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Title: Quantitative Trait Loci mapping of phenotypic plasticity and genotype: environment interactions in plant and insect performance

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Keywords: Aphid, phenotypic plasticity, Quantitative Trait Loci (QTL) mapping, rhizobacteria, species interactions.

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Running title: QTL mapping of phenotypic plasticity

27 **Abstract**

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

47

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

50 **Introduction**

51 Phenotypic plasticity is the ability of an organism or population to alter its phenotype according
52 to environmental variation (e.g. [1]). It enables an organism to continue to survive and reproduce
53 across variable environments, and is particularly important in organisms such as plants that live a
54 sessile life. Plant traits can exhibit plasticity to abiotic [2] and biotic (e.g. responses to herbivory)
55 environmental variation [3–5]. Phenotypic plasticity also occurs in insects and can influence both
56 individual morphology and population size [6–8] raising the question of whether both plants
57 and insects simultaneously show phenotypically plastic responses. However, reciprocal
58 phenotypic plasticity, i.e. whether plasticity in plant traits affects insect phenotype, has received
59 little attention [9]. Phenotypic plasticity becomes important in a community genetics framework
60 because effects caused by non-plastic traits (i.e. traits where the mapping of genotype to
61 phenotype is constant across environments) are likely to influence evolutionary trajectories in a
62 different fashion from effects caused by plastic traits. Non-plastic traits will have a consistent
63 influence on the structure of the associated community and the fitness of individual
64 interacting species, and thus a consistent influence on the evolutionary trajectories of these
65 species. Plastic traits will have a less consistent community genetic effect from each genotype
66 and thus a less consistent, although not necessarily weaker, influence on the evolution of
67 associated species. Here, we present a pilot study where we use existing methods of examining
68 the genetic basis of plasticity in individual species applied to a multi-species system. In nature,
69 plants interact with multiple aboveground and below-ground species, and these interactions could
70 be influenced by phenotypic plasticity [9]. Phenotypic plasticity can be visualized by plotting
71 trait values over environments, creating a norm of reaction. In this case, phenotypic plasticity

72 is the slope of the reaction norm, or the extent to which the trait value changes across
73 environments [10]. Genotype x environment interactions (GEIs) can occur if genetic variation
74 influences the slope of the reaction norm across environments, and can cause crossing of the
75 norms of reaction [11]. Phenotypic plasticity and GEIs can play an important role in species
76 interactions [7,9,12] and knowledge of the underlying genetic basis could provide further
77 explanation on how species have evolved within multi-species communities. Phenotypic
78 plasticity can be either adaptive when it confers a fitness advantage [1,7,10,13,14] or non-
79 adaptive as phenotypic plasticity also covers examples where phenotypic changes in response to
80 environmental heterogeneity may not enhance fitness [1,14]. GEIs explain that no single
81 phenotype can confer high fitness in all environments, therefore a species may have higher
82 tolerance to heterogeneous environments if its phenotype can change according to the
83 environment [10]. Genetic models have been presented to explain the genetic basis of plasticity
84 [10,15] and two main ideas prevail: (i) the mean trait (within each environment) and the plasticity
85 of the trait (difference in the mean phenotype over contrasting environments) may be influenced
86 by separate genes and each be subject to selection (the 'plasticity genes' could be thought of as
87 regulatory genes that influence which trait-associated genes are expressed [16]); (ii) plasticity is
88 influenced by environmentally sensitive loci directly influencing trait value in both environments
89 and may have evolved as a by-product of differential natural selection [10,16]. The quantitative
90 genetic basis of GEIs and phenotypic plasticity can be investigated using quantitative trait loci
91 (QTL) mapping, as reported in studies testing the effect of abiotic [2,16,17] heterogeneous
92 environments. Mapping populations are developed from two genotypes, and following many
93 generations of inbreeding, multiple lines are generated. The lines are almost genetically
94 homogeneous, except that each line differs at a few loci. Alleles at loci originate from either

95 parental genotype 1 or parental genotype 2 and ultimately the total population should be
96 constituted by lines that cover every possible combination of parental allele at each locus.
97 Therefore, a QTL is located when the difference in having the allele from either parent 1 or
98 parent 2 at each locus causes a significant change in the phenotype. In this way, we are
99 testing the association between the phenotype and the genotype at each locus. Where significant
100 QTL are found in one environment but not in the other, GEIs can then be tested using standard
101 analysis of variance (ANOVA) with QTL analysis to determine if genetic variation at that
102 location influences plasticity. QTL mapping can also be performed on environmentally
103 sensitive traits to test for plasticity between environments, by mapping the differences in mean
104 trait values between environments. This approach can locate different loci associated across all or
105 one environment, or whether separate loci are simply associated with the change in phenotype
106 across environments. If GEIs are present at a chromosomal location that also shows a significant QTL for
107 mean trait differences, this suggests that the loci controlling the trait value in the two
108 environments also determine the plasticity. However, if there is no significant QTL for mean
109 differences at a location showing a significant GEI, this suggests that other regulatory genes may
110 be controlling plasticity. Although plant–insect interactions have been previously mapped onto
111 the plant genome in terms of plant defence [18–20], plasticity in traits involved in the reciprocal
112 interaction has received little attention. Furthermore, plants interact with multiple above and
113 below-ground species, yet interactions among multiple species are rarely mapped. In this paper,
114 we present a ‘proof of concept’ study that applies established QTL mapping approaches for
115 plasticity (e.g. [16]) to multi-species interactions and indirect effects such as those described by
116 community genetics. We test the hypothesis that variation in the composition of the plant

117 rhizosphere could cause phenotypic plasticity in plant–insect interactions. We mapped reciprocal
118 barley–aphid interactions using a tri-trophic model ecosystem consisting of rhizobacterial
119 supplementation with *Pseudomonas aeruginosa* 7NSK2, barley (Hordeum vulgare) and cereal
120 aphid (*Sitobion avenae*). To test main effects and interactive effects of each species on barley and
121 aphid performance, we set up environments covering each possible combination of species (and
122 controls), with barley as the only species present in every environment. We used two doubled
123 haploid (DH) mapping populations, Steptoe x Morex (SxM) and Oregon Wolfe Barley
124 (OWB). Parental lines from the SxM population were previously investigated for the presence of
125 GEIs within the tri-trophic ecosystem used here [12]. The second mapping population (OWB)
126 was used to test whether GEIs and phenotypic plasticity resulting from multi-species interactions
127 are specific to mapping population or prevalent throughout both mapping populations. Our
128 objectives were to: (i) map root and shoot biomasses (plant traits involved in both aboveand
129 below-ground species interactions) and aphid fitness onto the barley genome in each
130 environment; (ii) test whether the environment has a significant effect on QTL expression (QTL
131 x Environment interaction); (iii) map phenotypic plasticity (as mean differences in trait values) of
132 plant root and shoot biomasses, and aphid fitness between environments; (iv) compare the
133 prevalence of GEIs and phenotypic plasticity across the two mapping populations.
134
135
136 **Materials and Methods**
137 *Quantitative trait loci mapping populations*
138 We mapped plant biomass onto the barley (H. vulgare) genome using two DH barley mapping
139 populations, derived from the parental genotypes SxM (population 1) and OWB dominant and

