New Phytologist

Research

Antagonistic epistasis for ecophysiological trait differences between *Solanum* species

Christopher D. Muir and Leonie C. Moyle

Department of Biology, Indiana University, 1001 East Third Street, Bloomington, IN 47401, USA

Author for correspondence:
Leonie C. Moyle
Tel: +1 812 856 7027
Email: lmoyle@indiana.edu

Received: *27 April 2009*
Accepted: *1 June 2009*

New Phytologist (2009) **183**: 789–802
doi: 10.1111/j.1469-8137.2009.02949.x

Key words: double-introgression lines, drought, epistasis, near-isogenic lines (NILs), quantitative trait loci (QTLs), *Solanum habrochaites*, *Solanum lycopersicum*, specific leaf area.

Summary

• Epistasis, the nonadditive interaction between loci, is thought to play a role in many fundamental evolutionary processes, including adaptive differentiation and speciation. Focusing on species differences in ecophysiological traits, we examined the strength and direction of pairwise epistatic interactions between target chromosomal regions from one species, when co-introgressed into the genetic background of a foreign species.

• A full diallel cross was performed using 15 near-isogenic lines (NILs) constructed between two tomato species (*Solanum habrochaites* and *Solanum lycopersicum*) to compare the phenotypic effects of each chromosomal region singly and in combination with each other region.

• We detected main effect quantitative trait loci (QTLs) for two of our three focal traits. Epistatic effects accounted for *c.* 25% of detected effects on trait means, depending on the trait. Strikingly, all but two interactions were antagonistic, with the combined effect of chromosomal regions acting in the opposite direction from that of one or both individual chromosomal regions.

• Our study is one of the few to systematically examine pairwise epistatic effects in a nonmicrobial system. Our results suggest that epistatic interactions can contribute substantially to the genetic basis of traits involved in adaptive species differentiation, especially highly complex, multivariate traits.

Introduction

Epistasis, the nonadditive interaction between loci, is thought to play a role in evolutionary processes as diverse as the evolution of sex and recombination (Peters & Lively, 1999; de Visser & Elena, 2007), the evolutionary dynamics of population polymorphism and genetic load (e.g. Kondrashov, 1994), and the nature and pace of adaptation and species divergence (e.g. Whitlock *et al.*, 1995). In terms of adaptive differentiation and speciation, epistasis might be important in maintaining natural variation (Barton & Keightley, 2002), and has been shown to contribute to local adaptation (e.g. Caicedo *et al.*, 2004; Gerke *et al.*, 2009). In addition, epistatic interactions among loci can influence the tempo of adaptation, such that antagonistic (less than additive) or synergistic (greater than additive) epistasis can retard or accelerate, respectively, the rate of adaptive change within a lineage (e.g. Wade, 2000). Postzygotic isolation caused by Dobzhansky–Muller incompatibilities also requires epistasis between two or more derived alleles (Coyne & Orr, 2004). Despite these observations, however, there is still considerable disagreement about the relative importance of epistatic versus additive genetic effects in adaptation and differentiation (e.g. Whitlock *et al.*, 1995; Malmberg & Mauricio, 2005; Hill *et al.*, 2008), a debate that stretches back to the contrasting theoretical approaches of R. A. Fisher and S. Wright (Fisher, 1930; Wright, 1931).

In the present study, we examine the genetic basis of species differences, focusing on the strength and direction of epistasis. Orr (2001) notes that observed epistasis between loci in divergent species does not necessarily imply that epistasis was important during species divergence, as alleles at disparate loci may fix sequentially. Nevertheless, understanding the prevalence of epistasis in between-species differences can still provide insights into their evolutionary origin. For example, if epistasis has no role in the present genetic basis of species differences, it is unlikely to have played a role during their evolutionary origin (Orr, 2001). Conversely, a significant role for epistasis in contemporary species differences establishes that nonadditive interactions might have contributed to their evolution. Further, the directionality of this epistasis can provide a first indication

© The Authors (2009)
Journal compilation © *New Phytologist* (2009)

of whether selection could have been facilitated (synergistic epistasis) or constrained (antagonistic epistasis) by the observed interactions, during the adaptive divergence of these traits. Understanding the genetic architecture of complex traits can also indicate future evolutionary consequences. For example, negative epistasis for ecologically relevant traits could contribute to extrinsic postzygotic isolation, where the examined species hybridize. Conversely, epistasis has been shown to contribute to transgressive phenotypes that could facilitate introgression or stabilize hybrid zones (Rieseberg *et al.*, 1999). Finally, classical and contemporary approaches to identifying epistasis can also be used to infer the molecular and mechanistic basis of genetic interactions and genetic pathway evolution (Demuth & Wade, 2006; Phillips, 2008; Rockman, 2008).

Despite its potential importance in evolutionary processes and for functional analysis, epistasis is often neglected in genome-wide dissections of complex traits because of limited statistical power to detect interaction effects within finite mapping populations (Carlborg & Haley, 2004). An alternative to genome-wide scans is to systematically measure epistasis between target loci within a small random sample of the genome. To date, this approach has primarily been limited to microorganisms (Segre *et al.*, 2005; Phillips, 2008 and references therein) with few notable exceptions in model plant and animal systems (e.g. Spickett & Thoday, 1966; Eshed & Zamir, 1996; Lukens & Doebley, 1999; Sambandan *et al.*, 2006; Yamamoto *et al.*, 2008). In the present study, we examine the strength and direction of pairwise epistatic interactions between target chromosomal regions from one species, when introgressed together into the genetic background of a foreign species. A full diallel cross involving 15 near-isogenic lines (NILs) constructed between two tomato species was used to create 105 double-introgression lines (DILs) (T. Nakazato, S. Josway, & L.C. Moyle, unpublished data). We used these lines to compare the phenotypic effects of each chromosomal region singly and in combination with each other region. Although we only examined a small portion of the genome, the advantages of our design are: first, that NILs are more powerful for detecting main effect quantitative trait loci (QTLs) compared with analyses that rely on recombinant mapping strategies; and second, in our sample, we can directly compare the relative contributions of additive and epistatic QTLs.

Our study focuses on ecophysiological traits that differentiate species in the tomato clade, *Solanum* section *Lycopersion* (Peralta & Spooner, 2001). Wild tomato species differ considerably from each other along multiple ecological axes (Moyle, 2008; Nakazato *et al.*, 2008; Peralta *et al.*, 2008). One prominent axis of differentiation is habitat aridity (e.g. mean annual rainfall) (Moyle, 2008, T. Nakazato, D. Warren, & L. C. Moyle, unpublished), implying that species are differentiated with respect to water deficit and drought response traits. This expectation is born out in patterns of intra- and inter-specific variation, where local variation in precipitation is strongly correlated with quantitative genetic variation for drought response traits (e.g. Nakazato *et al.*, 2008). More generally, ecophysiological traits are important foci of local adaptation and species divergence (Ackerly *et al.*, 2000) that can contribute significantly to ecologically mediated coexistence and community composition (Kraft *et al.*, 2008).

In our study of the genetics of species ecophysiological differences, we asked five questions.

- Are there significant main effect QTLs for the focal traits?
- Is there epistasis between target chromosomal regions? If so, are chromosomal regions with main effects more or less likely to be involved in epistatic interactions?
- What are the relative frequencies of nonadditive (epistatic) and additive interactions between target chromosomal regions?
- What is the direction of observed epistasis, synergistic or antagonistic?
- What is the relationship between QTLs for different ecophysiological traits and for morphology, and what does this indicate about indirect versus direct effects on whole-plant responses to drought?

Materials and Methods

Study system

Solanum section *Lycopersicon* is a relatively small plant group within the large and diverse Solanaceae family; the group consists of 14 closely related diploid species or subspecies, including the domesticated tomato, *Solanum lycopersicum* (Mill.) (D'Arcy, 1979; Peralta *et al.*, 2005, 2008; Spooner *et al.*, 2005). Although formerly classified as a separate genus (*Lycopersicon*), a recent taxonomic revision indicated that this group is a monophyletic clade nested within the genus *Solanum* and renamed *Lycopersicon* species accordingly (Peralta & Spooner, 2001). The two parental species analyzed here differ in several biologically significant features. *Solanum habrochaites* (S. Knapp & D.M. Spooner) (formerly *Lycopersicon hirustum*) is a wild, short-lived, herbaceous, perennial species that occurs predominantly from mid to high elevations in north-western South America, under relatively water-limited conditions (Moyle, 2008). Most populations of *S. habrochaites* are obligately outcrossing as a result of gametophytic self-incompatibility, and exhibit high nucleotide diversity (Miller & Tanksley, 1990; Stephan & Langley, 1998). By contrast, *Solanum lycopersicum* (Mill.) (formerly *Lycopersicon esculentum*) – the cultivated tomato – is a domesticated, self-pollinating species with comparatively low genetic variation. The putative wild progenitor of *S. lycopersicum* is also predominantly selfing (Miller & Tanksley, 1990; Kondo *et al.*, 2002), and self-compatibility is thought to have preceded domestication (Rick, 1995). In addition, there are substantial ecological and phenotypic differences between the two species that are relevant to abiotic stress, including water stress (Bloom *et al.*, 2004; Comstock *et al.*, 2005). For example, *S. habrochaites* has higher relative growth rates under drought stress conditions and less negative values of carbon isotope

