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Intraspecific competition reveals conditional fitness effects of single gene polymorphism at the *Arabidopsis* root growth regulator *BRX*

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Summary

• Intraspecific genetic variation for morphological traits is observed in many organisms. In *Arabidopsis thaliana*, alleles responsible for intraspecific morphological variation are increasingly being identified. However, the fitness consequences remain unclear in most cases.

• Here, the fitness effects of alleles of the *BRX* gene are investigated. A *brx* loss-of-function allele, which was found in a natural accession, results in a highly branched but poorly elongated root system.

• Comparison between the control accession *Sav-0* and an introgression of *brx* into this background *(brx^S)* indicated that, surprisingly, *brx* loss of function did not negatively affect fitness in pure stands. However, in mixed, well-watered stands *brx^S* performance and reproductive output decreased significantly, as the proportion of *Sav-0* neighbors increased. Additional comparisons between *brx^S* and a *brx^S* line that was complemented by a *BRX* transgene confirmed a direct effect of the loss-of-function allele on plant performance, as indicated by restored competitive ability of the transgenic genotype. Further, because plant height was very similar across genotypes and because the experimental setup largely excluded shading effects, the impaired competitiveness of the *brx* loss-of-function genotype likely reflects below-ground competition.

• In summary, these data reveal conditional fitness effects of a single gene polymorphism in response to intraspecific competition in *Arabidopsis*.

Key words: *Arabidopsis thaliana*, *BREVIS RADIX*, competition, density, environmental stress, population structure, replacement series, root morphology.

Introduction

In the model plant *Arabidopsis thaliana* (*Arabidopsis*), substantial intraspecific natural genetic variation has been found for most traits investigated so far. This includes life history, physiological and morphological traits, such as flowering time (Lempe *et al.*, 2005; Shindo *et al.*, 2005, 2006), seed germination (Bentsink *et al.*, 2006), ion acquisition rate (Rus *et al.*, 2006), leaf shape (Perez-Perez *et al.*, 2002) or root growth (Mouchel *et al.*, 2004; Sergeeva *et al.*, 2006). The growing characterization of the molecular genetic basis of natural intraspecific variation in *Arabidopsis* has also led to an increasing interest in its ecological significance (Mitchell-Olds & Schmitt, 2006; Shindo *et al.*, 2007). For instance, variation in germination timing has been demonstrated to influence

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fitness of *Arabidopsis* plants grown in the field (Donohue, 2002; Donohue *et al.*, 2005). However, in another study, which used artificially induced mutants, a photoreceptor controlling seedling phototropism has been shown to enhance fitness of *Arabidopsis* under field conditions (Galen *et al.*, 2004, 2007). This study is one of a few successful examples of how to evaluate the fitness value of a key gene controlling an adaptive phenotype. Still, case studies that address the fitness values of genetic variation between *Arabidopsis* natural accessions under relevant ecological conditions are rare. Because traits that display phenotypic plasticity in plants are often controlled by multiple genetic pathways, the fitness effects of a focal gene and genotype–environment interactions are often complex.

So far, most ecological analysis of genetic variation has focused on major life history traits, such as the timing of germination or flowering, or shade avoidance response (Schmitt *et al.*, 1995; Donohue, 2002; Galen *et al.*, 2004). One of the main reasons for this is that these traits can be easily measured and are relatively stable and replicable in controlled-growth conditions as well as in the field. By contrast, ecological analysis of root system phenotypes is less straightforward, primarily because development of the root system in response to the soil microenvironment is highly plastic and is difficult to observe *in situ* (Malamy, 2005; Osmont *et al.*, 2007). However, the impact of the root system on overall plant performance can often be monitored indirectly through analysis of shoot performance. This approach can also detect inter- as well as intraspecific below-ground competition, as has been observed in several plant species (Zobel & Zobel, 2002; Schenk, 2006; de Kroon, 2007; Murphy & Dudley, 2007).

In this study, we aimed to evaluate the ecological impact of root growth and morphology, by investigating the fitness effects of different root systems on plant performance under competitive conditions in different environments. We took advantage of a loss-of-function null allele of the *BREVIS RADIX* (*BRX*) gene (Briggs *et al.*, 2006), which has been found in the natural *Arabidopsis* accession Umkirch-1 (*Uk-1*) (Mouchel *et al.*, 2004). *BRX* belongs to a highly conserved gene family of higher plants. *brx* loss of function results in reduced root growth, owing to disturbed plant hormone signaling pathways (Mouchel *et al.*, 2006), eventually leading to a less deep but more compact and branched root system in plants grown in tissue culture as well as plants grown in soil (Mouchel *et al.*, 2004).

A frequent problem encountered when assessing fitness of natural allelic variation is the lack of genetically homogenous controls for different allele states. Therefore, in our analyses, we used the brx^S line, which was obtained from an introgression of the *Uk-1 brx* allele into the Slavice-0 (*Sav-0*) background (Mouchel *et al.*, 2004). In *Sav-0*, the *BRX* gene is functional. Additional experiments with a brx^S line complemented by a functional *BRX* transgene (BRX^S) enabled us to separate the fitness effect of this particular locus from genetic background noise. These three lines show very similar flowering time and plant morphology (Mouchel *et al.*, 2004), except for brx^S, which displays the *Uk-1* root system phenotype. Through competition experiments with intra- and intergenotype scales, we evaluated the relative fitness of the functional and the naturally occurring loss-of-function allele.