140 recessive (population 2). DH populations are used in many cereal crops and provide one of the
141 best methods to map QTL owing to the homozygous lines, produced using the bulbosum
142 technique [21]. The SxM population has a high-average map density of markers (5.6 cM);
143 chromosome (Ch) 1: 170 cM, 37 markers; Ch2: 181 cM, 37 markers; Ch3: 185 cM, 31 markers;
144 Ch4: 177 cM, 33 markers; Ch5: 151 cM, 29 markers; Ch6: 157 cM, 22 markers; Ch7: 202 cM, 34
145 markers. The OWB population has a similar map density (5.5 cM); Ch1: 136 cM, 29 markers;
146 Ch2: 180 cM, 35 markers; Ch3: 218 cM, 28 markers; Ch4: 125 cM, 31 markers; Ch5: 225 cM, 37
147 markers; Ch6: 167 cM, 35 markers; Ch7: 199 cM, 37 markers. Seeds for the two mapping
148 populations were supplied by P. Hayes (Oregon State University). The linkage maps for the SxM
149 population (consisting of 150 DH lines) and the OWB population (94 DH lines) are available on
150 the GrainGenes website: http://www.wheat.pw.usda.gov/GG2/index.shtml. In this study, a subset
151 of 50 lines from each population was chosen for phenotyping and subsequent mapping owing to
152 logistical constraints of phenotyping the full mapping populations. Using a subset can create two
153 experimental caveats, which are discussed below.
154

155 *The use of subsets in quantitative trait loci mapping*

156 The use of subsets of lines from mapping populations is known to limit QTL mapping in two
157 ways. Firstly, the ability to detect QTL–trait associations is limited, because QTL are only
158 detected where there is genetic variation at loci (between the two parental alleles), which has a
159 significant effect on the measured trait. Each line in the mapping population is designed to
160 contribute alleles from either parent, and each line will differ in which parental alleles they
161 contribute at a small number of loci [22]. Therefore, fewer lines results in fewer genetically
162 variable loci. Secondly, the detection of fewer QTL means that the QTL–trait association could

163 be over-exaggerated, owing to the nature of QTL analysis [22]. However, this does not
164 necessarily increase the likelihood of detecting false positives with the calculation of a threshold
165 value, which QTL must exceed to be significant. The calculation of threshold values used in this
166 study was based on a method that calculates the level of genetic variation within the 50-line
167 subset that we used [23]. Therefore, the threshold values that we calculated may have been higher
168 (limiting the number of QTL deemed significant) than if we had calculated values using the full
169 mapping population. The occurrence of false positives in QTL mapping can be affected by low
170 experimental power caused by several factors, including the method of QTL data analysis. This
171 study used composite interval mapping (CIM) [24], a high precision method that maps QTL by
172 testing the association between loci and trait, while simultaneously using flanking markers to
173 account for variance caused by other QTL located on the same chromosome. Therefore, this
174 method includes a control for the expected effects of QTL over exaggeration caused by the
175 detection of fewer QTL in mapping subsets, since each QTL is tested independently of other
176 QTL beyond the flanking markers [24]. A further concern for the power of QTL mapping is the
177 number of experimental replicates used, since the mean of the trait is mapped, ignoring any
178 standard deviation. In this study, the traits used to map QTL were the means calculated from four
179 experimental replicates. It is impossible to use the standard deviation of means within QTL
180 mapping to quantify the significance of results, therefore the number of replicates we used is
181 important, since the error distribution of the mean data collected from our four replicates is likely
182 to be smaller compared with studies that use fewer experimental replicates. A further source of
183 power in our analysis originates from the breeding design of the lines we used, and the number of
184 markers mapped onto each chromosome. We used DH lines produced using the 'bulbosum'
185 technique [21], with an average map density of 5.5 cm. This method results in lines that are

186 homozygous at each locus and thus no residual heterozygous individuals are involved in

187 mapping. To conclude, it is probably that the number of QTL detected in this study is a subset of

188 the total number that we would have detected had we mapped using the full population and that it

189 is probably that detection of 'false' QTL has been minimized.

190

191 *Experimental design*

192 We designed a fully factorial experiment with two biotic environmental factors (rhizobacteria, P.

193 aeruginosa 7NSK2 and cereal aphid, *S. avenae*) to map QTL resulting from both main effects and

194 interaction effects of the environmental factors on plant biomass. This gave four environmental

195 ecosystems: (i) control (P. aeruginosa 7NSK2 not supplemented, no aphids); (ii) P. aeruginosa

196 7NSK2 supplemented (no aphids); (iii) aphid infestation (P. aeruginosa 7NSK2 not

197 supplemented); and (iv) P. aeruginosa 7NSK2 supplemented, aphid infestation. We selected a 50

198 line subset of each mapping population (plus parental lines), and grew each line under all four

199 environments, and replicated four times, giving 832 plants per mapping population. We used a

200 randomized block design, with replicate as the block, and each line environment combination was

201 randomized within each treatment block.

202

203 *Plant phenotyping*

204 Plants were grown in a glasshouse at the Firs Experimental Research Station (The University of

205 Manchester) during June 2005 (SxM population) and June 2006 (OWB population).

206 Supplemental lights were used to provide a 16 L : 8 D regime and a daily temperature range of

207 16–30°C. To minimize the presence of non-experimental rhizobacteria, we sterilized seeds in 10

208 per cent sodium hypochlorite (followed by several washes with sterilized distilled water) and

209 germinated the seeds in sterile Petri dishes and filter paper for 5 days. Preparation of P.
210 aeruginosa 7NSK2 inoculum and inoculation onto barley roots were as previously described [12].
211 After inoculation, seedlings were planted into 10 cm pots containing heat sterilized horticultural
212 grade sharp sand. We had set up the rhizosphere system as a sterilised system, to minimize the
213 introduction of non-experimental micro-organisms pre-inoculation to aid the development of our
214 bacterial inoculum on plant roots. Post-plant transplantation, the rhizosphere was allowed to be
215 naturally colonized by non-experimental micro-organisms (e.g. via irrigation) therefore, the
216 rhizosphere treatment should be thought of as supplemented/not supplemented rather than
217 presence/absence of P. aeruginosa 7NSK2. Plants were watered once a week with 40 ml full
218 concentration Hoagland's solution [25]. Eleven days after transplantation, two adult aphids were
219 placed onto each plant and a plastic tube with mesh windows was fitted over each plant to
220 prevent aphids escaping. The total aphid population size was counted two weeks after infestation.
221 Plant shoots and roots were then separated, cleaned and dried at 808C for 3 days for dry biomass
222 measurements.
223
224 *Phenotypic plasticity*
225 Phenotypic plasticity was calculated using the character state approach [10]. In this method,
226 phenotypic plasticity is the difference in the mean phenotype between two environments, i.e. the
227 slope of the reaction norm. For plant biomass, we calculated phenotypic plasticity between five
228 environmental pairs: (i) aphid infested versus non-infested (P. aeruginosa 7NSK2 not
229 supplemented); (ii) aphid infested versus non-infested (P. aeruginosa 7NSK2 supplemented);

