

Research

Quantitative trait loci mapping of phenotypic plasticity and genotype–environment interactions in plant and insect performance

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Community genetic studies generally ignore the plasticity of the functional traits through which the effect is passed from individuals to the associated community. However, the ability of organisms to be phenotypically plastic allows them to rapidly adapt to changing environments and plasticity is commonly observed across all taxa. Owing to the fitness benefits of phenotypic plasticity, evolutionary biologists are interested in its genetic basis, which could explain how phenotypic plasticity is involved in the evolution of species interactions. Two current ideas exist: (i) phenotypic plasticity is caused by environmentally sensitive loci associated with a phenotype; (ii) phenotypic plasticity is caused by regulatory genes that simply influence the plasticity of a phenotype. Here, we designed a quantitative trait loci (QTL) mapping experiment to locate QTL on the barley genome associated with barley performance when the environment varies in the presence of aphids, and the composition of the rhizosphere. We simultaneously mapped aphid performance across variable rhizosphere environments. We mapped main effects, QTL × environment interaction (QTL × E), and phenotypic plasticity (measured as the difference in mean trait values) for barley and aphid performance onto the barley genome using an interval mapping procedure. We found that QTL associated with phenotypic plasticity were co-located with main effect QTL and QTL × E. We also located phenotypic plasticity QTL that were located separately from main effect QTL. These results support both of the current ideas of how phenotypic plasticity is genetically based and provide an initial insight into the functional genetic basis of how phenotypically plastic traits may still be important sources of community genetic effects.

Keywords: aphid; phenotypic plasticity; quantitative trait loci mapping; rhizobacteria; species interactions; genotype by environment interactions

1. INTRODUCTION

Phenotypic plasticity is the ability of an organism or population to alter its phenotype according to environmental variation (e.g. [1]). It enables an organism to continue to survive and reproduce across variable environments, and is particularly important in organisms such as plants that live a sessile life. Plant traits can exhibit plasticity to abiotic [2] and biotic (e.g. responses to herbivory) environmental variation [3–5]. Phenotypic plasticity also occurs in insects and can influence both individual morphology and population size [6–8], raising the question of whether both plants and insects simultaneously show phenotypically plastic responses. However, reciprocal phenotypic plasticity, i.e. whether plasticity in plant traits affects insect

phenotype, has received little attention [9]. Phenotypic plasticity becomes important in a community genetics framework because effects caused by non-plastic traits (i.e. traits where the mapping of genotype to phenotype is constant across environments) are likely to influence evolutionary trajectories in a different fashion from effects caused by plastic traits. Non-plastic traits will have a consistent influence on the structure of the associated community and the fitness of individual interacting species, and thus a consistent influence on the evolutionary trajectories of these species. Plastic traits will have a less consistent community genetic effect from each genotype and thus a less consistent, although not necessarily weaker, influence on the evolution of associated species. Here, we present a pilot study where we use existing methods of examining the genetic basis of plasticity in individual species applied to a multi-species system.

In nature, plants interact with multiple above-ground and below-ground species, and these interactions could be influenced by phenotypic plasticity [9]. Phenotypic plasticity can be visualized

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by plotting trait values over environments, creating a norm of reaction. In this case, phenotypic plasticity is the slope of the reaction norm, or the extent to which the trait value changes across environments [10]. Genotype \times environment interactions (GEIs) can occur if genetic variation influences the slope of the reaction norm across environments, and can cause crossing of the norms of reaction [11].

Phenotypic plasticity and GEIs can play an important role in species interactions [7,9,12], and knowledge of the underlying genetic basis could provide further explanation on how species have evolved within multi-species communities. Phenotypic plasticity can be either adaptive when it confers a fitness advantage [1,7,10,13,14] or non-adaptive, as phenotypic plasticity also covers examples where phenotypic changes in response to environmental heterogeneity may not enhance fitness [1,14]. GEIs explain that no single phenotype can confer high fitness in all environments; therefore a species may have higher tolerance to heterogeneous environments if its phenotype can change according to the environment [10]. Genetic models have been presented to explain the genetic basis of plasticity [10,15] and two main ideas prevail: (i) the mean trait (within each environment) and the plasticity of the trait (difference in the mean phenotype over contrasting environments) may be influenced by separate genes and each be subject to selection (the 'plasticity genes' could be thought of as regulatory genes that influence which trait-associated genes are expressed [16]); (ii) plasticity is influenced by environmentally sensitive loci directly influencing trait value in both environments and may have evolved as a by-product of differential natural selection [10,16].

The quantitative genetic basis of GEIs and phenotypic plasticity can be investigated using quantitative trait loci (QTL) mapping, as reported in studies testing the effect of abiotic [2,16,17] heterogeneous environments. Mapping populations are developed from two genotypes, and following many generations of inbreeding, multiple lines are generated. The lines are almost genetically homogeneous, except that each line differs at a few loci. Alleles at loci originate from either parental genotype 1 or parental genotype 2 and ultimately the total population should be constituted by lines that cover every possible combination of parental allele at each locus. Therefore, a QTL is located when the difference in having the allele from either parent 1 or parent 2 at each locus causes a significant change in the phenotype. In this way, we are testing the association between the phenotype and the genotype at each locus. Where significant QTL are found in one environment but not in the other, GEIs can then be tested using standard analysis of variance (ANOVA) with QTL analysis to determine if genetic variation at that location influences plasticity. QTL mapping can also be performed on environmentally sensitive traits to test for plasticity between environments, by mapping the differences in mean trait values between environments. This approach can locate different loci associated across all or one environment, or whether separate loci are simply associated with the change in phenotype across environments. If GEIs are present at a chromosomal

location that also shows a significant QTL for mean trait differences, this suggests that the loci controlling the trait value in the two environments also determine the plasticity. However, if there is no significant QTL for mean differences at a location showing a significant GEI, this suggests that other regulatory genes may be controlling plasticity.

Although plant–insect interactions have been previously mapped onto the plant genome in terms of plant defence [18–20], plasticity in traits involved in the reciprocal interaction has received little attention. Furthermore, plants interact with multiple above- and below-ground species, yet interactions among multiple species are rarely mapped.

In this paper, we present a 'proof of concept' study that applies established QTL mapping approaches for plasticity (e.g. [16]) to multi-species interactions and indirect effects such as those described by community genetics. We test the hypothesis that variation in the composition of the plant rhizosphere could cause phenotypic plasticity in plant–insect interactions. We mapped reciprocal barley–aphid interactions using a tri-trophic model ecosystem consisting of rhizobacterial supplementation with *Pseudomonas aeruginosa* 7NSK2, barley (*Hordeum vulgare*) and cereal aphid (*Sitobion avenae*). To test main effects and interactive effects of each species on barley and aphid performance, we set up environments covering each possible combination of species (and controls), with barley as the only species present in every environment. We used two doubled haploid (DH) mapping populations, Steptoe \times Morex (S \times M) and Oregon Wolfe Barley (OWB). Parental lines from the S \times M population were previously investigated for the presence of GEIs within the tri-trophic ecosystem used here [12]. The second mapping population (OWB) was used to test whether GEIs and phenotypic plasticity resulting from multi-species interactions are specific to mapping population or prevalent throughout both mapping populations. Our objectives were to: (i) map root and shoot biomasses (plant traits involved in both above- and below-ground species interactions) and aphid fitness onto the barley genome in each environment; (ii) test whether the environment has a significant effect on QTL expression (QTL \times environment (E) interaction); (iii) map phenotypic plasticity (as mean differences in trait values) of plant root and shoot biomasses, and aphid fitness between environments; (iv) compare the prevalence of GEIs and phenotypic plasticity across the two mapping populations.