© The Authors (2009)
Journal compilation © *New Phytologist* (2009)

Table 1 Near-isogenic lines (NILs) used to generate double-introgression lines and analyzed in the study (see also Moyle & Nakazato, 2009)

LA number[1]	QTL status[2]	Chromosome location	Introgression length (cM)	Proportion of genome
LA3975	None	3	12.1	0.0096
LA3968	None	12	14.1	0.0112
LA3964	None	10	22.5	0.0179
LA3957	None	9	44.8	0.0356
LA3947	None	6	8.6	0.0068
LA3956	Pollen	9	57.4	0.0456
LA3935	Pollen	4	53	0.0421
LA3950	Pollen	7	33.8	0.0268
LA3963	Pollen	10	30.3	0.0241
LA3948	Pollen	7	50.4	0.0400
LA3931	Seed	4	18.7	0.0149
LA3939	Seed	5	25.8	0.0205
LA3943	Seed	5	34	0.0270
LA3915	Seed	1	34.8	0.0276
LA3977	Seed	4	19	0.0151

[1]LA number: seed accession identifier (see tgrc.ucdavis.edu).
[2]QTL status: whether NILs had previously been shown to carry a quantitative trait locus (QTL) for partial hybrid sterility between these two species (Moyle & Graham, 2005).

discrimination (both ecophysiological responses consistent with elevated drought tolerance) in comparison to *S. lycopersicum* (Comstock *et al.*, 2005 and references therein). Nucleotide divergence between *S. lycopersicum* and *S. habrochaites* estimated from six independent noncoding regions averages 0.044 substitutions per base pair, indicating that these species are closely related (Nesbitt & Tanksley, 2002).

Generation of NILs and DILs

We selected 15 chromosomal regions for inclusion in the study (Table 1), drawing from a set of NILs previously developed between two plant species in the genus *Solanum* Section *Lycopersicon* (the tomato clade) (Monforte & Tanksley, 2000). Each NIL contains a unique short chromosomal region from the wild species *S. habrochaites* (SH) introgressed into the otherwise isogenic genetic background of the domesticated tomato, *S. lycopersicum* (SL) (Monforte & Tanksley, 2000; see also Moyle & Graham, 2005 for a previous summary). Full methods for generating DILs can be found in Moyle & Nakazato (2009) and in Supporting Information Methods S1. Briefly, to generate lines with two SH introgressed regions (DILs), a complete diallel cross was performed to combine each introgression with each other introgression for a total of 105 unique pairwise combinations of the 15 regions. Both heterozygote DILs (heterozygous for both SH introgressions) and homozygote DILs (homozygous for both SH introgressions) were generated. DILs were originally constructed to examine the strength and direction of epistasis between introgressions with and without interspecific pollen and seed sterility effects (Moyle & Nakazato, 2009; T. Nakazato, S. Josway, & L. C. Moyle, unpublished data), as previously identified in the genome-wide survey of hybrid incompatibility between these two species (Moyle & Graham, 2005). Therefore, five of the selected NILs are known to carry pollen sterility QTLs, and five are known to carry seed sterility QTLs; the remaining five NILs have no detected effects on hybrid pollen and seed fertility but have introgression lengths (cM) comparable to that of the sterility QTL NIL set. With respect to the present study, the NILs were selected with no prior knowledge of their effect on the morphological/physiological traits examined. Overall, our set of 15 NILs covers *c.* 36% of the total SH genome, in an SL genetic background.

Experimental genotypes

The following genotypes were examined in the experimental population: 15 NILs with the relevant SH introgression in either homozygous and heterozygous form (Hom NIL or Het NIL, respectively; 30 genotypes total); 104 DILs with either both SH introgressions in homozygous form, or both SH introgressions in heterozygous form (Hom DIL or Het DIL, respectively; 208 genotypes total); and the recurrent domesticated tomato parental genotype (SL). For each genotype, up to 25 seeds were germinated on wet sterile filter paper under artificial light. Because of variable seed germination and seedling survival, for the current study not all NIL or DIL genotypes had sufficient individuals (i.e. two or more) for analysis (see Table S1).

Plant cultivation

Once germinated, seedlings were hand-transplanted to cell-pack flats containing 'Metro' seedling mix, placed on benches under natural lighting in the Indiana University glasshouse facility, and misted daily for 2–3 wk. Mature seedlings were individually transplanted into 3.78-l pots containing a 50:50 mix of Metro-Mix growing medium and compost. Experimental plants were placed out on glasshouse benches in a fully randomized design. The total experimental size was 952 plants.

Trait measurements

We measured four traits on individuals, two morphological (plant height and leaf number at 5 wk) and two ecophysiological (specific leaf area (SLA) and time to wilting (TW) after experimental withholding of water). Plant height was measured as the distance from soil to the highest node. Leaf number included all leaves on the main stem and lateral branches. SLA is measured as leaf area divided by dry mass ($cm^2\ g^{-1}$), and is frequently used as one morphological indicator of water use efficiency. For example, a high SLA indicates relatively thin leaves and a 'water-hungry' resource use strategy (i.e. rapid biomass production at the expense of nutrient conservation). To obtain leaf area estimates, over 4 d during week 6, we scanned a single

© The Authors (2009)
Journal compilation © *New Phytologist* (2009)

fully expanded leaf (usually the 4th to 6th leaf from the base) immediately after removing it from the plant using an Epson GT-20000 flat bed scanner. We calculated leaf area using custom macros in Image J (Ambramoff *et al.*, 2004). Each leaf was then dried in a desiccating oven at 60°C and weighed using a Sartorius CP225D balance (Sartorius Corp., Edgewood, NY, USA). To estimate TW, at week 7, we stopped watering plants and recorded time to wilting, by visually assessing all experimental plants two or three times per day for the duration of the experiment. We considered a plant wilted when all leaves and branches showed loss of turgor. This method has been used successfully before to differentiate drought responses in tomato species (Nakazato *et al.*, 2008).

Data analysis

Transformation/manipulation Plant height and leaf number were positively correlated and nonnormally distributed (data not shown). Therefore, we used the first principal component of height and leaf number as a proxy for plant size (henceforth 'Size'). Size accounted for 69% of the variance in the principal component analysis, loaded strongly positively with both height and leaf number, and was more normally distributed than its component variables, although still negatively skewed. We natural log transformed SLA for normality. We did not transform TW. Before transformation and analysis we removed all plants with abnormal growth (most frequently 'blind' plants that failed to grow beyond the cotyledon stage, apparently because of a dysfunctional vegetative meristem). All transformations and analysis were performed in R version 2.7.2 (R Core Development Team, 2008).

Analysis for main effect QTLs We evaluated NILs (both Hom and Het NILs) for main effect QTLs using Welch's *t*-test (Welch, 1947). We interpreted a significant difference in trait means between a NIL and the domestic tomato (SL) genotype as evidence that the introgressed SH chromosomal region in the NIL contained a QTL for the relevant trait. We report the number of QTLs at a *P*-value of 0.05 and after a Bonferroni correction (Dunnett, 1955). As with all analyses that test more than one 'treatment' group (in this case, each NIL genotype) against a single control group (SL parent), the same control group is represented in all tests, and this approach has successfully identified QTLs for several quantitative traits in this species pair (e.g. Moyle & Nakazato, 2008). We also performed alternative analyses to evaluate the presence of main effects, including a full multi-way ANOVA that combined Hom and Het comparisons and evaluated main and interaction effects. While some specific results were influenced by the analysis method, the substantive findings of these different approaches agreed (data not shown). Therefore, we only report the results from the more conservative Welch's *t*-tests below.

We also evaluated DILs (both Hom and Het DILs) for significant effects on each trait (i.e. QTL), using Welch's *t*-tests (Welch, 1947) which compared trait means for each DIL to that of the SL parent. Even with our delimited experimental design, correction for multiple testing in these models prohibits significance given our sample sizes. Therefore, we report all significant ($P < 0.05$) coefficients and corresponding false discovery rates (FDRs; Benjamini & Hochberg, 1995). We report the FDR as the expected number of false positives ($N\alpha$) divided by the number of significant tests, where N is the number of tests performed and α is the Type I error rate. An FDR $\ll 1$ indicates that there were many more significant results than expected by chance.

