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Floral Genetic Architecture: An Examination of QTL Architecture Underlying Floral (Co)Variation Across Environments

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ABSTRACT

Genetic correlations are expected to be high among functionally related traits and lower between groups of traits with distinct functions (*e.g.*, reproductive *vs.* resource-acquisition traits). Here, we explore the quantitative-genetic and QTL architecture of floral organ sizes, vegetative traits, and life history in a set of *Brassica rapa* recombinant inbred lines within and across field and greenhouse environments. Floral organ lengths were strongly positively correlated within both environments, and analysis of standardized **G**-matrices indicates that the structure of genetic correlations is ~80% conserved across environments. Consistent with these correlations, we detected a total of 19 and 21 additive-effect floral QTL in the field and the greenhouse, respectively, and individual QTL typically affected multiple organ types. Interestingly, QTL × QTL epistasis also appeared to contribute to observed genetic correlations; *i.e.*, interactions between two QTL had similar effects on filament length and two estimates of petal size. Although floral and nonfloral traits are hypothesized to be genetically decoupled, correlations between floral organ size and both vegetative and life-history traits were highly significant in the greenhouse; **G**-matrices of floral and vegetative traits as well as floral and life-history traits differed across environments. Correspondingly, many QTL (45% of those mapped in the greenhouse) showed environmental interactions, including approximately even numbers of floral and nonfloral QTL. Most instances of QTL × QTL epistasis for floral traits were environment dependent.

EVOLUTIONARY responses to selection are dependent on genetic architecture. The proportion of phenotypic variation with a heritable genetic basis affects the response to selection, as does the structure of genetic correlations among selected traits. For example, an evolutionary response will be constrained if selection favors an increase in the value of two traits that are negatively correlated; *i.e.*, a negative correlation is antagonistic to the joint vector of selection. Alternatively, if the vector of selection is parallel to the genetic correlation, then trait covariation is reinforcing and the population mean may more rapidly approach favored trait values (Etterson and Shaw 2001; Merilä and Björklund 2004). One measure of genetic architecture is the **G**-matrix (Lynch and Walsh 1998), which is composed of genetic variances (diagonal matrix elements) and genetic covariances among traits (off-diagonal matrix elements). **G**-matrices have been shown to vary across environments (Donohue *et al.* 2000; Conner *et al.* 2003; Brock and Weinig 2007), indicating that the molecular-genetic underpinnings of matrix elements (*e.g.*, identity and/or relative effect of additive and epistatic loci, degree of pleiotropy, etc.) and the traits' evolutionary potential vary across environments. Few studies, however, have related matrix and QTL architectures; and, therefore, the molecular-genetic underpinnings of quantitative-genetic estimates remain unclear (but see Gardner and Latta 2007; Kelly 2009).

In angiosperms, covariances between floral whorls (*e.g.*, petal and stamen length) are frequently positive among functionally related traits. These positive correlations can arise from pollinator-mediated (or pollination-mediated) selection for specific allometric relationships among floral traits and ensuing linkage disequilibrium (LD) among causal loci (Berg 1959, 1960; also referred to as phenotypic integration, see Pigliucci 2003; Klingenberg 2008). For example, in outcrossing species, male fitness may be more dependent on the frequency and efficiency of pollinator visitation than

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female fitness (Bell 1985; but see Hodgins and Barrett 2008). Anther placement relative to the corolla opening can affect the efficiency of pollen dissemination (Conner and Via 1993; Morgan and Conner 2001); in addition, comparative work indicates that petal–stamen length correlations are stronger than stamen–pistil length correlations in outcrossers, whereas species that reproduce via autogamous selfing show the opposite pattern (Ushimaru and Nakata 2002). Alternatively, strong floral integration could be attributed to the developmental hypothesis that genetic correlations arise due to pleiotropic genes coregulating floral whorls (Herrera 2001; Herrera *et al.* 2002). Strong correlations resulting from linkage disequilibrium or from developmentally based pleiotropy may constrain the evolution of novel reproductive morphologies when biotic or abiotic factors (and selection) change (Cheverud 1984; Clark 1987; Smith and Rausher 2008; Agrawal and Stinchcombe 2009).

Similar to genetic covariances among floral traits, covariances between floral and nonfloral traits could also alter the evolutionary response of reproductive traits. In contrast to hypotheses regarding the adaptive significance of floral-trait integration, genetic correlations between floral and nonfloral traits (*e.g.*, vegetative or phenological traits) are hypothesized to be disadvantageous (Berg 1960). More specifically, floral allometry may be shaped by selection for reproductive success, as described above, whereas vegetative morphology is shaped primarily by selection to optimize other functions, such as light capture. If floral and nonfloral traits have a common genetic basis, then selection on phenological or morphological traits may result in maladaptive expression of floral organ size. As a result, functionally integrated floral traits are predicted to be genetically decoupled from vegetative and phenological traits (Berg 1960).

QTL mapping provides a powerful tool to explore the genetic architecture of evolutionarily important traits. The QTL architecture of interspecific floral traits has been explored in diverse systems (Bradshaw *et al.* 1995; Fishman *et al.* 2002; Goodwillie *et al.* 2006; Bouck *et al.* 2007; Moyle 2007); however, insight into the molecular genetic basis of intraspecific floral variation comes almost exclusively from *Arabidopsis thaliana* (Juenger *et al.* 2000, 2005) and *Mimulus guttatus* (Hall *et al.* 2006). Floral traits in these intraspecific crosses are polygenic with a majority of detected QTL being of small to moderate effect size. Consistent with other quantitative-genetic studies (reviewed in Ashman and Majetic 2006), floral traits in *A. thaliana* and *M. guttatus* mapping populations exhibited moderate to high genetic correlations. In both systems, mapped QTL often affected multiple floral traits. In the few cases where QTL underlying intraspecific floral morphology have been evaluated, only a single growth environment was used; estimation of floral quantitative genetics across environments and subsequent comparison with the QTL architecture underlying observed across-environment patterns are lacking.

Using a segregating progeny of *Brassica rapa* (recombinant inbred lines, RILs) and a small sample of crop and wild accessions, we examine the quantitative-genetic and QTL architecture of floral traits under field and greenhouse environments. Specifically, we address the following questions: (1) Does this RIL population express significant genetic (co)variation for floral traits when growing in the field or greenhouse? (2) Is there significant genetic variation for vegetative traits and days to flowering in field and greenhouse environments, and is there evidence for genetic correlations between floral and nonfloral traits? (3) Does the genetic architecture of floral and nonfloral traits, as measured by the **G**-matrix, differ across environments? (4) What is the number and effect size of additive and epistatic QTL in field and greenhouse environments? (5) What is the relationship between mapped QTL and quantitative genetic estimates of trait (co)variation within and between floral and nonfloral traits? And (6) what is the relationship between the quantitative-genetic architecture of floral traits in the RILs *vs.* in the accessions?

MATERIALS AND METHODS

B. rapa L. (syn: *B. campestris*) is an annual (occasionally biennial) crop plant native to Eurasia that has been introduced in the Americas (Holm *et al.* 1997); naturalized populations typically develop where it is cultivated. Plants produce an erect stem with alternating cauline leaves and secondary branches. Flowers of *B. rapa* are yellow, perfect, and borne on indeterminate racemes, and individual flowers are composed of four sepals, four petals, six stamens (four long plus two short), and a compound pistil of two carpels. Although *B. rapa* plants are commonly obligate outcrossers due to a well-characterized sporophytic self-incompatibility system (Bateman 1955), some genotypes are capable of self-fertilization.

A segregating progeny of RILs was created utilizing two self-compatible genotypes of *B. rapa* (yellow sarson, R500, and the rapid cycling IMB211) (Iniguez-Luy *et al.* 2009, genotypic data of RILs available as supplementary material therein; RIL seeds publically available, http://www.brassica.info/resource/plants/mapping-populations.php). IMB211 was derived from the Wisconsin Fast Plant (WFP) population, a rapid-cycling population produced from 10 generations of selection for early flowering and rapid generation time (Williams and Hill 1986). The IMB211 genotype was produced by intermating WFPs and selecting for self-compatibility and high fecundity. This selection regime was followed by seven generations of selfing and single-seed descent. The artificial selection for rapid generation time and high fecundity in IMB211 is similar to that experienced by naturalized populations and agricultural weeds of this species (Dorn and Mitchell-Olds 1991; Mitchell-Olds 1996). Moreover, self-compatibility arises frequently in the subspecific clade to which R500 belongs (Zhao *et al.* 2005), a pattern also observed in ruderal/weed species (Baker 1965). The yellow sarson (R500) genotype is a cultivar planted in India for at least 3000 years (Hinata and Prakash 1984). In comparison with IMB211, R500 delays flowering and attains substantially greater biomass. Although

they are often self-compatible (Zhao *et al.* 2005), yellow sarson plants produce flowers similar in size to self-incompatible varieties (Hinata *et al.* 1994). Sarson varieties significantly increase fruit and seed production in the presence of visiting insects (Mishra *et al.* 1988; Mishra and Kaushik 1992), suggesting moderate to high levels of outcrossing. In many regards, the IMB211 and R500 parental genotypes have experienced selection similar to other weed and crop *B. rapa* populations, and genetic variation segregating in the RILs used here may resemble that segregating in crop × wild hybrids found in nature (Adler *et al.* 1993).

The two parental genotypes were crossed to produce an F_1 individual that was subsequently selfed. An F_2 generation of 160 plants was established and advanced to the S5 generation via self-fertilization and single-seed decent with an expectation of at least 93.75% homozygosity on average genome-wide. Seeds of this S5 generation (used in two QTL mapping experiments described below) were bulked by selfing replicate plants of each RIL during January 2004 and 2007, depending on the experiment. In each bulking generation, plants were raised in 13-cm-diameter clay pots containing MetroMix 200 (Scotts, Marysville, OH) and ~0.68 g of fertilizer (14-14-14 Osmocote; Scotts) in the University of Minnesota (St. Paul) greenhouses under supplemental lighting [12 hr light (L):12 hr dark (D)]. Flowers were self-pollinated by hand, and fruits were allowed to ripen for 2 weeks following plant senescence.

Field environment: As part of a larger QTL mapping project exploring plant responses to variation in local density, seeds of 150 RILs were planted into density treatments in the agricultural fields at the University of Minnesota (April 19–21, 2004; St. Paul). Here, we report data from the competitive treatment (see below); for discussion of additional treatments see Dechaine *et al.* (2007). Each RIL was randomly assigned to 1 of 150 planting locations that were divided evenly among three subplots in each of three spatial blocks (*i.e.*, 50 RILs per subplot × three subplots per block). We planted seeds of each RIL in a three-column by six-row configuration with 5 cm separating each of the 18 plants and 20 cm separating the next adjacent planting. All data were collected on the four central plants of the middle column (*i.e.*, rows 2–5 for column 2). This design was chosen so that competing neighbors were of the same genotype, a situation common in natural populations due to passive seed dispersal as well as in crop settings. However, because replicates within the same subplot were grouped spatially, observed phenotypes are not statistically independent, and the group is referred to as replicate cluster in *Statistical analyses*. In sum, there are 12 replicate plants per RIL (three blocks × 4 replicates per block).