230 (iii) P. aeruginosa 7NSK2 supplemented versus non-supplemented (aphid non-infested); (iv) P.
231 aeruginosa 7NSK2 supplemented versus non-supplemented (aphid infested); and (v) aphid
232 infested and P. aeruginosa 7NSK2 supplemented versus non-infested and non supplemented.
233 For aphid population size, phenotypic plasticity was calculated as the difference in population
234 size between environments with and without P. aeruginosa 7NSK2 supplementation.

235

236 *Data analysis*

237 ANOVA was performed on the trait data using the GLM method in MINITAB (v. 15), treating
238 line as a random factor. We used three approaches to map the effects of genotype and
239 environment on QTL–trait association. Firstly, we mapped main effects (trait values) onto the
240 genotypic data for each of the four environments. Secondly, we tested the effect of the
241 environment at each locus where we had located a significant QTL to look for QTL by
242 environment interactions. As this was a single-site analysis, the ANOVA of QTLxE is a test of
243 whether the association between loci and trait value significantly changes between contrasting
244 environments. Thirdly, we mapped phenotypic plasticity (difference between mean trait value),
245 which effectively maps the GEIs onto the genotypic data. We mapped main effects and
246 phenotypic plasticity for barley and aphid performance onto the barley genome using the CIM
247 procedure in QTL Cartographer [26]. CIM tests the association between marker sites and trait
248 values at 2 cM intervals along each chromosome. At each 2 cM test site, the analysis includes
249 background markers as cofactors, to control variance caused by QTL at non-target loci outside
250 flanking markers determined by the 'window size' [24]. We used a window size of 10 cM around
251 the target loci. The location of a QTL associated with a significant phenotypic effect was defined
252 as the point where the likelihood probability ratio (LPR) exceeded the threshold value. Threshold

253 values were calculated genome wide and for each chromosome in each mapping population
254 following the method of Li & Ji [23]. Chromosome significance threshold were used to interpret
255 results but genome wide significance is also reported in table 3. This method involves calculation
256 of the effective number of marker loci using results from principal components analysis (PCA) of
257 the marker data. Values for r2 (% phenotypic variation explained by a QTL) and additive genetic
258 effect were generated by QTL Cartographer [26]. Tests for QTLxE interactions were conducted
259 for all significant QTL. We performed a single marker site QTLxE analysis where QTL had been
260 located, using SAS [27], and conducted Bonferroni corrections for multiple testing.
261
262
263 **Results**
264 *Phenotypic effect of environmental factors*
265 Barley shoot and root biomass and aphid population size were influenced by both environmental
266 and genetic (mapping line) variation (figure 1). Furthermore, for SxM mapping lines, a
267 significant line x environment interaction influenced barley root and shoot biomasses (table 1).
268 Across lines of both mapping populations, aphid population size increased, decreased or
269 remained constant when the rhizosphere was supplemented with P. aeruginosa 7NSK2 compared
270 with the control. Compared with the control environment, P. aeruginosa 7NSK2 supplementation
271 resulted in a reduction of the aphid population size in 60 per cent (SxM population) and 54 per
272 cent (OWB population) of lines, and an increase in 26 (SxM population) and 31 per cent (OWB
273 population) of lines (table 2). Similarly, the effect of environmental factors on shoots and root
274 biomass varied across mapping lines. For both populations, P. aeruginosa 7NSK2
275 supplementation led to a reduction in biomass in 67–72% of lines, and an increase in 15–22% of

276 lines. Aphid infestation tended to reduce biomass, in up to 82 per cent of lines. The combination
277 of both P. aeruginosa 7NSK2 supplementation and aphids led to reduced biomass in 94–96% of
278 lines, and increased biomass in 2–4% of lines. The aphid environment had a negative effect on
279 root and shoot biomasses for more lines than the environment with P. aeruginosa 7NSK2
280 (P.a) supplementation, indicated by the aphid–aphid and P.a, and the P.a–aphid and P.a
281 comparisons (table 2). We observed that the mean traits for the lines exceeded the mean value for
282 either parental line (figure 1). This is a general observation in QTL mapping studies, and this
283 transgressive variation can be caused by epistatic interactions, or by the accumulation of
284 complementary alleles in the DH lines [11].
285
286 *Quantitative trait loci mapping*
287 We mapped main effects (direct association between phenotype and loci) across environments,
288 and phenotypic plasticity (difference in trait means between two environments) onto the barley
289 genome. This analysis produced plots showing the association between loci and trait, measured
290 as LPR value (figures 2 and 3). When we have a significant association we see a QTL peak on
291 the graph, and this means that at that locus there is a high probability that swapping the allele
292 from parent 1 (Steptoe/OWB D) with the allele from parent 2 (Morex/OWB R) will significantly
293 affect the trait. The level of probability of QTL–trait association ranged from 2.3 LPR (the
294 minimum threshold level) to 5.8 LPR (table 3), and each QTL explained between 9 and 31 per
295 cent of phenotypic variance. The additive genetic effect (A.G.E., table 3) is a measure of the
296 magnitude of the QTL effect (i.e. the effect of swapping the allele for parent 1–parent 2) on the
297 trait, and the polarity indicates which parental allele results in a greater trait value, for example,
298 bigger aphid population size, or greater shoot biomass. The largest A.G.E. (262.62) was for

299 a QTL on chromosome 4 of the SxM population. This means that when the Morex allele was
300 contributing to this locus, shoot biomass is higher than when the Steptoe allele was contributing
301 to this locus. This QTL also has the highest LPR (5.81), making it highly probable that this is a
302 significant QTL (not a false positive). It also explains a high percentage variance (31%) of shoot
303 biomass. Therefore, it is highly likely that swapping the Steptoe allele for the Morex allele at this
304 locus resulted in a large increase in barley shoot biomass when barley was grown with P.
305 aeruginosa 7NSK2 supplementation and aphid infestation (aph and P.a, table 3).
306

307 *Steptoe x morex, main effect and phenotypic plasticity quantitative trait loci*

