

The genetics of intra- and interspecific competitive response and effect in a local population of an annual plant species

Etienne Baron^{1,2,3}, Julien Richirt¹, Romain Villoutreix¹, Laurent Amsellem¹ and Fabrice Roux^{*,1,2,3}

¹Laboratoire Génétique et Evolution des Populations Végétales, UMR CNRS 8198, Université des Sciences et Technologies de Lille – Lille 1, F-59655 Villeneuve d'Ascq Cedex, France; ²INRA, Laboratoire des Interactions Plantes-Microorganismes (LIPM), UMR441, F-31326 Castanet-Tolosan, France; and ³CNRS, Laboratoire des Interactions Plantes-Microorganismes (LIPM), UMR2594, F-31326 Castanet-Tolosan, France

Summary

1. While competition is recognized as a major factor responsible for plant community dynamics, the genetics of intra- and interspecific competitive ability of a target species (i.e. level of intra-population genetic variation, identity of phenotypic traits under selection and genetic bases) still deserves a deeper investigation at the local spatial scale by considering both numerous genotypes and several interacting species.

2. In this study, we tested whether the genetics of competitive response and effect in *Arabidopsis thaliana* was dependent on the competitive environment at both the intraspecific and interspecific levels. We used a mapping population of 48 accessions (i) that maximize the genetic diversity of a local population of *A. thaliana* and (ii) that have been genotyped for 168 503 single nucleotide polymorphisms.

3. In a common garden experiment, those 48 accessions were grown in six competitive environments: the absence of competition, intraspecific competition and interspecific competition with four species frequently associated with *A. thaliana* in natural plant communities (i.e. *Poa annua*, *Stellaria media*, *Trifolium repens* and *Veronica arvensis*). A suite of nine phenotypic traits, including a proxy of fitness, were scored on each target *A. thaliana* plant and the above-ground dry biomass of its corresponding competitor was estimated.

4. We first showed that crossing reaction norms of competitive response (*A. thaliana* performance) and effect (competitor biomass) might promote maintenance of genetic variation in a local population of *A. thaliana* and species coexistence at a fine spatial scale. By estimating genotypic gradients of selection, we then demonstrated that the optimal phenotypic strategies in response to competition depend on the identity of the competitor species. Finally, a genome-wide association mapping approach highlighted that genomic regions associated with direct genetic effects were (i) dependent on the competitor species and (ii) different from genomic regions associated with interspecific indirect genetic effects.

5. While a first step, this study highlighted the power of adding ecology to genomics in *A. thaliana* to identify genetic bases underlying micro-geographic adaptation to competition. Next-generation sequencing technologies will undoubtedly facilitate the discovery of molecular and genetic mechanisms underlying competitive ability in other plant species, and thereby the prediction of evolutionary trajectories of plant communities.

Key-words: competition, ecological genomics, fitness-related traits, genome-wide association mapping, gradient of natural selection, reaction norms

*Correspondence author. E-mail: fabrice.roux@toulouse.inra.fr

Introduction

Among the processes described in community assembly theories (Martorell & Freckleton 2014), competition is recognized as a major factor responsible for plant community structure (Golberg & Barton 1992), diversity (Chesson 2000) and dynamics (Tilman 1985) by governing coexistence between species. Competition may affect metacommunity-level dynamics in interaction with evolutionary forces (Urban 2006). For instance, resources available for one plant can be limited by competition, which may decrease effective size of the population and thus its rate of adaptation to a potentially new or changing environment (Johansson 2008), like those expected under global change (Lau *et al.* 2014).

While the integration of both ecology and evolution appears as a priority to understand adaptive dynamics of local populations in the presence of competition (Post & Palkovacs 2009), three intertwined points related to the genetics of competition (level of intra-population genetic variation, identity of phenotypic traits under selection and genetic bases, i.e. genomic regions associated with natural variation of competitive ability and the associated underlying genetic variants) still deserves a deeper investigation. First, competitive response (target plant performance as a function of a neighbour plant) and effect (influence of a target plant on the performance of a neighbour) have been shown to be highly genotype-dependent at both the intraspecific and interspecific levels (e.g. Cahill, Kembel & Gustafson 2005; Bossdorf, Shuja & Banta 2009; Willis, Brock & Weinig 2010). However, despite the short distance within which plants interact with neighbours (Purves & Law 2002), only a small proportion of studies considered genotypes collected in the same local community (e.g. Turkington & Harper 1979; Aarssen & Turkington 1985; Aarssen 1989; Vavrek 1998; Fridley, Grime & Bilton 2007). While informative, these studies were, however, generally based on (i) few genotypes (generally <10) potentially impeding the estimate of the extent of the natural variation for competitive ability of the target species at a small spatial scale and/or (ii) few interacting species. By growing, in the presence of several competitor species, genotypes that maximize the genetic diversity of a local population, one can estimate the extent of natural variation for competitive response and effect, which may in turn help to fully estimate the adaptive potential of this local population in its local community.

Secondly, the identity of genotypes with the best fitness may depend on both the presence of intra- and interspecific competition and the identity of the competitor species (Vavrek 1998; Dorn, Hammond Pyle & Schmitt 2000; Weinig *et al.* 2006; Fridley, Grime & Bilton 2007; Bossdorf, Shuja & Banta 2009; Brachi *et al.* 2012), suggesting the presence of genotypes that are well adapted to interactions with neighbour plants as well as biotic specialization of some genotypes to a given competitor

species. Further tests at the genotypic level are required to determine whether the identity of the traits under selection in a natural plant population – as well as the strength and nature of selection acting on those traits – varies among competitive environments. If reaction norms for fitness of target genotypes cross among competitive environments, variation in the identity of the traits under selection should favour the maintenance of genetic diversity of functional traits in local plant populations.

Thirdly, identifying genes associated with natural variation of intra- and interspecific competitive response and effect is recognized as an important step to predict and understand adaptive dynamics and evolutionary trajectories of plant communities (Pierik, Mommer & Voesenek 2013). While molecular mechanisms of plant competition started to be elucidated by laboratory mutant analysis in model species like the mouse-ear cress *Arabidopsis thaliana* (L.) Heynh (Pierik, Mommer & Voesenek 2013), identification of natural genetic variants associated with competitive ability offers both (i) a complementary approach to unravel molecular mechanisms associated with either neighbour detection or response strategies and (ii) an exciting opportunity to further study the traces of selection acting on those variants (Bergelson & Roux 2010). However, only few studies have dealt with the identification of quantitative trait loci (QTL) involved in plant–plant interactions. Notably, by growing a mapping population of 411 recombinant inbred lines (RILs) of *A. thaliana*, Mutic & Wolf (2007) detected that most QTLs directly associated with the response of *A. thaliana* target plants (direct genetic effects, DGEs) to intraspecific competition also had an indirect effect on the expression of developmental traits of their neighbour plants (indirect genetic effects, IGEs; Moore, Brodie & Wolf 1997; Genung, Bailey & Schweitzer 2013). This suggests a pleiotropic relationship between DGE and IGE loci in a conspecific neighbourhood. To our knowledge, no study has been designed to identify QTLs underlying plant–plant interactions in a natural population at a community scale. Whether QTLs associated with DGEs in the target species are identical to QTLs having an indirect effect on the phenotypic expression of neighbours in a heterospecific context (interspecific indirect genetic effects, IIGEs; Genung, Bailey & Schweitzer 2013) remains an open question. Because genome-wide association (GWA) mapping has been demonstrated to be an efficient tool to finely map genes involved in plant–pathogen interactions (e.g. Huard-Chauveau *et al.* 2013), GWA mapping may appear promising to identify molecular underpinnings of other biotic interactions such as intra- and interspecific competitive response and effect (Bergelson & Roux 2010).