Over the growing season, we censused the four central plants for a variety of vegetative and reproductive traits, including hypocotyl length at bolting (stem length from the ground to cotyledons), longest leaf length at bolting, and flowering date. During flowering, we collected up to two newly opened flowers per focal plant; we predominantly sampled floral nodes four and five to limit developmental variation. Flowers were sampled between 1000 and 1300 hr, during which petals reflexed and anthers dehisced. Flowers were preserved in 70% ethanol and later processed in the lab (see below). Once plants had finished flowering and were ripening fruits (>50% of fruits were yellow), we measured primary inflorescence height and longest secondary branch length (hereafter, branch length). (For phenotypic data, see supporting information, File S1.)

Flowers were dissected under a stereomicroscope [Nikon (Tokyo) SMZ-800], and petals, stamens, and pistils were arranged flat on the stage. A digital image was recorded via a sideport-attached camera, and from these images, we measured petal length and width, midpoint length (base of the petal to the point at which the petal blade reflexes, *i.e.*, the transition point between petal claw and petal limb), filament length (base of the filament to the anther), anther length, ovary length (from receptacle to the top of the ovary), and combined style–stigma length (from the top of the ovary to the top of the stigma). All floral-image measurements were processed with ImageJ (ver 1.31; Wayne Rasband, National Institutes of Health, Bethesda, MD).

Floral morphology in cultivated and wild B. rapa accessions: To explore the level of genetic (co)variation in floral traits within cultivated and wild *B. rapa* populations during the common-garden field experiment, we obtained 20 accessions from the National Genetic Resources Program (for the 13 accessions that flowered, see Table S1). These accessions originate from the native range of *B. rapa*, including India and neighboring Eurasian countries (Table S1). Each accession was integrated into the common-garden field experimental design, and seeds were randomly planted with identical replication as described above. Flowers were collected and measured with the same methodology as for RILs (see above).

Greenhouse environment: To examine the expression of floral and vegetative traits (and the covariation among traits) in a second, more benign environment, we raised RILs in the greenhouses at the University of Minnesota. On March 14, 2007, we planted seeds of each RIL into eight 7.6-cm square pots that were randomized across six blocks, such that each block had at least one replicate (but no more than two) of each RIL. Each block consisted of plants surrounded by a 314 × 72 × 70-cm rectangular polyvinylchloride frame on which laminated filters (Full Tough Spun filter, no. 214; LEE Filters, Burbank, CA) were suspended. These filters reduced photosynthetically active radiation by ~40%, simulating the light available to plants during early development when grown under field conditions. Following 1 month of growth, plants were censused for a variety of early vegetative and phenological traits; here, we present hypocotyl length and the length and width of the second true leaf.

On April 17, 2007, we planted a second cohort of RILs in the greenhouse with the same design as above; however, each RIL was replicated in only six pots, one pot per block. In this second cohort, plants were raised under ambient light for 2 weeks prior to being placed under light filters. Plants were censused for flowering date, and newly opened flowers from nodes four and five were removed, preserved in 70% ethanol, and measured as described previously. Primary inflorescence height was measured when at least 50% of fruits were yellowing, indicating that plants were close to senescence.

Statistical analyses: *Quantitative genetic analysis of B. rapa traits:* Variation in the expression of floral vegetative and phenological traits in the field environment was analyzed using ANOVA (PROC MIXED, SAS v. 8.02). We included the following random factors in each model: line, subplot (as a spatial blocking term), and replicate cluster nested in the line × subplot term. The replicate cluster term was a unique identifier assigned to each planting location for each RIL and was included to account for lack of independence among the four replicate plants in one column. Some traits failed to meet assumptions of ANOVA, and prior to analysis, we utilized the Box–Cox transformation to identify transformations that improved normality. Using this ANOVA model, we calculated RIL best linear unbiased predictors (BLUPs) for each trait with significant genetic variation. These BLUPs were subsequently used to estimate bivariate genetic correlations between all traits measured in the field experiment (PROC CORR, SAS v. 8.02). Floral traits measured from wild and cultivated *B. rapa* accessions were analyzed with ANOVA models identical to those presented for RILs above. In our initial analyses, replicate cluster and subplot were nonsignificant for all floral traits and were subsequently

pooled with the error term. Genotypic BLUPs were estimated for all floral traits and used to estimate bivariate genetic correlations between all floral traits (PROC CORR).

We applied the same statistical approach to data from *B. rapa* plants raised in the greenhouse environment. Using ANOVA, variation in measured traits was partitioned among the random factors block and line (PROC MIXED). Traits were again transformed on the basis of the Box–Cox procedure and, for each trait, BLUPs were estimated for the line term as RIL genotypic values. We calculated correlation matrices for all greenhouse traits as described above.

To determine if RILs differed in floral and nonfloral trait expression across field and greenhouse environments, we used a mixed-model ANOVA (PROC MIXED) to partition variation between the fixed effect, growth environment, and the following random factors: line and the line × environment interaction. Due to the different experimental designs used in the field and greenhouse environments, analyses were performed on plant residuals after accounting for spatial blocking factors within each environment.

To examine variation in the genetic architecture of *B. rapa* traits across environments, we tested for significant differences in standardized **G**-matrix structure across the field and greenhouse environments. We tested three separate hypotheses. First, we tested for differences in "floral" correlation matrices across environments; matrices were estimated using the following traits: petal width and the length of petal, filament, anther, ovary, and style–stigma. Second, we tested for differences between "floral–vegetative" correlation matrices estimated from floral traits (listed above), leaf length, and primary inflorescence height. Finally, we tested for differences between "floral–flowering phenology" correlation matrices, using floral traits (listed above) and days to flower. We include these specific nonfloral traits because they were measured in both environments and at comparable developmental stages; however, we test them in separate analyses because of the distinct functions of vegetative *vs.* phenological traits and because we were interested in identifying the traits underlying any matrix differences.

We employed Flury's hierarchical common principal components (CPC) analysis to test for differences between matrices (Flury 1988; Phillips and Arnold 1999). This procedure transforms **G**-matrices into eigenvectors and eigenvalues and then compares hierarchically related matrices. From the lowest hierarchical level, the procedure explores the following: unrelated structure, matrices do not share any eigenstructure; CPC(1), matrices share the first principal component; CPC(2), matrices share first and second principal components; . . .; CPC(p-2), where p is the number of traits per $p \times p$ matrix; full CPC, where matrices share all principal components but not eigenvalues (the variance explained by each principal component axis); proportional, where matrices share principal components but all eigenvalues differ by a single constant; and equality, where matrices share all elements of eigenstructure. We use the "jump-up" method for interpreting results (Phillips and Arnold 1999). This approach compares each level in the hierarchy of relatedness against a matrix of unrelated structure, and because each level in the hierarchy includes lower levels, testing stops when a significant difference is detected.

CPC matrix comparisons can be performed on either nonstandardized (covariance) or standardized (correlation) **G**-matrices. However, utilizing correlation matrices may be preferable when measurement scales differ among traits or across environments (Phillips and Arnold 1999). Here, variation in measured traits differed dramatically across environments, and because our interest was in exploring trait covariances, we standardized our data to genetic correlations. For example, flowering phenology variation in the greenhouse was 17.7 times greater than that expressed in the field, which could potentially overshadow differences across environments in floral–flowering phenology covariation. Finally, results utilizing the correlation matrix were more conservative (*i.e.*, detected more matrix similarity) than when using variance–covariance matrices, which were all significantly different at the first principal component. For these reasons, we present analyses of standardized **G**-matrices. Because CPC analysis can be sensitive to normality, we confirmed initial findings with CPCrand (Phillips and Arnold 1999), which determines test significance via a randomization procedure. CPCrand and CPC findings were always identical; we present the CPC results. To corroborate our CPC results, we estimated multivariate **G**-matrices (PROC MIXED) from phenotypic data and tested whether a common **G**-matrix provided a better fit to the data than separate **G**-matrices for the field and greenhouse environments (*e.g.*, Stinchcombe *et al.* 2009). Results of the mixed-model analyses were consistent with those from the CPC analyses, and therefore only the latter analyses are presented.

The CPC and mixed-model analyses examine differences in the multivariate relationship among traits. We used Fisher's Z-tests to identify bivariate correlations that differed significantly across field and greenhouse environments and, therefore, contributed to matrix differences.

Linkage map construction and QTL mapping: We used JoinMap 3.0 (Van Ooijen and Voorrips 2001) to estimate the initial linkage map ($N = 215$ markers) in all 160 RILs of *B. rapa* (for genotypic data of RILs, see Iniguez-Luy *et al.* 2009). Linkage groups were determined using a LOD score between 5 and 8 and a recombination fraction of 0.4. We used the Kosambi mapping function when estimating mapping distances. Due to the loss of RILs in both the field and the greenhouse environments, we reestimated the marker locations (in centimorgans) for RILs that were present in either of the two environments ($N = 153$) using R/qtl (Broman *et al.* 2003), which maintains the original marker order as estimated for the whole population in JoinMap.

We identified QTL affecting floral, vegetative, and phenological traits using composite interval mapping (Zeng 1994) as estimated in QTL cartographer (QTLcart v. 2.5; Wang *et al.* 2007). The CIM procedure tests the hypothesis that a QTL resides in the interval defined by two adjacent markers, while statistically controlling for additional QTL segregating elsewhere in the genome. All QTL mapping analyses (here and below) were performed on genotypic BLUP values (or back-transformed BLUPs when appropriate). We used a CIM testing interval (walking speed) of 2 cM and a window size for excluding background QTL (*i.e.*, nearest markers) of 5 cM. Background QTL were determined using the forward–backward stepwise regression method (P-values set to 0.05) under the standard model (model 6). A genome-wide significance threshold (type I error rate, $\propto = 0.05$) was estimated for each trait with 2000 random permutations of the data set (Doerge and Churchill 1996). We calculated 2-LOD support limit intervals for the location of each significant QTL (*i.e.*, the peak likelihood ratio) as the flanking markers where the LR dropped by ~9.22 (Van Ooijen 1992). To formally test for possible QTL × environment interaction effects (*i.e.*, a shift in the detection of, or magnitude of effect of, QTL across environments), we selected the closest markers to significant QTL in each environment and included all markers as well as two-way marker × environment interactions as main effects in an ANOVA model for each trait (PROC GLM, SAS v. 8.02). Specifically, a QTL may have a significant phenotypic effect in a genome-wide scan in one environment, but have only a minor and statistically undetectable effect in a second envi-

ronment due to low power. The ANOVA described above distinguishes true QTL × environment interactions from statistical artifacts.