Materials and Methods

Plant materials

The *Arabidopsis* lines used in the competition analysis, *Sav-0*, brx^S, and brx^S complemented by a functional *BRX* transgene (the *BRX* open reading frame expressed under control of the *BRX* promoter) (BRX^S), have been described previously (Mouchel *et al.*, 2004, 2006). Importantly, the timing of germination and flowering were completely synchronized between the experimental lines. The new Umkirch accessions (*UkA, UkB, UkC, UkD*) were collected in June 2006 along the banks of the old Dreisam river near Umkirch, Germany. Other natural accessions used in this study were obtained from the Nottingham *Arabidopsis* Stock Centre.

Competition analyses – experimental setup

For competition analyses, genotype performance was compared in glasshouse experiments with the following variables: pure stands vs mixtures of variable frequency, low versus high density, abundant watering versus limited watering (drought). Plants were grown in plastic trays (16.2 × 24.3 cm) containing 2 l of sieved, uniformly mixed soil ('Treff BF4', GVZ-Bolltec, Zurich, Switzerland) containing 60% humus, 1% clay and 6% silt. Soil pH was 5.7, electrolytic conductivity 1157 μS cm^{-1}. Water extraction (1 : 1.5 v/v) revealed the following concentrations of water-soluble nutritive elements: NO_3^-, 7382 μM; NH_4^+, 40 μM; N, 7422 μM; P, 694 μM; K, 1488 μM; Ca, 3040 μM; Mg, 1651 μM; Fe, 279 μM; Mn, 110 μM; Cu, 6.8 μM; Zn, 13.9 μM. The soil in each plastic tray was leveled to a height of 5 cm and sprinkled with 500 ml water. A grid of 54 squares of 2.7 × 2.7 cm size was drawn on to the soil surface and three to five seeds soaked in distilled water were placed in the center of each square (or of a subset of six squares in the low-density treatment). Trays were covered with lids and incubated at 4°C for 3 d to synchronize germination. At day 4, trays were moved to glasshouse benches under natural light with 16 h of supplemental lighting per day. Seeds germinated 3 d later and extra seedlings were removed to have one seedling per square. Densities were either high (54 plants per tray, corresponding to 1372 plants m^{-2}), or low (six plants per tray, corresponding to 152 plants m^{-2}). Trays were covered with clingfilm to prevent the soil surface from drying out for a further 4 d. After cling film removal, two different watering regimes (wet vs dry) were initiated for a further 50 d, until all lines had completed flowering and started senescence. During

this time, soil water content of each tray was monitored daily (by weighing the tray) and adjusted to 1800 g (wet) or 800–840 g (dry) (Supplementary material, Fig. S1). That is, well-watered plants were watered daily, while plants in dry conditions were only watered sporadically after day 14 and maintained at a substantially lower soil moisture level. To minimize the possibility of shading between individual plants, the stems of adult plants were supported by grid lines made from strings anchored on the top edges of trays. Finally, high-density trays were replicated with mixtures of the two genotypes at various ratios (1 : 1, 1 : 8, 8 : 1; Fig. 2). The position of trays within the glasshouse was randomized daily within tables, and table position was changed weekly.

Competition analyses – data collection and analysis

To determine plant performance, we measured end-of-life fruit number, fruit mass, plant height, branch number, lateral branch number, shoot mass and above-ground total mass of four plants per genotype and tray, randomly selected excluding the edges of the tray. All treatment combinations were tested in duplicate trays. Branch number counted basal branches emerging from the rosette, and lateral branch number counted all side branches of basal branches. Fruit mass was the gross weight of siliques including seeds, collected at mature stage just before bursting and dried for 1 month before weighing. All weight measurements represent dry mass after senescence. For the effect of density and watering on performance of *Sav-0* and *brx*^{S} in pure stands, a total of 64 plants were measured in two watering regimes × two densities × two genotypes × two replicate trays. For the effect of relative genotype frequency and watering regime on *Sav-0* and *brx*^{S} competition under high density, we measured a total of 128 plants in two watering regimes × four frequencies × two genotypes × two replicate trays. For the effect of genotype frequency and identity of competing genotype under high density and high watering, 120 plants were measured. For analysis, since treatments (watering, density, frequency) were applied at the level of the tray, we ran linear mixed model analysis (LMM) with tray as random factor and REML estimation (SPSS 13.0, SPSS Inc., Chicago, IL, USA). Data are given as means ± SE, unless specified otherwise.