2. MATERIAL AND METHODS

(a) Quantitative trait loci mapping populations

We mapped plant biomass onto the barley (*H. vulgare*) genome using two DH barley mapping populations, derived from the parental genotypes S \times M (population 1) and OWB dominant and recessive (population 2). DH populations are used in many cereal crops and provide one of the best methods to map QTL owing to the homozygous lines, produced using the bulbosum technique [21]. The S \times M population has a high-average map density of markers (5.6 cM); chromosome (Ch) 1: 170 cM, 37 markers;

Ch2: 181 cM, 37 markers; Ch3: 185 cM, 31 markers; Ch4: 177 cM, 33 markers; Ch5: 151 cM, 29 markers; Ch6: 157 cM, 22 markers; Ch7: 202 cM, 34 markers. The OWB population has a similar map density (5.5 cM); Ch1: 136 cM, 29 markers; Ch2: 180 cM, 35 markers; Ch3: 218 cM, 28 markers; Ch4: 125 cM, 31 markers; Ch5: 225 cM, 37 markers; Ch6: 167 cM, 35 markers; Ch7: 199 cM, 37 markers. Seeds for the two mapping populations were supplied by P. Hayes (Oregon State University). The linkage maps for the S×M population (consisting of 150 DH lines) and the OWB population (94 DH lines) are available on the GrainGenes website: <http://www.wheat.pw.usda.gov/GG2/index.shtml>. In this study, a subset of 50 lines from each population was chosen for phenotyping and subsequent mapping owing to logistical constraints of phenotyping the full mapping populations. Using a subset can create two experimental caveats, which are discussed below.

(b) *The use of subsets in quantitative trait loci mapping*

The use of subsets of lines from mapping populations is known to limit QTL mapping in two ways. Firstly, the ability to detect QTL–trait associations is limited, because QTL are only detected where there is genetic variation at loci (between the two parental alleles), which has a significant effect on the measured trait. Each line in the mapping population is designed to contribute alleles from either parent, and each line will differ in which parental alleles they contribute at a small number of loci [22]. Therefore, fewer lines results in fewer genetically variable loci. Secondly, the detection of fewer QTL means that the QTL–trait association could be over-exaggerated, owing to the nature of QTL analysis [22]. However, this does not necessarily increase the likelihood of detecting false positives with the calculation of a threshold value, which QTL must exceed to be significant. The calculation of threshold values used in this study was based on a method that calculates the level of genetic variation within the 50-line subset that we used [23]. Therefore, the threshold values that we calculated may have been higher (limiting the number of QTL deemed significant) than if we had calculated values using the full mapping population.

The occurrence of false positives in QTL mapping can be affected by low experimental power caused by several factors, including the method of QTL data analysis. This study used composite interval mapping (CIM) [24], a high precision method that maps QTL by testing the association between loci and trait, while simultaneously using flanking markers to account for variance caused by other QTL located on the same chromosome. Therefore, this method includes a control for the expected effects of QTL over exaggeration caused by the detection of fewer QTL in mapping subsets, since each QTL is tested independently of other QTL beyond the flanking markers [24]. A further concern for the power of QTL mapping is the number of experimental replicates used, since the mean of the trait is mapped, ignoring any standard deviation. In this study, the traits used

to map QTL were the means calculated from four experimental replicates. It is impossible to use the standard deviation of means within QTL mapping to quantify the significance of results; therefore the number of replicates we used is important, since the error distribution of the mean data collected from our four replicates is likely to be smaller compared with studies that use fewer experimental replicates. A further source of power in our analysis originates from the breeding design of the lines we used, and the number of markers mapped onto each chromosome. We used DH lines produced using the ‘bulbosum’ technique [21], with an average map density of 5.5 cM. This method results in lines that are homozygous at each locus and thus no residual heterozygous individuals are involved in mapping. To conclude, it is probable that the number of QTL detected in this study is a subset of the total number that we would have detected had we mapped using the full population and likely that detection of ‘false’ QTL has been minimized.

(c) *Experimental design*

We designed a fully factorial experiment with two biotic environmental factors (rhizobacteria, *P. aeruginosa* 7NSK2 and cereal aphid, *S. avenae*) to map QTL resulting from both main effects and interaction effects of the environmental factors on plant biomass. This gave four environmental ecosystems: (i) control (*P. aeruginosa* 7NSK2 not supplemented, no aphids); (ii) *P. aeruginosa* 7NSK2 supplemented (no aphids); (iii) aphid infestation (*P. aeruginosa* 7NSK2 not supplemented); and (iv) *P. aeruginosa* 7NSK2 supplemented, aphid infestation. We selected a 50 line subset of each mapping population (plus parental lines), grew each line under all four environments, and replicated four times, giving 832 plants per mapping population. We used a randomized block design, with replicate as the block, and each line–environment combination was randomized within each treatment block.

(d) *Plant phenotyping*

Plants were grown in a glasshouse at the Firs Experimental Research Station (University of Manchester) during June 2005 (S×M population) and June 2006 (OWB population). Supplemental lights were used to provide a 16 L:8 D regime and a daily temperature range of 16–30°C. To minimize the presence of non-experimental rhizobacteria, we sterilized seeds in 10 per cent sodium hypochlorite (followed by several washes with sterilized distilled water) and germinated the seeds in sterile Petri dishes and filter paper for 5 days. Preparation of *P. aeruginosa* 7NSK2 inoculum and inoculation onto barley roots were as previously described [12]. After inoculation, seedlings were planted into 10 cm pots containing heat sterilized horticultural grade sharp sand. We had set up the rhizosphere system as a sterilised system, to minimize the introduction of non-experimental micro-organisms pre-inoculation to aid the development of our bacterial inoculum on plant roots. Post transplantation, the rhizosphere was allowed to be naturally colonized by non-experimental

Table 1. ANOVA results for plant (shoot and root biomasses) and aphid performance for the Steptoe × Morex (S×M) and Oregon Wolfe Barley (OWB) mapping populations.