Quantifying epistasis We used a linear regression model to test for epistasis. We fit the following model for Het and Hom comparisons separately:

$$Y = \mu + SH1 + SH2 + SH1 \times SH2 + \varepsilon \qquad \text{Eqn 1}$$

(Y, the trait being considered; μ, the trait mean of the SL genotype; SH1 and SH2, the two NILs containing the SH chromosomal regions being compared.) We inferred epistasis in each case where the coefficient of the interaction term in a linear model differed significantly from zero. A significant interaction term indicates that chromosomal regions interacted nonadditively, meaning that the sum of two NIL phenotypes did not predict the phenotype of their composite DIL. We evaluated epistasis separately in Hom and Het DILs because all the genotypes (heterozygous at one region and homozygous at the other) that would be necessary to infer the effect of epistasis on the dominance relationship were not included in this experiment. Once again, the number of tests made Bonferroni-corrected *P*-values prohibitive. We report all significant ($P < 0.05$) interactions as well as corresponding FDRs.

Epistatic versus additive interactions The number of significant interaction terms in the linear regression models is not a direct evaluation of the relative frequency of biologically (rather than statistically) significant additive and epistatic relationships between chromosomal regions. This is because chromosomal regions with no main effect may interact only in the trivial sense of producing DILs that also did not significantly alter the phenotype. To more directly gauge the prevalence of additivity versus epistasis specifically underlying species trait differences, we only considered cases where at least one NIL or their corresponding DIL differed significantly from SL. Within this subset, we compared the number of times there was a significant interaction to the number of times there was no significant interaction. In addition, considering only comparisons with significant interaction terms, we evaluated the relative frequency with which epistasis involved SH introgressions with or without main effects.

Directionality of epistasis Considering only significant epistatic interactions as defined above, the sign of the interaction

© The Authors (2009)
Journal compilation © *New Phytologist* (2009)

coefficient from the linear regression was used to indicate whether epistasis is antagonistic (interaction coefficient is opposite of main effect) or synergistic (interaction coefficient is same as main effect). We determined that a false signature of antagonistic epistasis could be generated if the main effect detected in one of NILs was itself a false positive (see Notes S1 and Fig. S1 for a formal demonstration). Heterogeneity of sample sizes and variance precluded exact calculation of probabilities for our experimental data, so we re-sampled the data with replacement to ask: how many significant interactions do we get, and are those interactions antagonistic or synergistic? The re-sampled data were used as a null distribution against which we compared our observed data.

Mechanistic associations between traits If two traits are mechanistically related, then these traits should be genetically correlated and significant genetic effects for the two traits should be associated more often than expected by chance. We assessed the strength of trait correlations among Size, SLA and TW, using Pearson's product-moment correlation on genotype means. In addition, based on frequently observed associations between leaf and plant size traits affecting whole-plant drought response (Fitter & Hay, 2002), we assessed two hypotheses about the specific associations between traits.

1) QTLs affect TW through altered SLA, as more succulent leaves will lose water more slowly.
2) QTLs affect TW through Size, as smaller plants have less surface area for evaporation.

To evaluate these hypotheses, for each trait we counted the number of times that a significant trait means test for a NIL or DIL coincided with an equivalent significant test for each other trait. The number of 'shared' significant tests between TW and SLA, and TW and Size were used to assess hypotheses 1 and 2, respectively. We assessed the significance of overlap using a one-tailed Fisher's exact test.

Results

Main effect QTLs for ecophysiological and morphological traits

Table 2 summarizes our findings for main effect QTLs, evaluated at a nominal $P = 0.05$ and after a Bonferroni correction for multiple comparisons. Two homozygous NILs (LA3915 and LA3977) had insufficient replicates to assess significance, and were excluded from this analysis. We found at least two significant QTLs in both Het and Hom NIL groups for TW and SLA, but not Size. SLA was the most 'complex' trait, with up to eight QTLs detected, followed by TW (up to two QTLs), and Size (no QTLs). However, none of these main effects remained significant after Bonferroni correction. In addition, fewer QTLs were detected in the Hom NIL group than in the Het NIL group (Table 2). This might be a consequence of more limited power to detect QTLs in Hom NILs, as sample sizes for these genotypes were consistently smaller than for Het NILs (as a result of differential germination success or early seedling survival). Differences in power might also explain why, in most cases, there was limited correspondence between genotypes that were significant in the Het vs Hom NIL groups, with the exception of NIL LA3943 for TW and LA3931 for SLA (Table 2). There was also no overlap between significant NILs for different traits (see 'Mechanistic associations between traits' results below). Note that previous studies show that *S. habrochaites* has larger values for SLA in comparison to *S. lycopersicum* (Comstock *et al.*, 2005), which agrees with the overall direction of effects of SH introgressions in our study, as well as the direction of our QTLs. By contrast, although SH is more drought-tolerant than SL (as measured in terms of other physiological traits, such as carbon isotope discrimination; Comstock *et al.*, 2005), results indicate that all our detected main effect QTLs are for decreased TW, and there was a trend for SH chromosomal introgressions in NILs to generally decrease TW (Fig. 1).

Table 2 Heterozygous and homozygous near-isogenic lines (NILs) revealing significant trait mean differences from the parental *Solanum lycopersicum* (SL) genotype, indicating the presence of additive quantitative trait loci (QTLs) for the three traits: Size, specific leaf area (SLA), and time to wilting (TW)

	Heterozygotes		Homozygotes	
	$P < 0.05$	$P < 0.0018$[1]	$P < 0.05$	$P < 0.0018$[1]
TW	LA3939 LA3943	None	LA3943	None
SLA	LA3931 LA3935 LA3948 LA3950 LA3956 LA3963 LA3964 LA3958	None	LA3931 LA3957	None
Size			None	None

[1]Bonferroni-corrected *P*-value for 28 (15 heterozygous plus 13 homozygous NILs) comparisons, to give an experiment-wise alpha of 0.05. Note that one heterozygous NIL and two homozygous NILs had insufficient replicates to assess significance, and are excluded from this analysis.

Table 3 summarizes results from the analysis of trait mean differences between DILs and the SL parent. We detected multiple significant effects for each of the three traits at $P = 0.05$ (Table S2), with a wide range of corresponding FDRs (Table 3). (Note that 16 of 105 and 39 of 105 Het and Hom DILs, respectively, did not have enough replicates for statistical testing and were excluded from this analysis.) Overall, many (especially Het) DILs differed from the SL parent genotype in SLA and to a lesser extent TW, but not Size, at a liberal statistical threshold. The finding of fewer significant results for Hom DILs probably reflects lower biological replication, and hence statistical power. In addition, in most cases > 50% of

© The Authors (2009)
Journal compilation © *New Phytologist* (2009)