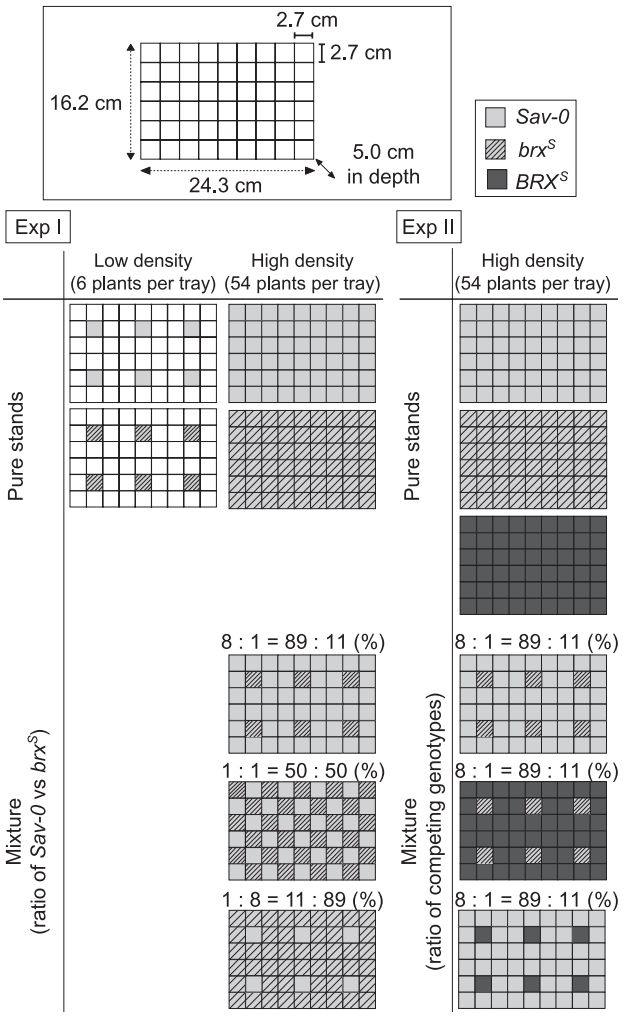

Fig. 1 Experimental design of the competition experiments to test the effects of genetic variation at the *BRX* locus on plant competitive ability and fitness. Top panel, dimensions of trays and grid. Exp I, competition of *brx*^{S} (null mutant) and *Sav-0* plants in pure stands (100%) or mixtures with ratios of focal : competing genotypes: 8 : 1, 1 : 1, 1 : 8 (i.e. frequencies of the focal genotype of 89, 50 and 11%, respectively). The experiment was replicated on duplicate tables, each containing all treatments. Exp II, competition analysis of *brx*^{S} (null mutant), *Sav-0*, and *brx*^{S} complemented by a *BRX* transgene (*BRX*^{S}) plants in pure stands (100%) and 8 : 1 mixtures (frequency of the focal genotype, 89%).

Estimation of population structure

The *Uk-1* line has been maintained in stock centers for > 40 yr (Roebbelen, 1965). It thus appeared possible that mutations, such as *brx* loss of function, may have occurred during post-collection maintenance. However, all plants, whether from a single seed descent line or the bulked stock, displayed short roots, and genotyping of > 24 individuals from bulked stock always detected the *brx* allele in a homozygous state, suggesting that the allele was present in the originally collected plants. We went back to Umkirch (48°2′N, 7°46′E) to collect a total of 17 plants at four points (200–400 m apart from each other), along the banks of the old Dreisam river. Progeny of collected plants was then obtained by selfing. Genomic DNA was isolated using DNeasy kits (Qiagen, Hilden, Germany). Single nucleotide polymorphism (SNP) genotyping was performed for the progeny of the field-collected plants, a representative sample of worldwide accessions and four distinct *Uk* lines from the stock center with a set of 149 markers that are evenly spread over the genome (Warthmann

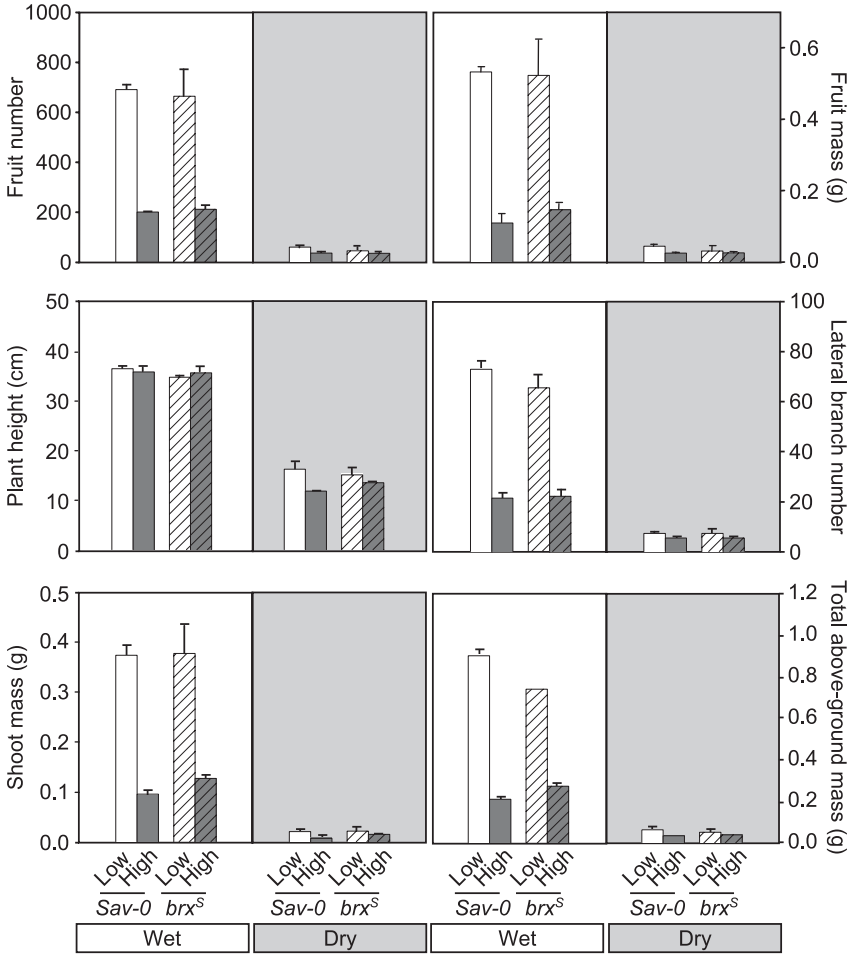

Fig. 2 Mean (± SE) reproductive output (total number of fruits produced, total fruit mass) and growth traits of *Sav-0* and *brx*^{S} genotypes in pure stands, at either low (six plants per tray) or high (54 plants per tray) density, in well–watered or drought simulation conditions. To account for tray effects, the mean of the four plants measured in each tray was used as independent observation to calculate treatment mean ± SE.