We also tested for the influence of epistatic interactions (*i.e.*, QTL × QTL interaction effects) on each trait using the *scantwo* procedure in R/qtl (Broman *et al.* 2003). We used the Haley–Knott regression method (Haley and Knott 1992) to test all possible marker × marker interactions on all traits using a 2-cM testing interval. To determine a genome-wide significance threshold, we permuted the data 1000 times per trait. We also used *Epistacy* (Holland 1998) to test for QTL × QTL epistasis and confirm results of the *scantwo* procedure; this program tests the influence of all possible pairwise combinations of markers on each trait in SAS (PROC GLM). To control for multiple comparisons, the authors of *Epistacy* recommend adjusting the *P*-value threshold to $\propto = 0.05/[g(g-1)/2]$ (Holland 1998), where g is the number of linkage groups (for *B. rapa* $N = 10$ and $\propto = 0.001$). A slightly more conservative threshold ($\propto = 0.0001$) resulted in comparable results between these two methods; we report the *scantwo* results.

For significant epistatic interactions, we formally tested the hypothesis that QTL × QTL interactions differ across environments. We used ANOVA (PROC GLM), and for each significant epistatic interaction, we included markers closest to epistatic QTL positions, the two-way marker × marker interaction, and the three-way marker × marker × environment interaction (*i.e.*, the QTL × QTL × *E* interaction).

Contribution of main-effect QTL to genetic correlations: To compare the genetic architecture estimated by the **G**-matrix to architecture estimated from QTL mapping, we calculated the bivariate genetic correlation (r_Q) attributable to QTL affecting trait pairs (Falconer and Mackay 1996). We follow the calculation of r_Q described in Gardner and Latta (2007) (see also Kelly 2009) as

$$r_{Q(Z_1,Z_2)} = \frac{\sum_{i=1}^{n} 2p_i q_i a_{i(Z_1)} a_{i(Z_2)}}{\sqrt{\sum_{i=1}^{n} 2p_i q_i a_{i(Z_1)}^2 \cdot \sum_{i=1}^{n} 2p_i q_i a_{i(Z_2)}^2}},$$

where p and q are the frequencies of alleles at a QTL locus (i), $a_{i(Z_1)}$ and $a_{i(Z_2)}$ are the additive effects of QTL locus i on the two traits (Z_1 and Z_2), and these estimates of covariance (numerator) and trait variances (denominator) are summed across all QTL that affect one (or both) of the traits in the bivariate pair. Our inclusion of QTL detected for only one of the two traits allows for incorporation of possible "minor" QTL that were not formally significant at the genome-wide threshold. This estimate of genetic correlation assumes no dominance (an assumption met in RILs where heterozygotes are not present), no linkage disequilibrium between QTL, and no epistasis. We estimated r_Q for all trait pairs with significant genetic correlations. We calculated the correlation between r_G and r_Q in each environment (PROC CORR) to evaluate the extent to which mapped QTL account for genotypic correlations.

RESULTS

Quantitative genetics: We detected significant genetic variation in this population of RILs for all measured traits under both field and greenhouse conditions (Table 1; for full ANOVA results see Table S2 and Table S3). Similarly, we detected significant line × environment interactions for all measured traits (Table 1; for full ANOVA results, see Table S4), indicating genetic variation for plasticity of floral and nonfloral traits. In general, RILs growing in the field produced smaller floral and vegetative organs and flowered later in comparison to those raised in the greenhouse (Figure 1). For all traits, we detected evidence of transgressive segregation; that is, the range of trait expression among RILs exceeded that of the parental lines (*e.g.*, hypocotyl length, Figure 1). Broad-sense heritability was lower when estimated from RILs growing in the field (floral mean 0.30, range 0.23–0.41; nonfloral mean 0.20, range 0.07–0.35; Table 1) than from RILs growing under greenhouse conditions (floral mean 0.43, range 0.36–0.55; nonfloral mean 0.49, range 0.36–0.62; Table 1).

CPC comparisons of correlation matrices composed of floral traits were marginally significant for the fourth principal component [CPC4, $\chi^2 = 22.8$, d.f. = 14, $P \leq 0.0638$; *i.e.*, matrices share the first three principal components (PCs) but differ at the fourth of six PCs]. The first three PCs that are shared between field and greenhouse environments explain 80% of the variation among the traits. Thus, the correlation matrices differ across field and greenhouse environments, but only once significant variation is explained. Genetic correlation matrices of floral–vegetative traits differed across environments (CPC5, $\chi^2 = 44.4$, d.f. = 27, $P = 0.0188$; *i.e.*, matrices share four of eight PCs), and a large number of bivariate correlations differed across environments (see Fisher's *Z*-tests below). Finally, the floral–flowering time correlation matrices differed significantly at the second principal component across environments (CPC2, $\chi^2 = 19.6$, d.f. = 11, $P \leq 0.0506$; *i.e.*, matrices share only one of seven PCs), and nearly half of the bivariate correlations differed across environments.

We detected strong positive genetic correlations among floral traits within the field and greenhouse environments (Table 2). Floral–floral genetic correlations were largely similar across environments with only 3 significant differences (of 21 possible) in the floral correlation matrix (Table 2). Similarly, the vegetative traits, leaf length and primary inflorescence height, were positively correlated in this RIL population and did not significantly differ across environments. Interestingly, anther length was positively correlated with two vegetative traits (leaf length and primary inflorescence height) when grown in the field, as were, to a lesser degree, midpoint and style–stigma length (Table 2, top right, italics). Contributing to matrix differences detected in CPC analyses, significant floral–vegetative correlations were more numerous and stronger for plants growing in the greenhouse (Table 2, bottom left, italics), including all floral traits except petal width. Fisher's *Z*-tests indicate that 9 of 14 possible pairwise correlations between floral traits and either leaf length or inflorescence height differed significantly across environments. Also consistent with CPC results, correlations between floral traits and flowering phenology were nonsignificant in the field, but with the exception of petal width, all floral traits were significantly positively

TABLE 1

ANOVA results partitioning variation in Box–Cox-transformed phenotypic traits among lines (*i.e.*, RILs; for full ANOVA see Table S2 and Table S3) and associated quantitative genetic parameters of variance components (V_G and V_P) and broad sense heritability (H^2) for *B. rapa* RILs growing in field and greenhouse environments

	Field environment				Greenhouse environment					
Trait	Line significance	V_G	V_P	H^2	Line significance	V_G	V_P	H^2	Line × environment	Across-environment correlation
Hypocotyl Ln	***	0.06	0.71	0.09	*****	27.88	59.95	0.47	*****	0.42*****
Leaf Ln	*****	0.53	2.16	0.25	*****	0.0045	0.012	0.39	***	0.62*****
Leaf Wd	NA	NA	NA	NA	*****	0.0049	0.014	0.36	NA	NA
Branch Ln	*****	4.31	18.27	0.24	NA	NA	NA	NA	NA	NA
Primary inflorescence height	*****	5.49	15.79	0.35	*****	271.68	462.79	0.59	****	0.59*****
Days to flower	**	2.37	31.80	0.07	*****	13.04	21.01	0.62	*****	0.17*
Petal Ln	*****	0.29	0.91	0.32	*****	0.0092	0.025	0.38	****	0.48*****
Midpoint Ln	*****	0.040	0.16	0.25	*****	0.022	0.061	0.36	****	0.42*****
Petal Wd	*****	0.0024	0.0073	0.33	*****	0.016	0.029	0.55	***	0.65*****
Filament Ln	*****	0.68	2.34	0.29	*****	0.37	1.0	0.37	*****	0.39*****
Anther Ln	*****	0.039	0.096	0.41	*****	0.48	0.90	0.53	*****	0.56*****
Ovary Ln	*****	0.0083	0.029	0.29	*****	0.59	1.38	0.43	*****	0.38*****
Style–stigma Ln	*****	0.030	0.13	0.23	*****	0.0033	0.0079	0.42	****	0.43*****

From an additional ANOVA, the line × environment interaction (for full ANOVA see Table S4) tests for evidence of genetic variation in trait plasticity across environments (minimum number of RILs common to both environments; $N = 112$). *$P = 0.0779$; **$P = 0.0548$; ***$P < 0.01$; ****$P < 0.001$; *****$P < 0.0001$. Ln, length; Wd, width (throughout tables).

correlated with phenology in the greenhouse. Fisher's *Z*-tests indicate that 3 of 7 possible bivariate correlations between floral traits and phenology were significant (including midpoint and anther length as well as petal width due to the reversal in sign across environments).

QTL mapping: We list all QTL identified for floral, vegetative, and phenology traits in Tables 3 and 4 for field and greenhouse environments, respectively (Figure 2; see Table S5 for markers closest to QTL in Tables 3 and 4). We detected a total of 27 main-effect QTL in the field environment; 8 QTL influenced multiple traits, of which 3 QTL influenced floral and vegetative traits (FQTL 3-2, which affected inflorescence and anther lengths; FQTL 6-1, which affected branch length, filament length, petal width, and petal length; and FQTL 6-2, which affected inflorescence and anther lengths). In the greenhouse, we detected 29 total main-effect QTL; 8 QTL affected multiple phenotypic traits, of which 1 QTL (GQTL 6-3) coregulated floral and nonfloral traits (inflorescence length, leaf width and length, and anther length).

In total for each trait, QTL in the field account for between 20.1 and 37.5% (style–stigma length and anther length, respectively; floral mean 29.0%) of the total genetic variation in floral traits and between 8.8 and 27.0% (days to flower and hypocotyl length, respectively; nonfloral mean 21.2%) of the variation in nonfloral traits. For RILs growing in the greenhouse, QTL together account for between 12.4 and 52.2% (style–stigma length and midpoint length, respectively; floral mean 29.2%) of the total genetic variation in floral traits and between 27.6 and 45.9% (leaf width and primary inflorescence height, respectively; nonfloral mean 34.5%) of the variation in nonfloral traits.

The effect of 21 QTL regulating floral and nonfloral traits varied across the field and greenhouse environments (QTL × *E* interaction; Tables 3 and 4). In 17 instances, QTL affected traits in only one environment (4 and 13 environment-specific QTL from the field and the greenhouse, respectively). For example, FQTL 3-1 and FQTL 8-1 regulated ovary length only in the field. In four cases, a QTL regulating the same trait [*e.g.*, petal width QTL (FQTL 3-5 and GQTL 3-5)] was detected in both the field and the greenhouse environments; however, the degree of influence of the QTL differed across environments. Interestingly, slightly different loci contribute to positive floral–vegetative genotypic correlations observed in both environments. For instance, GQTL 6-3 shows a significant QTL × *E* interaction for leaf length, indicating that this QTL contributes to the observed correlation with anther length only in the greenhouse environment.