308 In the SxM population, we located 22 main effect QTL; eight shoot, nine root and five aphid
309 population size (table 3). QTL were detected over all seven chromosomes. Most QTL were
310 located in environments with either aphids, P. aeruginosa 7NSK2 supplementation, or both
311 aphids + P. aeruginosa 7NSK2 supplementation. Only three QTL were detected in the control
312 environment, suggesting that the majority of QTL were associated with the plant response to
313 aphids and or P. aeruginosa 7NSK2 supplementation. There was no overlap between root
314 and shoot QTL, showing that above-ground and below-ground biomass are associated with
315 separate loci under these conditions. One aphid population size QTL overlapped with a shoot
316 biomass QTL on chromosome 6, and two aphid population size QTL overlapped with root
317 biomass QTL, indicating that aphid fitness was influenced by the same loci associated
318 with above- and below-ground plant biomass. Of the 22 QTL, nine exhibited QTLxE interactions
319 across one or more environments (table 3), indicating that these QTL–trait associations were
320 significantly influenced by the environment. Phenotypic plasticity was mapped as the difference

321 in mean trait values over two environments (e.g. control–aphid, etc), resulting in six sets of
322 plasticity data per trait. Overall, we located 25 plasticity QTL (using chromosome wide
323 significance levels) 10 shoot, 12 root biomass and three aphid fitness (table 3). Shoot and root
324 plasticity QTL were located across all chromosomes, except for chromosome 2. Aphid
325 population size plasticity QTL were located on chromosomes 4 and 5. Each QTL was specific to
326 plasticity between two-paired environments. We observed that main effects and phenotypic
327 plasticity QTL were either co-located (located at the same loci), or were located separately.
328 Seven phenotypic plasticity QTL co-located with main effect QTL. For example, a main effect
329 shoot QTL on chromosome 1 (mapped in the P. aeruginosa 7NSK2-supplemented environment)
330 was co-located with a shoot QTL associated with plasticity between the P. aeruginosa 7NSK2
331 and P. aeruginosa 7NSK2 + aphid environments. The main trait QTL also had significant QTLxE
332 interactions with all other environments. This gives strong evidence that these loci are
333 specifically associated with shoot biomass when P. aeruginosa 7NSK2 is supplemented, and the
334 additional presence of aphids altered the QTL–trait association. On chromosome 3, we detected a
335 QTL associated with root plasticity between the aphid and P. aeruginosa 7NSK2 + aphid
336 environments that co-located with a root main trait QTL in the aphid environment. Six
337 phenotypic plasticity QTL mapped the plasticity between the control–P. aeruginosa 7NSK2-
338 supplemented environments, which demonstrates the influence of P. aeruginosa 7NSK2
339 supplementation on QTL–trait association compared with the control environment, as expected.
340 Four QTL associated with plasticity of root and shoot biomasses between control–
341 aphid environments were detected. We were also able to locate two QTL for plasticity between
342 the aphid–P. aeruginosa 7NSK2-supplemented environments associated with shoot and root
343 biomass plasticity. The majority of phenotypic plasticity QTL, however, was located separately

344 from main effect QTL (18 of 23). This indicates that there are QTL associated with the plasticity
345 of a trait between environments that are not directly associated with a significant difference in a
346 trait across genotypes. Therefore, genetic variation (between the two parents' alleles) at these loci
347 directly influences how plastic a trait is across environments, but is not associated with the trait
348 within any one environment.
349
350 *Oregon Wolfe Barley main effect and phenotypic plasticity quantitative trait loci*
351 In the OWB population, we located 20 main effect QTL; nine shoot, six root and five aphid
352 population size (table 3). QTL were located over all seven chromosomes. Most QTL were located
353 in the aphid, P. aeruginosa 7NSK2 supplemented, or aphid + P. aeruginosa 7NSK2-supplemented
354 environments and three QTL were located in the control environment. This is similar to the
355 results for the SxM mapping population, and indicates that we were mainly detecting QTL
356 associated with the plant response to the presence of interacting species. The QTL located in the
357 control environment on chromosome 6 was associated with root and shoot biomasses, indicating
358 that this QTL is associated with plant growth, rather than with a plant response to interacting
359 species. Root and shoot QTL also collocated on chromosome 4 in the aphid environment. The
360 QTL on chromosome 7 (in the control environment) was only associated with shoot biomass,
361 indicating that this QTL may be specific for aboveground growth. Two shoot QTL were located
362 in multiple environments—on chromosomes 2 and 4. The aphid QTL on chromosome 3 was also
363 located in multiple environments. QTL detection in multiple environments indicates that these
364 QTL are robust to environmental variation, and the traits associated with those QTL may be less
365 plastic across environmental variation. Most QTL were located in only one environment, and six

366 QTL exhibited significant QTLxE interactions, demonstrating that the environment had a
367 significant influence on QTL expression. We located 16 QTL associated with plasticity of
368 traits; seven associated with plasticity in shoot biomass, eight associated with plasticity in root
369 biomass and one associated with plasticity in aphid population size (table 3). QTL were detected
370 across all seven chromosomes. Phenotypic plasticity QTL were located separately from main
371 effect QTL in all but one example. The aphid population size QTL on chromosome 1 was
372 mapped in the aphid environment (i.e. without P. aeruginosa 7NSK2 supplementation) and
373 exhibited QTLxE. This also co-located with a phenotypic plasticity QTL for aphid fitness. In
374 addition, a root biomass QTL for plasticity between the P. aeruginosa 7NSK2-supplemented and
375 combined environments co-located at the same site. This suggests that this is an important site for
376 (i) barley response to aphids, which is significantly altered by P. aeruginosa 7NSK2
377 supplementation and gives rise to significant phenotypic plasticity, (ii) plasticity in root biomass
378 caused by combined P. aeruginosa 7NSK2 supplementation and aphid presence compared with P.
379 aeruginosa 7NSK2 supplementation without aphids.
380
381
382 **Discussion**
383 *Phenotypic effect of environmental factors*
384 We investigated the effect of rhizosphere supplementation with P. aeruginosa 7NSK2 and aphid
385 infestation on plant biomass across lines of two mapping populations of barley. Both mapping
386 populations gave similar patterns of phenotypic effects, in that biomass could be increased,
387 decreased or unaffected by environmental factors (aphid infestation and rhizobacterial
388 supplementation) compared with the control. This agrees with our previous results [12] on this

