or an inert soil component, under the developmental constraints established by the species root architecture. We propose that the ability of some species to respond to gradients of genetic relatedness (e.g. *Plantago lanceolata* in Tonsor 1989; *Ambrosia dumosa* in Mahall & Callaway 1996; *C. edentula* in Dudley & File 2007) might be a by-product of the essential ability of distinguishing itself from others. Thus, contrary to Callaway & Mahall (2007), root recognition may not be the core of evidence for kin selection but, perhaps, simply a side-effect of self recognition. This seems to us a more parsimonious explanation. It should be noted, however, that self root recognition, like kin recognition and kin selection, is not of universal nature (see Semchenko *et al.* 2007). Indeed, heritable phenotypic variation should occur if recognition is to be a target of natural selection. In this sense, it is of interest that we found some degree of among-family variation in this experiment. This signals the possibility that different genotypes respond differently to a sibling/stranger encounter, which provides ground for natural selection to select on this phenotypically variable trait.

Regarding the group level of analyses, improved performance of groups of full strangers was detected for above-ground vegetative biomass and number of flowers, although this did not result in higher seed or fruit number. In any case, groups of siblings or moderate strangers never outperformed groups of full strangers. These results agree with those found at the individual level, where improved performance of full strangers was detected in most fitness components. Similarly, in an experiment addressing multilevel selection in *C. edentula*, Donohue (2003) also found a high match among responses at two integration scales: positive selection on focal plants with heavy stems, and positive selection on focal individuals growing in the vicinity of heavy-stemmed neighbours.

We did not find consistent significant effects of sowing distance on individual or group performance in our pots. Sowing at two distances was conceived as a way of forcing roots to interact at different developmental stages. Roots of plants sown at 2 cm from each other consistently interacted with neighbouring roots throughout almost their whole lifetimes, while plants sown 10 cm apart, near the edge of pots, probably came into contact at a later stage, when at least primary root architecture was already defined. In any case, earlier or later root contact did not significantly affect fitness, which further indicates that kin recognition was of minor relevance to plant performance in this experiment. Callaway & Mahall (2007) suggested that results from pot experiments, such as reported by Dudley and File (2007), should be taken with care as to interpret kin selection effects of root recognition. This assertion stems from the assumption that after roots recognize each other and initiate putative mechanisms of root contact avoidance, they cannot explore the substrate much farther due to restrained soil volume in pots. We were careful here to select a pot volume (8 l) that was by far larger than the volume annual *Lupinus* roots can fill in a single growing season at the established planting density (Milla 2007, personal observation from essays in preceding years). In any case, large pots did not hinder plant competition, as shown by the higher performance of single cultures (fig. (a) in the electronic supplementary material) and the intense intermingling of root remains after the final harvest of the experiment (Milla 2007, personal observation).

In summary, several case studies have shown that kin recognition and kin selection may occur in some species under some circumstances. The present study, along with others, provides ground for making two annotations to this assertion. First, kin selection seems far from being comprehensive and of general scope for the plant kingdom, in light of the little available evidence (which includes the results of this paper). In this sense, we hypothesize that the existence of kin recognition in several plant genera might be a by-product of self versus non-self recognition mechanisms. Second, when kin recognition is present, its importance compared to that of other factors that determine the outcome of competition might be small (Griffin & West 2002). Although frequently neglected, evaluation of relative importance is essential when we move from mechanisms (e.g. kin recognition) to further competitive outcomes or evolutionary patterns (e.g. kin selection; Brooker & Kikvidze 2008). In any case, renewed interest in the genetic determinants of intraspecific competition is timely, and the above should not be viewed as proof that genetics are irrelevant to this process. Instead, we hope this work stimulates further research in this area. As stressed earlier, we are just beginning to grasp the astonishing ability of plants to recognize the genetic identity and degree of relatedness of neighbours. More research is clearly needed to advance our knowledge of the comprehensiveness of this striking phenomenon, its mechanisms and its role in ecological and evolutionary processes.

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