source	shoot biomass			root biomass			aphid performance			
	d.f.	F	p	d.f.	F	p	d.f.	F	p	
StxMo	line	49	2.18	<0***	49	3.48	<0***	49	2.23	0.003**
	environment	3	50.87	<0***	3	42.32	<0***	1	7.25	0.01**
	line × environment	147	1.60	<0***	147	1.40	0.004**	49	0.62	0.979
	error	567			556			275		
OWB	line	47	4.02	<0***	47	3.16	<0***	47	1.33	0.167
	environment	3	42.82	<0***	3	47.74	<0***	1	3.85	0.055
	line × environment	141	1.07	0.286	141	0.81	0.940	47	1.00	0.476
	error	519			520			253		

** $p \leq 0.01$.*** $p \leq 0.001$.

micro-organisms (e.g. via irrigation); therefore, the rhizosphere treatment should be thought of as supplemented/not supplemented rather than presence/absence of *P. aeruginosa* 7NSK2. Plants were watered once a week with 40 ml full concentration Hoagland's solution [25]. Eleven days after transplantation, two adult aphids were placed onto each plant and a plastic tube with mesh windows was fitted over each plant to prevent aphids escaping. The total aphid population size was counted two weeks after infestation. Plant shoots and roots were then separated, cleaned and dried at 80°C for 3 days for dry biomass measurements.

(e) Phenotypic plasticity

Phenotypic plasticity was calculated using the character state approach [10]. In this method, phenotypic plasticity is the difference in the mean phenotype between two environments, i.e. the slope of the reaction norm. For plant biomass, we calculated phenotypic plasticity between five environmental pairs: (i) aphid infested versus non-infested (*P. aeruginosa* 7NSK2 not supplemented); (ii) aphid infested versus non-infested (*P. aeruginosa* 7NSK2 supplemented); (iii) *P. aeruginosa* 7NSK2 supplemented versus non-supplemented (aphid non-infested); (iv) *P. aeruginosa* 7NSK2 supplemented versus non-supplemented (aphid infested); and (v) aphid infested and *P. aeruginosa* 7NSK2 supplemented versus non-infested and non-supplemented. For aphid population size, phenotypic plasticity was calculated as the difference in population size between environments with and without *P. aeruginosa* 7NSK2 supplementation.

(f) Data analysis

ANOVA was performed on the trait data using the GLM method in MINITAB (v. 15), treating line as a random factor. We used three approaches to map the effects of genotype and environment on QTL–trait association. Firstly, we mapped main effects (trait values) onto the genotypic data for each of the four environments. Secondly, we tested the effect of the environment at each locus where we had located a significant QTL to look for QTL by environment interactions. As this was a single-site analysis, the ANOVA of QTL×E is a test of whether the association

between loci and trait value significantly changes between contrasting environments. Thirdly, we mapped phenotypic plasticity (difference between mean trait value), which effectively maps the GEIs onto the genotypic data. We mapped main effects and phenotypic plasticity for barley and aphid performance onto the barley genome using the CIM procedure in QTL Cartographer [26]. CIM tests the association between marker sites and trait values at 2 cM intervals along each chromosome. At each 2 cM test site, the analysis includes background markers as cofactors, to control variance caused by QTL at non-target loci outside flanking markers determined by the 'window size' [24]. We used a window size of 10 cM around the target loci. The location of a QTL associated with a significant phenotypic effect was defined as the point where the likelihood probability ratio (LPR) exceeded the threshold value. Threshold values were calculated genome wide and for each chromosome in each mapping population following the method of Li & Ji [23]. Chromosome significance threshold were used to interpret results but genome wide significance is also reported in table 3. This method involves calculation of the effective number of marker loci using results from principal components analysis (PCA) of the marker data. Values for r^2 (% phenotypic variation explained by a QTL) and additive genetic effect were generated by QTL Cartographer [26].

Tests for QTL×E interactions were conducted for all significant QTL. We performed a single marker site QTL×E analysis where QTL had been located, using SAS [27], and conducted Bonferroni corrections for multiple testing.

3. RESULTS

(a) Phenotypic effect of environmental factors

Barley shoot and root biomass and aphid population size were influenced by both environmental and genetic (mapping line) variation (figure 1). Furthermore, for S×M mapping lines, a significant line × environment interaction influenced barley root and shoot biomasses (table 1). Across lines of both mapping populations, aphid population size increased, decreased or remained constant when the rhizosphere was supplemented with *P. aeruginosa* 7NSK2

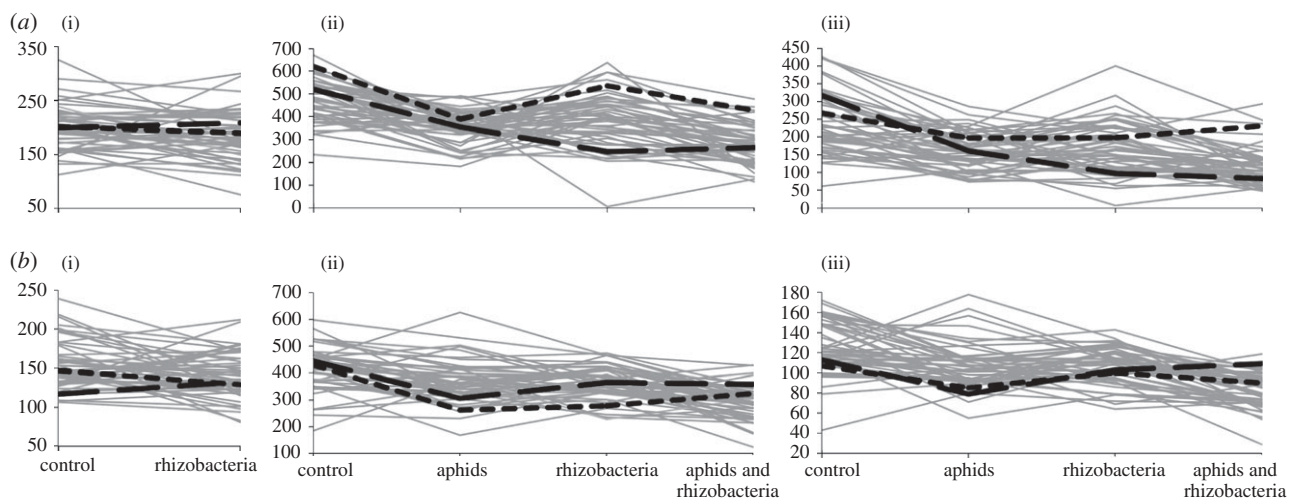


Figure 1. Reaction norms for (i) aphid population size, (ii) stem biomass and (iii) root biomass in two mapping populations; (a) Steptoe \times Morex and (b) Oregon Wolfe Barley. The long dashed line represents parental lines (a) OWB-rec and (b) Morex; the short dashed line represents parental lines (a) OWB-dom and (b) Steptoe.