389 experimental system. A decrease in barley biomass was observed in more lines in the aphid
390 environment compared with the P. aeruginosa 7NSK2-supplemented environment (compared
391 with the control), and the combined aphid and P. aeruginosa 7NSK2-supplemented environment
392 led to a decrease in biomass in the greatest proportion of lines. This indicates that aphid
393 infestation and P. aeruginosa 7NSK2 supplementation influence biomass via separate
394 mechanisms, since lines that were positively influenced by either aphid infestation or P.
395 aeruginosa 7NSK2-supplemented environment were negatively affected in the combined aphid
396 and P. aeruginosa 7NSK2-supplemented environment. We simultaneously investigated the effect
397 of P. aeruginosa 7NSK2 supplementation on aphid population size. Pseudomonas aeruginosa
398 7NSK2 supplementation reduced aphid population size on the majority of lines, indicating that P.
399 aeruginosa 7NSK2 supplementation enhances plant defence or reduces availability of nutrients to
400 the aphids. The root and shoot biomasses of most barley lines were reduced by the combination
401 of P. aeruginosa 7NSK2 supplementation and aphid infestation compared with only aphid
402 infestation. These effects were observed in both mapping populations. Therefore, it seems more
403 probably that the reduction of aphid population size when P. aeruginosa 7NSK2 was
404 supplemented is due to the reduction in barley host quality rather than host defence, which would
405 also explain the reduction in barley biomass [28].
406
407 *Quantitative trait loci mapping of environmentally sensitive main effects and phenotypic*
408 *plasticity*
409 We located multiple QTL associated with root and shoot biomasses in both mapping populations.
410 For aphid performance, we located five QTL in the SxM population, and five QTL in the OWB

411 population. The difference in the number of QTL for plant biomass and aphid population size
412 may be due to the continuous distribution of plant biomass in contrast to the more categorical
413 effect of plant traits (e.g. defence traits) that influence aphid population size. This would also lead
414 one to expect multiple phenotypic plasticity QTL for root and shoot biomasses, since the
415 plasticity of a quantitative trait such as biomass could be due to phenotypic variation (across
416 environments) in any one, or combination of, those main traits. All barley biomass QTL
417 displayed environmental sensitivity, since none were detected in more than two of the four
418 environments. Similarly, most aphid population size QTL were detected in only one
419 environment, with the exception of one QTL that was detected in both the P. aeruginosa 7NSK2-
420 supplemented and non-supplemented environments. However, not all QTL showing sensitivity
421 had significant QTLxE interactions. In the SxM and OWB populations, 45 and 30 per cent,
422 respectively, of QTL exhibited significant QTLxE interactions. Variation in occurrence of
423 QTLxE interactions is common among studies investigating similar traits [16,29]. The
424 occurrence of QTL that lacked significant QTLxE interactions indicates that those QTL had an
425 association with the trait in other environments, however, the association was not significant
426 enough for the QTL to be detected. In this case, QTLxE can be said to test whether the
427 environment has significantly altered the QTL–trait association (significant QTLxE), or whether
428 the environment has merely increased the effect of QTL on the phenotype (environmentally
429 sensitive but statistically non-significant QTLxE). It is unlikely that the detection of QTL in one
430 environment but not others was caused by the use of mapping population subsets in this study.
431 While it is true that the use of mapping subsets does limit the ability to detect QTL [22], this is
432 due to the limited number of loci with genetic variation (between the two parental alleles)

433 included in the subset. In the full mapping population more lines are included, providing genetic
434 variation at a greater number of loci. Since we used the same 50 mapping lines in all
435 environments, we would have detected any environmental variation that altered the QTL–trait
436 associations that we could locate. In the SxM population, two main effect QTL that exhibited
437 QTLxE were co-located with phenotypic plasticity QTL. In the OWB mapping population,
438 one main effect QTL with QTLxE co-located with phenotypic plasticity. The co-location of
439 multiple environmentally sensitive main effect QTL (i.e. those exhibiting QTLxE) and
440 phenotypic plasticity QTL lends strong support to the idea that a trait can be influenced by
441 multiple loci, with some loci only expressed in certain environments [13]. Whitham &
442 Agrawal [7] propose that the presence of phenotypic plasticity implies that a genotype does not
443 determine a set phenotype, but a range of possible phenotypes, that are influenced by the
444 environment. Our data indicate that a range of possible phenotypes is influenced by a range of
445 different loci expressed in certain environments. Weinig et al. [30] propose that the presence
446 of QTLxE 'shows that variation at specific loci is only available to selection in some
447 environments'. We also located phenotypic plasticity QTL separately from main effect QTL.
448 This indicates that there are loci that indirectly affect the phenotype by regulating the plasticity in
449 QTL–phenotype associations. The presence of both co-locating and separate phenotypic
450 plasticity QTL may indicate that there are many loci that can influence a phenotype (but are only
451 expressed in certain environments), and the expression of those different loci may be regulated by
452 separate loci (that are not directly associated with the trait). These two ideas were originally
453 proposed by Scheiner [31] in models 2 and 3 for the genetic basis of plasticity. Even though our
454 ability to locate different QTL was limited by the use of subsets of lines, this is unlikely to have
455 affected the conclusion of whether main affects and phenotypic plasticity were co-located. If

456 phenotypic plasticity was caused by the same loci as the main trait, these would have been
457 mapped together, since the same dataset from the same lines was used to map both. Given the
458 power of our analysis, the QTL–trait associations and interactions we located, this study may be
459 viewed as a proof of concept that phenotypic plasticity caused by species interactions can be
460 mapped onto specific loci.
461
462 *Plasticity and evolution in species interactions within multi-species communities*
463 We simultaneously mapped QTL association with both plant biomass and aphid performance
464 onto the barley genome, to locate main effect and plasticity QTL affecting both species' traits. Of
465 the five aphid performance QTL mapped in the OWB population, one was co-located with a root
466 biomass plasticity QTL (chromosome 1). In the SxM population, aphid QTL were co-located
467 with two root QTL (chromosomes 3 and 5), a root plasticity QTL (chromosome 5) and a shoot
468 biomass QTL (chromosome 6). The location of aphid and plant biomass QTL indicates that these
469 loci could be involved in plant defence in the environment that they were
470 mapped in. The locations cited are the marker location for the peak value of the loci–phenotype
471 association, and the flanking markers of the full QTL do overlap between aphid and shoot
472 biomass QTL. However, co-location of main effect and plasticity QTL may not mean that the
473 same genes are involved in both species traits, since the confidence interval of each estimated
474 position is likely to contain hundreds of genes [32]. QTL mapped in this study can highlight areas
475 of interest for future high-resolution mapping studies investigating the plasticity of species
476 interactions focusing on specific areas of a chromosome, as is demonstrated by high-resolution
477 mapping and nearisogenic lines (NILs) [33]. High-resolution mapping combined with analyses of
478 candidate gene mutants and gene silencing could identify genes involved in phenotypic plasticity