In this study, we tested whether the genetics of competitive response and effect (i.e. level of genetic variation, identity of traits under selection and genetic bases) in *A. thaliana* was dependent on the competitive environment

at both the intraspecific and interspecific levels. As previously advised, mapping populations used to identify genomic regions associated with ecologically relevant traits should be chosen according to the scale of adaptive phenotypic variation (Bergelson & Roux 2010; Brachi *et al.* 2013). Because plants interact with neighbours over short distances (i.e. the adaptive spatial scale should be small), we focused on a mapping population of 48 accessions (i) that maximize the genetic diversity of a local population of *A. thaliana* and (ii) that have been genotyped for 168 503 single nucleotide polymorphisms (SNPs).

In a common garden experiment, those 48 accessions were grown in six competitive environments: absence of competition, intraspecific competition and interspecific competition with four species with different growth forms and frequently associated with *A. thaliana* in natural plant communities (i.e. *Poa annua*, *Stellaria media*, *Trifolium repens* and *Veronica arvensis*). *A. thaliana* target plants were phenotyped for a suite of nine phenotypic traits, including seed production as a proxy for plant fitness, while performance of competitors was estimated by above-ground dry biomass. Specifically, we first tested for biotic specialization by describing the ‘accession × competitive environment’ interactions for both seed production of target *A. thaliana* plants and performance of competitors. Then, by estimating genotypic gradients of selection, we tested whether the identity of traits under selection in our local population of *A. thaliana* as well as the strength and nature of selection acting on them vary between the six competitive environments. Finally, by a GWA mapping approach, we tested whether genomic regions associated with DGEs (i) were dependent on the competitive environment and/or (ii) were identical to genomic regions associated with IIGEs.

Materials and methods

PLANT MATERIAL

We focused on a set of 48 accessions of *A. thaliana* collected in 2002 in the TOU-A population, located under a 350 m electric fence separating two permanent meadows that experience cycles of periodic grazing by cattle. This local mapping population has been previously demonstrated to be powerful enough to fine-map genomic regions associated with natural phenotypic variation (Appendix S1, Supporting information).

We used four competitor species commonly associated with *A. thaliana* in natural plant communities in France (<http://junon.u-3mrs.fr/msc41www/PSHTM/PS2135.htm>) and observed in the TOU-A plant community (F. Roux, personal observation). These species are the meadow grass *P. annua* (Poaceae) with a low spreading growth form, the haresfoot clover *Trifolium arvense* (Fabaceae) with an erect bearing, the chickweed *S. media* (Caryophyllaceae) and the speedwell *V. arvensis* (Scrophulariaceae) both with a crawling growth form. Seeds for these four species were ordered to the company Herbiseeds (<http://www.herbiseed.com/home.aspx>).

COMMON GARDEN EXPERIMENT

An experiment of 1152 target plants was set up at the University of Lille 1 (North, France) in March 2011 using a split-plot design arranged as a randomized complete block design (RCBD) with six treatments nested within four blocks (Fig. S1, Supporting information). The six treatments correspond to (i) one control treatment without competition (treatment C), (ii) one intraspecific competition treatment (treatment A) and (iii) four interspecific competition treatments using the four species *P. annua*, *S. media*, *T. arvense* and *V. arvensis*; hereafter named treatments P, S, T and V, respectively. In addition, the growth behaviour of the four interspecific competitor species was estimated by growing *P. annua*, *S. media*, *T. arvense* and *V. arvensis* in the absence of *A. thaliana* (Fig. S1). Details of the common garden experiment are provided in Appendix S2, Supporting information.

PHENOTYPIC CHARACTERIZATION

A total of eight phenotypic traits related to phenology (bolting time BT, interval between bolting and flowering INT and length of reproductive period RP, resource accumulation (maximum diameter of the rosette FT_diam), architecture and seed dispersal (number of basal branches RAMBB, number of primary branches with fruits on the main stem RAMPB_S and height from soil to the first fruit on the main stem HIS), response strategy to competition (ratio HD as HIS/FT_diam; high and low values of HD would correspond to an escape and aggressive strategy, respectively) were scored on each target *A. thaliana* plant.

In addition, total seed production as a good proxy of lifetime fitness for a selfing annual species like *A. thaliana* (Roux, Gasquez & Reboud 2004) was estimated by total fruit length (FITTOT) on each target *A. thaliana* plant.

At the time when an *A. thaliana* target plant was harvested, performance of competitors was estimated by above-ground dry biomass (COMP). At the end of the experiment, we also estimated the above-ground dry biomass of plants of *P. annua*, *S. media*, *T. arvense* and *V. arvensis* grown in the absence of *A. thaliana*.

Details on the measuring of all phenotypic traits are provided in Appendix S3 (Supporting information).

DATA ANALYSIS

Natural variation and reaction norms

To explore the genetic variation of competitive response among the 48 TOU-A accessions, we used the following statistical model (PROC MIXED procedure, REML method, SAS 9.3; SAS Institute Inc., Cary, NC, USA):

$$Y_{ijk} = \mu_{\text{trait}} + \text{block}_i + \text{competition}_j + \text{block}_i \times \text{competition}_j + \text{accession}_k + \text{competition}_j \times \text{accession}_k + \varepsilon_{ij}, \quad \text{eqn 1}$$

where ‘Y’ is one of the nine phenotypic traits scored on target *A. thaliana* plants, ‘μ’ is the overall phenotypic mean; ‘block’ accounts for differences in microenvironment among the four experimental blocks; ‘competition’ corresponds to effect of the six competition treatments (C, A, P, T, S and V); ‘accession’ measures the effect of the 48 accessions; the interaction term ‘competition × accession’ accounts for genetic variation in reaction norms across the six competition treatments; and ‘ε’ is the residual term. Model (1) was also run to explore the genetic variation of competitive effect of *A. thaliana* on competitor species, with ‘Y’ corresponding to the COMP trait and ‘competition’ that only considers the treatments A, P, S, T and V. All factors were treated as fixed

effects because levels of no factor were random samples from a population to which we intended to extrapolate (Appendix S4, Supporting information). Given the split-plot design used in this study, terms were tested over their appropriate denominators for calculating *F*-values. Two phenotypic traits HD and COMP were log-transformed to satisfy the normality and equal variance assumptions of linear regression.