Table 2. Comparisons of means (phenotypic plasticity) between paired environments. Lines expressing increase or decrease have more than 5% change in environment 2 compared with environment 1 (shows trends in polarity change).

trait	environment 1 ^a	environment 2 ^a	% lines expressing change in mean trait			
			decrease	increase	no change	
S \times M	aphid	<i>Pa</i>	60	26	14	
	shoot; root	control	aphid	82; 82	6; 14	12; 4
		control	<i>Pa</i>	72; 70	20; 22	8; 6
		control	aphid + <i>Pa</i>	94; 96	2; 4	4; 0
		aphid	aphid + <i>Pa</i>	68; 72	20; 20	12; 8
		<i>Pa</i>	aphid + <i>Pa</i>	80; 78	8; 18	12; 4
OWB	aphid	<i>Pa</i>	54	31	15	
	shoot; root	control	aphid	69; 69	14; 17	17; 15
		control	<i>Pa</i>	71; 67	15; 19	15; 15
		control	aphid + <i>Pa</i>	94; 94	4; 4	2; 2
		aphid	aphid + <i>Pa</i>	79; 83	8; 10	13; 6
		<i>Pa</i>	aphid + <i>Pa</i>	83; 85	10; 8	6; 6

^a*Pa* refers to *Pseudomonas aeruginosa* 7NSK2.

compared with the control. Compared with the control environment, *P. aeruginosa* 7NSK2 supplementation resulted in a reduction of the aphid population size in 60 per cent (S \times M population) and 54 per cent (OWB population) of lines, and an increase in 26 (S \times M population) and 31 per cent (OWB population) of lines (table 2). Similarly, the effect of environmental factors on shoots and root biomass varied across mapping lines. For both populations, *P. aeruginosa* 7NSK2 supplementation led to a reduction in biomass in 67–72% of lines, and an increase in 15–22% of lines. Aphid infestation tended to reduce biomass, in up to 82 per cent of lines. The combination of both *P. aeruginosa* 7NSK2 supplementation and aphids led to reduced biomass in 94–96% of lines, and increased biomass in 2–4% of lines. The aphid environment had a negative effect on root and shoot biomasses for more lines than the environment with *P. aeruginosa* 7NSK2 (*Pa*) supplementation, indicated by the aphid–aphid and *Pa*, and the *Pa*–aphid and *Pa* comparisons (table 2).

We observed that the mean traits for the lines exceeded the mean value for either parental line (figure 1). This is a general observation in QTL mapping studies, and this transgressive variation can be caused by epistatic interactions, or by the accumulation of complementary alleles in the DH lines [11].

(b) Quantitative trait loci mapping

We mapped main effects (direct association between phenotype and loci) across environments, and phenotypic plasticity (difference in trait means between two environments) onto the barley genome. This analysis produced plots showing the association between loci and trait, measured as LPR value (figures 2 and 3). When we have a significant association we see a QTL peak on the graph, and this means that at that locus there is a high probability that swapping the allele from parent 1 (Steptoe/OWB D) with the allele from parent 2 (Morex/OWB R) will significantly affect the trait. The level of probability of QTL–trait association ranged from 2.3 LPR (the minimum

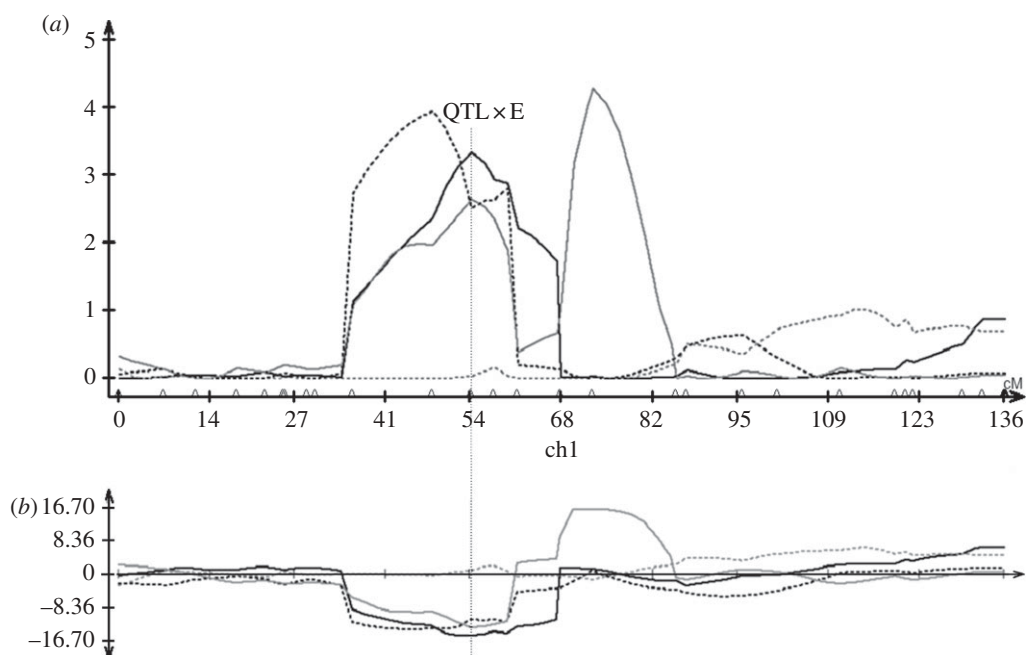


Figure 2. QTL plot for chromosome 1 of the OWB mapping population. The QTL plot shows (a) the association between the trait and loci (LPR, *y*-axis), and (b) the additive genetic effect along the chromosome (cM, *x*-axis). The positions of markers are indicated by small triangles along the chromosome (*x*-axis, plot a). Four lines are plotted: solid black and dashed grey, aphid fitness when *P. aeruginosa* 7NSK2 was not/was supplemented, respectively; dashed black, plasticity in aphid fitness across the two aphid environments; solid grey, plasticity in root biomass between *P. aeruginosa* 7NSK2-supplemented and combined aphid + *P. aeruginosa* 7NSK2-supplemented environments.