New Phytologist

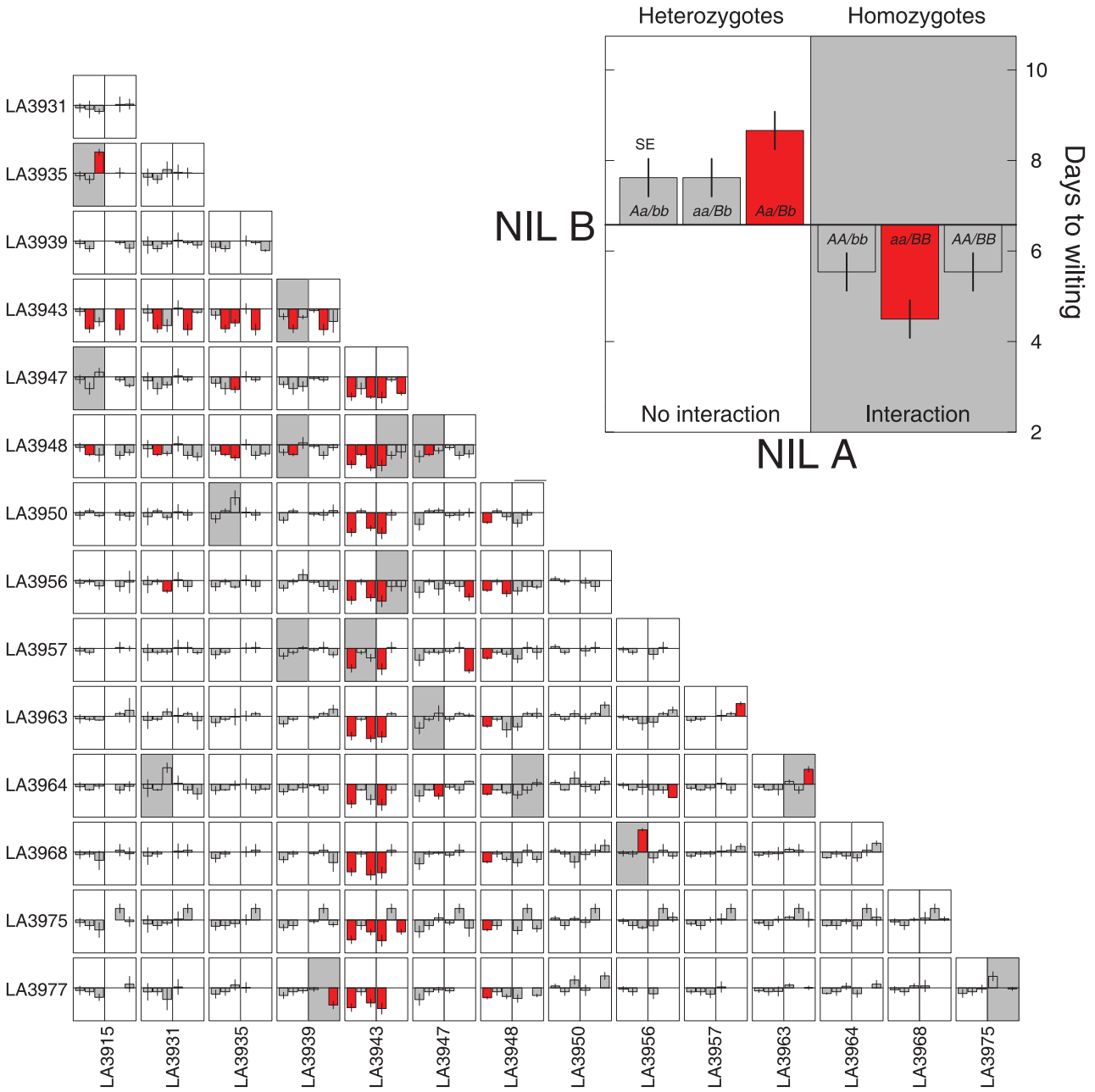

Fig. 1 Time to wilting (TW) for all near-isogenic lines (NILs) and double-introgression lines (DILs) following experimentally imposed drought. The key (top right) is an idealized but to-scale version for the purpose of explanation. The left and right halves of each figure are for heterozygous and homozygous introgressions, respectively. Within each half, the order from left to right is NIL A, NIL B, DIL A-B. The height of the bars represents the deviation for *Solanum lycopersicum* (SL), while the top/bottom of each bar is the mean trait value. Error bars are ±1 SD from the mean. Light gray bars indicate no significant difference from the SL trait mean, while red bars indicate a significant difference ($P < 0.05$). A gray background indicates a significant epistatic term ($P < 0.05$). NILs are identified by their LA number: seed accession identifier (see tgrc.ucdavis.edu).

DILs that had significantly different trait means did not include an NIL with a main effect for that trait (Table 3). There are two plausible explanations for this result: these QTLs are a product of synergistic epistatic interactions between NILs that have no main effects; or NILs interact additively, but we did not have enough power to detect main effects. We investigated these possibilities using linear regression below.

Evidence for epistasis

Fitting a linear model to detect significant interactions between SH chromosomal regions required that there be replicates from both NILs and their composite DIL. We had sufficient data to fit 90 of 105 heterozygote and 68 of 105 homozygote models (Figs 1, 2). For TW we found that 11 of 90 (FDR =

© The Authors (2009)
Journal compilation © *New Phytologist* (2009)

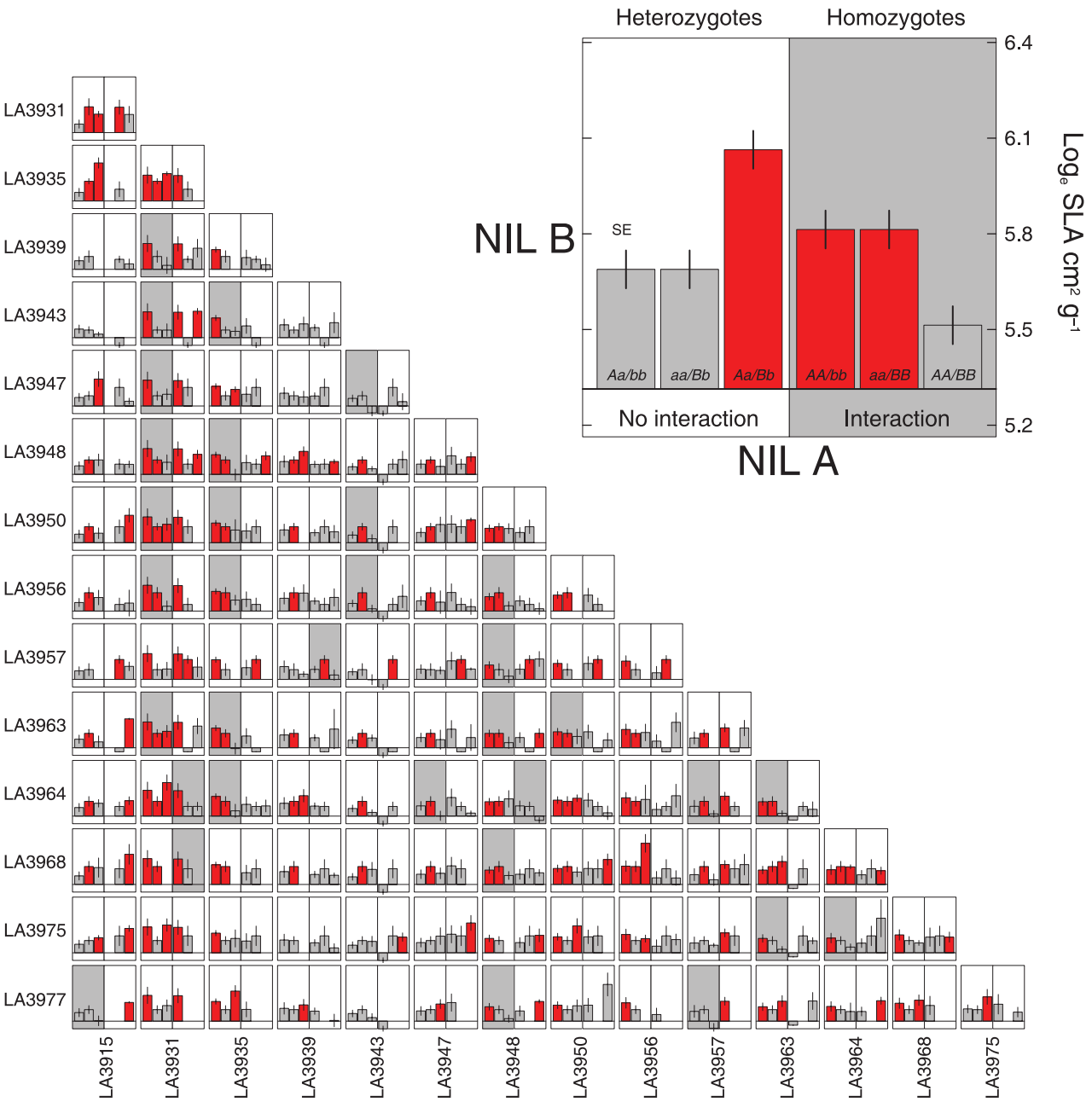

Fig. 2 Specific leaf area (SLA) for all near-isogenic lines (NILs) and double-introgression lines (DILs) following experimentally imposed drought. Details are the same as in Fig. 1.

0.41) and 6 of 68 (FDR = 0.57) Het and Hom DILs had phenotypes consistent with epistasis between their component SH introgressions. For SLA, there were 29 of 90 (FDR = 0.16) and 4 of 68 (FDR = 0.85) significant interactions in Het and Hom DILs, respectively. For size, there were 5 of 90 (FDR = 0.9) and 8 of 68 (FDR = 0.43) significant interactions in Het and Hom DILs, respectively. Correspondingly, we observed significantly more epistatic interactions between Het introgressions for SLA than in the re-sampled data ($P = 0.004$; Fig. 3b). We observed marginally more significant interactions between Het introgressions for TW ($P = 0.091$; Fig. 3a) than in the re-sampled data. There was no excess of interactions in the observed data relative to the re-sampled data for other comparisons (Fig. 3c–f). Differences between the proportion of significant Het and Hom interactions were probably attributable to differences in biological replication and therefore statistical power, although we cannot rule out more complex forms of dominance.

© The Authors (2009)
Journal compilation © *New Phytologist* (2009)