For each treatment, least-square means (LSmeans) for each phenotypic trait were obtained for each accession using the following model in the *R* environment (R Core Team 2013).

$$Y_{ik} = \mu_{\text{trait}} + \text{block}_i + \text{accession}_k + \varepsilon_{ik}. \quad \text{eqn 2}$$

Because *A. thaliana* is a highly selfing species, LSmeans correspond to genotypic values of accessions. For the competition treatments A, P, S, T and V, the relationship between competitive response and competitive effect was tested by estimating a non-parametric Spearman coefficient of correlation between FITTOT and COMP.

To quantify phenotypic plasticity of the 48 accessions across the six treatments, a coefficient of variation $CV_{6\text{treatments}}$ was calculated for each 'accession \times phenotypic trait' combination based on genotypic values (Valladares, Sanchez-Gomez & Zavala 2006).

Genotypic gradients of selection in *A. thaliana*

To identify the traits under linear and nonlinear selection in each of the six treatments, we run genotypic selection analyses (Rauscher 1992) based on genotypic values with the following polynomial regression (PROC GLM procedure; SAS 9.3, SAS Institute Inc.):

$$\text{Relative fitness}_i = \mu_{\text{relative fitness}} + \text{trait}1_i + \text{trait}1_i^2 + \dots + \text{trait}8_i + \text{trait}8_i^2 + \varepsilon_i, \quad \text{eqn 3}$$

where 'Relative fitness' is the relative fitness within each treatment computed as the fitness estimate divided by the mean fitness estimate within that treatment, ' μ ' is the constant, 'trait1' to 'trait8' correspond to the eight phenotypic traits (BT, INT, RP, FT_diam, H1S, HD, RAMPB_S and RAMBB) standardized within each treatment, and ε is the residual term. Linear selection gradients were estimated by linear partial regression coefficients, whereas quadratic regression coefficients were doubled to correctly estimate nonlinear selection gradients (Stinchcombe *et al.* 2008).

For each treatment, we discriminated between different types of nonlinear selection (i.e. curvilinear and stabilizing/disruptive selection; Appendix S4). In addition, we also tested for significant differences in linear and nonlinear selection gradients between the six treatments; in particular between each interspecific competition treatment and either the control treatment or the intraspecific competition treatment (Appendix S4).

Genome-wide association mapping

Phenotype-genotype associations were tested at each SNP by linear regression based on LSmeans. Given the number of accessions ($n = 48$), linear regression was run at each SNP with a minor allele relative frequency (MARF) $> 20\%$ ($n = 87\,160$). Significant SNPs were identified according to the permuting scheme described in Alkorta-Aranburu *et al.* (2012). Details on the GWA mapping analysis performed in this study are provided in Appendix S4.

In the vicinity of the most associated significant SNPs (i.e. within 20 kb; Brachi *et al.* 2010), we searched for candidate genes belonging to one of the four following biological processes: light-quality signals, nutrient levels, soluble root exudates and

volatile organic compounds (VOCs; Pierik, Mommer & Voisenek 2013).

Results

NATURAL GENETIC VARIATION WITHIN THE TOU-A POPULATION

Competitive response

A significant 'competition treatment' effect was detected for four phenotypic traits scored on *A. thaliana* target plants, including total seed production (Table S1, Supporting information, Fig. 1). TOU-A accessions produced on average less seeds in the presence than in the absence of competition (except in the presence of *T. arvense*; Fig. 1). Based on total seed production, the competitive hierarchy was as follows: *S. media* $>$ *A. thaliana* $>$ *V. arvensis* $>$ *P. annua* $>$ *T. arvense* (Fig. 1).

Highly significant genetic variation was found for the nine traits scored on *A. thaliana* target plants (Table S1). More importantly, as evidenced by highly significant 'Competition treatment \times Accession' interactions, strong genetic variation of reaction norms was found for most traits (Table S1), such as bolting time and total seed production (Fig. 2a,b).

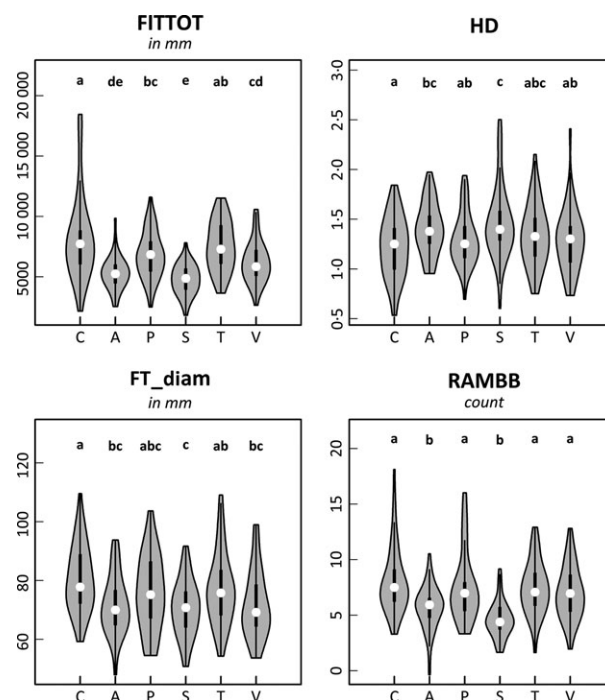


Fig. 1. Violin plots (i.e. box-and-whisker plot overlaid with a kernel density plot) of genetic natural variation across the six competition treatments (C, A, P, S, T and V) for total seed production (FITTOT), maximum rosette diameter at flowering (FT_diam), the ratio 'height from soil to the first fruit on the main stem/maximum rosette diameter at flowering' (HD) and the number of basal branches (RAMBB). For each trait, different letters indicate different groups after pairwise comparisons using a *P*-value threshold of 0.01.