In genome-wide scans, we detected significant QTL × QTL epistasis in the field and the greenhouse (Table 5; see Table S6 for markers closest to QTL). In the field, six epistatic interactions influenced four traits (one floral and three vegetative), individually accounting for between 9.5 and 12.5% of the variation for these traits. For RILs grown in the greenhouse, we detected eight epistatic interactions for seven measured traits (five floral and two vegetative); QTL × QTL interactions

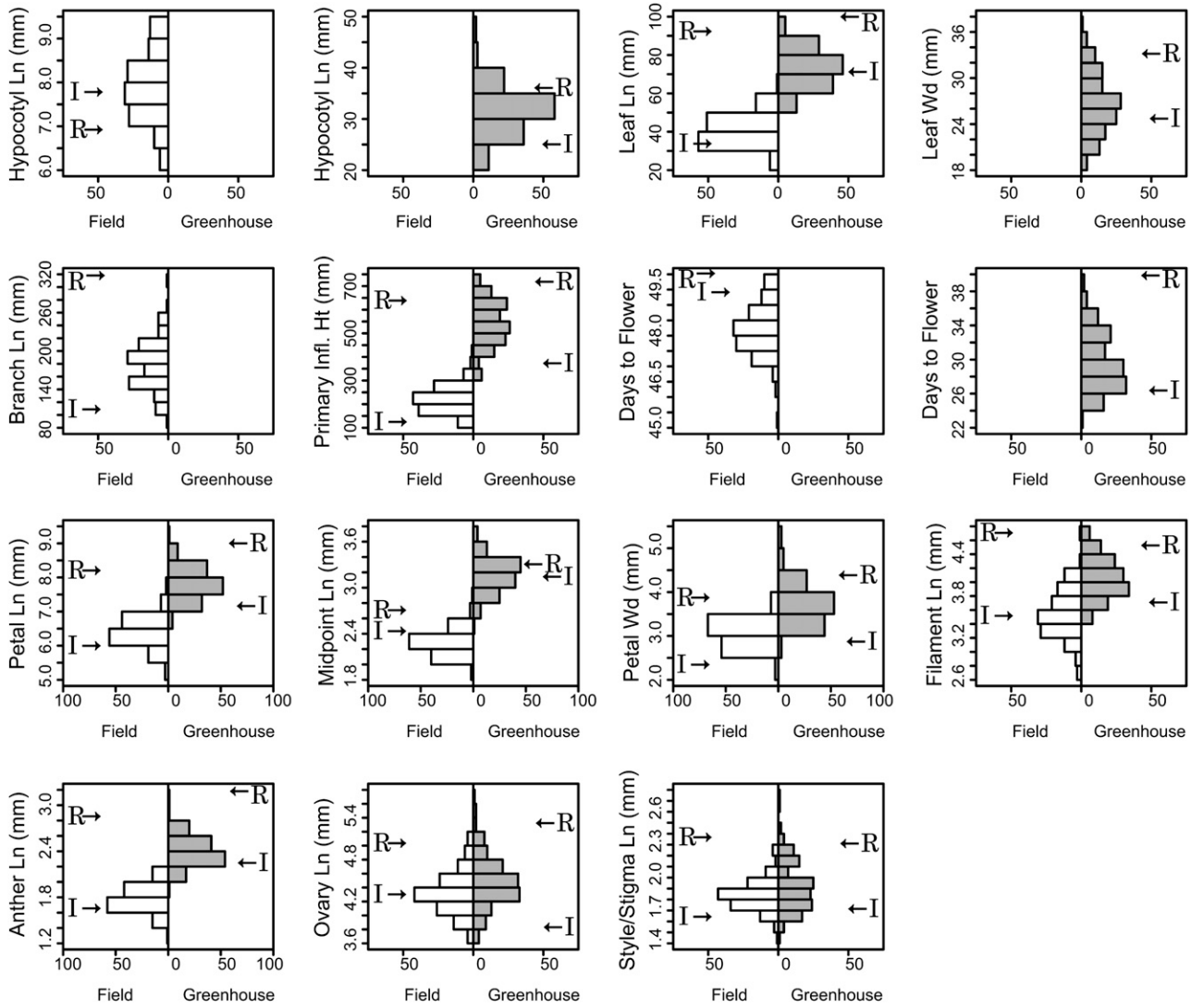

FIGURE 1.—Back-to-back histograms of genotypic trait values of *B. rapa* RILs when grown in the field (left side, open bars) and greenhouse (right side, shaded bars) environments. Parental trait means are designated by arrows (I, IMB211; R, R500). A few plots have data for only one side, because some trait distributions could not be illustrated on the same axis or because a few traits were measured in only one environment.

individually account for between 10.5 and 13.9% of the trait variation. Interestingly, the epistatic interactions between markers on chromosome 2 and chromosome 10 influenced four floral traits (petal, midpoint, filament, and ovary lengths), suggesting that in the greenhouse these traits are partially regulated by pleiotropic epistatic interactions (Figure S1). Three of these pleiotropic epistatic interactions differed significantly across environments (QTL × QTL × *E* interaction; Table 5). We also detected significant QTL × QTL × *E* interactions for primary inflorescence height and anther length, indicating that epistatic interactions influenced these traits in only the field and the greenhouse, respectively.

Contribution of main-effect QTL to genetic correlations: Genetic correlations based on identified QTL (r_Q; Table 6) were strongly positively correlated with those estimated from trait genotypic means (Table 2; field, $r = 0.81$, $P < 0.0001$; greenhouse, $r = 0.78$, $P < 0.0001$); that is, mapped QTL largely account for bivariate genotypic correlations. Despite the strong association between r_Q and r_G for many trait pairs, some r_Q under- or overestimate r_G, presumably because some QTL of small effect were not identified or because epistasis and LD are not accounted for in the calculation of r_Q.

Floral morphology in cultivated and wild B. rapa accessions: We detected significant genetic variation for all floral traits measured in 13 accessions of *B. rapa* (all *P*-values ≤ 0.05; see Table S7 for ANOVA). Floral trait means estimated among the accessions [petal length, 8.8 ± 0.3 mm (BLUP ± SE); midpoint length, 3.1 ± 0.1 mm; petal width, 4.5 ± 0.2 mm; filament length, 5.3 ± 0.1 mm; anther length, 2.6 ± 0.1 mm; ovary length, 5.0 ± 0.1 mm; and style–stigma length, 2.3 ± 0.1 mm]

TABLE 2

Genotypic correlations of floral, vegetative, and phenological traits for *B. rapa* RILs raised in the field (above diagonal) and greenhouse (below diagonal) environments

r_G	Hy L	Lf L	Br L	PI H	DTF	Pet L	Mid L	Pet W	Fil L	An L	Ov L	Sty L
Hypocotyl Ln (Hy L)		−0.08	0.02	0.04	−0.14	*0.02*	*0.02*	*0.00*	*−0.08*	***0.01***	*−0.01*	*−0.20**
Leaf Ln (Lf L)	0.17		0.54**	0.64**	**−0.23***	***0.14***	***0.26****	*−0.06*	*0.05*	***0.29*****	***0.04***	***0.11***
Branch Ln (Br L)	NA	NA		0.77**	−0.41**	*0.20**	*0.21**	*0.18**	*0.07*	*0.40***	*0.14*	*0.28**
Primary inflorescence height (PI H)	0.15	0.61**	NA		**−0.38****	***0.11***	***0.21****	*−0.01*	***0.04***	*0.41***	***0.07***	*0.18**
Days to flower (DTF)	−0.33**	**0.35****	NA	**0.52****		*0.13*	***0.08***	***0.11***	*0.14*	***−0.16***	*0.04*	*0.16*
Petal Ln (Pet L)	*0.08*	***0.40*****	*NA*	***0.46*****	*0.35***		0.75**	**0.67****	0.75**	0.55**	0.62**	0.38**
Midpoint Ln (Mid L)	*0.09*	***0.47*****	*NA*	***0.46*****	***0.39*****	0.79**		**0.34****	0.64**	0.51**	0.54**	0.31*
Petal Wd (Pet W)	*0.02*	*0.14*	*NA*	*0.09*	***−0.15***	**0.41****	**0.05**		0.41**	0.31**	0.37**	0.22*
Filament Ln (Fil L)	*0.03*	*0.21**	*NA*	***0.29*****	*0.26**	0.75**	0.74**	0.20*		0.55**	0.49**	0.23*
Anther Ln (An L)	***0.27****	***0.5*****	*NA*	*0.57***	***0.23****	0.67**	0.50**	0.33**	0.39**		**0.39****	0.35**
Ovary Ln (Ov L)	*0.17*	***0.47*****	*NA*	***0.48*****	*0.22**	0.73**	0.60**	0.24*	0.60**	**0.58****		0.26*
Style–stigma Ln (Sty L)	*0.03*	***0.36*****	*NA*	*0.34***	*0.38***	0.53**	0.46**	0.20*	0.42**	0.41**	0.35**	

Bivariate correlation significance is indicated with superscripts and significant differences in bivariate correlations across environments (Fisher's *Z*-tests) are indicated in boldface type. Italics designate floral–nonfloral correlations; $*P < 0.05$; $**P < 0.001$. Boldface type indicates Fisher's *Z*-test significant at $P < 0.05$.

were 37% (range 16–50%) larger on average than those of RILs. Despite the small sample size ($N = 13$), we detected significant positive genetic correlations among all petal traits (range 0.68–0.89), between filament length and both petal and midpoint length (0.80 and 0.83, respectively), and between style–stigma length and each of petal, midpoint, and filament lengths (range 0.64–0.85; see Table S8 for all correlation coefficients), and many correlations were of similar or greater magnitude relative to the RILs.

DISCUSSION

Plant reproductive success is contingent upon floral morphology. Floral organ size and allometry are targeted by selection due effects on, for instance, pollinator attraction (Conner and Rush 1996), physiological function (Galen 2000; Herrera 2005), and response to herbivore damage (Strauss 1997). For example, in this *B. rapa* RIL population, stigma–anther separation (herkogamy) explained significant variation in outcrossing rate (M. T. Brock and C. Weinig, unpublished results), which can in turn affect the quantity or quality of offspring (Charlesworth and Charlesworth 1987). Yet, despite the selective importance of intraspecific floral variation, the genetic dissection of floral traits has been largely explored through interspecific comparisons (Bradshaw *et al.* 1995; Fishman *et al.* 2002; Goodwillie *et al.* 2006; Bouck *et al.* 2007; Moyle 2007; but see Juenger *et al.* 2000, 2005; Hall *et al.* 2006). Using *B. rapa* RILs, we detect significant genetic variation and additive and/or epistatic QTL for floral and nonfloral traits of RILs raised under field and greenhouse conditions. Within environments, floral traits exhibited strong genotypic correlations indicative of phenotypic integration, and mapped QTL correspondingly regulated multiple floral traits. Floral genetic architecture, as estimated by genetic correlations, showed only modest differences across these distinct environments. Surprisingly and in contrast to prior studies (Conner and Via 1993; Conner and Sterling 1996; Juenger *et al.* 2005), floral traits showed a moderate to high level of phenotypic integration with vegetative and phenological traits in the more favorable greenhouse environment; genotypic correlations between these functional trait groups were less frequent and of lesser magnitude in the field.