New Phytologist

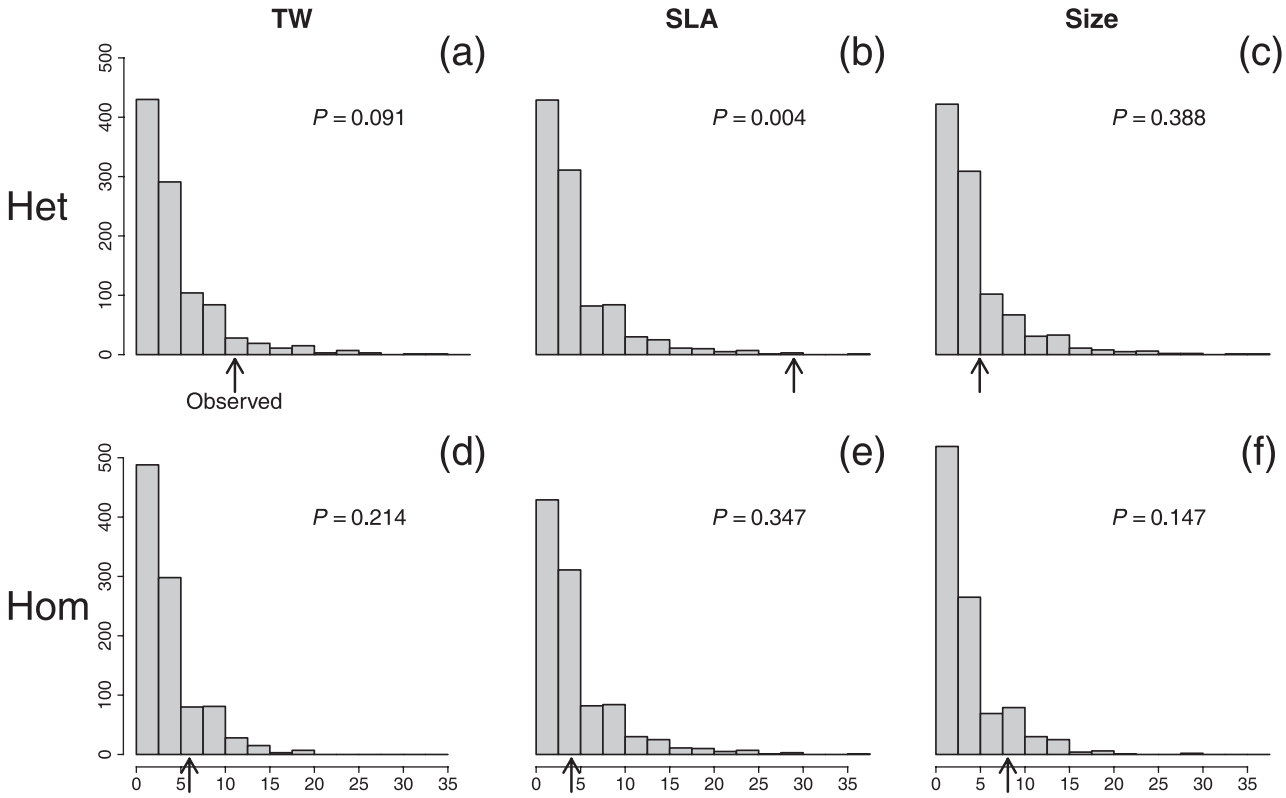

Fig. 3 Histogram of the number of significant epistatic interactions in 1000 re-sampled data sets for Het (a–c) and Hom (d–f) comparisons, broken down by trait (a, d, time to wilting (TW); b, e, specific leaf area (SLA); c, f, Size). Arrows below the *x*-axis refer to the number of significant interactions observed in the actual data. The *P*-value (upper right of each plot) refers to the proportion of re-sampled data points with as many or more significant interactions compared with the actual data.

Table 3 Double-introgression lines (DILs) with trait mean differences from the parental *Solanum lycopersicum* (SL) genotype for three traits: Size, specific leaf area (SLA), and time to wilting (TW)

	Heterozygotes			Homozygotes		
	$P < 0.05$	FDR	Number with main effect NIL	$P < 0.05$	FDR	Number with main effect NIL
TW	16	0.28	8	8	0.41	2
SLA	24	0.19	19	21	0.16	2
Size	4	> 1	0	4	0.83	0
Number of tests	89[1]			66[1]		

[1]Note that 16 heterozygous (Het) DILs and 39 homozygous (Hom) DILs had insufficient replicates to assess significance, and are excluded from this analysis.
FDR, false discovery rate.

SH chromosomal regions act both additively and epistatically

Many genotypes had no measurable effect on the phenotype. Accordingly, the proportion of significant interaction terms in the linear regression model does not necessarily reveal the relative biological importance of additive and epistatic gene action for our focal traits. When we excluded all cases where neither NIL nor their corresponding DIL significantly altered the trait mean from SL, we found that 21–67% of SH chromosomal regions behaved epistatically (Table 4). Het NIL LA3943 had a strong effect on TW (Table 1), which could singularly inflate the prevalence of epistasis for that trait. The frequency of epistasis did not change substantially when we removed LA3943 (Table 4). A smaller proportion of SH chromosomal regions affecting TW and SLA acted epistatically compared with those affecting Size, but this was probably because there were so few significant effects on Size. Overall, then, SH

© The Authors (2009)
Journal compilation © *New Phytologist* (2009)

Table 4 Relative frequencies of additive and epistatic relationships between chromosomal regions after removal of cases where neither near-isogenic line (NIL) nor the double-introgression line (DIL) differed significantly from *Solanum lycopersicum* (SL)

	Heterozygotes		Homozygotes		
	Additive	Epistatic	Additive	Epistatic	Total % epistatic
TW	25 (12)[1]	6 (5)	8 (4)	3 (1)	21% (27%)
SLA	50 (46)	26 (22)	20 (18)	2 (2)	29% (27%)
Size	1 (1)	3 (3)	1 (1)	1 (1)	67% (67%)

[1]Numbers in parentheses are the results after removal of all comparisons involving LA3943 (see Results for explanation).
TW, time to wilting; SLA, specific leaf area.

chromosomal regions frequently behaved epistatically for all three traits. Finally, when considering all significant interactions (whether or not they included significant NILs or DILs), 7 of 17, 29 of 33, and 0 of 13 significant epistatic interactions involved SH chromosomal regions that also had significant main effects for TW, SLA, and Size, respectively. The proportion of epistatic interactions involving main effect QTLs differed significantly among traits (Fisher's exact test, $P \leq 0.01$).

Epistasis between chromosomal regions is primarily antagonistic

When SH chromosomal regions interact nonadditively, epistasis may enhance (synergistic) or retard (antagonistic) their individual effect. To determine the directionality of epistasis we compared the sign of the sum of the main effects with that of the interaction effect. Of all significant interactions, all but two were antagonistic (Table 5). These analyses also indicate that DILs with significant effects that are composed of introgressions without significant main effects (see above) are mostly consistent with additivity (i.e. two 'weak' (nonsignificant) introgressions combine to give an overall significant effect when co-introgressed). Otherwise, these cases would have yielded significant synergistic epistatic interactions. In addition, in three cases where we detected epistasis between chromosomal regions without main effects, one or both introgressions individually (but not significantly) reduced TW whereas the DIL containing both SH introgressions significantly increased TW (Fig. 1: interactions between LA3915 and LA3935, LA3956 and LA3968, and LA3963 and LA3964), consistent with sign epistasis (Weinreich *et al.*, 2005).

We have formally demonstrated that a false main effect can lead to a false signature of antagonistic epistasis (Notes S1). Briefly, this expectation is based on the intuition that, in cases where neither the DIL nor its two contributing NILs are biologically different from the recurrent parent, but a false positive main effect QTL is detected for one NIL (as is expected $(100 \times \alpha)$% of times), the interaction term will regress towards the mean, thereby giving a false signal of antagonism.

Table 5 Direction of epistatic interactions in heterozygous double-introgression line (DIL) and homozygous DIL comparisons

	Heterozygous DILs		Homozygous DILs	
	Antagonistic	Synergistic	Antagonistic	Synergistic
TW	11	0	6	0
SLA	29	0	4	0
Size	3	2	8	0

TW, time to wilting; SLA, specific leaf area.

As expected, in the re-sampled data > 96% of epistatic interactions were antagonistic. This artifact may explain the preponderance of antagonistic epistasis detected in some of our comparisons. However, it cannot fully explain the severe excess of antagonistic epistasis detected in the Het DIL SLA data, because: (i) it is highly unlikely that all eight (out of 15) Het SH introgressions with significant main effects were false positives; and (ii) we observed many more epistatic interactions compared with the re-sampled data for this trait (Fig. 3b). It is also likely that the main effect of LA3943 on TW is not a false positive as it had a *P*-value (0.004) approaching the Bonferroni cut-off for multiple comparisons (0.0018), indicating that the significant antagonistic epistatic interactions involving this introgression (LA3939–LA3943 and LA3943–LA3957) are probably real. Nevertheless, given the statistical bias toward antagonistic epistasis, lack of evidence for synergistic epistasis should be interpreted with caution.

Little evidence for mechanistic associations between traits

There was a modest, but highly significant correlation between genotype means for all three traits (TW and SLA, $r = 0.45$; TW and Size, $r = -0.37$; SLA and Size, $r = -0.41$; df = 187; $P < 0.0001$ for all correlations). Despite these significant correlations, there was little evidence for a direct mechanistic relationship between traits as they shared few significant main or epistatic effects in common. In total, the number of shared significant effects between TW and SLA (Fisher's exact test, $P = 1$) as well as TW and Size (Fisher's exact test, $P = 0.31$) was not greater than expected by chance.

Discussion

Epistasis figures prominently in much evolutionary theory and may be an important genetic component of population and species differences. Although challenging, documenting the prevalence and kind of epistasis underlying between-species differences is a necessary prerequisite to dissecting its evolutionary significance. In the present study, our goal was to quantify the relative contributions of additive and nonadditive interactions between target chromosomal regions of *S. habrochaites* (SH)

© The Authors (2009)
Journal compilation © *New Phytologist* (2009)

in the background of *S. lycopersicum* (SL), focusing on three traits that might contribute to adaptive differentiation between these species. Here we address the implications of our results for each of our five questions. We conclude with a discussion of the possible implications for understanding ecophysiological differentiation among species in this system, and for understanding the evolution of adaptive differentiation in general.