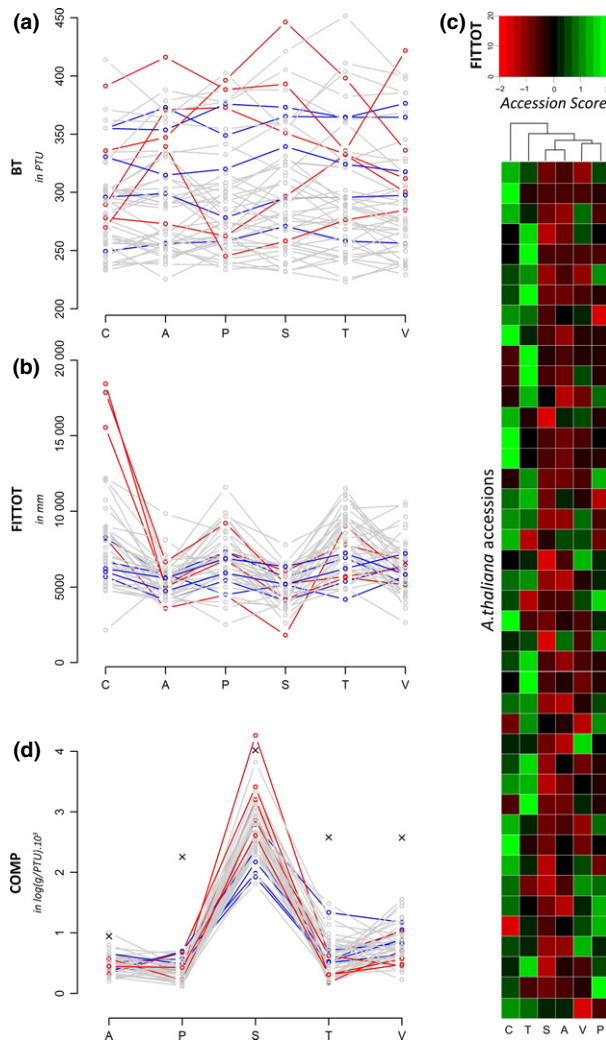


Fig. 2. Natural genetic variation of reaction norms of the 48 TOU-A accessions. (a) Bolting time (BT). (b) Total seed production (FITTOT). (c) Heatmap of total seed production of each accession according to the six competition treatments (package plot R). Each line corresponds to one of the 48 accessions. For each accession, the six competition treatments (C, A, P, S, T and V) were ranked according to FITTOT, with the light red and light green squares indicating the treatments with the lowest and the highest seed production, respectively. (d) Above-ground dry biomass of competitors (COMP). For each trait (BT, FITTOT and COMP), the five accessions with the highest phenotypic plasticity across the competition treatments and the five accessions with the lowest phenotypic plasticity across the competition treatments are represented by blue and red reaction norms, respectively. In panel 'd', crosses indicate the mean above-ground dry biomass of competitors grown in the absence of *A. thaliana*.

Interestingly, for each trait, we identified both genotypes with high phenotypic plasticity across the six competition treatments and genotypes with a nearly flat reaction norm for response to competition (Fig. 2a,b). In addition, the ranking of accessions for total seed production differed with competition treatment (Fig. 2b,c), with some accessions producing more seeds in competition with either *P. annua*, *T. arvense* or *V. arvensis* than in the absence of competition (Fig. 2b). Based on genotypic values, the cross-environment

Pearson's coefficient of genetic correlation for total seed production ranged from 0.04 (CI: $-0.26, 0.33$) for the 'Control – *P. annua*' environments to 0.41 (CI: $0.13, 0.63$) for the '*A. thaliana* – *S. media*' environments.

Competitive effect

For each interspecific competitor species, above-ground dry biomass was on average reduced by the presence of *A. thaliana*, suggesting a potentially strong competitive effect of the target plants on neighbour plants (Fig. 2d). In addition, a significant 'Competition \times Accession' interaction effect was detected for the effect of *A. thaliana* target plants on neighbour competitors (Table S1), with the ranking of accessions for their effect on above-ground dry biomass that largely differed with competitor species (Fig. 2d). Similarly to competitive response, we identified accessions with a high phenotypic plasticity for competitive effect. In this case, some accessions with almost no competitive effect on *S. media* were among the accessions with the strongest competitive effect on both *T. arvense* and *V. arvensis* (Fig. 2d).

Relationships between competitive response and competitive effect

While a slightly positive relationship between competitive response and competitive effect was found for the intraspecific competition treatment (Spearman $\rho = 0.33$, $P = 0.023$), no significant relationship between competitive response and competitive effect was found in the four interspecific competition treatments (*P. annua*: Spearman $\rho = 0.11$, $P = 0.443$; *S. media*: Spearman $\rho = 0.13$, $P = 0.399$; *T. arvense*: Spearman $\rho = -0.02$, $P = 0.882$; *V. arvensis*: Spearman $\rho = -0.01$, $P = 0.929$).

GENOTYPIC GRADIENTS OF SELECTION

The identity of the traits under selection as well as the strength and nature of selection acting on them largely differed between the six competition treatments (Table 1). First, different optimal phenotypic strategies were identified between the absence and presence of competition. For instance, while a longer reproductive period is favoured in the absence of competition, no significant relationship between the length of the reproductive period and relative fitness was detected in the intraspecific, *P. annua* and *T. arvense* competition treatments. In contrast, while a significant stabilizing selection was observed for the interval between bolting and flowering in the *P. annua* competition treatment, no significant relationship between the interval between bolting and flowering and relative fitness was detected in the absence of competition.

Secondly, we identified different optimal phenotypic strategies between intraspecific and interspecific competition treatments (Table 1). While early-flowering was favoured in the intraspecific competition treatment, a higher number of

Table 1. Genotypic selection analysis with linear (β) and nonlinear (γ) selection gradients for eight phenotypic traits in each competition treatment

	Competition treatments														
	C		A		P		S		T		V				
Traits	E	P	E	P	E	P	E	P	E	P	E	P			
Linear selection gradients (β)															
BT	-0.40	*	-0.23	*	-0.07	ns	-0.24	**	-0.36	***	-0.15	ns			
INT	-0.11	ns	-0.05	ns	0.05	ns	-0.06	ns	-0.04	ns	-0.07	ns			
FT_diam	0.38	ns	-0.01	ns	-0.32	ns	0.38	**	0.23	ns	0.23	ns			
RP	0.20	*	-0.04	ns	0.02	ns	0.10	*	0.00	ns	0.09	ns			
RAMBB	0.21	*	-0.04	ns	0.19	***	0.06	ns	0.11	*	0.06	ns			
RAMPB_S	0.01	ns	0.02	ns	0.03	ns	0.12	**	-0.16	*	0.09	*			
H1S	-0.06	ns	0.18	ns	0.63	*	-0.57	**	0.02	ns	-0.16	ns			
HD	0.17	ns	-0.15	ns	-0.59	*	0.78	**	0.15	ns	0.19	ns			
Nonlinear selection gradients (γ)															
BT ²	-0.32	ns	0.03	ns	-0.03	ns	0.21	ns	-0.08	ns	-0.03	ns			
INT ²	0.13	ns	-0.08	ns	-0.11	S	**	-0.05	ns	-0.05	ns	0.04	ns		
FT_diam ²	0.24	ns	0.02	ns	0.14	D	*	-0.12	ns	0.04	ns	0.01	ns		
RP ²	0.05	ns	-0.04	ns	-0.01	ns	-0.21	S	*	-0.18	S	*	-0.20	S	*
RAMBB ²	-0.19	ns	0.05	ns	-0.08	ns	-0.10	ns	-0.11	ns	-0.13	ns			
RAMPB_S ²	0.04	ns	-0.03	ns	-0.14	S	**	0.09	ns	0.13	ns	0.08	ns		
H1S ²	0.08	ns	-0.10	ns	-0.14	C	*	-0.14	ns	-0.06	ns	0.00	ns		
HD ²	-0.14	ns	0.11	ns	0.17	D	*	-0.03	ns	0.00	ns	-0.07	ns		

BT, Bolting time; INT, flowering interval; RP, reproductive period; FT_diam, maximum rosette diameter at flowering; H1S, height from soil to the first fruit on the main stem; HD, ratio H1S/FT_diam; RAMBB, number of basal branches; RAMP_BS, number of primary branches with fruits on the main stem; ns, non-significant.