Quantitative genetics of floral morphology: Genetic variances can fluctuate across environments (Hoffmann and Merila 1999; Charmantier and Garant 2005), with a common expectation that estimates of genetic parameters are greater in benign environments (Conner *et al.* 2003). We detected substantial genetic variation for all measured floral traits in both the greenhouse and the field environments (Table 1), but average estimates of genetic variances and broad-sense heritabilities were in fact greater in the greenhouse. Our estimates of floral broad-sense heritabilities in the RILs are commensurate with reported values in other mapping studies (*e.g.*, Hall *et al.* 2006) as well as average heritabilities of floral traits from natural populations (reviewed in Ashman and Majetic 2006). Genetic variances in mapping populations may be lower than those in natural populations (in part, due to the fact that only two alternative alleles segregate in such experimental populations) or greater than those in natural populations (due to independent assortment and the potential that some RILs harbor alleles of predominantly positive/negative effect at contributing QTL). Nevertheless, our survey of floral morphology in publicly available *B. rapa* accessions (Table S7) supports the hypothesis of standing genetic variation for floral traits in cultivated

TABLE 3

QTL identified for floral, vegetative, and phenological traits for *B. rapa* RILs grown under field conditions

QTL	Trait	Chr	cM	LOD	2-LOD range (cM)	PVE	a_0	QTL × *E*
FQTL1-1	Anther Ln	1	20.2	4	(15.2, 28.3)	8.4	−0.049	NS
	Petal Wd	1	21.7	2.9	(16.5, 26.3)	6.3	−0.091	NS
FQTL1-2	Days To flower	1	32.8	3.2	(26.3, 46.3)	8.8	0.24	NS
FQTL2-1	Leaf Ln	2	2.0*	2.7	(0.0, 4.0)	6.2	−1.87	NS
FQTL2-2	Leaf Ln	2	52.9	2.9	(47.4, 66.4)	7	−2.04	NS
FQTL3-1	Ovary Ln	3	19.2	3.5	(0, 25.5)	6.9	0.08	0.0039
FQTL3-2	Primary inflorescence height	3	33.3	4.4	(25.5, 37.8)	13.1	−21.26	0.0398
	Anther Ln	3	33.3	6.9	(27.1, 39.8)	9	−0.052	NS
FQTL3-3	Anther Ln	3	77.1	4.8	(72.6, 79.4)	9.7	−0.056	NS
	Petal Ln	3	77.1	3.7	(72.6, 85.0)	7.6	−0.13	NS
	Filament Ln	3	79.1	2.8	(66.9, 85.0)	5.5	−0.088	NS
FQTL3-4[a]	Midpoint Ln	3	86.5	2.9	(79.1, 93.7)	5.5	−0.038	NS
FQTL3-5[a]	Petal Wd	3	91	3.1	(86.5, 101.7)	6.7	0.074	0.0083
FQTL5-1	Style–stigma Ln	5	0	4.0	(0.0, 12.0)	7.5	−0.04	NS
FQTL5-2	Petal Wd	5	21.4	3.4	(14.0, 28.4)	7.4	−0.076	NS
FQTL6-1	Filament Ln	6	22.8	6.0	(17.1, 31.6)	5.7	−0.087	0.0571
	Branch Ln	6	22.8	2.9	(17.1, 27.6)	13.1	−14.78	—
	Petal Wd	6	24.3	3.3	(15.4, 32.1)	7.4	−0.077	0.0196
	Petal Ln	6	24.3	3.3	(15.4, 32.1)	7	−0.12	NS
FQTL6-2	Primary inflorescence height	6	44.8	3.9	(39.5, 61.0)	6.7	−15.06	NS
	Anther Ln	6	46.8	5.1	(40.6, 69.0)	10.4	−0.058	NS
FQTL7-1	Branch Ln	7	0	4.3	(0.0, 10.0)	8.7	−13.62	—
FQTL7-2[a]	Ovary Ln	7	23.5	3.8	(15.4, 29.2)	7.5	0.085	NS
FQTL7-3[a]	Filament Ln	7	29.2	3.0	(23.5, 37.8)	6	0.087	0.0624
	Midpoint Ln	7	29.2	4.3	(17.7, 37.8)	8.5	0.047	NS
FQTL7-4[a]	Petal Ln	7	39.8	3.9	(37.2, 43.5)	9.1	0.17	NS
FQTL7-5	Hypocotyl Ln	7	58.5†	2.6	(51.8, 59.3)	7.3	−0.25	NS
FQTL8-1	Ovary Ln	8	10.4	4.6	(0.4, 26.8)	11.9	0.11	0.0248
FQTL8-2	Style–stigma Ln	8	34.5	2.8	(28.0, 34.5)	5.6	−0.033	0.0495
FQTL9-1[a]	Petal Ln	9	7.6	4.2	(0.0, 12.5)	9.7	−0.14	NS
FQTL9-2[a]	Midpoint Ln	9	18.5	3.1	(11.6, 23.1)	6	−0.039	NS
FQTL9-3[a]	Primary inflorescence heightt	9	22.7	3.7	(14.5, 28.6)	6.7	−15.72	NS
FQTL9-4	Hypocotyl Ln	9	37.3	3.8	(31.5, 44.8)	10.1	0.26	NS
	Leaf Ln	9	38.7	3.8	(35.3, 44.8)	8.5	−3.03	NS
FQTL9-5	Ovary Ln	9	61.8	3.1	(52.3, 66.4)	5.9	−0.083	NS
	Midpoint Ln	9	61.8	2.8	(52.3, 66.4)	6.1	−0.039	NS
	Filament Ln	9	63.3	4.1	(52.3, 66.4)	8.5	−0.1	NS
FQTL10-1	Hypocotyl Ln	10	0	3.6	(0.0, 11.2)	9.6	0.25	NS
FQTL10-2	Style–stigma Ln	10	29.4	3.2	(21.9, 34.7)	7	−0.039	NS

Traits influenced by each QTL are listed along with chromosome, position, LOD score, 2-LOD support limits, percentage of variance explained by each QTL, additive effect (positive values indicate that IMB211 alleles increase trait means), and *P*-values from ANOVA that tested if QTL differ in their effect on a trait across environments. *Genome-wide significance threshold $P < 0.075$.

[a] QTL assignment based on 1-LOD confidence intervals.

and wild populations. Taken together, these results suggest that floral morphology can respond to natural (or artificial) selection across a broad range of environmental conditions.

Genetic correlations among all measured floral traits were strongly positive in both greenhouse and field environments (Table 2), consistent with prior studies in both natural and controlled environments (reviewed in Ashman and Majetic 2006). This strong floral phenotypic integration is the result of either pleiotropic loci that regulate the development of multiple floral whorls [*e.g.*, the ABC model of floral development (Coen and Meyerowitz 1991)] or linkage disequilibrium between multiple causal loci. In both environments, the strongest interwhorl correlations were observed between petal length (or midpoint length) and filament length in the RILs. Despite the small sample, we also detect genotypic correlations of similar or greater magnitude between petal and filament lengths in wild and cultivated *B. rapa* accessions relative to the RILs (Table S8). This correlation influences the degree to which anthers protrude from the corolla opening (*i.e.*, anther exsertion), a trait that contributes to efficient pollen dispersal (Morgan and Conner 2001). Conner *et al.* (2009)

TABLE 4

QTL identified for floral, vegetative, and phenological traits for *B. rapa* RILs grown in the greenhouse

QTL	Trait	Chr	cM	LOD	2-LOD range (cM)	PVE	a_0	QTL × *E*
GQTL1-1[a]	Petal Wd	1	13.2	4.1	(8.3, 18.4)	7.2	−0.13	NS
GQTL1-2[a]	Anther Ln	1	18.4	4.0	(13.2, 26.3)	8.5	−0.056	NS
GQTL1-3	Primary inflorescence height	1	30.3	6.1	(24.9, 36.3)	10.7	−33.7	0.0484
	Leaf Wd	1	32.1*	2.8	(24.9, 48.7)	5.4	−0.97	—
GQTL2-1	Anther Ln	2	26.4	3.6	(19.2, 35.6)	8.3	−0.056	NS
	Midpoint Ln	2	27.5	3.3	(20.0, 35.6)	6.1	−0.058	0.021
GQTL2-2[a]	Style–stigma Ln	2	43.8	3.6	(36.9, 55.1)	6.9	−0.066	0.0128
GQTL2-3[a]	Leaf Wd	2	55.4	4.1	(42.0, 65.1)	7	−1.1	—
GQTL2-4	Hypocotyl Ln	2	76.7*	2.7	(63.1, 87.3)	6.7	1.3	0.0164
GQTL3-1	Petal Ln	3	19.2	2.9	(6.0, 25.5)	7.3	−0.13	0.0398
	Anther Ln	3	23.2	4.8	(8.0, 35.8)	10.2	−0.063	NS
GQTL3-2	Primary inflorescence height	3	40	4.0	(33.8, 47.6)	6.5	−28.11	0.0398
GQTL3-3	Anther Ln	3	66.1	3.2	(55.2, 72.6)	8.3	−0.057	NS
GQTL3-4[a]	Petal Ln	3	79.4	2.8	(74.0, 105.7)	6.3	−0.13	NS
GQTL3-5[a]	Petal Wd	3	91	3.9	(86.5, 103.7)	14	0.18	0.0083
	Midpoint Ln	3	91	7.4	(83.4, 97.7)	7.3	−0.065	NS
GQTL4-1	Midpoint Ln	4	31.7	3.4	(24.3, 36.5)	6.7	−0.061	NS
GQTL4-2	Hypocotyl Ln	4	57.4	2.8	(47.3, 65.7)	7.9	−1.4	0.0222
GQTL6-1[a]	Petal Wd	6	22.4	3.1	(15.4, 32.1)	5.3	−0.11	0.0196
GQTL6-2[a]	Anther Ln	6	36.1	3.2	(31.6, 43.0)	8.2	−0.056	NS
	Ovary Ln	6	37	3.4	(31.6, 42.3)	7.2	−0.1	NS
GQTL6-3[a]	Anther Ln	6	40.3	4.1	(39.6, 43.0)	10	−0.063	NS
	Leaf Wd	6	42.3	7.1	(37.5, 47.0)	15.1	−1.6	—
	Primary inflorescence height	6	42.3	6.2	(39.5, 57.0)	10.3	−33.87	NS
	Leaf Ln	6	46.8	11.5	(40.3, 55.0)	25.4	−5.4	0.0088
GQTL7-1	Petal Wd	7	15.4	3.2	(6.0, 23.0)	5.6	−0.11	0.0125
GQTL7-2	Midpoint Ln	7	41.5	5.3	(29.2, 44.9)	9.9	0.081	NS
GQTL7-3	Midpoint Ln	7	58.5	4.8	(51.8, 59.3)	9.7	−0.08	NS
GQTL8-1	Petal Wd	8	10.4*	2.8	(0, 18.8)	5.5	0.11	NS
GQTL8-2	Style–stigma Ln	8	30.5	2.9	(18.8, 34.5)	5.5	−0.056	0.0495
GQTL9-1	Days to flower	9	2.0*	2.6	(0, 5.6)	5	−0.77	0.0034
GQTL9-2[a]	Primary inflorescence height	9	22	6.2	(12.5, 27.1)	10.4	−34.42	NS
GQTL9-3[a]	Petal Wd	9	33.3	3.1	(23.1, 38.0)	5.5	−0.11	NS
GQTL9-4[a]	Midpoint Ln	9	37.3	3.8	(35.3, 44.7)	7.2	−0.094	NS
GQTL9-5[a]	Ovary Ln	9	46	4.2	(38.7, 53.8)	9	−0.12	NS
	Filament Ln	9	52.3	5.5	(38.7, 66.4)	13.2	−0.11	NS
GQTL10-1	Midpoint Ln	10	2.0*	2.7	(0.0, 11.2)	5.2	−0.055	0.0282
GQTL10-2	Days to flower	10	25.4	11.7	(17.9, 29.4)	26.1	−1.77	<0.0001
	Primary inflorescence height	10	29.4	2.9	(27.1, 35.8)	8.07	−29.83	0.0003
	Leaf Ln	10	29.4	4.8	(13.9, 29.7)	8.1	−4.3	NS
	Hypocotyl Ln	10	30.4	8.9	(17.9, 34.7)	19.8	2.3	0.0003