et al., 2007) (Sequenom Inc., San Diego, CA, USA). Population structure was estimated by K cluster analysis using STRUCTURE, version 2.0 (Pritchard *et al.*, 2000). Three trials were performed with different values of burn-in period and iterations (1000 and 100 000; 50 000 and 20 000; and 1000 and 1000, respectively). In each trial, 10 independent runs were calculated for K values from 2 to 7. $K = 5$ was the most appropriate value (Evanno *et al.*, 2005).

Results

Experimental design for competition analyses

The primary goal of our experiments was to ascertain whether *brx* loss of function has a negative effect on plant performance and fitness *per se* (i.e. in pure stands); whether effects of *brx* loss of function depend on environmental variables; and whether *brx* loss of function affects intraspecific competitive ability. To this end, we first compared pure stands of *brx*^{S} and *Sav-0* in glasshouse experiments with plants grown in trays at low vs high density, with abundant watering vs limited watering (simulating drought conditions, see Materials and Methods section and Fig. S1). The timing of germination and flowering time were completely synchronized between the two lines. For competition analysis, high-density trays were replicated with mixtures of the two genotypes at various ratios (Fig. 1). To measure plant performance, we determined fruit number, fruit mass, plant height, stem number, lateral branch number, shoot mass (excluding fruits) and total above-ground mass over the entire life cycle.

High plant density leads to competition in pure stands

Plant performance and reproductive output were significantly affected by density, watering and their interaction (or watering × density × genotype interaction for plant height) in *brx*^{S} as well as *Sav-0* (Table 1, Fig. 2). As expected, plants grew better at low density, where they produced roughly three times more fruits (for a very similar plant height) than at high density, and this difference was significantly more pronounced under wet conditions. This suggests that high density resulted in more intense competition among individual plants for limited resources than low density. We therefore used high-density conditions in between-genotype competition experiments (see discussion later).

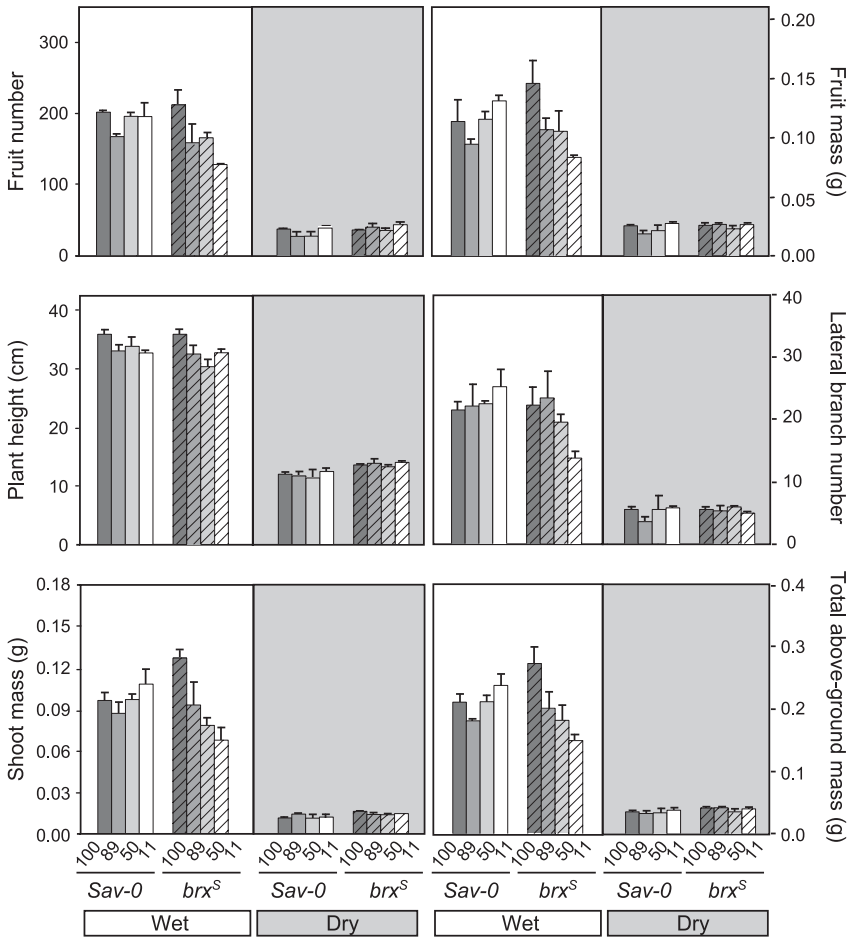

Fig. 3 Mean (± SE) reproductive output (total number of fruits produced, total fruit mass) and growth traits of *Sav-0* and *brx*^{S} genotypes in pure stands (100%) and mixtures with variable frequency (1 : 8, 1 : 1, and 8 : 1, i.e. frequencies of the focal genotype of 89, 50 and 11%, respectively), at constant plant density (54 plants per tray), in well-watered or drought simulation conditions. To account for tray effects, the mean of the four plants measured in each tray was used as independent observation to calculate treatment mean ± SE.