'S' and 'D' denote stabilizing ($\gamma < 0$) and disruptive ($\gamma > 0$) selection, respectively. Curvilinear selection was notified as 'C'. Bold values indicate a significant difference with the control treatment. Italic values indicate a significant difference with the intraspecific competition treatment.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

basal branches and a smaller height from soil to the first fruit on the main stem were favoured in the *P. annua* and *S. media* competition treatments, respectively.

Finally, the optimal strategy of *A. thaliana* was also found to depend on the identity of the competitor in a heterospecific context (Table 1). For instance, opposite response strategies were favoured between the *P. annua* and *S. media* competition treatments. While an escape strategy (high HD value) was favoured in the presence of *S. media*, accessions with an aggressive strategy (low HD value) were favoured in the presence of *P. annua* (contrast test; $F = 10.56$, $P = 0.001$).

IDENTIFICATION OF GENOMIC REGIONS ASSOCIATED WITH NATURAL VARIATION OF COMPETITIVE RESPONSE AND EFFECT

We identified five genomic regions significantly associated with competitive response, that is, DGEs (Fig. S2, Supporting information). The first association peak located at the bottom of chromosome IV was shared across the six competition treatments and was associated with bolting time variation, with the most significant SNPs located in the vicinity of the candidate gene *FRIGIDA* (Fig. S3a, Supporting information). In contrast, the second and third association peaks were specific to one specific

'trait x competition treatment' combination, that is, SNP 1_14164428 on chromosome I for the height from soil to the first fruit on the main stem scored in the absence of competition and SNP 5_3021231 on chromosome V for the number of basal branches scored in competition with *P. annua* (Fig. S3b and S3c). While no obvious candidate gene was identified in the vicinity of SNP 1_14164428, the closest candidate gene to SNP 5_3021231 (i.e. 751 bp apart) encodes the histone acetyltransferase HAM2 (*AT5G09740*). Interestingly, the fourth and fifth association peaks were both identified for the length of reproductive period, but were located in two separate genomic regions on chromosome V and were specific to an interspecific competition treatment (Fig. 3a). SNP 5_9317055 explains 39.2% of natural variation of the reproductive period length in the presence of *S. media* but has no detectable effect in the other competition treatments (Fig. S4a, Supporting information). SNP 5_26491091 explains 43.4% of natural variation of the reproductive period length in the presence of *V. arvensis* but has almost no detectable effect in the other competition treatments (Fig. S4b). The closest candidate gene to SNP 5_9317055 (i.e. 1.2 kb apart) encodes a pectinacetyl-lesterase (*AT5G26670*), while the closest candidate gene to SNP 5_26491091 (i.e. 13.3 kb apart) encodes a SHI (for Short Internodes) protein (*AT5G66350*).