Traits influenced by each QTL are listed along with chromosome, position, LOD score, 2-LOD support limits, percentage of variance explained by each QTL, additive effect (positive values indicate that IMB211 alleles increase trait means), and *P*-values from ANOVA that tested if QTL differ in their effect on a trait across environments. *Genome-wide significance threshold $P < 0.075$.

[a] QTL assignment based on 1-LOD confidence intervals.

argue that correlational selection for the joint expression of petal and filament length in *Raphanus raphanistrum* (and other related Brassicaceous species) produces significantly stronger correlations between petal and filament lengths than between other pairs of floral traits. While the occurrence of this petal–filament correlation in the accessions is consistent with the hypothesis of past selection and enhanced LD, the similar patterning of floral trait correlations in our *B. rapa* RIL population, in which LD arising from selection is at least somewhat disrupted, suggests that significant petal–filament correlations may also arise from floral developmental genetics in this species (*i.e.*, from pleiotropic effects of floral regulatory loci). The strong phenotypic integration of floral organ dimensions should result in consistent expression of floral allometry across environments, but these genetic correlations could also constrain the evolution of novel floral shapes and sizes if trait correlations are not parallel to the joint vector of selection (Smith and Rausher 2008).

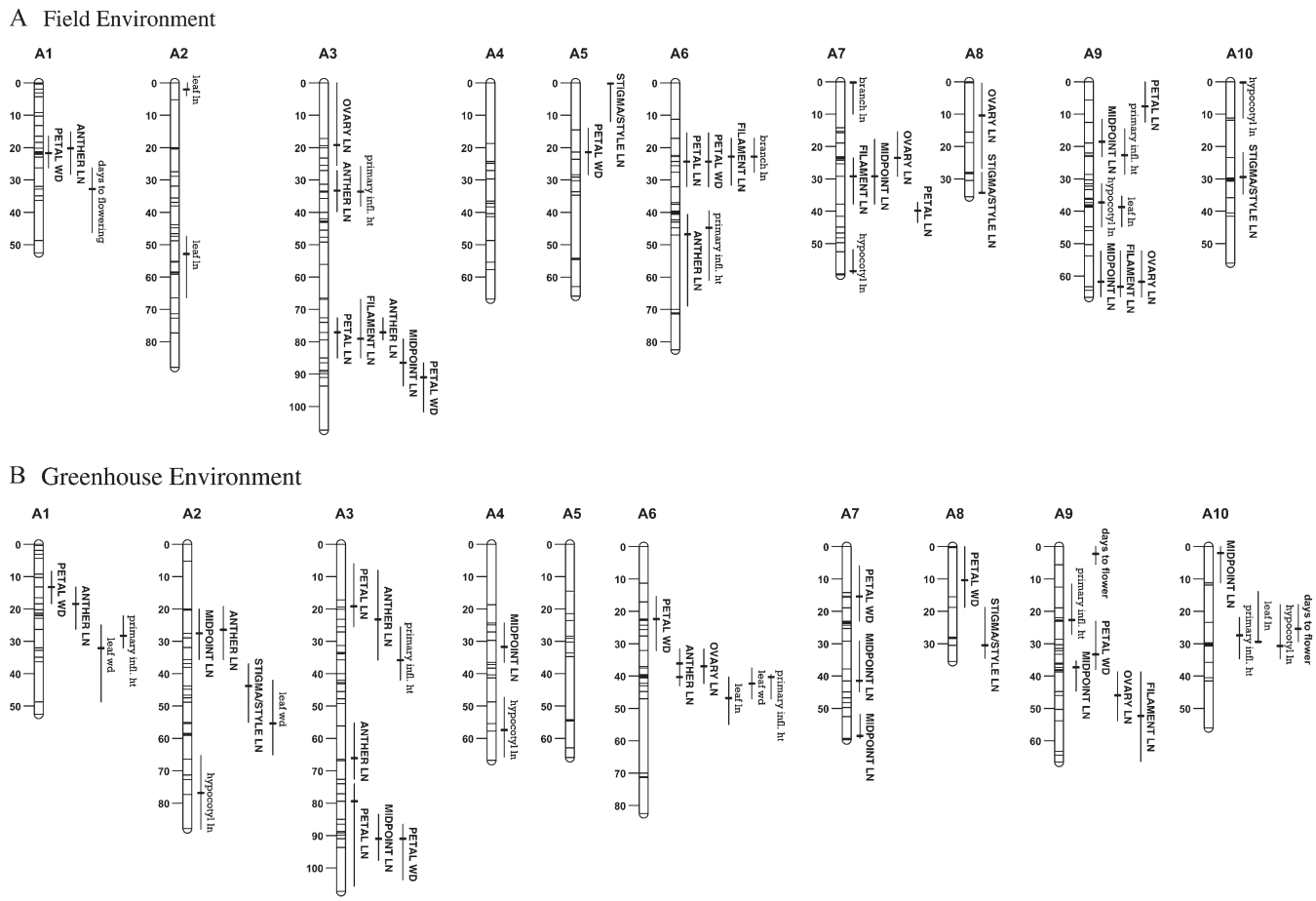

Figure 2.—Genomic locations of QTL identified for floral, vegetative, and phenological traits of *B. rapa* RILs raised in (A) field and (B) greenhouse environments. Chromosome number (A1–A10, international nomenclature) is listed at the top of each linkage group and position (in centimorgans) is detailed on the left. Phenotypic traits are listed next to estimated QTL position and 2-LOD support limits. Floral traits are in uppercase and nonfloral traits are in lowercase.

The genetic architecture of floral traits, as measured by the correlation matrix, was subtly but significantly different across environments. Multivariate CPC analysis detected differences between correlation matrices, but at least 80% of the variance in floral correlations did not differ across environments. The similarity of individual **G**-matrix elements (*i.e.*, only 2 of 15 correlations in the floral–matrix CPC comparison differed significantly across environments) is consistent with the multivariate view of environmentally invariant floral correlations. A growing number of studies have explored **G**-matrix constancy across a variety of environmental and taxonomic categories; however, **G**-matrix behavior, including that of across-environment floral morphological matrices (Conner *et al.* 2003; Brock and Weinig 2007; Brock *et al.* 2009), can be variable (Steppan *et al.* 2002; Björklund 2004). Similar to the findings presented here, Conner *et al.* (2003) detected differences in higher-level principal component axes of unstandardized floral **G**-matrices of *R. raphanistrum* raised in field and greenhouse environments. We should note that our initial CPC analyses of unstandardized **G**-matrices revealed fundamental differences across field and greenhouse environments for this population of *B. rapa* RILs (*i.e.*, differences in the first principal component axis). However, we chose to standardize these matrices due to the dramatic difference in trait variances across environments (see Figure 1). As a result, our matrix comparisons suggest that the structures of genetic correlations are relatively similar across environments, but that differences in trait variances across environments could alter the magnitude of floral morphological responses to selection.

QTL architecture of floral morphology: We detected numerous main-effect floral QTL in both field and greenhouse environments (Tables 3 and 4). On average, floral QTL segregating in this population are of moderate to small effect size [average percent variance explained (PVE) in the field and the greenhouse, 7.5 and 7.9, respectively]. Further, additive-effect QTL detected for individual floral traits explain on average only 29% of the genetic variation in each environment (although significant epistatic-effect QTL in these selfed RILs also explain on average 15% of floral trait variation). Our results suggest that floral morphological variation is controlled by many genes of small to

TABLE 5

Epistatic interactions influencing floral and vegetative traits detected in A) a common garden field experiment and B) a greenhouse experiment using *B. rapa* RILs

Trait	Marker A Chr	Marker A Position (cM)	Marker B Chr	Marker B Position (cM)	PVE (%)	$4i$	$\bar{x}_{00}$	$\bar{x}_{02}$	$\bar{x}_{20}$	$\bar{x}_{22}$	QTL × QTL × E
						Field					
Leaf Ln	5	34	7	8	12.5	10.8	53.6 (±1.4)	49.4 (±1.8)	47.7 (±1.4)	54.2 (±1.5)	NS
Branch Ln	2	20	5	8	9.5	−14.5	213.0 (±8.1)	166.6 (±8.1)	167.8 (±6.4)	173.1 (±5.3)	—
Branch Ln	2	36	8	28	11.1	−54.2	163.8 (±8.7)	198.6 (±6.7)	185.1 (±6.4)	165.7 (±5.7)	—
Branch Ln	3	8*	6	72*	10.5	11.8	187.0 (±6.5)	166.9 (±6.2)	167.4 (±7.5)	201.3 (±9.0)	—
Primary inflorescence height	3	4	6	72	12.4	83.5	237.1 (±9.1)	204.6 (±8.8)	201.7 (±10.6)	252.6 (±12.7)	0.0009
Style–stigma Ln	4	48*	9	66*	12.1	−0.2	1.8 (±0.03)	1.9 (±0.02)	1.9 (±0.02)	1.8 (±0.02)	NS
						Greenhouse					
Leaf Ln	4	4	8	34	10.9	13.7	74.8 (±1.5)	70.1 (±1.8)	68.0 (±2.0)	77.1 (±2.0)	NS
Leaf Wd	3	34	9	56	12.6	5.9	29.4 (±0.7)	25.6 (±0.7)	24.8 (±0.8)	26.9 (±0.6)	—
Petal Ln	2	78	10	46	13.7	−0.7	7.6 (±0.08)	7.9 (±0.08)	8.1 (±0.10)	7.7 (±0.09)	0.0508
Petal Ln	4	56	5	50	11.4	0.6	8.0 (±0.08)	7.8 (±0.08)	7.6 (±0.10)	8.0 (±0.07)	NS
Midpoint Ln	2	76	10	42	13.6	−0.4	3.1 (±0.04)	3.2 (±0.04)	3.3 (±0.05)	3.1 (±0.04)	0.0108
Filament Ln	2	78	10	48	10.5	−0.4	4.0 (±0.05)	4.0 (±0.05)	4.3 (±0.06)	3.9 (±0.05)	NS
Anther Ln	3	86	4	64	15.0	−0.3	2.4 (±0.03)	2.6 (±0.04)	2.4 (±0.03)	2.3 (±0.03)	0.0437
Ovary Ln	2	78	10	42	13.9	−0.6	4.3 (±0.06)	4.6 (±0.06)	4.7 (±0.08)	4.4 (±0.07)	0.0014