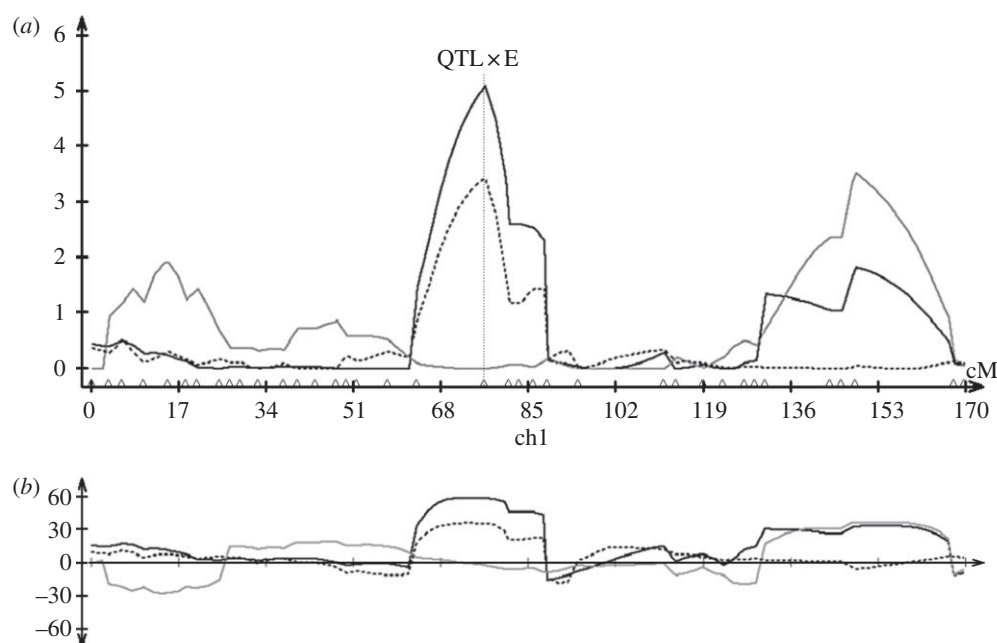


Figure 3. QTL plot for chromosome 1 of the S × M mapping population. The QTL plot shows (a) the association between the trait and loci (LPR, *y*-axis), and (b) the additive genetic effect along the chromosome (cM, *x*-axis). The positions of markers are indicated by small triangles along the chromosome (*x*-axis, plot a). Three lines are plotted: solid black, shoot biomass when *P. aeruginosa* 7NSK2 was supplemented; dashed line, plasticity in shoot biomass across *P. aeruginosa* 7NSK2 supplemented and combined environments; grey line, shoot biomass in the combined environment.

threshold level) to 5.8 LPR (table 3), and each QTL explained between 9 and 31 per cent of phenotypic variance. The additive genetic effect (A.G.E., table 3) is a measure of the magnitude of the QTL effect (i.e. the effect of swapping the allele for parent 1–parent 2) on the trait, and the polarity indicates

which parental allele results in a greater trait value; for example, bigger aphid population size, or greater shoot biomass. The largest A.G.E. (–62.62) was for a QTL on chromosome 4 of the S × M population. This means that when the Morex allele was contributing to this locus, shoot biomass is higher than when

Table 3. QTLs located for plant (shoot and root biomasses) and aphid performance for the S×M and Oregon Wolfe Barley (OWB) mapping populations.

location (cM)	trait ^a	environment ^b	LPR ^c	% variance	A.G.E. ^d	QTL×E ^e
S×M						
Ch1, 76.31	shoot	<i>Pa</i>	5.11***	23.85	59.46	0.0284 ^f (CvD); 0.0052 ^f (BvC); 0.0040 ^f (AvC)
	shoot PP	<i>Pa</i> –aph and <i>Pa</i>	3.43**	15.60	36.28	
Ch1, 111.21	root	<i>Pa</i>	4.61***	21.05	35.37	
	root PP	<i>Pa</i> –aph and <i>Pa</i>	2.91**	16.35	22.57	
Ch1, 117.51	root	<i>Pa</i>	4.26***	20.67	35.06	
	root PP	<i>Pa</i> –aph and <i>Pa</i>	3.69**	19.02	23.58	
Ch1, 148.51	shoot	aph and <i>Pa</i>	3.52**	17.40	37.19	0.0184 ^f (BvD)
Ch2, 3.41	root	<i>Pa</i>	2.31*	9.19	23.50	
Ch3, 29.81	root	control	5.00***	30.45	49.86	0.0105 ^f (AvD)
	root PP	control–aph and <i>Pa</i>	3.57**	16.50	36.01	
	aphid	aph	2.73*	15.15	16.71	
Ch3, 123.81	aphid	aph and <i>Pa</i>	3.27**	24.29	31.93	
Ch3, 139.91	root	aph	2.49*	13.70	–27.07	
Ch3, 151.01	shoot	aph	2.51**	14.35	43.79	
Ch3, 158.51	root	aph	2.85*	18.54	29.77	
	root PP	aph–aph and <i>Pa</i>	2.78*	15.79	19.56	
Ch3, 171.11	shoot PP	aph–aph and <i>Pa</i>	2.33*	14.50	31.51	
Ch3, 172.11	root	aph and <i>Pa</i>	3.25**	16.75	–22.38	0.0022 ^f (CvD)
Ch3, 182.61	root PP	control–aph	2.98**	18.44	–24.89	
Ch4, 11.41	shoot	aph and <i>Pa</i>	5.81***	30.67	–62.62	
Ch4, 34.51	shoot	aph and <i>Pa</i>	2.20*	10.19	31.23	
Ch4, 36.51	aphid	aph and <i>Pa</i>	3.43**	17.37	–20.37	0.0296 ^f
Ch4, 96.61	shoot PP	aph– <i>Pa</i>	2.46*	12.75	–33.25	
Ch4, 122.21	aphid PP	aph–aph and <i>Pa</i>	2.72*	13.99	–17.85	
Ch4, 143.91	aphid PP	aph–aph and <i>Pa</i>	4.68***	26.44	25.33	
Ch5, 13.61	root	<i>Pa</i>	3.00**	12.33	26.97	0.0482 ^f (AvC)
Ch5, 22.41	root PP	control– <i>Pa</i>	2.28*	11.90	–26.81	
Ch5, 28.41	shoot	<i>Pa</i>	2.84*	12.71	42.97	0.0113 ^f (BvC); 0.0083 ^f (AvC)
Ch5, 31.51	shoot PP	control–aph and <i>Pa</i>	3.04**	19.59	–56.37	
Ch5, 50.21	root PP	control–aph	2.39*	13.09	–21.44	
Ch5, 58.61	shoot PP	control– <i>Pa</i>	2.71*	15.86	–53.21	
Ch5, 84.41	shoot PP	control– <i>Pa</i>	2.65*	14.69	50.26	
Ch5, 102.71	aphid PP	aph–aph and <i>Pa</i>	3.12**	16.30	–14.09	
	root PP	control– <i>Pa</i>	3.49**	19.29	34.27	
Ch5, 106.71	root PP	aph–aph and <i>Pa</i>	2.71*	16.68	20.12	
Ch5, 112.51	shoot PP	aph–aph and <i>Pa</i>	2.40*	14.19	32.92	
Ch5, 130.41	root PP	aph– <i>Pa</i>	2.58*	15.61	29.83	
Ch5, 148.01	aphid	aph	2.48*	13.53	–15.91	
	root	control	2.34*	13.68	–31.59	
Ch6, 26.61	shoot PP	control–aph	3.07**	18.84	38.80	
Ch6, 42.61	shoot	<i>Pa</i>	2.81*	12.72	43.62	0.0820 ^f (BvC)
	aphid	aph	2.49*	13.63	15.20	
Ch6, 47.11	shoot PP	control–aph	5.17***	28.95	45.89	
Ch6, 55.11	shoot	control	2.21*	17.75	38.99	0.0376 ^f (AvB)
Ch6, 105.81	shoot PP	control– <i>Pa</i>	2.41*	11.12	–32.96	
Ch7, 48.11	root PP	control–aph and <i>Pa</i>	2.28*	12.34	27.04	
Ch7, 78.31	root PP	control– <i>Pa</i>	3.21**	17.68	33.36	
OWB						
Ch1, 54.11	aphid	aph	3.34**	17.78	–14.93	0.0157 ^f
	aphid PP	aph–aph and <i>Pa</i>	3.95**	23.49	–13.15	
	root PP	<i>Pa</i> –aph and <i>Pa</i>	2.65*	17.74	–12.77	
Ch1, 72.77	root PP	<i>Pa</i> –aph and <i>Pa</i>	4.28***	28.93	16.73	
Ch1, 116.79	shoot	aph	3.07*	17.16	–38.47	
Ch2, 65.23	root	<i>Pa</i>	2.50*	10.10	5.81	
Ch2, 93.88	shoot	<i>Pa</i>	2.77*	10.17	20.73	
Ch2, 141.26	shoot	<i>Pa</i>	3.89**	16.36	–26.08	
	aph	aph	4.86***	18.50	–35.35	
Ch2, 164.55	root PP	aph– <i>Pa</i>	3.34**	20.12	–7.21	
Ch3, 0.00	aphid	aph and <i>Pa</i>	4.10***	17.90	12.97	