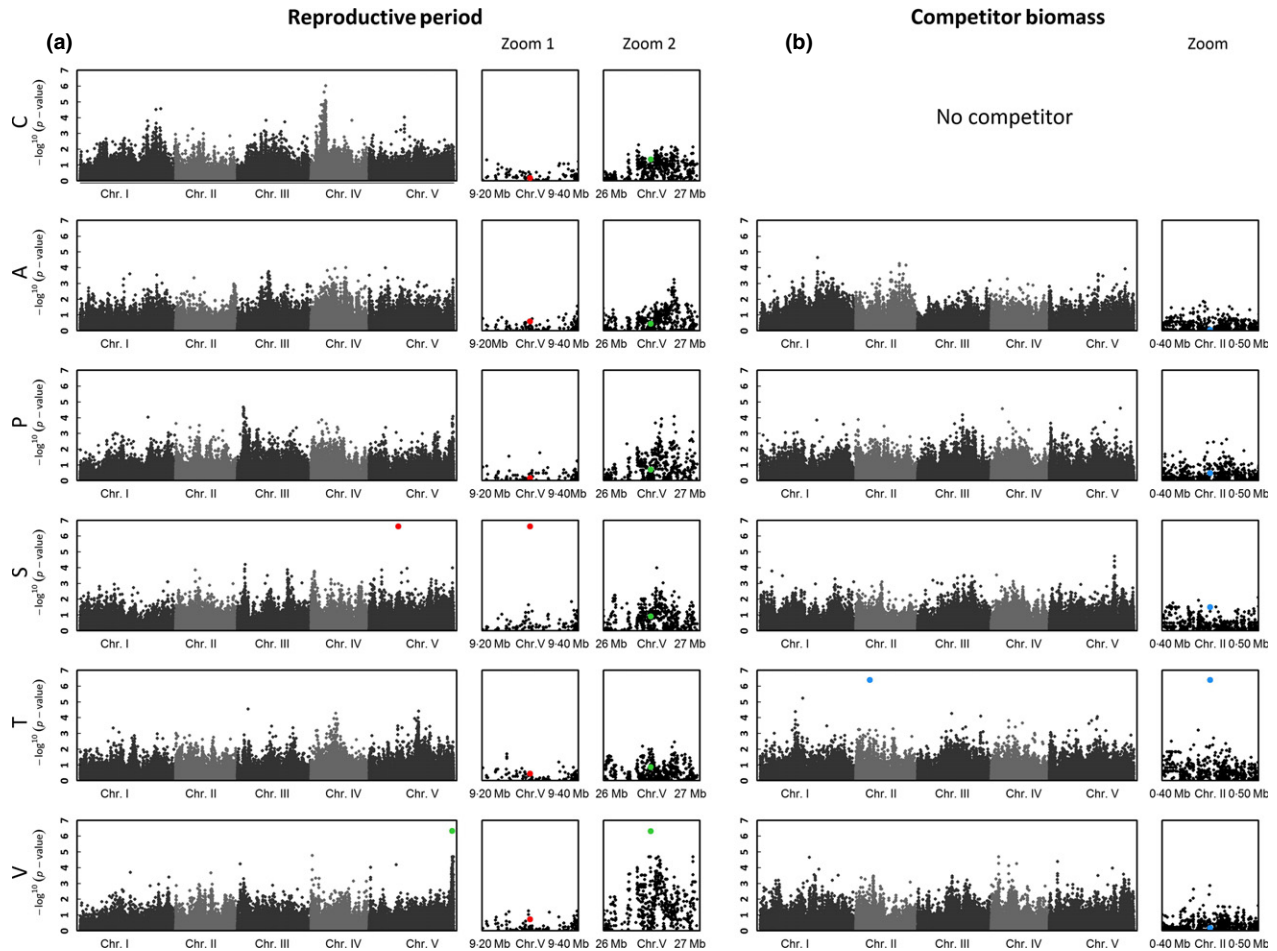


Fig. 3. Genomic regions associated with phenotypic variation in each of the six competition treatments (C, A, P, S, T and V). (a) Length of reproductive period (RP). Left panel: Manhattan plots of the genome-wide association mapping results (MARF > 20%). Red and green dots indicate the most associated SNPs with RP natural variation when *A. thaliana* accessions were grown in the presence of *S. media* (SNP_5_9317055) and *V. arvensis* (SNP_5_26491091), respectively. Mid-panel: Zoom on the most associated SNP with RP natural variation in the *S. media* treatment (plotting window 200 kb). Right panel: Zoom on the most associated SNP with RP natural variation in the *V. arvensis* treatment (plotting window 1 Mb). (b) Above-ground dry biomass of competitor (COMP). Left panel: Manhattan plot of the genome-wide association mapping results (MARF > 20%). The blue dot indicates the most associated SNP with above-ground dry biomass of *T. arvense* in response to 48 *A. thaliana* accessions (SNP_2_4537845). Right panel: Zoom on the most associated SNP with above-ground dry biomass of *T. arvense* in response to 48 *A. thaliana* accessions (plotting window 100 kb).

We identified a single significant association peak located on chromosome II that was associated with the competitive effect of *A. thaliana* on above-ground dry biomass of neighbour competitors, that is, IIGEs (Fig. 3b). SNP 2_4537845 in *A. thaliana* explains 45.5% of above-ground dry biomass of *T. arvense* but has no detectable indirect effect on the other competitor species (Fig. S4c). No obvious candidate gene was identified in the vicinity of SNP 2_4537845.

Discussion

By growing 48 *A. thaliana* accessions in six competitive environments, we aimed at conducting a multidisciplinary approach to study the genetics of competitive response and effect in a natural population at a community scale. First, we suggest that crossing reaction norms of competitive ability of *A. thaliana* can promote maintenance of

both its genetic variation at a local spatial scale and coexistence between its competitor species. By estimating genotypic gradients of selection, we then showed that the optimal phenotypic strategies in response to competition depend on the identity of the competitor species. Finally, a GWA mapping approach was adopted to identify QTLs underlying competitive ability. We found that genomic regions associated with DGEs were (i) dependent on the competitor species and (ii) different from genomic regions associated with interspecific indirect genetic effects (IIGEs).

CROSSING REACTION NORMS OF COMPETITIVE ABILITY MIGHT PROMOTE MAINTENANCE OF GENETIC VARIATION AND SPECIES COEXISTENCE

Competition is known to promote the maintenance of genetic diversity in local plant populations (Fridley, Grime

& Bilton 2007; Matesanz *et al.* 2011) through biotic specialization and ultimately co-evolution in a restricted neighbourhood (Turkington 1989; Vellend 2006). Because habitat preferences related to competition are clearly different among the 48 accessions used in this study, with no accession that is maximally fit across the five competitor species, micro-geographic adaptation to competition may be suggested in the local TOU-A population.

In agreement with Cahill, Kembel & Gustafson (2005), we found that competitive response may evolve independently from competitive effect in *A. thaliana*, especially in a heterospecific neighbourhood. In addition, while *A. thaliana* is considered as a ruderal species, we found crossing reaction norms for competitive effect which might (i) promote coexistence among species by the use of different resources (Urban 2006; Fridley, Grime & Bilton 2007; Lankau & Strauss 2007; Johnson *et al.* 2008; Matesanz *et al.* 2011) and (ii) facilitate phenotypic evolution in response to selection by different competitors (Vellend 2006; Johnson *et al.* 2008) in the local TOU-A plant community.

OPTIMAL PHENOTYPIC STRATEGY DEPENDS ON THE IDENTITY OF THE COMPETITOR SPECIES

The difference between the six competitive environments in the identity of the traits under selection as well as the strength and nature of selection acting on them may result from the different growth forms of the competitor species. We observed an escape strategy in response to *S. media* with a crawling growth form and an aggressive response strategy to *P. annua* with a low spreading growth form. While a crawling growth form may have led to an increased competition for light in *A. thaliana*, we cannot rule out that optimal phenotypic strategies estimated on above-ground traits did not result from below-ground plant–plant interactions (Bossdorf, Shuja & Banta 2009). For instance, *S. media* has been described to produce allelopathic water-soluble phenolics in the soil (Inderjit & Dakshini 1998), which may have affected *A. thaliana* growth in our study. Physiological experiments are clearly needed to disentangle the resources (such as light, nutrients and water) and/or chemical substances (such as soluble root exudates) limiting the growth of *A. thaliana* in a heterospecific competitive environment (Pierik, Mommer & Voesenek 2013).

The presence of different optimal phenotypic strategies between the six competitive environments suggests that competition can maintain functional diversity in *A. thaliana* at a small spatial scale and consequently, increase the phenotypic space associated with a niche expansion of the local population studied here (Agashe & Bolnick 2010). Numerous studies already demonstrated that both intra-specific and interspecific competitions allow niche expansion of a local population, by favouring the use of novel resources (Bolnick *et al.* 2010), and by facilitating genetic variation within the population (Agashe & Bolnick 2010).

As a positive feedback, intra-population genetic variation of response to different competitors may reduce intraspecific competition, which will in turn increase biotic specialization at a micro-spatial scale (Johnson *et al.* 2008; Whitlock *et al.* 2011). Because plants are often surrounded by multiple species, further experiments are required to test in the local TOU-A population whether optimal strategies in a multispecific context may be predicted by the additivity of optimal strategies observed in a monospecific context.

DIFFERENT GENOMIC REGIONS ASSOCIATED WITH COMPETITIVE ABILITY IN *A. THALIANA*

In this study, we adopted a GWA mapping approach to finely map genes underlying competitive ability, allowing us to test the relationships between DGEs and either IGEs or IIGEs. We identified five genomic regions significantly associated with natural variation of competitive response, that is, DGEs. In addition, we identified a single genomic region significantly associated with the competitive effect of *A. thaliana* on the above-ground dry biomass of *T. arvense* competitors (i.e. IIGEs), but no genomic region significantly associated with a competitive effect of *A. thaliana* on conspecific neighbours (i.e. IGEs).

To our knowledge, this study is the first report of QTLs associated with either DGE or IIGE in an interspecific neighbourhood in a wild plant species. The dependence on the competitor species identity of the genomic regions associated with competitive ability is in line with the maintenance of genetic variation in *A. thaliana* due to biotic specialization in the context of plant–plant interactions.

In a mapping population of 411 RILs of *A. thaliana*, Mutic & Wolf (2007) detected many small QTLs associated with competitive response to intraspecific competition and suggested a pleiotropic relationship between DGE and IGE loci. In contrast, in our study, we identified (i) one major association peak for either competitive response or competitive effect and (ii) in agreement with the absence of relationship between competitive response and competitive effect in a heterospecific context, no pleiotropic relationship between DGE and IIGE loci. Other QTL mapping studies are clearly required to fully describe the genetic architecture of competitive ability as well as the relationship between DGE and either IGE or IIGE loci in *A. thaliana*.