479 of multi-species interactions. Good targets for such a study interested in phenotypic plasticity are
480 the QTL we mapped on chromosome 1 in the SxM population. Here, we located the shoot and
481 root biomasses' main effects mapped at separate locations in the P. aeruginosa 7NSK2-
482 supplemented environment (contributed by Morex alleles). At the same marker site, we mapped
483 shoot and root biomasses' phenotypic plasticity QTL for plasticity between P. aeruginosa
484 7NSK2-supplemented and combined environments (contributed by Morex alleles). The co-
485 location of these QTL indicates that phenotypic plasticity is caused by extreme environmental
486 sensitivity of alleles. The QTL was associated with shoot biomass when P. aeruginosa 7NSK2
487 was supplemented, and the additional presence of aphids reduced the association. QTL mapping
488 using three species is uncommon; therefore this may be the first example of two interacting
489 species with antagonistic effects on QTL–trait association in an intermediate species. It is
490 proposed that multi-species interactions that have strong phenotypic effects could alter
491 evolutionary trajectories depending on how their interactions influence the polarity of trait values
492 [34]. Traits involved in plant–insect interactions have previously been mapped, however previous
493 studies have focused on traits from one of the two species [18,19]. Plants interact with a plethora
494 of above- and below-ground species, and it is possible that interacting individuals reciprocally
495 respond to each other over ecological time [9]. Reciprocal interactions imply continuous back
496 and forth responses, as are postulated by co-evolutionary arms races between plants and insects
497 [5,6,9]. Peppe & Lomonaco [35] state that 'when plasticity contributes positively to fitness, it can
498 be considered adaptive, and constitutes an important advantage in exploiting heterogenous
499 environments'. However, when applying this to antagonistic species interactions, a positive
500 contribution to fitness for one species could result in a negative contribution to fitness for the
501 interacting species, and is likely to lead to the interacting species phenotypically responding with

502 its plasticity genes contributing to its fitness. Phenotypic plasticity in aphids is known to be
503 triggered by host quality and secondary plant substances[6,36], which can be genetically based
504 [28,36]. The aim of community genetics is to address the phenomenon of how genetic variation
505 and species presence may influence the phenotypes of associated species within a community,
506 and over time influence the evolution of those species. This study has provided a proof of
507 concept that genetic variation at multiple loci within the barley genome can alter the effect of
508 rhizobacterial supplementation and aphid infestation on barley biomass and of rhizobacterial
509 supplementation on aphid fitness. If such genetically based phenotypic effects were to pervade
510 over time within natural communities, they would be likely to alter coevolutionary trajectories
511 [34].
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613 **Figure legends**

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615 Figure 1: Reactions norms for aphid population size, stem biomass, and root biomass in two
616 mapping populations; Steptoe x Morex (A) and Oregon Wolfe Barley (B). The long dashed line
617 represents parental lines OWB-rec (A) and Morex (B); the short dashed line represents parental
618 lines OWB-dom (A) and Steptoe (B).

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620 Figure 2 / 3: QTL plots for chromosome 1 of the OWB (fig. 2) and SxM mapping populations
621 (fig. 3). Each QTL plot shows the association between the trait and loci (LOD, y-axis) across the
622 length of the chromosome (cM, x-axis) (A), and the Additive Genetic Effect along the
623 chromosome (B). The positions of markers are indicated by small triangles along the
624 chromosome (x-axis, plot A). For the OWB plot, four lines are plotted; solid black and dashed
625 gray = aphid fitness when *P. aeruginosa* 7NSK2 was not / was supplemented respectively;
626 dashed black = plasticity in aphid fitness across the two aphid environments; solid gray =
627 plasticity in root biomass between *P. aeruginosa* 7NSK2 supplemented and combined aphid + *P.*
628 *aeruginosa* 7NSK2 supplemented environments. For the SxM plot, three lines are plotted; solid
629 black = shoot biomass when *P. aeruginosa* 7NSK2 was supplemented; dashed line = plasticity in
630 shoot biomass across *P. aeruginosa* 7NSK2 supplemented and combined environments; gray line
631 = shoot biomass in the combined environment.

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636 **Tables**

637 Table 1: ANOVA results for plant (shoot and root biomass) and aphid performance for the

638 Steptoe x Morex (SxM) and Oregon Wolf Barley (OWB) mapping populations.

		Shoot biomass			Root biomass			Aphid performance		
	Source	DF	F	P	DF	F	P	DF	F	P
StxMo	Line	49	2.18	<.000***	49	3.48	<.000***	49	2.23	.003**
	Environment	3	50.87	<.000***	3	42.32	<.000***	1	7.25	.01**
	Line x environment	147	1.60	<.000***	147	1.40	.004**	49	0.62	.979
	Error	567			556			275		
OWB	Line	47	4.02	<.000***	47	3.16	<.000***	47	1.33	.167
	Environment	3	42.82	<.000***	3	47.74	<.000***	1	3.85	.055
	Line x environment	141	1.07	.286	141	0.81	.940	47	1.00	.476
	Error	519			520			253		

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640 Notes: p-levels: *** = p≤0.001; ** = p≤0.01;

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

	Trait	Environment 1*	Environment 2*	Percentage of lines expressing change in mean trait		
				Decrease	Increase	No change
SxM	Aphid	Control	*P.a*	60	26	14
	Shoot; Root	Control	Aphid	82; 82	6; 14	12; 4
		Control	*P.a*	72; 70	20; 22	8; 6
		Control	Aphid + *P.a*	94; 96	2; 4	4; 0
		Aphid	Aphid + *P.a*	68; 72	20; 20	12; 8
		P.a	Aphid + *P.a*	80; 78	8; 18	12; 4
OWB	Aphid	Control	*P.a*	54	31	15
	Shoot; Root	Control	Aphid	69; 69	14; 17	17; 15
		Control	*P.a*	71; 67	15; 19	15; 15
		Control	Aphid + *P.a*	94; 94	4; 4	2; 2
		Aphid	Aphid + *P.a*	79; 83	8; 10	13; 6
		P.a	Aphid + *P.a*	83; 85	10; 8	6; 6

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653 Notes: * *P.a* refers to *Pseudomonas auruginosa* 7NSK2

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661 Table 3: QTLs located for plant (shoot and root biomass) and aphid performance for the Steptoe
662 x Morex (SxM) and Oregon Wolf Barley (OWB) mapping populations