To dissect the density × watering treatment interaction effect, we examined the effects of density within amounts of watering treatment. Under wet conditions, density significantly affected all traits (Fig. 2, Table 1). Plant height was marginally affected by genotype × density effects in both wet and dry conditions (Table 1). Under dry conditions, only shoot mass was significantly affected by density (Fig. 2, Table 1). The fact that density effects were generally nearly not detectable under dry conditions was probably because of the severely limited performance. Indeed, under dry conditions plants only grew to about one-third of the height reached under wet conditions, and produced only about one-tenth of the fruits (Fig. 2).

In the pure stands, both genotypes performed equally well in reproductive output and most measures of performance (Table 1, Fig. 2), suggesting that *brx* loss of function did not have a negative impact on plant performance *per se* in pure stands.

Variable genotype frequency affects plant performance in high-density mixture plots

Plants in mixture plots were grown at high density, that is, under competitive conditions (see earlier discussion). Because of the strong difference between wet and dry conditions, these were analyzed separately. Under dry conditions, the only significant effect was for genotype identity × genotype frequency on plant height and on shoot mass (Table 2). By contrast, under wet conditions, there were significant genotype identity × genotype frequency effects on all measured traits of plant performance and reproductive output (Table 2, Fig. 3). This indicates that under wet conditions, genotypes responded differently to the varying frequency of competition from the other genotype, that is, to the genetic composition of competitors in their plot. In fact, under wet conditions, *brx*^{S} plants performed less well as the frequency of *brx*^{S} decreased compared with *Sav-0* for all measures of plant performance (Fig. 3). Thus the *brx*^{S} genotype suffered more when competing against *Sav-0* than against itself, indicating that *brx*^{S} is competitively inferior to *Sav-0*.

Sensitivity of *brx*^{S} to intergenotypic competition depends on the *BRX* locus

A genome-wide SNP genotyping (see later) of the *Sav-0* and *brx*^{S} lines used in this study revealed that the introgression of the *brx* loss-of-function allele of *Uk-1* into the *Sav-0*

Table 1 Effect of density (six plants per tray vs 54 plants per tray, i.e. 152 vs 1372 plants m^{-2}) and watering treatment (wet vs dry conditions) on plant performance in pure stands of *Arabidopsis Sav-0* and *brx*^{S} genotype

		Fruit number			Fruit mass			Plant height			Lateral branch number			Shoot mass			Total above-ground mass		
Source	df	MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*
Water	1	6.32E+05	183.68	< **0.001**	3.53E+05	127.29	< **0.001**	1806.25	1032.14	< **0.001**	6241	484.74	< **0.001**	2.05E+05	197.13	< **0.001**	8.33E+05	1087	< **0.001**
Density	1	2.37E+05	69.04	< **0.001**	1.67E+05	60.25	< **0.001**	6.25	3.57	*0.095*	2450.25	190.31	< **0.001**	7.30E+04	70.39	< **0.001**	3.21E+05	418.45	< **0.001**
Genotype	1	2.81E+02	0.08	0.782	2.03E+01	0.01	0.934	0.25	0.14	0.715	12.25	0.95	0.358	3.52E+02	0.34	0.577	2.71E+03	3.54	0.102
Water × density	1	2.07E+05	60.14	< **0.001**	1.48E+05	53.25	< **0.001**	12.25	7	**0.029**	2070.25	160.8	< **0.001**	6.52E+04	62.79	< **0.001**	2.80E+05	364.58	< **0.001**
Water × genotype	1	6.30E-02	0	0.997	3.24E+02	0.12	0.741	2.25	1.29	0.29	12.25	0.95	0.358	2.18E+02	0.21	0.659	1.98E+03	2.59	0.152
Density × genotype	1	6.63E+02	0.19	0.672	7.56E+02	0.27	0.616	6.25	3.57	0.095	16	1.24	0.297	2.03E+02	0.2	0.67	1.32E+04	17.19	**0.004**
Water × density × genotype	1	1.27E+02	0.04	0.853	1.96E+02	0.07	0.797	0.25	0.14	0.715	16	1.24	0.297	1.50E+02	0.15	0.714	9.94E+03	12.97	**0.009**
Error	8	3.44E+03			2.78E+03			1.75			12.875			1.04E+03			7.67E+02		
Total	16																		

Linear mixed models (LMM) were run on plant performance for a total of 64 plants in two watering regimes × two densities × two genotypes × two replicate trays.