Several candidate genes overlapped by significant association peaks have been detected in this study. For the number of basal branches in the presence of *P. annua*, the histone acetyltransferase HAM2 (*AT5G09740*) is known to regulate the expression of *FLOWERING LOCUS C* (Xiao *et al.* 2013), a flowering time repressor gene which also regulates branching patterns in *A. thaliana* (Huang *et al.* 2013). For the length of reproductive period, we identified two candidate genes with different molecular mechanisms. The pectinacetylesterase gene *AT5G26670* detected in the presence of *S. media* is a cell wall modification gene

regulated by VOCs emitted by the rhizobacteria *Bacillus subtilis* (Zhang *et al.* 2007). VOCs are now well established as reliable signals for neighbour detection (Pierik, Mommer & Voesebeke 2013). The SHI (for Short Inter-nodes) protein (*AT5G66350*) detected in the presence of *V. arvensis* is involved in the perception of or in the response to gibberellin (Fridborg *et al.* 1999), a growth-promoting hormone typically enhanced in shade conditions. While informative, these candidate genes remain speculative until functional validations based on mutant and transgenic approaches are performed to identify true ‘genetic variant–phenotypic variation’ associations.

Because plants interact with neighbours over short distances, we focused on a local GWA mapping population of *A. thaliana*. Whether the genetics of competitive ability (i.e. level of intra-population genetic variation, identity of phenotypic traits under selection and genetic bases) is similar in other natural populations of *A. thaliana* remains an open question. Because plant communities largely differ among natural populations of *A. thaliana* (Brachi *et al.* 2013), we hypothesize that the genetics of competitive ability should be different in other local populations. Testing this hypothesis certainly represents the next step to start establishing a genomic map of local adaptation to interspecific competition in *A. thaliana*. A complementary step would be to clone a gene associated with competitive ability in a local plant community (i) to better disentangle the molecular mechanisms of plant competition and (ii) to provide information for understanding and predicting evolutionary trajectories of plant communities.

Acknowledgements

Special thanks are given to Cédric Glorieux, Nathalie Faure and Angélique Bourceaux for their assistance during the common garden experiment. This work was funded by a PhD fellowship from the University of Lille 1 – Région Nord-Pas-de-Calais to EB, PhD fellowship from the University of Lille 1 to RV and a Région Nord-Pas-de-Calais grant (project Bio-impact : Impacts des changements globaux sur la distribution de la Biodiversité en Région Nord-Pas-de-Calais). This study was also supported by the LABEX TULIP (ANR-10-LABX-41; ANR-11-IDEX-0002-02).

Data accessibility

Phenotypic data are available in the Dryad Digital Repository <http://dx.doi.org/10.5061/dryad.5p2b1> (Baron *et al.* 2015)

References

- Aarssen, L.W. (1989) Competitive ability and species coexistence: a ‘plant’s-eye’ view. *Oikos*, **56**, 386–401.
- Aarssen, L.W. & Turkington, R. (1985) Biotic specialization between neighboring genotypes in *Lolium perenne* and *Trifolium repens* from a permanent pasture. *Journal of Ecology*, **73**, 605–614.
- Agashe, D. & Bolnick, D.I. (2010) Intraspecific genetic variation and competition interact to influence niche expansion. *Proceedings of the Royal Society B: Biological Sciences*, **277**, 2915–2924.
- Alkorta-Aranburu, G., Beall, C.M., Witosky, D.B., Gebremedhin, A., Pritchard, J.K. & Di Rienzo, A. (2012) The genetic architecture of adaptations to high altitude in Ethiopia. *PLoS Genetics*, **8**, e1003110.
- Baron, E., Richier, J., Villoutreix, R., Amsellem, L. & Roux, F. (2015) Data from: The genetics of intra- and interspecific competitive response and effect in a local population of an annual plant species. *Dryad Digital Repository*, <http://dx.doi.org/10.5061/dryad.5p2b1>.
- Bergelson, J. & Roux, F. (2010) Towards identifying genes underlying ecologically relevant traits in *Arabidopsis thaliana*. *Nature Reviews Genetics*, **11**, 867–879.
- Bolnick, D.I., Ingram, T., Stutz, W.E., Snowberg, L.K., Lau, O.L. & Paul, J.S. (2010) Ecological release from interspecific competition leads to decoupled changes in population and individual niche width. *Proceedings of the Royal Society B: Biological Sciences*, **277**, 1789–1797.
- Bosdorf, O., Shuja, Z. & Banta, A. (2009) Genotype and maternal environment affect belowground interactions between *Arabidopsis thaliana* and its competitors. *Oikos*, **118**, 1541–1551.
- Brachi, B., Faure, N., Horton, M., Flahauw, E., Vazquez, A., Nordborg, M. *et al.* (2010) Linkage and association mapping of *Arabidopsis thaliana* flowering time in nature. *PLoS Genetics*, **6**, e1000940.
- Brachi, B., Aimé, C., Glorieux, C., Cuguen, J. & Roux, F. (2012) Adaptive value of phenological traits in stressful environments: predictions based on seed production and laboratory natural selection. *PLoS One*, **7**, e32069.
- Brachi, B., Villoutreix, R., Faure, N., Nautekète, N., Piquot, Y., Pauwels, M. *et al.* (2013) Investigation of the geographical scale of adaptive phenological variation and its underlying genetics in *Arabidopsis thaliana*. *Molecular Ecology*, **22**, 4223–4240.
- Cahill, J.F., Kembel, S.W. & Gustafson, D.J. (2005) Differential genetic influences on competitive effect and response in *Arabidopsis thaliana*. *Journal of Ecology*, **93**, 958–967.
- Chesson, P. (2000) Mechanisms of maintenance of species diversity. *Annual Review of Ecology and Systematics*, **31**, 343–366.
- Dorn, L.A., Hammond Pyle, E. & Schmitt, J. (2000) Plasticity to light cues and resources in *Arabidopsis thaliana*: testing for adaptive value and cost. *Evolution*, **54**, 1982–1994.
- Fridborg, I., Kuusk, T., Moritz, T. & Sundberg, E. (1999) The *Arabidopsis* dwarf mutant *shi* exhibits reduced gibberellin responses conferred by overexpression of a new putative zinc finger protein. *The Plant Cell*, **11**, 1019–1031.
- Fridley, J.D., Grime, J.P. & Bilton, M. (2007) Genetic identity of interspecific neighbours mediates plant responses to competition and environmental variation in a species-rich grassland. *Journal of Ecology*, **95**, 908–915.
- Genung, M.A., Bailey, J.K. & Schweitzer, J.A. (2013) Belowground interactions shift the relative importance of direct and indirect genetic effects. *Ecology and Evolution*, **3**, 1692–1701.
- Golberg, D.E. & Barton, A.M. (1992) Patterns and consequences of interspecific competition in natural communities: a review of field experiments with plants. *American Naturalist*, **139**, 771–801.
- Huang, X., Ding, J., Effen, S., Turck, F. & Koornneef, M. (2013) Multiple loci and genetic interactions involving flowering time genes regulate stem branching among natural variants of *Arabidopsis*. *New Phytologist*, **199**, 843–857.
- Huard-Chauveau, C., Percheval, L., Debieu, M., Rivas, S., Kroj, T., Kars, I. *et al.* (2013) An atypical kinase under balancing selection confers broad-spectrum disease resistance in *Arabidopsis*. *PLoS Genetics*, **9**, e1003766.
- Inderjit, S. & Dakshini, K. M. M. (1998) Allelopathic interference of chickweed, *Stellaria media* with seedling growth of wheat (*Triticum aestivum*). *Canadian Journal of Botany*, **76**, 1317–1321.
- Johansson, J. (2008) Evolutionary responses to environmental changes: how does competition affect adaptation. *Evolution*, **62**, 421–435.
- Johnson, M.T.J., Dinnage, R., Zhou, A.Y. & Hunter, M.D. (2008) Environmental variation has stronger effects than plant genotype on competition among plant species. *Journal of Ecology*, **96**, 947–955.
- Lankau, R.A. & Strauss, S.Y. (2007) Mutual feedbacks maintain both genetic and species diversity in a plant community. *Science*, **317**, 1561–1563.
- Lau, J.A., Shaw, R.G., Reich, P.B. & Tiffin, P. (2014) Indirect effects drive evolutionary responses to global change. *New Phytologist*, **201**, 335–343.
- Martorell, C. & Freckleton, R.P. (2014) Testing the roles of competition, facilitation and stochasticity on community structure in a species-rich assemblage. *Journal of Ecology*, **102**, 74–85.
- Matesanz, S., Gimeno, T.E., de la Cruz, M., Escudero, A. & Valladares, F. (2011) Competition may explain the fine-scale spatial patterns and genetic structure of two co-occurring plant congeners. *Journal of Ecology*, **99**, 838–848.
- Moore, A.J., Brodie, E.D. & Wolf, J.B. (1997) Interacting phenotypes and the evolutionary process. I. Direct and indirect genetic effects of social interactions. *Evolution*, **51**, 1352–1362.