(Continued.)

Table 3. (Continued.)

location (cM)	trait ^a	environment ^b	LPR ^c	% variance	A.G.E. ^d	QTL×E ^e
		aph	3.01**	15.82	14.02	
Ch3, 38.74	shoot PP	control- <i>Pa</i>	3.26**	18.22	25.93	
Ch3, 73.01	shoot	<i>Pa</i>	3.70**	14.39	-23.58	0.0367 ^f (BvC)
Ch3, 164.58	root PP	aph-aph and <i>Pa</i>	3.35**	18.12	28.73	
Ch3, 167.96	shoot PP	control-aph and <i>Pa</i>	3.93**	26.1	34.61	
Ch3, 171.80	root PP	control-aph and <i>Pa</i>	4.10***	24.28	10.92	
Ch 4, 38.74	shoot PP	control-aph	2.39*	18.22	25.93	
Ch 4, 67.48	shoot PP	aph- <i>Pa</i>	4.11***	18.75	-22.53	
Ch4, 111.96	root	aph	3.00**	11.60	7.64	
	shoot	aph	2.60*	12.65	28.35	
	shoot	aph and <i>Pa</i>	4.00***	21.70	29.04	
Ch5, 11.35	aphid	aph and <i>Pa</i>	2.78*	10.87	-10.21	
Ch5, 118.87	shoot PP	control- <i>Pa</i>	2.45*	13.67	28.87	
Ch5, 134.15	shoot PP	control- <i>Pa</i>	4.42***	26.21	-40.69	
Ch5, 140.56	root	<i>Pa</i>	3.52**	14.56	6.99	
Ch5, 158.55	shoot	aph and <i>Pa</i>	3.40**	15.89	-24.84	0.0283 ^f (CvD)
Ch5, 197.35	root PP	aph- <i>Pa</i>	4.01**	25.58	-7.18	
Ch6, 0.02	shoot PP	control- <i>Pa</i>	4.01***	23.25	-28.40	
Ch6, 44.85	aphid	aph and <i>Pa</i>	3.64**	15.29	-15.15	
Ch6, 48.85	root PP	control-aph	3.38**	21.87	10.35	
Ch6, 51.10	root	control	4.33***	21.62	13.46	
	shoot	control	3.06**	16.22	34.97	
Ch6, 68.00	aphid	aph and <i>Pa</i>	4.38***	19.13	17.76	
Ch6, 105.43	root PP	control-aph	3.88**	27.48	-11.14	
Ch6, 137.18	root	<i>Pa</i>	4.93***	28.44	-12.62	0.0321 ^f (BvC)
Ch6, 142.64	root	aph	4.00***	22.40	16.40	0.0129 ^f (BvC); 0.0317 ^f (BvD)
Ch7, 103.26	shoot	control	2.95*	14.50	33.07	0.0477 ^f (AvB); 1.0359 ^f (AvD)

^aTraits associated with a QTL; main effects for barley (shoot, root) and aphid, and phenotypic plasticity (PP) of each of the three main traits.

^b*Pa* refers to *Pseudomonas aeruginosa* 7NSK2.

^cLPR, Likelihood probability ratio is the likelihood of a significant loci-phenotype association. QTL listed are those that had a LPR exceeding the chromosome-wide threshold level; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; bold values are significant at a genome-wide level $p < 0.05$.

^dAdditive genetic effect (A.G.E.) illustrates which parental allele causes a greater trait value compared with the alternative parental allele. In the S×M population, a positive A.G.E. corresponds to a higher trait value when the allele from Morex is contributed to that locus, compared with when Steptoe is contributed, and vice versa for the negative A.G.E. In the OWB population, positive A.G.E. corresponds to the contribution of the allele of OWB-D influencing higher trait value compared with OWB-R, and vice versa.

^eQTL×E, p -values. Letters in brackets denote environments for QTL×E; A, control; B, aphid; C, *Pseudomonas aeruginosa* 7NSK2 supplemented; D, aphid and *P. aeruginosa* 7NSK2 supplemented. Asterisks indicate significant QTL × environment interactions between the two traits plotted, following the levels.

^fSignificant after Bonferroni correction ($\alpha = 0.1$). All other QTL×E interactions listed here were significant in a single-site analysis.

the Steptoe allele was contributing to this locus. This QTL also has the highest LPR (5.81), making it highly probable that this is a significant QTL (not a false positive). It also explains a high percentage variance (31%) of shoot biomass. Therefore, it is highly likely that swapping the Steptoe allele for the Morex allele at this locus resulted in a large increase in barley shoot biomass when barley was grown with *P. aeruginosa* 7NSK2 supplementation and aphid infestation (aph and *Pa*, table 3).

(c) Steptoe × Morex, main effect and phenotypic plasticity quantitative trait loci

In the S×M population, we located 22 main effect QTL: eight shoot, nine root and five aphid population size (table 3). QTL were detected over all seven chromosomes. Most QTL were located in environments with either aphids, *P. aeruginosa* 7NSK2 supplementation, or both aphids + *P. aeruginosa*

7NSK2 supplementation. Only three QTL were detected in the control environment, suggesting that the majority of QTL were associated with the plant response to aphids and/or *P. aeruginosa* 7NSK2 supplementation. There was no overlap between root and shoot QTL, showing that above-ground and below-ground biomass are associated with separate loci under these conditions. One aphid population size QTL overlapped with a shoot biomass QTL on chromosome 6, and two aphid population size QTL overlapped with root biomass QTL, indicating that aphid fitness was influenced by the same loci associated with above- and below-ground plant biomass. Of the 22 QTL, nine exhibited QTL×E interactions across one or more environments (table 3), indicating that these QTL-trait associations were significantly influenced by the environment.