Table 2 Effect of genotype and their relative frequency on plant performance in mixtures of the two *Arabidopsis* genotypes (*Sav-0* and *brx*^{S}) at 1 : 8, 1 : 1, and 8 : 1 ratios (i.e. frequencies of the focal genotype of 89, 50 and 11%, respectively) analyzed separately with linear mixed models (LMM) for wet vs dry conditions

		Fruit number			Fruit mass			Plant height			Lateral branch number			Shoot mass			Total above-ground mass		
	df	MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*	MS	*F*	*P*
Watering = wet																			
Frequency	3	1711.15	4.12	**0.048**	659.97	2.08	0.181	11.01	4.63	**0.037**	8.21	0.65	0.604	564.81	3.23	*0.082*	2338.55	3.46	*0.071*
Genotype	1	2328.06	5.61	**0.045**	43.89	0.14	0.719	4.63	1.95	0.2	37.76	3	0.122	154.19	0.88	0.375	362.62	0.54	0.485
Frequency × genotype	3	1162.95	2.8	0.108	1220.15	3.85	*0.056*	2.59	1.09	0.408	33.28	2.64	0.121	968.46	5.54	**0.024**	4317.44	6.39	**0.016**
Error	8	414.88			316.63			2.38			12.59			174.7			675.44		
Total	16																		
Watering = dry																			
Frequency	3	68.69	1.78	0.229	21.59	1.15	0.385	0.74	0.82	0.518	1.36	0.77	0.541	1.57	0.38	0.77	24.13	0.63	0.616
Genotype	1	116.48	3.02	0.121	19.69	1.05	0.335	12.82	14.24	**0.005**	0.39	0.22	0.65	19.69	4.79	*0.06*	78.77	2.05	0.19
Frequency × genotype	3	38.14	0.99	0.446	15.9	0.85	0.505	0.23	0.26	0.852	1.19	0.68	0.589	0.95	0.23	0.872	11.31	0.3	0.828
Error	8	38.63			18.72			0.9			1.76			4.11			38.34		
Total	16																		

A total of 128 plants were measured for two watering regimes × four frequencies × two genotypes × two replicate trays.

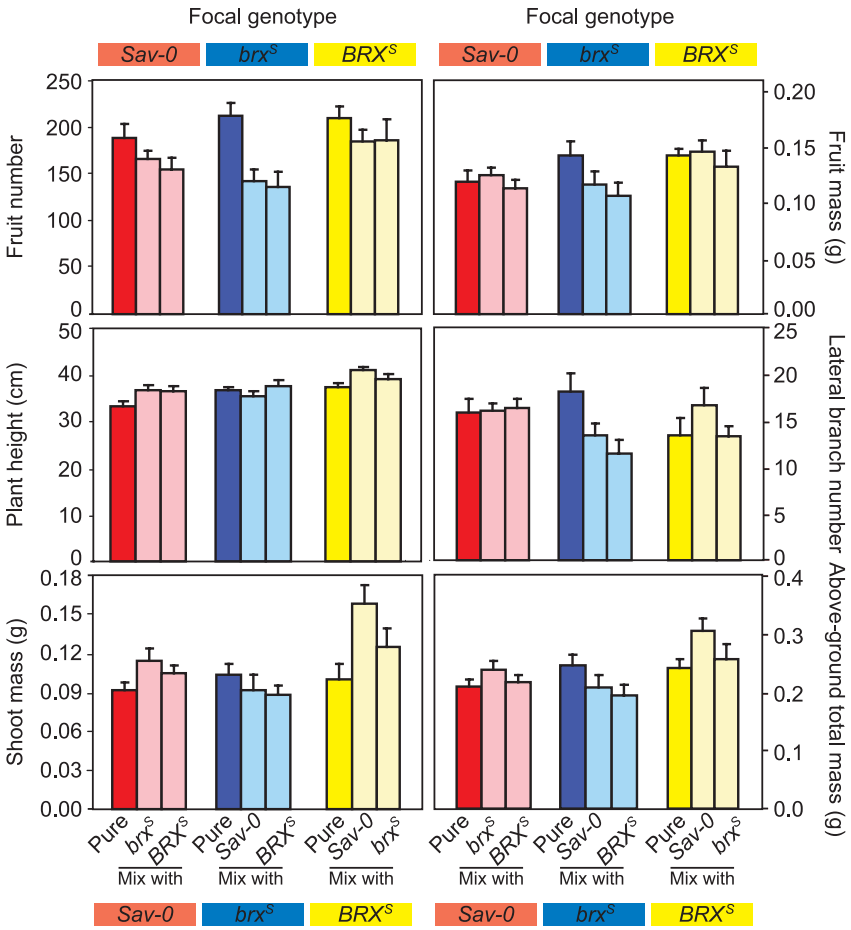

Fig. 4 Mean (± SE) reproductive output (total number of fruits produced, total fruit mass) and growth traits of brx^S, *Sav-0* and brx^S complemented by a *BRX* transgene (BRX^S) genotypes. brx^S was in competition with both its introgression parent *Sav-0* and the transgenically complemented BRX^S line, and plant performance was compared with controls (pure stands for each genotype, and mixture *Sav-0*/BRX^S). Plant density, 54 plants per tray. Genotype frequencies in mixtures, 8 : 1. Phenotypes and units of measurement as in Figs 2, 3.