- Mutic, J.J. & Wolf, J.B. (2007) Indirect genetic effects from ecological interactions in *Arabidopsis thaliana*. *Molecular Ecology*, **16**, 2371–2381.
- Pierik, R., Mommer, L. & Voesenek, L.A.C.J. (2013) Molecular mechanisms of plant competition neighbour detection and response strategies. *Functional Ecology*, **27**, 841–853.
- Post, D.M. & Palkovacs, E.P. (2009) Eco-evolutionary feedbacks in community and ecosystem ecology interactions between the ecological theatre and the evolutionary play. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **364**, 1629–1640.
- Purves, D.W. & Law, R. (2002) Fine-scale spatial structure in a grassland community: quantifying the plant's-eye view. *Journal of Ecology*, **90**, 121–129.
- R Core Team (2013) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>.
- Rausher, M.D. (1992) The measurement of selection on quantitative traits: biases due to environmental covariances between traits and fitness. *Evolution*, **46**, 616–626.
- Roux, F., Gasquez, J. & Reboud, X. (2004) The dominance of the herbicide resistance cost in several *Arabidopsis thaliana* mutant lines. *Genetics*, **166**, 449–460.
- Stinchcombe, J.R., Agrawal, A.F., Hohenlohe, P.A., Arnold, S.J. & Blows, M.W. (2008) Estimating-nonlinear-selection gradients using quadratic regression coefficients double or nothing? *Evolution*, **62**, 2435–2440.
- Tilman, D. (1985) The resource ratio hypothesis of succession. *American Naturalist*, **125**, 827–852.
- Turkington, R. (1989) The growth, distribution and neighbor relationships of *Trifolium repens* in a permanent pasture. V. The coevolution of competitors. *Journal of Ecology*, **77**, 717–733.
- Turkington, R. & Harper, J.L. (1979) The Growth, Distribution and Neighbour Relationships of *Trifolium repens* in a Permanent Pasture: IV. Biotic differentiation. *Journal of Ecology*, **67**, 245–254.
- Urban, M.C. (2006) Maladaptation and mass effects in a metacommunity: consequences for species coexistence. *American Naturalist*, **168**, 28–40.
- Valladares, F., Sanchez-Gomez, D. & Zavala, M.A. (2006) Quantitative estimation of phenotypic plasticity: bridging the gap between the evolutionary concept and its ecological applications. *Journal of Ecology*, **94**, 1103–1116.
- Vavrek, M.C. (1998) Within-population genetic diversity of *Taraxacum officinale* Asteraceae: differential genotype response and effect on interspecific competition. *American Journal of Botany*, **85**, 947–954.
- Vellend, M. (2006) The consequences of genetic diversity in competitive communities. *Ecology*, **87**, 304–311.
- Weinig, C., Johnston, J., German, Z.M. & Demink, L.M. (2006) Local and global costs of adaptive plasticity to density in *Arabidopsis thaliana*. *American Naturalist*, **167**, 826–836.
- Whitlock, R.A.J., Bilton, M., Grime, J.P. & Burke, T. (2011) Fine-scale community and genetic structure are tightly linked in species-rich grasslands. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **366**, 1346–1357.
- Willis, C.G., Brock, M.T. & Weinig, C. (2010) Genetic variation in tolerance of competition and neighbour suppression in *Arabidopsis thaliana*. *Journal of Evolutionary Biology*, **23**, 1412–1424.
- Xiao, J., Zhang, H., Xing, L., Xu, S., Liu, H., Chong, K. et al. (2013) Requirement of histone acetyltransferase HAM1 and HAM2 for epigenetic modification of FLC in regulating flowering in *Arabidopsis*. *Journal of Plant Physiology*, **170**, 444–453.
- Zhang, H., Mi-Seong, K., Krishnamachari, V., Payton, P., Sun, Y., Grimson, M. et al. (2007) Rhizobacterial volatile emissions regulate auxin homeostasis and cell expansion in *Arabidopsis*. *Planta*, **226**, 839–851.

Received 9 September 2014; accepted 31 January 2015

Handling Editor: Alison Bennett

Supporting Information

Additional Supporting information may be found in the online version of this article:

Appendix S1. Plant material.

Appendix S2. Common garden experiment.

Appendix S3. Phenotypic characterization.

Appendix S4. Data analysis.

Fig. S1. Experimental split-plot design.

Fig. S2. Quantile-Quantile plots of *P*-values representing the excess of strong associations for traits with QTLs.

Fig. S3. Genomic regions associated with phenotypic variation in each of the six competition treatments.

Fig. S4. Box-plots illustrating for each competition treatment the effects of three top SNPs individually identified in a specific competition treatment.

Table S1. Natural variation of nine phenotypic traits scored on *A. thaliana* target plants and one trait scored on competitors.