Phenotypic plasticity was mapped as the difference in mean trait values over two environments (e.g. control-aphid, etc.), resulting in six sets of plasticity

data per trait. Overall, we located 25 plasticity QTL (using chromosome wide significance levels): 10 shoot, 12 root biomass and three aphid fitness (table 3). Shoot and root plasticity QTL were located across all chromosomes, except for chromosome 2. Aphid population size plasticity QTL were located on chromosomes 4 and 5. Each QTL was specific to plasticity between two-paired environments. We observed that main effects and phenotypic plasticity QTL were either co-located (located at the same loci), or were located separately.

Seven phenotypic plasticity QTL co-located with main effect QTL. For example, a main effect shoot QTL on chromosome 1 (mapped in the *P. aeruginosa* 7NSK2-supplemented environment) was co-located with a shoot QTL associated with plasticity between the *P. aeruginosa* 7NSK2 and *P. aeruginosa* 7NSK2 + aphid environments. The main trait QTL also had significant QTL×E interactions with all other environments. This gives strong evidence that these loci are specifically associated with shoot biomass when *P. aeruginosa* 7NSK2 is supplemented, and the additional presence of aphids altered the QTL–trait association. On chromosome 3, we detected a QTL associated with root plasticity between the aphid and *P. aeruginosa* 7NSK2 + aphid environments that co-located with a root main trait QTL in the aphid environment. Six phenotypic plasticity QTL mapped the plasticity between the control–*P. aeruginosa* 7NSK2-supplemented environments, which demonstrates the influence of *P. aeruginosa* 7NSK2 supplementation on QTL–trait association compared with the control environment, as expected. Four QTL associated with plasticity of root and shoot biomasses between control–aphid environments were detected. We were also able to locate two QTL for plasticity between the aphid–*P. aeruginosa* 7NSK2-supplemented environments associated with shoot and root biomass plasticity.

The majority of phenotypic plasticity QTL, however, were located separately from main effect QTL (18 of 23). This indicates that there are QTL associated with the plasticity of a trait between environments that are not directly associated with a significant difference in a trait across genotypes. Therefore, genetic variation (between the two parents' alleles) at these loci directly influences how plastic a trait is across environments, but is not associated with the trait within any one environment.

(d) Oregon Wolfe Barley main effect and phenotypic plasticity quantitative trait loci

In the OWB population, we located 20 main effect QTL: nine shoot, six root and five aphid population size (table 3). QTL were located over all seven chromosomes. Most QTL were located in the aphid, *P. aeruginosa* 7NSK2 supplemented, or aphid + *P. aeruginosa* 7NSK2-supplemented environments, and three QTL were located in the control environment. This is similar to the results for the S×M mapping population, and indicates that we were mainly detecting QTL associated with the plant response to the presence of interacting species. The QTL located in the control environment on

chromosome 6 was associated with root and shoot biomasses, indicating that this QTL is associated with plant growth, rather than with a plant response to interacting species. Root and shoot QTL also co-located on chromosome 4 in the aphid environment. The QTL on chromosome 7 (in the control environment) was only associated with shoot biomass, indicating that this QTL may be specific for above-ground growth. Two shoot QTL were located in multiple environments—on chromosomes 2 and 4. The aphid QTL on chromosome 3 was also located in multiple environments. QTL detection in multiple environments indicates that these QTL are robust to environmental variation, and the traits associated with those QTL may be less plastic across environmental variation. Most QTL were located in only one environment, and six QTL exhibited significant QTL×E interactions, demonstrating that the environment had a significant influence on QTL expression.

We located 16 QTL associated with plasticity of traits: seven associated with plasticity in shoot biomass, eight associated with plasticity in root biomass and one associated with plasticity in aphid population size (table 3). QTL were detected across all seven chromosomes. Phenotypic plasticity QTL were located separately from main effect QTL in all but one example. The aphid population size QTL on chromosome 1 was mapped in the aphid environment (i.e. without *P. aeruginosa* 7NSK2 supplementation) and exhibited QTL×E. This also co-located with a phenotypic plasticity QTL for aphid fitness. In addition, a root biomass QTL for plasticity between the *P. aeruginosa* 7NSK2-supplemented and combined environments co-located at the same site. This suggests that this is an important site for (i) barley response to aphids, which is significantly altered by *P. aeruginosa* 7NSK2 supplementation and gives rise to significant phenotypic plasticity, and (ii) plasticity in root biomass caused by combined *P. aeruginosa* 7NSK2 supplementation and aphid presence compared with *P. aeruginosa* 7NSK2 supplementation without aphids.

4. DISCUSSION

(a) Phenotypic effect of environmental factors

We investigated the effect of rhizosphere supplementation with *P. aeruginosa* 7NSK2 and aphid infestation on plant biomass across lines of two mapping populations of barley. Both mapping populations gave similar patterns of phenotypic effects, in that biomass could be increased, decreased or unaffected by environmental factors (aphid infestation and rhizobacterial supplementation) compared with the control. This agrees with our previous results [12] on this experimental system. A decrease in barley biomass was observed in more lines in the aphid environment compared with the *P. aeruginosa* 7NSK2-supplemented environment (compared with the control), and the combined aphid and *P. aeruginosa* 7NSK2-supplemented environment led to a decrease in biomass in the greatest proportion of lines. This indicates that aphid infestation and *P. aeruginosa* 7NSK2 supplementation influence biomass via

separate mechanisms, since lines that were positively influenced by either aphid infestation or *P. aeruginosa* 7NSK2-supplemented environment were negatively affected in the combined aphid and *P. aeruginosa* 7NSK2-supplemented environment.

We simultaneously investigated the effect of *P. aeruginosa* 7NSK2 supplementation on aphid population size. *Pseudomonas aeruginosa* 7NSK2 supplementation reduced aphid population size on the majority of lines, indicating that *P. aeruginosa* 7NSK2 supplementation enhances plant defence or reduces availability of nutrients to the aphids. The root and shoot biomasses of most barley lines were reduced by the combination of *P. aeruginosa* 7NSK2 supplementation and aphid infestation compared with only aphid infestation. These effects were observed in both mapping populations. Therefore, it seems more probable that the reduction of aphid population size when *P. aeruginosa* 7NSK2 was supplemented is due to the reduction in barley host quality rather than host defence, which would also explain the reduction in barley biomass [28].

(b) Quantitative trait loci mapping of environmentally sensitive main effects and phenotypic plasticity

We located multiple QTL associated with root and shoot biomasses in both mapping populations. For aphid performance, we located five QTL in the S×M population, and five QTL in the OWB population. The difference in the number of QTL for plant biomass and aphid population size may be due to the continuous distribution of plant biomass in contrast to the more categorical effect of plant traits (e.g. defence traits) that influence aphid population size. This would also lead one to expect multiple phenotypic plasticity QTL for root and shoot biomasses, since the plasticity of a quantitative trait such as biomass could be due to phenotypic variation (across environments) in any one, or combination of, those main traits.