background was accompanied by significant introgression drag of linked loci (Table S1), despite four backcrosses to the *Sav-0* parent (Mouchel *et al.*, 2004). Thus, it appeared possible that the observed performance decrease of brx^S plants in competitive situations might reflect genetic differences other than *brx* loss of function. To determine whether or not this is the case, we conducted another set of competition analyses that also included the BRX^S line, that is, brx^S complemented by a functional *BRX* transgene. First, we assessed plant performance in pure stands for *Sav-0*, brx^S and BRX^S. As in the previous experiment, this confirmed that all three genotypes produced a similar number of fruits in pure stands, since genotype identity had generally no significant effect on fitness components and plant performance, with only significant among-genotype differences for plant height ($F_{2,18} = 5.575$, $P = 0.013$; *Sav-0* was slightly smaller than both other genotypes; Fig. 4). Further, we compared performance of brx^S in mixed stands (brx^S : *competing genotype*) in which the competing genotype was either *Sav-0* or BRX^S. In an additional setup, we compared performance of *Sav-0* mixed stands (*Sav-0* : *competing genotype*) in which the competing genotype was either brx^S or BRX^S. For each genotype, we compared performance in the two competitive situations and the pure stand. Neither the number of fruits produced (linear mixed models: $F_{2,30} = 1.762$, $P = 0.189$) nor any other measure of *Sav-0* performance differed significantly when comparing pure stands and plots in which it competed against either brx^S or BRX^S (linear mixed models for the effect of competing genotype, with tray as random effect, all $P > 0.10$; Fig. 4). This suggests that the two lines with the functional *BRX* allele were insensitive to whether they were competing with their own genotype (pure stand), the other functional genotype or the null-allele line. By contrast, brx^S produced significantly more fruits (linear mixed models: $F_{2,32} = 5.70$, $P = 0.008$) and more lateral branches ($F_{2,31} = 3.478$, $P = 0.043$) in pure stands than when competing against either BRX^S or *Sav-0* (Fig. 4). This is consistent with the idea that decreased fitness of brx^S in the competitive situations tested resulted from a single gene polymorphism at the *BRX* locus.

Analysis of extant *Arabidopsis* population from Umkirch

At the reported *Uk-1* sampling site (Umkirch, Germany, along the banks of the old Dreisam river), we found a total of 17 *A. thaliana* plants in four separate locations (see the Materials

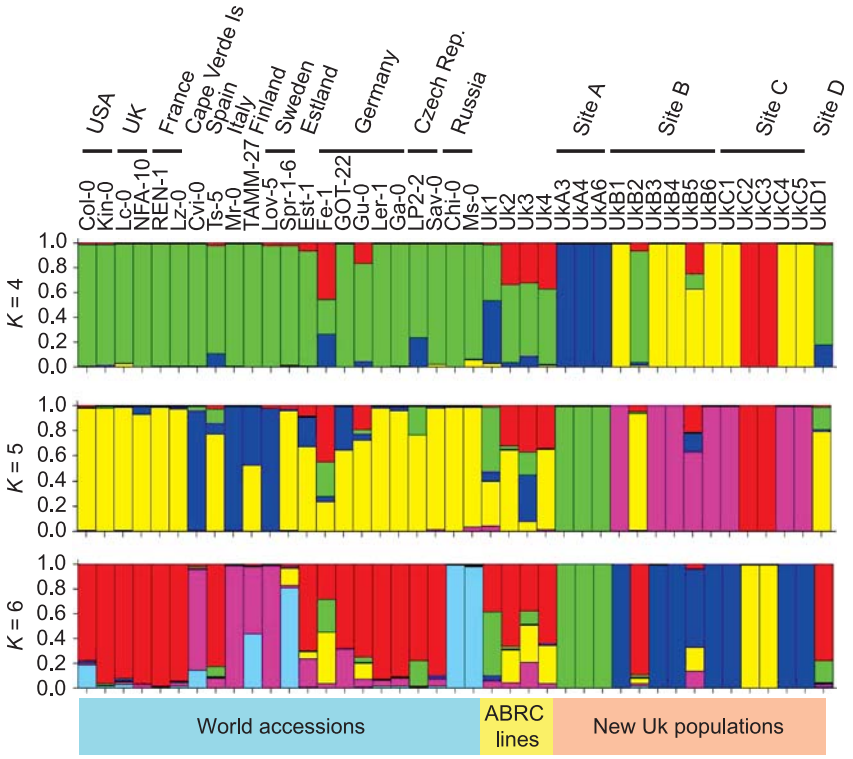

Fig. 5 *K* cluster analysis to estimate population structure in a sample of natural *Arabidopsis thaliana* accessions, based on genotyping data for 149 single nucleotide polymorphism markers spread throughout the *Arabidopsis* genome. Each accession is represented as a vertical bar; the height of colours in each bar signifies the accession's fractional assignment to a given cluster. The analysis was run for $K = 4$, 5 and 6 clusters, whereby maximum log-likelihood was reached at $K = 5$ .World accessions, representative worldwide sample of lines obtained from the *Arabidopsis* stock center; ABRC lines, Umkirch lines (*Uk1-4*) obtained from the *Arabidopsis* stock center. *Uk-1* carries the *brx* loss-of-function allele. New *Uk* populations, novel lines collected on the banks of the old Dreisam river near Umkirch in June 2006. See the Materials and Methods section for details.

and Methods section) none of which carried the *brx* loss-of-function allele. For 15 of those plants, we obtained selfed progeny, which, together with the four distinct *Uk* lines from the stock center and a representative sample of worldwide accessions, was genotyped for a set of 149 SNP markers that are evenly spread over the genome (Warthmann *et al.*, 2007; Table S1). Cluster analysis of these data indicates a unique profile of genetic structure among our new *Uk* collection (Fig. 5). The data also indicate that *Uk-1* is rather distinct from the *Uk-2*, *-3* and *-4* lines, which share a very similar profile. Within the new *Uk* lines, those collected at sample point A represent highly related individuals, which are more closely related to *Uk-1* than any of the other lines investigated. Together, these lines form a clearly genetically distinct cluster with respect to all other lines. This suggests that *Uk-1* indeed originates from the sampled region, although the *brx* loss-of-function allele might have disappeared from the extant population or is present at too low a frequency to be detected in our sample.