Location (cM)	Trait[∞]	Environment[+]	LOD[†]	% Variance	A.G.E[‼]	QTLxE[$]
SxM						
Ch1, 76.31	Shoot	*P.a*	5.11***	23.85	59.46	.0284* (CvD) .0052* (BvC) .0040* (AvC)
	Shoot PP	*P.a* – Aph & *P.a*	3.43**	15.60	36.28	
Ch1, 111.21	Root	*P.a*	4.61***	21.05	35.37	
	Root PP	*P.a* – Aph & *P.a*	2.91**	16.35	22.57	
Ch1, 117.51	Root	*P.a*	4.26***	20.67	35.06	
	Root PP	*P.a* – Aph & *P.a*	3.69**	19.02	23.58	
Ch1, 148.51	Shoot	Aph & *P.a*	3.52**	17.40	37.19	.0184* (BvD)
Ch2, 3.41	Root	*P.a*	2.31*	9.19	23.50	
Ch3, 29.81	Root	Control	5.00***	30.45	49.86	.0105* (AvD)
	Root PP	Control - Aph & *P.a*	3.57**	16.50	36.01	
	Aphid	Aph	2.73*	15.15	16.71	
Ch3, 123.81	Aphid	Aph & *P.a*	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 & *P.a*	2.78*	15.79	19.56	
Ch3, 171.11	Shoot PP	Aph – Aph & *P.a*	2.33*	14.50	31.51	
Ch3, 172.11	Root	Aph & *P.a*	3.25**	16.75	-22.38	0.0022** (CvD)
Ch3, 182.61	Root PP	Control - Aph	2.98**	18.44	-24.89	
Ch4, 11.41	Shoot	Aph & *P.a*	5.81***	30.67	-62.62	
Ch4, 34.51	Shoot	Aph & *P.a*	2.20*	10.19	31.23	
Ch4, 36.51	Aphid	Aph & *P.a*	3.43**	17.37	-20.37	.0296* (v)
Ch4, 96.61	Shoot PP	Aph – *P.a*	2.46*	12.75	-33.25	
Ch4, 122.21	Aphid PP	Aph – Aph & *P.a*	2.72*	13.99	-17.85	
Ch4, 143.91	Aphid PP	Aph – Aph & *P.a*	4.68***	26.44	25.33	
Ch5, 13.61	Root	*P.a*	3.00**	12.33	26.97	.0482* (AvC)
Ch5, 22.41	Root PP	Control – *P.a*	2.28*	11.90	-26.81	
Ch5, 28.41	Shoot	*P.a*	2.84*	12.71	42.97	.0113* (BvC) .0083* (AvC)
Ch5, 31.51	Shoot PP	Control – Aph & *P.a*	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 – *P.a*	2.71*	15.86	-53.21	
Ch5, 84.41	Shoot PP	Control – *P.a*	2.65*	14.69	50.26	
Ch5, 102.71	Aphid PP	Aph – Aph & *P.a*	3.12**	16.30	-14.09	
	Root PP	Control - *P.a*	3.49**	19.29	34.27	
Ch5, 106.71	Root PP	Aph – Aph & *P.a*	2.71*	16.68	20.12	

Ch5, 112.51	Shoot PP	Aph – Aph & *P.a*	2.40*	14.19	32.92	
Ch5, 130.41	Root PP	Aph – *P.a*	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	*P.a*	2.81*	12.72	43.62	.0820* (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	.0376* (AvB)
Ch6, 105.81	Shoot PP	Control – *P.a*	2.41*	11.12	-32.96	
Ch7, 48.11	Root PP	Control – Aph & *P.a*	2.28*	12.34	27.04	
Ch7, 78.31	Root PP	Control – *P.a*	3.21**	17.68	33.36	
OWB						
*Ch1, 54.11	Aphid	Aph	3.34**	17.78	-14.93	.0157*
	Aphid PP	Aph – Aph & *P.a*	3.95**	23.49	-13.15	
	Root PP	*P.a* – Aph & *P.a*	2.65*	17.74	-12.77	
Ch1, 72.77	Root PP	*P.a* – Aph & *P.a*	4.28***	28.93	16.73	
Ch1, 116.79	Shoot	Aph	3.07*	17.16	-38.47	
Ch2, 65.23	Root	*P.a*	2.50*	10.10	5.81	
Ch2, 93.88	Shoot	*P.a*	2.77*	10.17	20.73	
Ch2, 141.26	Shoot	*P.a*	3.89**	16.36	-26.08	
		Aph	4.86***	18.50	-35.35	
Ch2, 164.55	Root PP	Aph – *P.a*	3.34**	20.12	-7.21	
Ch3, 0.00	Aphid	Aph & *P.a*	4.10***	17.90	12.97	
		Aph	3.01**	15.82	14.02	
Ch3, 38.74	Shoot PP	Control – *P.a*	3.26**	18.22	25.93	
Ch3, 73.01	Shoot	*P.a*	3.70**	14.39	-23.58	.0367* (BvC)
Ch3, 164.58	Root PP	Aph – Aph & *P.a*	3.35**	18.12	28.73	
Ch3, 167.96	Shoot PP	Control – Aph & *P.a*	3.93**	26.1	34.61	
Ch3, 171.80	Root PP	Control – Aph & *P.a*	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 – *P.a*	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 & *P.a*	4.00***	21.70	29.04	
Ch5, 11.35	Aphid	Aph & *P.a*	2.78*	10.87	-10.21	
Ch5, 118.87	Shoot PP	Control – *P.a*	2.45*	13.67	28.87	
Ch5, 134.15	Shoot PP	Control – *P.a*	4.42***	26.21	-40.69	
Ch5, 140.56	Root	*P.a*	3.52**	14.56	6.99	
Ch5, 158.55	Shoot	Aph & *P.a*	3.40**	15.89	-24.84	.0283* (CvD)
Ch5, 197.35	Root PP	Aph – *P.a*	4.01**	25.58	-7.18	
Ch6, 0.02	Shoot PP	Control – *P.a*	4.01***	23.25	-28.40	

Ch6, 44.85	Aphid	Aph & *P.a*	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 & *P.a*	4.38***	19.13	17.76	
Ch6, 105.43	Root PP	Control - Aph	3.88**	27.48	-11.14	
Ch6, 137.18	Root	*P.a*	4.93***	28.44	-12.62	.0321* (BvC)
Ch6, 142.64	Root	Aph	4.00***	22.40	16.40	.0129* (BvC) .0317* (BvD)
Ch7, 103.26	Shoot	Control	2.95*	14.50	33.07	.0477* (AvB) .0359* (AvD)

663 Notes: ∞ Traits associated with a QTL; main effects for barley (shoot, root) and aphid, and

664 phenotypic plasticity (PP) of each of the three main traits.

665 + *P.a* refers to *Pseudomonas auruginosa* 7NSK2.

666

667 † LPR = Likelihood Probabiliy Ratio is the likelihood of a significant loci – phenotype

668 association. QTL listed are those that had a LPR exceeding the threshold level; *=p<0.05;

669 **=p<0.01; ***=p<0.001.

670 $QTLxE, p-values. Letters in brackets denote environments for QTLxE; A = control, B =

671 aphid, C = *Pseudomonas aeruginosa* 7NSK2 supplemented, D = aphid & *P. aeruginosa*

672 7NSK2 supplemented. Asterisks indicate significant QTL x environment interactions

673 between the two traits plotted, following the levels:

674 * = significant in a single site analysis; ** = significant after bonferroni correction (α =

675 0.1).