Chromosome and positions of interacting QTL are listed along with the percentage of variance explained by the interaction, the epistatic effect ($4i$), and traits means (±SE) of each combination of IMB211 alleles (designated as 2) and R500 alleles (designated as 0). The final column tests the hypothesis that epistatic interactions differ in their effect on traits across environments (*i.e.,* QTL × QTL × E). *P-value <0.07.

moderate additive effect, a pattern observed in both environments. Many of the mapped QTL are likely floral-specific regulators of organ size, as opposed to QTL that simply influence overall plant size: we observed similar QTL for floral traits when we mapped residuals that accounted for plant size (*i.e.*, plant size-corrected floral traits, data not shown). The exception, of course, is that pleiotropic QTL that influenced both floral traits and the size of vegetative organs differed between the original and the residuals-based analysis of QTL.

In total, we detected 19 main-effect floral QTL in the field and 21 QTL in the greenhouse, of which 5 QTL in each environment influenced multiple floral traits. Instances of such "pleiotropic" QTL and/or QTL in close proximity contribute to the strong genetic correlations observed among floral whorls (Table 2). In both environments, estimates of genetic correlations based on QTL additive effects (r_Q) (Table 6) strongly correlate with those based on RIL trait means (r_G). For example, petal and filament length have two pleiotropic QTL (FQTL 3-3 and FQTL 6-1) and one instance of physically proximate QTL (FQTL 7-3 and FQTL 7-4) under field conditions (Figure 2), at which alleles affect petal and filament morphology in the same direction (Table 3). Correspondingly, petal–filament length r_Q closely approximates r_G (0.8 and 0.75, respectively), suggesting that mapped QTL largely account for the genetic correlation. (It is, however, worth noting that r_Q for many other trait pairs underestimates r_G, a pattern potentially attributable to undetectable QTL of small effect). Intriguingly, other features of the QTL architecture may explain the absence of significant floral–floral correlations. Greenhouse QTL 3–5 influenced petal width and midpoint length in opposing directions (Table 4). This case of antagonistic pleiotropy may counter other QTL that have parallel additive effects on petal width and midpoint length (*e.g.*, GQTL 9-3 and 9-4), resulting in the nonsignificant genetic correlation observed under greenhouse conditions (Table 2).

A majority of the main-effect QTL had significant effects on floral traits in both environments; however, we did detect significant QTL × E interactions that arose either because QTL had statistically significant effects in only one environment or because QTL had statistically significant phenotypic effects in both environments, but the magnitude of effect differed across environments (Tables 3 and 4). Although the overall similarity in floral **G**-matrices across environments may seem at odds with the extent of QTL × E, those QTL whose phenotypic effects differed across environments typically regulated floral organs for which bivariate correlations likewise differed across environments. For example, QTL regulating petal traits (petal width as well as petal and midpoint lengths) and ovary length accounted for a majority of the detected QTL × E interactions, and it is genetic correlations involving these traits that differed across environments (Table 2).

In spite of the statistical hurdles, an increasing number of studies illustrate the important role of epistasis in regulating trait expression and potentially maintain-

TABLE 6

Genetic correlations based on the additive effect of QTL (r_Q) identified for vegetative, phenological, and floral traits of *B. rapa* RILs raised in the field (above diagonal) and in the greenhouse (below diagonal)

r_Q	Hy L	Lf L	Br L	PI H	DTF	Pet L	Mid L	Pet W	Fil L	An L	Ov L	Sty L
Hypocotyl Ln (Hy L)		—	—	—	—	—	—	—	—	—	—	*−0.15*
Leaf Ln (Lf L)	—		0.51	0.75	−0.41	—	*−0.07*	—	—	*0.33*	—	—
Branch Ln (Br L)	NA	NA		0.51	−0.38	*0.34*	*0.21*	*0.70*	—	*0.27*	—	*0.03*
Primary inflorescence height (PI H)	—	0.78	NA		−0.55	—	*0.13*	—	—	*0.81*	—	*−0.17*
Days to flower (DTF)	−0.63	0.68	NA	0.62		—	—	—	—	—	—	—
Petal Ln (Pet L)	—	*0.18*	*NA*	*0.61*	*0.62*		0.78	0.71	0.80	0.61	0.61	0.33
Midpoint Ln (Mid L)	—	*0.26*	*NA*	*0.25*	*0.24*	0.58		0.23	0.82	0.19	0.46	0.31
Petal Wd (Pet W)	—	—	*NA*	—	—	0.11	—		0.47	0.61	0.24	0.25
Filament Ln (Fil L)	—	*0.53*	*NA*	*0.22*	*0.33*	0.44	0.70	−0.10		0.35	0.53	0.58
Anther Ln (An L)	*0.26*	*0.38*	*NA*	*0.46*	*0.28*	0.91	0.36	0.49	0.17		−0.07	−0.08
Ovary Ln (Ov L)	—	*0.46*	*NA*	*0.64*	*0.08*	0.88	0.50	−0.13	0.81	0.75		0.11
Style–stigma Ln (Sty L)	—	*0.30*	*NA*	*0.13*	*0.39*	0.57	0.43	0.05	0.03	0.38	0.09	

Dashes indicate that r_Q was not estimated because the correlation estimated with genotypic trait means was not significant (Table 2). Italics designate floral–nonfloral correlations.

ing genetic variation (Kroymann and Mitchell-Olds 2005; Malmberg *et al.* 2005). In genome-wide scans, we detected epistatic interactions in both the field (one interaction) and the greenhouse (six interactions) that regulated floral phenotypes (see PVE in Table 5). The small number of two-way epistatic interactions we detected across both environments should be viewed as a minimum estimate, a measure constrained by the stringent significance levels needed to properly account for multiple comparisons and by the limited ability to detect epistatic interactions of small effect. As with pleiotropic main-effect QTL, epistatic interactions contributed to the observed integration among floral whorls. Illustrating this last point, the interaction between QTL on chromosome 2 (~78 cM) and chromosome 10 (~46 cM) detected in the greenhouse affected multiple floral traits (Table 5, Figure S1). Finally, we note that most floral epistatic interactions differed significantly across environments; however, the small number of detected epistatic interactions limits our ability to determine the generality of this pattern.

Floral–vegetative correlations within multiple environments: Floral and vegetative traits in natural populations are hypothesized to be genetically decoupled (Berg 1960; reviewed in Murren 2002), a genetic independence that has arisen despite the developmental homology between shoots and flowers and between leaves and floral organs (Goethe 1790; Frohlich and Chase 2007). This decoupling should facilitate independent evolutionary responses to environmental heterogeneity of functionally divergent trait groups. In the field, floral traits were indeed less integrated with nonfloral traits than with other floral traits; instances of significant correlations between floral and nonfloral traits were less frequent and weaker than those between floral traits.

We did, however, find evidence of significant floral–vegetative genetic correlations in both environments. For example, anther length was positively correlated with primary inflorescence height in the field, which in turn was supported by the detection of pleiotropic QTL on chromosomes 3 and 6 (FQTL 3-2 and FQTL 6-2, respectively) that influenced these traits in a coordinated manner (Table 6). When RILs were raised in the greenhouse, apparent integration estimated from **G**-matrices increased significantly between floral and vegetative traits and, to a greater extent, between floral and phenological traits. All greenhouse floral traits, except petal width, were significantly positively correlated with later-staged vegetative traits and days to flower (Tables 2 and 6). Of the floral–vegetative correlations, anther length again exhibited the strongest genetic correlations with nonfloral traits, and QTL regulating anther length overlapped or neighbored those influencing primary inflorescence height (*e.g.*, chromosomes 1, 3, and 6). However, much of the increased integration between floral and nonfloral traits in the greenhouse was not reflected in the underlying QTL architecture, suggesting that undetected small-effect QTL and/or higher-order epistatic interactions coregulate floral and nonfloral traits under these more benign environmental conditions.

The detection of floral–vegetative and floral–phenological genetic correlations in our *B. rapa* population (especially those in the greenhouse) stands in contrast to studies of the close relatives *A. thaliana* (Juenger *et al.* 2005) and *R. raphanistrum* (Conner and Via 1993; Conner and Sterling 1996). In the Brassicaceous systems, strong genetic correlations were detected within floral and vegetative modules, but correlations between floral and vegetative traits were much weaker and rarely significant (Conner and Via 1993; Juenger

et al. 2005). Furthermore in *A. thaliana*, pleiotropic QTL were most commonly observed to influence either vegetative or floral trait groups, but not both (Juenger *et al.* 2005). Other studies examining the independence of floral–vegetative correlations are more equivocal (Armbruster *et al.* 1999; Hall *et al.* 2006). In *M. guttatus*, Hall *et al.* (2006) detected QTL that jointly affected floral, vegetative, and phenological traits. It is possible that genetic correlations between floral and nonfloral traits in this last QTL study (and our own) are more a consequence of the very divergent populations used in the experimental cross [*e.g.*, annual *vs.* perennial (Hall and Willis 2006; Hall *et al.* 2006)] than a reflection of floral–vegetative correlations within populations experiencing more natural levels of migration and gene flow. Nevertheless, utilizing segregating progeny from divergent populations is fundamental for understanding genetic mechanisms underlying complex traits. Moreover, gene flow between weedy and cultivated genotypes of *B. rapa* (Heenan and Dawson 2005; Warwick *et al.* 2008) and of other species of agricultural importance (Ellstrand *et al.* 1999; Pilson and Prendeville 2004), as well as between native and exotic populations (*e.g.*, Culley and Hardiman 2009), argues that similar crosses between distinct populations are frequent in nature.