All barley biomass QTL displayed environmental sensitivity, since none were detected in more than two of the four environments. Similarly, most aphid population size QTL were detected in only one environment, with the exception of one QTL that was detected in both the *P. aeruginosa* 7NSK2-supplemented and non-supplemented environments. However, not all QTL showing environmental sensitivity had significant QTL×E interactions. In the S×M and OWB populations, 45 and 30 per cent, respectively, of QTL exhibited significant QTL×E interactions. Variation in occurrence of QTL×E interactions is common among studies investigating similar traits [16,29]. The occurrence of QTL that lacked significant QTL×E interactions indicates that those QTL had an association with the trait in other environments; however, the association was not significant enough for the QTL to be detected. In this case, QTL×E can be said to test whether the environment has significantly altered the QTL–trait association (significant QTL×E), or whether the environment has merely increased the effect of QTL on the phenotype (environmentally sensitive but statistically

non-significant QTL×E). It is unlikely that the detection of QTL in one environment but not others was caused by the use of mapping population subsets in this study. While it is true that the use of mapping subsets does limit the ability to detect QTL [22], this is due to the limited number of loci with genetic variation (between the two parental alleles) included in the subset. In the full mapping population more lines are included, providing genetic variation at a greater number of loci. Since we used the same 50 mapping lines in all environments, we would have detected any environmental variation that altered the QTL–trait associations that we could locate.

In the S×M population, two main effect QTL that exhibited QTL×E were co-located with phenotypic plasticity QTL. In the OWB mapping population, one main effect QTL with QTL×E co-located with phenotypic plasticity. The co-location of multiple environmentally sensitive main effect QTL (i.e. those exhibiting QTL×E) and phenotypic plasticity QTL lends strong support to the idea that a trait can be influenced by multiple loci, with some loci only expressed in certain environments [13]. Whitham & Agrawal [7] propose that the presence of phenotypic plasticity implies that a genotype does not determine a set phenotype, but a range of possible phenotypes, that are influenced by the environment. Our data indicate that a range of possible phenotypes is influenced by a range of different loci expressed in certain environments. Weinig *et al.* [30, p. 1153] propose that the presence of QTL×E ‘shows that variation at specific loci is only available to selection in some environments’.

We also located phenotypic plasticity QTL separately from main effect QTL. This indicates that there are loci that indirectly affect the phenotype by regulating the plasticity in QTL–phenotype associations. The presence of both co-locating and separate phenotypic plasticity QTL may indicate that there are many loci that can influence a phenotype (but are only expressed in certain environments), and the expression of those different loci may be regulated by separate loci (that are not directly associated with the trait). These two ideas were originally proposed by Scheiner [31] in models 2 and 3 for the genetic basis of plasticity. Even though our ability to locate different QTL was limited by the use of subsets of lines, this is unlikely to have affected the conclusion of whether main effects and phenotypic plasticity were co-located. If phenotypic plasticity was caused by the same loci as the main trait, these would have been mapped together, since the same dataset from the same lines was used to map both. Given the power of our analysis, the QTL–trait associations and interactions we located, this study may be viewed as a proof of concept that phenotypic plasticity caused by species interactions can be mapped onto specific loci.

(c) Plasticity and evolution in species interactions within multi-species communities

We simultaneously mapped QTL association with both plant biomass and aphid performance onto the barley genome, to locate main effect and plasticity QTL affecting both species’ traits. Of the five aphid

performance QTL mapped in the OWB population, one was co-located with a root biomass plasticity QTL (chromosome 1). In the S×M population, aphid QTL were co-located with two root QTL (chromosomes 3 and 5), a root plasticity QTL (chromosome 5) and a shoot biomass QTL (chromosome 6). The location of aphid and plant biomass QTL indicates that these loci could be involved in plant defence in the environment in which they were mapped. The locations cited are the marker location for the peak value of the loci–phenotype association, and the flanking markers of the full QTL do overlap between aphid and shoot biomass QTL. However, co-location of main effect and plasticity QTL may not mean that the same genes are involved in both species traits, since the confidence interval of each estimated position is likely to contain hundreds of genes [32]. QTL mapped in this study can highlight areas of interest for future high-resolution mapping studies investigating the plasticity of species interactions focusing on specific areas of a chromosome, as is demonstrated by high-resolution mapping and near isogenic lines (NILs) [33]. High-resolution mapping combined with analyses of candidate gene mutants and gene silencing could identify genes involved in phenotypic plasticity of multi-species interactions. Good targets for such a study of phenotypic plasticity are the QTL we mapped on chromosome 1 in the S×M population. Here, we located the shoot and root biomasses' main effects mapped at separate locations in the *P. aeruginosa* 7NSK2-supplemented environment (contributed by Morex alleles). At the same marker site, we mapped shoot and root biomasses' phenotypic plasticity QTL for plasticity between *P. aeruginosa* 7NSK2-supplemented and combined environments (contributed by Morex alleles). The co-location of these QTL indicates that phenotypic plasticity is caused by extreme environmental sensitivity of alleles. The QTL was associated with shoot biomass when *P. aeruginosa* 7NSK2 was supplemented, and the additional presence of aphids reduced the association. QTL mapping using three species is uncommon; therefore this may be the first example of two interacting species with antagonistic effects on QTL–trait association in an intermediate species. It is proposed that multi-species interactions that have strong phenotypic effects could alter evolutionary trajectories depending on how their interactions influence the polarity of trait values [34].

Traits involved in plant–insect interactions have previously been mapped; however, previous studies have focused on traits from one of the two species [18,19]. Plants interact with a plethora of above- and below-ground species, and it is possible that interacting individuals reciprocally respond to each other over ecological time [9]. Reciprocal interactions imply continuous back and forth responses, as are postulated by co-evolutionary arms races between plants and insects [5,6,9]. Peppe & Lomonaco [35, p. 193] state that 'when plasticity contributes positively to fitness, it can be considered adaptive, and constitutes an important advantage in exploiting heterogeneous environments'. However, when applying this to antagonistic species interactions, a positive contribution to

fitness for one species could result in a negative contribution to fitness for the interacting species, and is likely to lead to the interacting species phenotypically responding with its plasticity genes contributing to its fitness. Phenotypic plasticity in aphids is known to be triggered by host quality and secondary plant substances [6,36], which can be genetically based [28,36].

The aim of community genetics is to address the phenomenon of how genetic variation and species presence may influence the phenotypes of associated species within a community, and over time influence the evolution of those species. This study has provided a proof of concept that genetic variation at multiple loci within the barley genome can alter the effect of rhizobacterial supplementation and aphid infestation on barley biomass and of rhizobacterial supplementation on aphid fitness. If such genetically based phenotypic effects were to pervade over time within natural communities, they would be likely to alter co-evolutionary trajectories [34].

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