676 ‼ A.G.E. (Additive Genetic Effect) illustrates which parental allele causes a greater trait

677 value compared to the alternative parental allele. In the SxM population, a positive A.G.E

678 corresponds to a higher trait value when the allele from Morex is contributed to that locus,

679 compared to when Steptoe is contributed, and vice versa for the negative A.G.E. In the

680 OWB population, positive A.G.E. corresponds to the contribution of the allele of OWB-D

681 influencing higher trait value compared to OWB-R, and vice versa.

1 Figure 1:

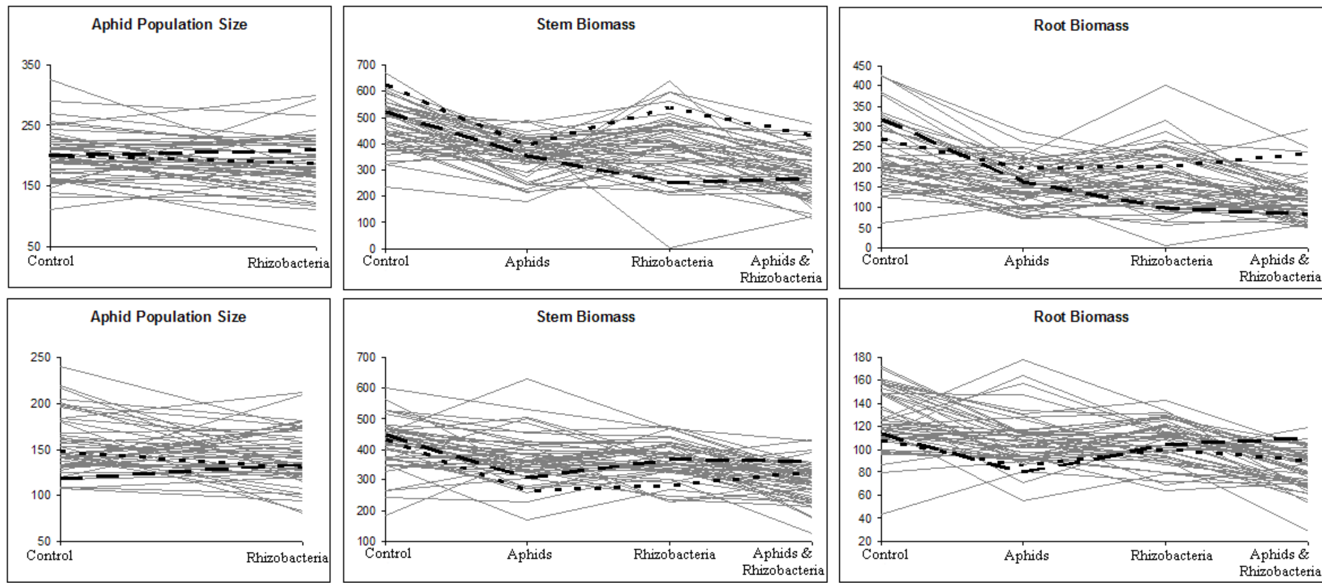

Figure 2

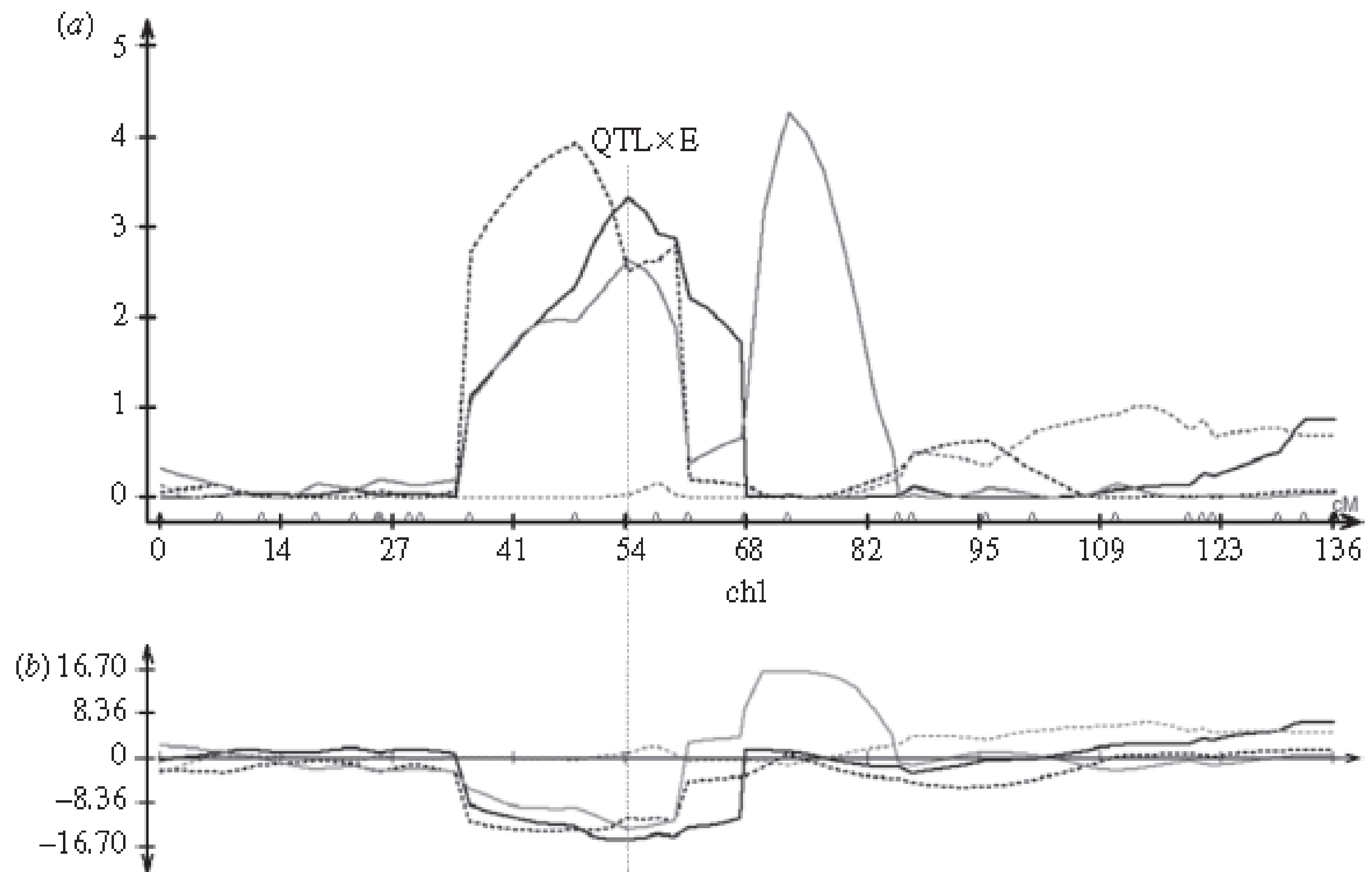

Figure 3