Discussion

Variation at the *BRX* locus directly affects competitive ability

In this study, we investigated the consequences of genetically controlled variation in root system morphology on plant performance and fitness. We investigated plants that differed at the *BRX* locus (either harboring the loss-of-function null allele or a functional allele, while controlling for genetic background) that were grown in pure stands or two-genotype mixtures at variable frequencies. Our results revealed that in pure stands, high densities lead to competitive conditions for genotypes with or without the functional allele. However, in mixtures under well-watered, competitive conditions, plants with the loss-of-function null allele suffered a significantly stronger reduction in reproductive output and in all measures of performance the higher the frequency of competing plants with the functional allele. Because both genotypes performed similarly in pure stands, this difference between genotypes in mixed, dense and well-watered stands must be directly ascribed to competition effects between genotypes. These results were further confirmed by transgenic complementation of *brx* loss of function (BRX^S), because brx^S produced more fruits in pure stands than when competing against either functional line, while *Sav-0* was apparently insensitive to the competing genotype. This indicates that variation at the *BRX* locus is directly affecting competitive ability of genotypes.

Across genotypes used in this study, plant height and above-ground morphology were very uniform. This is in line with our experimental setup, which largely excluded shading effects because of synchronized germination, equidistant plant distribution and supplemental artificial lighting directly from above. Therefore, our results also suggest that the impaired competitiveness of the *brx* loss-of-function genotype likely reflects below-ground competition.

The fate of the natural *brx* loss-of-function allele

The present study indicates that *brx* loss of function does not reduce plant performance in pure stands, but renders individuals competitively inferior in intergenotype competition. Although intergenotype competition might not always occur in natural conditions, because of the high degree of inbreeding in *Arabidopsis*, genetically diverse patches likely exist. This is, for instance, indicated by the genome-wide SNP genotyping of our newly sampled *Uk* lines. Genetic homogeneity was only observed in one (*UkA* lines) out of three plots from which multiple plants could be obtained, while the two other plots contained either individuals that had hybridized with other genotypes (*UkB* lines) or represented a mix of two clearly distinct genotypes (*UkC* lines). In such plots harboring more than one genotype, competitive exclusion of plants with the *brx* loss-of-function allele, as isolated in *Uk-1*, might have occurred. This scenario is consistent with our survey of the *BRX* locus of the novel accessions from the Umkirch collection site, which, although related to *Uk-1* (based on SNP genotyping), did not harbor the loss-of-function null allele, suggesting that this allele may be absent or present only at low frequencies at this site. Notably, it has been shown recently that crosses of *Uk-1* to at least one of the other stock center *Uk* lines are conditionally incompatible, resulting in nonviable progeny (Bomblies *et al.*, 2007). Since outcrossing is more frequent in *Arabidopsis* in field conditions than originally anticipated (Nordborg *et al.*, 2005; Bakker *et al.*, 2006), this reproductive isolation might have contributed to the disappearance of the *Uk-1 brx* allele by suppressing the propagation and maintenance of the allele in a heterozygous state, via both a mating and a competitive disadvantage.

Advantage of elongated root system in competitive situations

Variation in root system architecture is important for water and nutrient uptake (Linkohr *et al.*, 2002; Schenk, 2006). This is particularly true for annual plants that do not form symbioses with soil organisms for nutrient acquisition, such as *Arabidopsis*. Although root system size generally does not appear to limit the acquisition of important mobile macronutrients (Robinson, 1996), such as nitrate, exploration of a larger soil volume by the root system is advantageous when immobile macronutrients, such as phosphate, or water availability, are limiting growth. This becomes particularly apparent in competitive situations, where a faster-growing, more branched and/or more elongated root system can exploit a larger soil volume per time unit (Schenk, 2006). An effect of root morphology on nutrient uptake has been conclusively demonstrated in a study that compared the artificial *Arabidopsis* mutant *axr4*, which displays significantly decreased amounts of root branching, with its wild-type background (Fitter *et al.*, 2002). In these experiments, *axr4* performance and reproductive success was generally reduced across conditions. As a competitor in 1 : 1 mixture plots, *axr4* and wild-type were equally effective, except when phosphate concentrations were low. Thus in future studies, it would be interesting to identify the exact mechanism mediating relative competitive ability of plants that differ at the *BRX* locus through similar experimental manipulations of resource availability.

Finally, substantial phenotypic plasticity has been reported for many genotypes (Pigliucci, 2002). Because of this, and because of differential gene expression in different environments, we cannot exclude the possibility that *brx* may confer an advantage under some environmental conditions, other than those tested in the experiment, either through its direct effects on root phenotype or because of pleiotropic effects.

Conclusion

In conclusion, our results demonstrate that under competitive conditions of high density, single gene polymorphism at the *BRX* locus has a conditional impact on plant performance and fitness. Our approach may be widely applicable for the functional analysis of fitness consequences of natural variation with known genetic basis in *Arabidopsis*.

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Supplementary Material

The following supplementary material is available for this article online:

Fig. S1 Watering regimes for dry and wet conditions.

Table S1 Single nucleotide polymorphism marker genotypes for accessions as indicated in the Materials and Methods section