Main effects for ecophysiological traits

A long-standing evolutionary question concerns the number and effect size of loci typically underlying adaptation (Gottlieb, 1984; Orr & Coyne, 1992). Although we assessed less than 40% of the total genome of SH in the background of SL, we still detected QTLs for two of our three focal traits. Overall, main effects were generally not strong (none was significant after a conservative Bonferroni correction), but nevertheless provide a first assessment of genomic locations containing genes for these potentially adaptive traits. Interestingly, both our main effects for TW were in a direction that indicated reduced drought tolerance. The transgressive direction of single SH introgressions suggests that substantial background effects are operating for this trait.

There are no previous QTL analyses of these particular traits between SH and SL. However, studies have examined ecophysiological differences between SL and other wild tomato species (Martin *et al.*, 1989; Foolad *et al.*, 2003; Xu *et al.*, 2008). In particular, Xu *et al.* (2008) examined carbon isotope discrimination differences between SL and *Solanum pennellii* (SP), also using NILs. SP is known to be substantially more drought resistant than SL. Across the whole genome, this study found one QTL consistent with higher water use efficiency (WUE) but at least five QTLs (six NILs in total) for which carbon isotope discrimination was significantly more negative (consistent with reduced WUE) than that of both SL and SP parents. That is, the frequency of transgression for this ecophysiological trait, as for our TW, appears to be high. In addition, three of the transgressive QTLs in Xu *et al.* (2008) occur at chromosomal regions that were also represented in our study. None corresponds with our main effect QTLs; however, two of these regions (on chromosomes 9 and 12) are involved in many epistatic interactions influencing TW, including interactions with our main effect loci (see next section).

Several of our main effect QTLs or significantly epistatic regions also appear to coincide with QTLs detected in other tomato analyses. Monforte *et al.* (2001) found significant antagonistic epistasis for fruit traits between SH introgressions on chromosomes 1 and 4 in regions that overlap with a significant interaction for TW between LA3915 and LA3925. All of our introgressions with a significant main effect on SLA overlapped with significant leaf shape QTLs from SP (Holtan & Hake, 2003). Finally, chromosome 9 introgressions (LA3956 and LA3957) with significant main effects on SLA and epistatic effects on TW encompass a locus found to confer chilling tolerance through shoot turgor maintenance (Truco *et al.*, 2000; Goodstal *et al.*, 2005).

Frequency of epistasis and involvement of main effect QTLs

We found more epistatic interactions between Het chromosomal regions than we expected by chance, particularly for SLA. There were fewer significant interactions between Hom DILs, probably as a consequence of reduced power or perhaps complex dominance (see Results). Approximately half of all epistatic interactions were between chromosomal regions with no detected main effects. Our study therefore suggests that simply evaluating the individual (main) effect of loci could lead to a substantial underestimate of loci influencing a complex trait. For example, two of our SH chromosomal regions – on chromosomes 9 and 12 – have previously been shown to have main effects on WUE responses differentiating SL and SP (Xu *et al.*, 2008). Our analysis suggests that these regions do not have strong individual effects but significantly change the drought response phenotype (in our case, TW) when found in combination with each other. Interestingly, we find that these regions act to increase apparent drought resistance (time to wilting) when found in combination; their individual main effects in the Xu *et al.* (2008) study were to decrease WUE.

Relative frequencies of epistatic and additive interactions

Studies have suggested that epistatic QTLs often underlie ecologically relevant variation in plant species (Malmberg & Mauricio, 2005). We found that chromosomal regions often interact epistatically for our three traits, although generally less often than they do additively (Table 4). The relative prevalence of epistatic and additive interactions impacts the amount of constraint and path-dependence natural selection faces, as new mutations may only be beneficial in some genetic backgrounds. For example, Weinreich *et al.* (2006) found that epistasis prevented all but a few mutational paths from being favored by natural selection in an experimental microbial population. If traits or suites of traits systematically differ in their proclivity for additive and epistatic interactions, then selection on them could be more or less constrained and/or path-dependent.

Directionality of epistasis

Epistasis may enhance (synergistic) or diminish (antagonistic) the main effects of loci. Our results are striking in that we found that all but two significant epistatic interactions were antagonistic (Table 5). Generally, this meant that an additional SH introgression reduced, or even changed the sign of, the main effect (if any) of a chromosomal region acting alone. In the case of TW, because both our main effects were in the direction of reducing drought tolerance, this result was consistent with

© The Authors (2009)
Journal compilation © *New Phytologist* (2009)

two SH introgressions 'restoring' a more drought-resistant phenotype. Indeed, the only cases in which we detected significant increases in drought resistance (greater times to wilting compared with SL) in our experiment involved DILs (four cases; Fig. 1), indicating that the combined effect of at least two SH introgressions was required for an 'SH-typical' (more drought-resistant) response to water stress.

Evolutionarily, antagonistic epistasis can reduce selection coefficients on later mutations, altering their probability of fixation. Interestingly, other studies from tomato have found that antagonistic epistasis is much more common than synergistic epistasis for fruit and yield traits (Eshed & Zamir, 1996; Causse *et al.*, 2007). By contrast, studies from several other systems do not report a systematic bias in the direction of epistasis (Segre *et al.*, 2005; Sambandan *et al.*, 2006; Yamamoto *et al.*, 2008). This difference is intriguing, but hard to interpret without additional data on the nature of the antagonism for these interactions in tomato. Antagonistic epistasis is, for example, consistent with evolutionary changes along a relatively linear pathway. A possible example of such a pathway is abscisic acid-mediated drought response. When a plant is stressed by drought, a cellular signal is tranduced, inducing abscisic acid biosynthesis, which in turn up-regulates transcription factors, which up-regulate numerous downstream regulatory and stress-responsive genes (Bray, 2002).

Finally, while there is strong evidence that several of our antagonistic epistatic interactions are biologically real, our general finding that antagonistic epistasis was far more prevalent than synergistic epistasis should be interpreted in light of our demonstration that an inflated signature of antagonistic epistasis can result where main effect QTLs are false positives. Interestingly, all similar experimental designs, comparing the strengths of effect of single versus pairwise combinations of mutations or introgressions, are open to the same experimental hazard. In these cases, data re-sampling will also be important in establishing that bias in the directionality of epistasis exceeds random expectations.

Relationship between traits

Despite a significant, experiment-wide correlation among TW, SLA, and Size, we did not find strong evidence that our traits were mechanistically linked; in particular, whole-plant responses to acute drought stress (TW phenotypes) did not appear to be attributable to differences in SLA or Size. Because these traits are high-dimensional composites of many underlying factors, we expect that they are mechanistically associated with additional, unmeasured traits (see below).

Implications for genetic basis and evolution of ecophysiological differentiation in *Solanum*

Whole-plant drought responses are known to be highly complex traits. Net WUE, for example, is the combined product of numerous phenological, structural, and molecular responses to water stress, many of which can be highly environment-dependent (McKay *et al.*, 2003; Tuberosa & Salvi, 2006; Collins *et al.*, 2008). Even with substantial recent advances in our understanding of the genetic architecture of drought-related traits (e.g. Hausmann *et al.*, 2005; Juenger *et al.*, 2005; McKay *et al.*, 2008), these complexities explain in part why drought response has remained such a challenging trait to dissect.

SH is perhaps a good example of this complexity of drought response traits. High SLA (relatively thin leaves) is generally associated with low WUE. SH clearly has higher SLA than the domesticated tomato but nonetheless greater overall WUE and more resistant drought responses under a range of conditions (e.g. Comstock *et al.*, 2005). This apparent inconsistency is probably explained by the mediating effects of other trait differences between SH and SL. For example, the leaves of SH are densely covered with trichomes (whereas SL leaves are largely glabrous), which could also contribute to reduced transpirational water loss, and SH is known to have superior stomatal regulation under water deficits in comparison to SL (Bloom *et al.*, 2004). In this species, these other traits could have an ameliorating effect on the overall WUE consequences of high SLA, a trait that responds to many other selection pressures (Poorter *et al.*, 2009). Accordingly, a suite of traits can produce a net 'water thrifty' phenotype in combination, even when individual components might not maximize water conservation.

The complexity, and within-species 'fine-tuning', of these mechanisms underlying drought response might also explain why this and other studies (Lexer *et al.*, 2005; Xu *et al.*, 2008) have found notable negative transgression for drought and other physiological responses in interspecific crosses. This fine-tuning might be particularly susceptible to disruption in species hybrids when multiple, genetically independent components are necessary for appropriate functional responses, as seems to be the case for complex ecophysiological traits. QTLs that alter phenotypes in the 'wrong' direction are frequently reported in species crosses (e.g. *Helianthus*, Lexer *et al.*, 2005; tomato, Lippman *et al.*, 2007), and many are thought to be a result of the disruption of appropriate genetic interactions and/or trait combinations. Whether the same patterns will be detected for other trait classes between our two species remains to be seen. We are currently examining pollen and seed traits in NILs and DILs of the same population to assess this expectation for interspecific sterility (L.C. Moyle, T. Nakazato & S. Josway, unpublished).

Implications for epistasis in adaptive evolution

Our study is one of the few to systematically measure epistasis in a randomly sampled portion of the genome in a nonmicrobial system. Our results therefore provide an interesting and unique snapshot of the prevalence of epistasis in between-species

© The Authors (2009)
Journal compilation © *New Phytologist* (2009)

differences. Documenting epistasis in between-species crosses is a prerequisite for investigating whether epistasis could have played a role in species divergence. Theory and empirical data strongly implicate a role for epistasis in speciation (Dobzhansky–Muller interactions), but the expectation for 'normal' species differences is more equivocal (Orr, 2001). Given their rapid ecological divergence into arid habitats (Moyle, 2008; Chetelat *et al.*, 2009), the evolution of drought response and allied traits in *Solanum* is a promising system in which to study the genetic architecture of adaptively important complex traits. Overall, our results here suggest that the differentiation between species could be strongly influenced by the strength and nature of genetic interactions underlying adaptive phenotypic differences, especially where these phenotypes are the product of multiple, complex underlying mechanisms.

Acknowledgements

We thank T. Nakazato for generating the DILs used in this experiment, and S. Josway, E. Josephs, M. Fryska, K. Wolt, E. Lines, and J. Krantz for assistance in the glasshouse. Three anonymous referees provided helpful and constructive suggestions on earlier versions of the manuscript. CDM is supported by an NSF Predoctoral Fellowship. This work was supported in part by NSF Division of Environmental Biology grant 0532097 to LCM.

References

Ackerly DD, Dudley SA, Sultan SE, Schmitt J, Coleman JS, Linder CR, Sandquist DR, Geber MA, Evans AS, Dawson TE *et al.* 2000. The evolution of plant ecophysiological traits: recent advances and future directions. *Bioscience* **50**: 979–995.

Ambramoff MD, Magelhaes PJ, Ram SJ. 2004. Image processing with ImageJ. *Biophotonics International* **11**: 36–42.

Barton NH, Keightley PD. 2002. Understanding quantitative genetic variation. *Nature Reviews Genetics* **3**: 11–21.

Benjamini Y, Hochberg Y. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society Series B (Methodological)* **57**: 289–300.

Bloom AJ, Zwieniecki MA, Passioura JB, Randall LB, Holbrook NM, St. Clair DA. 2004. Water relations under root chilling in a sensitive and tolerant tomato species. *Plant, Cell & Environment* **27**: 971–979.

Bray EA. 2002. Abscisic acid regulation of gene expression during water-deficit stress in the era of the arabidopsis genome. *Plant, Cell & Environment* **25**: 153–161.

Caicedo AL, Stinchcombe JR, Olsen KM, Schmitt J, Purugganan MD. 2004. Epistatic interaction between arabidopsis FRI and FLC flowering time genes generates a latitudinal cline in a life history trait. *Proceedings of the National Academy of Sciences, USA* **101**: 15670–15675.

Carlborg O, Haley CS. 2004. Epistasis: too often neglected in complex trait studies? *Nature Reviews Genetics* **5**: 618–625.

Causse M, Chaïb J, Lecomte L, Buret M, Hospital F. 2007. Both additivity and epistasis control the genetic variation for fruit quality traits in tomato. *Theoretical and Applied Genetics* **115**: 429–442.

Chetelat RT, Pertuzé RA, Faúndex L, Graham EB, Jones CM. 2009. Distribution, ecology and reproductive biology of wild tomatoes and related nightshades from the Atacama desert region of northern Chile. *Euphytica* **167**: 77–93.

Collins NC, Tardieu F, Tuberosa R. 2008. Quantitative trait loci and crop performance under abiotic stress: where do we stand? *Plant Physiology* **147**: 469–486.

Comstock JP, McCouch SR, Martin BC, Tauer CG, Vision TJ, Xu YB, Pausch RC. 2005. The effects of resource availability and environmental conditions on genetic rankings for carbon isotope discrimination during growth in tomato and rice. *Functional Plant Biology* **32**: 1089–1105.

Coyne JA, Orr HA. 2004. *Speciation*. Sunderland, MA, USA: Sinauer Associates.

D'Arcy WG. 1979. The classification of the Solanaceae. In: Lester RN, Skelding AD, eds. *The biology and taxonomy of the Solanaceae*. London, UK: Academic Press, 3–47.

Demuth JP, Wade MJ. 2006. Experimental methods for measuring gene interactions. *Annual Review of Ecology Evolution and Systematics* **37**: 289–316.

Dunnett CW. 1955. A multiple comparison procedure for comparing several treatments with a control. *Journal of the American Statistical Association* **50**: 1096–1121.

Eshed Y, Zamir D. 1996. Less-than-additive epistatic interactions of quantitative trait loci in tomato. *Genetics* **143**: 1807–1817.

Fisher RA. 1930. *The genetical theory of natural selection*. Oxford, UK: Oxford University Press.

Fitter A, Hay R. 2002. *Environmental physiology of plants*. San Francisco, CA, USA: Academic Press.

Foolad MR, Zhang LP, Subbiah P. 2003. Genetics of drought tolerance during seed germination in tomato: inheritance and QTL mapping. *Genome* **46**: 536–545.

Gerke J, Lorenz K, Cohen B. 2009. Genetic interactions between transcription factors cause natural variation in yeast. *Science* **323**: 498–501.

Goodstal FJ, Kohler GR, Randall LB, Bloom AJ, St. Clair DA. 2005. A major QTL introgressed from wild *Lycopersicon hirsutum* confers chilling tolerance to cultivated tomato (*Lycopersicon esculentum*) *Theoretical and Applied Genetics* **111**: 898–905.

Gottlieb LD. 1984. Genetics and morphological evolution in plants. *American Naturalist* **123**: 681–709.

Hausmann NJ, Juenger TE, Sen S, Stowe KA, Dawson TE, Simms EL. 2005. Quantitative trait loci affecting delta c-13 and response to differential water availability in *Arabidopsis thaliana*. *Evolution* **59**: 81–96.

Hill WG, Goddard ME, Visscher PM. 2008. Data and theory point to mainly additive genetic variance for complex traits. *PLoS Genetics* **4**: e1000008. doi:10.1371/journal.pgen.1000008

Holtan HEE, Hake S. 2003. Quantitative trait locus analysis of leaf dissection in tomato using *Lycopersicon pennellii* segmental introgression lines. *Genetics* **165**: 1541–1550.

Juenger TE, McKay JK, Hausmann N, Keurentjes JJB, Sen S, Stowe KA, Dawson TE, Simms EL, Richards JH. 2005. Identification and characterization of QTL underlying whole-plant physiology in *Arabidopsis thaliana*: delta c-13, stomatal conductance and transpiration efficiency. *Plant, Cell & Environment* **28**: 697–708.

Kondo K, Yamamoto M, Itahashi R, Sato T, Egashira H, Hattori T, Kowyama Y. 2002. Insights into the evolution of self-compatibility in Lycopersicon from a study of stylar factors. *Plant Journal* **30**: 143–153.

Kondrashov AS. 1994. Mullers ratchet under epistatic selection. *Genetics* **136**: 1469–1473.

Kraft NJB, Valencia R, Ackerly DD. 2008. Functional traits and niche-based tree community assembly in an Amazonian forest. *Science* **322**: 580–582.

Lexer C, Rosenthal DM, Raymond O, Donovan LA, Rieseberg LH. 2005. Genetics of species differences in the wild annual sunflowers, *Helianthus annuus* and *H. petiolaris*. *Genetics* **169**: 2225–2239.

Lippman ZB, Semel Y, Zamir D. 2007. An integrated view of quantitative trait variation using tomato interspecific introgression lines. *Current Opinion in Genetics & Development* **17**: 545–552.

© The Authors (2009)
Journal compilation © *New Phytologist* (2009)

Lukens LN, Doebley J. 1999. Epistatic and environmental interactions for quantitative trait loci involved in maize evolution. *Genetical Research* **74**: 291–302.

Malmberg RL, Mauricio R. 2005. QTL-based evidence for the role of epistasis in evolution. *Genetical Research* **86**: 89–95.

Martin B, Nienhuis J, King G, Schaefer A. 1989. Restriction fragment length polymorphisms associated with water-use efficiency in tomato. *Science* **243**: 1725–1728.

McKay JK, Richards JH, Mitchell-Olds T. 2003. Genetics of drought adaptation in *Arabidopsis thaliana*: I. Pleiotropy contributes to genetic correlations among ecological traits. *Molecular Ecology* **12**: 1137–1151.

McKay JK, Richards JH, Nemali KS, Sen S, Mitchell-Olds T, Boles S, Stahl EA, Wayne T, Juenger TE. 2008. Genetics of drought adaptation in *Arabidopsis thaliana* II. QTL analysis of a new mapping population, kas-1 x tsu-1. *Evolution* **62**: 3014–3026.

Miller JC, Tanksley SD. 1990. RFLP analysis of phylogenetic relationships and genetic variation in the genus *Lycopersicon*. *Theoretical and Applied Genetics* **80**: 437–448.

Monforte AJ, Friedman E, Zamir D, Tanksley SD. 2001. Comparison of a set of allelic QTL-NILs for chromosome 4 of tomato: deductions about natural variation and implications for germplasm utilization. *Theoretical and Applied Genetics* **102**: 572–590.

Monforte AJ, Tanksley SD. 2000. Development of a set of near isogenic and backcross recombinant inbred lines containing most of the *Lycopersicon hirsutum* genome in a *L. esculentum* genetic background: a tool for gene mapping and gene discovery. *Genome* **43**: 803–813.

Moyle LC. 2008. Ecological and evolutionary genomics in the wild tomatoes (*Solanum* sect. *Lycopersicon*). *Evolution* **62**: 2995–3013.

Moyle LC, Graham EB. 2005. Genetics of hybrid incompatibility between *Lycopersicon esculentum* and *L. hirsutum*. *Genetics* **169**: 355–373.

Moyle LC, Nakazato T. 2008. Comparative genetics of hybrid incompatibility: sterility in two Solanum species crosses. *Genetics* **179**: 1437–1453.

Moyle LC, Nakazato T. 2009. Complex epistasis for Dobzhansky–Muller hybrid incompatibility in *Solanum*. *Genetics* **181**: 347–351.

Nakazato T, Bogonovich M, Moyle LC. 2008. Environmental factors predict adaptive phenotypic differentiation within and between two wild Andean tomatoes. *Evolution* **62**: 774–792.

Nesbitt TC, Tanksley SD. 2002. Comparative sequencing in the genus *Lycopersicon*: implications for the evolution of fruit size in the domestication of cultivated tomatoes. *Genetics* **162**: 365–379.

Orr HA. 2001. The genetics of species differences. *Trends in Ecology & Evolution* **16**: 343–350.

Orr HA, Coyne JA. 1992. The genetics of adaptation – a reassessment. *American Naturalist* **140**: 725–742.

Peralta IE, Knapp SK, Spooner DM. 2005. New species of wild tomatoes (*Solanum* section *Lycopersicon*: *Solanaceae*) from northern Peru. *Systematic Botany* **30**: 424–434.

Peralta IE, Spooner DM. 2001. Granule-bound starch synthase (gbssi) gene phylogeny of wild tomatoes (*Solanum* l. Section Lycopersicon [Mill.] Wettst. Subsection *Lycopersicon*). *American Journal of Botany* **88**: 1888–1902.

Peralta IE, Spooner DM, Knapp S. 2008. Taxonomy of wild tomatoes and their relatives (*Solanum* sect. *Lycopersicoides*, sect. *Juglandifolia*, sect. *Lycopersicon*; Solanaceae) *Systematic Botany Monographs* **84**: 1–186.

Peters AD, Lively CM. 1999. The red queen and fluctuating epistasis: a population genetic analysis of antagonistic coevolution. *American Naturalist* **154**: 393–405.

Phillips PC. 2008. Epistasis – the essential role of gene interactions in the structure and evolution of genetic systems. *Nature Reviews Genetics* **9**: 855–867.

Poorter H, Niinemets U, Poorter L, Wright IJ, Villar R. 2009. Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. *New Phytologist* **182**: 565–588.

R Core Development Team. 2008. *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing.

Rick CM. 1995. Tomato. In: Smartt J, Simmonds NW, eds. *Evolution of Crop Plants. 2nd edn*. London, UK: Longman Scientific & Technical, 452–457.

Rieseberg LH, Archer MA, Wayne RK. 1999. Transgressive segregation, adaptation and speciation. *Heredity* **83**: 363–372.

Rockman MV. 2008. Reverse engineering the genotype-phenotype map with natural genetic variation. *Nature* **456**: 738–744.

Sambandan D, Yamamoto A, Fanara JJ, Mackay TFC, Anholt RRH. 2006. Dynamic genetic interactions determine odor-guided behavior in *Drosophila melanogaster*. *Genetics* **174**: 1349–1363.

Segre D, DeLuna A, Church GM, Kishony R. 2005. Modular epistasis in yeast metabolism. *Nature Genetics* **37**: 77–83.

Spickett SG, Thoday JM. 1966. Regular responses to selection 3. Interaction between located polygenes. *Genetical Research* **7**: 96–121.

Spooner DM, Peralta IE, Knapp S. 2005. Comparison of AFLPs with other markers for phylogenetic inference in wild tomatoes [*Solanum* l. Section *Lycopersicon* (Mill.) Wettst.]. *Taxon* **54**: 43–61.

Stephan W, Langley CH. 1998. DNA polymorphism in lycopersicon and crossing-over per physical length. *Genetics* **150**: 1585–1593.

Truco MJ, Randall LB, Bloom AJ, St. Clair DA. 2000. Detection of QTLs associated with shoot wilting and root ammonium uptake under chilling temperatures in an interspecific backcross population from *Lycopersicon esculentum* × *L. hirsutum*. *Theoretical and Applied Genetics* **101**: 1082–1092.

Tuberosa R, Salvi S. 2006. Genomics-based approaches to improve drought tolerance of crops. *Trends in Plant Science* **11**: 405–412.

de Visser J, Elena SF. 2007. The evolution of sex: empirical insights into the roles of epistasis and drift. *Nature Reviews Genetics* **8**: 139–149.

Wade MJ. 2000. Epistasis as a genetic constraint within populations and an accelerant of adaptive divergence among them. In: Wolf JB, Brodie ED III, Wade MJ, eds. *Epistasis and the evolutionary process*. Oxford, UK: Oxford University Press.

Weinreich DM, Delaney NF, DePristo MA, Hartl DL. 2006. Darwinian evolution can follow only very few mutational paths to fitter proteins. *Science* **312**: 111–114.

Weinreich DM, Watson RA, Chao L. 2005. Perspective: sign epistasis and genetic constraint on evolutionary trajectories. *Evolution* **59**: 1165–1174.

Welch BL. 1947. The generalization of 'student's' problem when several different population variances are involved. *Biometrika* **34**: 28–35.

Whitlock MC, Phillips PC, Moore FBG, Tonsor SJ. 1995. Multiple fitness peaks and epistasis. *Annual Review of Ecology and Systematics* **26**: 601–629.

Wright S. 1931. Evolution in Mendelian populations. *Genetics* **16**: 97–159.

Xu X, Martin B, Comstock JP, Vision TJ, Tauer CG, Zhao B, Pausch RC, Knapp S. 2008. Fine mapping a QTL for carbon isotope composition in tomato. *Theoretical and Applied Genetics* **117**: 221–233.

Yamamoto A, Zwarts L, Callaerts P, Norga K, Mackay TFC, Anholt RRH. 2008. Neurogenetic networks for startle-induced locomotion in *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences, USA* **105**: 12393–12398.

Supporting Information

Additional supporting information may be found in the online version of this article.

Methods S1 Generation of near-isogenic lines (NILs) and double-introgression lines (DILs).

© The Authors (2009)
Journal compilation © *New Phytologist* (2009)

Notes S1 Formal demonstration that an inflated signature of antagonistic epistasis can result when the main effect of a near-isogenic line (NIL) is a false positive.

Fig. S1 The probability density function of *U*, the random variable for antagonistic epistasis between near-isogenic lines (NILs) conditional on one of them being a false positive.

Table S1 Sample sizes for all genotypes in the study

Table S2 Significant differences in trait means between double-introgression lines (DILs) and the parental *Solanum lycopersicum* (SL) genotype

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting information supplied by the authors. Any queries (other than missing material) should be directed to the *New Phytologist* Central Office.

About *New Phytologist*

- *New Phytologist* is owned by a non-profit-making **charitable trust** dedicated to the promotion of plant science, facilitating projects from symposia to open access for our Tansley reviews. Complete information is available at **www.newphytologist.org**.
- Regular papers, Letters, Research reviews, Rapid reports and both Modelling/Theory and Methods papers are encouraged. We are committed to rapid processing, from online submission through to publication 'as-ready' via *Early View* – our average submission to decision time is just 29 days. Online-only colour is **free**, and essential print colour costs will be met if necessary. We also provide 25 offprints as well as a PDF for each article.
- For online summaries and ToC alerts, go to the website and click on 'Journal online'. You can take out a **personal subscription** to the journal for a fraction of the institutional price. Rates start at £139 in Europe/$259 in the USA & Canada for the online edition (click on 'Subscribe' at the website).
- If you have any questions, do get in touch with Central Office (**newphytol@lancaster.ac.uk**; tel +44 1524 594691) or, for a local contact in North America, the US Office (**newphytol@ornl.gov**; tel +1 865 576 5261).

© The Authors (2009)
Journal compilation © *New Phytologist* (2009)