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Floral Genetic Architecture: An Examination of QTL Architecture Underlying Floral (Co)Variation Across Environments

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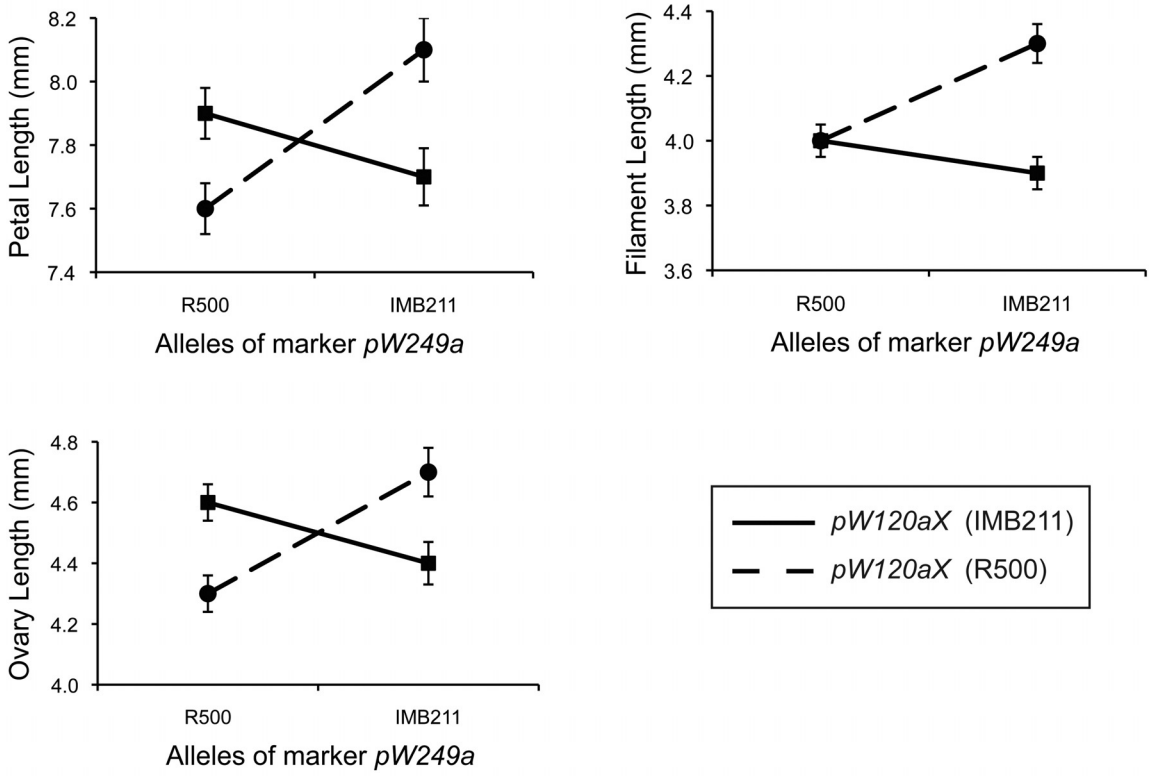

FIGURE S1.—Epistatic interactions for floral traits (petal, filament, and ovary length) of *B. rapa* RILs raised in the greenhouse. Interactions are illustrated using markers closest to detected QTL locations (see Table 5 for QTL positions). Least-squares means ± 1 standard error are shown.

File S1

Phenotypic data

File S1 is available for download as an Excel file at http://www.genetics.org/cgi/content/full/genetics.110.119982/DC1.

TABLE S1

List of *Brassica rapa* accessions obtained from the National Genetic Resources Program that were raised in the field at the University of Minnesota, St. Paul.

a) Country of origin of each accession and average (BLUP ± SE) expression of floral traits

Accession no.	Species	subspecies	variety	Country	Improvement Status
PI 116021	*Brassica rapa*			India	NA
PI 135871	*Brassica rapa*			Pakistan	NA
PI 173846	*Brassica rapa*		Sarson	India	NA
PI 173847	*Brassica rapa*	trilocularis		India	wild
PI 173852	*Brassica rapa*		Sarson	India	NA
PI 173864	*Brassica rapa*			Nepal	NA
PI 174793	*Brassica rapa*		Sarson	India	NA
PI 250004	*Brassica rapa*			Egypt	NA
PI 250129	*Brassica rapa*			Pakistan	NA
PI 469895	*Brassica rapa*			South Korea	NA
PI 597831	*Brassica rapa*	oleifera		Egypt	wild
PI 633161	*Brassica rapa*			Italy	cultivated
PI 633165	*Brassica rapa*	chinensis		China	wild

a) cont.

Accession no.	Petal Ln (mm)	Midpoint Ln (mm)	Petal Wd (mm)	Filament Ln (mm)	Anther Ln (mm)	Ovary Ln (mm)	Style/Stigma Ln (mm)
PI 116021	8.4 (± 0.41)	3.0 (± 0.19)	4.8 (± 0.28)	4.9 (± 0.24)	2.6 (± 0.11)	5.1 (± 0.21)	2.3 (± 0.19)
PI 135871	8.3 (± 0.41)	3.0 (± 0.19)	4.6 (± 0.28)	5.1 (± 0.24)	2.5 (± 0.11)	4.5 (± 0.21)	2.1 (± 0.19)
PI 173846	8.3 (± 0.52)	2.9 (± 0.24)	4.0 (± 0.36)	4.8 (± 0.30)	2.3 (± 0.14)	5.2 (± 0.26)	2.7 (± 0.24)
PI 173847	10.3 (± 0.42)	4.2 (± 0.20)	5.2 (± 0.29)	6.1 (± 0.25)	2.5 (± 0.11)	4.8 (± 0.21)	3.6 (± 0.20)
PI 173852	9.1 (± 0.39)	3.4 (± 0.19)	4.4 (± 0.27)	5.7 (± 0.23)	3.1 (± 0.11)	5.4 (± 0.20)	2.3 (± 0.19)
PI 173864	7.6 (± 0.66)	2.7 (± 0.32)	3.9 (± 0.44)	5.1 (± 0.36)	2.3 (± 0.19)	4.7 (± 0.31)	2.0 (± 0.32)
PI 174793	9.1 (± 0.46)	3.2 (± 0.21)	4.4 (± 0.32)	5.4 (± 0.27)	2.8 (± 0.12)	5.3 (± 0.23)	2.5 (± 0.21)
PI 250004	8.7 (± 0.42)	2.9 (± 0.20)	4.2 (± 0.29)	5.4 (± 0.25)	2.7 (± 0.11)	4.7 (± 0.21)	1.9 (± 0.20)
PI 250129	8.9 (± 0.41)	3.2 (± 0.19)	4.8 (± 0.28)	5.3 (± 0.24)	2.5 (± 0.11)	4.9 (± 0.21)	2.4 (± 0.19)
PI 469895	8.7 (± 0.44)	2.8 (± 0.20)	4.6 (± 0.30)	5.1 (± 0.26)	2.6 (± 0.12)	5.0 (± 0.22)	1.8 (± 0.20)
PI 597831	9.0 (± 0.37)	3.5 (± 0.17)	4.6 (± 0.26)	5.2 (± 0.22)	2.4 (± 0.10)	5.3 (± 0.19)	2.5 (± 0.18)

PI 633161	7.8 (± 0.38)	2.8 (± 0.18)	3.8 (± 0.26)	5.1 (± 0.22)	2.2 (± 0.10)	4.8 (± 0.19)	1.9 (± 0.18)
PI 633165	9.7 (± 0.38)	3.4 (± 0.18)	5.3 (± 0.26)	5.3 (± 0.22)	2.7 (± 0.10)	5.5 (± 0.19)	2.1 (± 0.18)

Included in this table are countries of origin and best linear unbiased predictors (± 1 SE) of floral traits.

TABLE S2

ANOVA of phenotypic traits measured in a population of *B. rapa* RILs grown in a field experiment at the University of Minnesota.

	T Hypocotyl Ln		T Leaf Ln		T Branch Ln		T Flower Ht		T Primary Infl. Ht		Days to Flower	
Sources of Variation	Z value	*P* value	Z value	*P* value	Z value	*P* value	Z value	*P* value	Z value	*P* value	Z value	*P* value
Subplot	1.29	0.098	1.81	**0.035**	1.71	**0.0434**	1.73	**0.0418**	1.79	**0.0369**	1.23	0.11
RC (Line × Subplot)	2.12	**0.0172**	4.14	**< 0.0001**	3.92	**< 0.0001**	3.97	**< 0.0001**	4.43	**< 0.0001**	3.67	**0.0001**
Line	2.43	**0.0076**	5.33	**< 0.0001**	4.35	**< 0.0001**	5.9	**< 0.0001**	5.56	**< 0.0001**	1.6	0.0548

	Petal Ln		Midpoint Ln		T Petal Wd		T Filament Ln		Anther Ln		T Ovary Ln	
Sources of Variation	Z value	*P* value	Z value	*P* value	Z value	*P* value	Z value	*P* value	Z value	*P* value	Z value	*P* value
Subplot	1.6	0.0553	1.57	0.0578	1.41	0.0789	1.15	0.13	1.56	0.0595	0.17	0.43
RC (Line × Subplot)	0.67	0.25	2.74	**0.0031**	0.92	0.18	0.15	0.44	0.9	0.18	0.17	0.43
Line	5.46	**< 0.0001**	4.55	**< 0.0001**	5.49	**< 0.0001**	5.17	**< 0.0001**	6.06	**< 0.0001**	4.95	**< 0.0001**

	Style-Stigma Ln	
Sources of Variation	Z value	*P* value
Subplot	0.72	0.24
RC (Line × Subplot)	0.46	0.32
Line	4.56	**< 0.0001**

Variation in traits (T designates transformed via Box-Cox procedure) is partitioned among spatial subplots, line (*i.e.*, RIL), and replicate cluster (RC) nested within the line × subplot term. See MATERIALS AND METHODS for description of planting design and statistical methodology.

TABLE S3

ANOVA of floral and non-floral traits measured in a population of *B. rapa* RILs raised in a greenhouse experiment.

	Hypocotyl Ln		T Leaf Ln		T Leaf Wd		T Primary Infl. Ht		Days to Flower	
Sources of Variation	Z value	*P* value	Z value	*P* value	Z value	*P* value	Z value	*P* value	Z value	*P* value
Block	1.55	0.0602	1.57	0.0584	1.55	0.0601	1.2	0.12	1.42	0.0781
Line	7.26	**< 0.0001**	7.22	**< 0.0001**	6.88	**< 0.0001**	7.19	**< 0.0001**	7.36	**< 0.0001**

	T Petal Ln		T Midpoint Ln		T Petal Wd		T Filament Ln		T Anther Ln		T Ovary Ln	
Sources of Variation	Z value	*P* value	Z value	*P* value	Z value	*P* value	Z value	*P* value	Z value	*P* value	Z value	*P* value
Block	1.44	0.0746	1.43	0.076	1.42	0.0771	1.24	0.11	1.3	0.0966	1.44	0.075
Line	6.24	**< 0.0001**	6.19	**< 0.0001**	7.04	**< 0.0001**	6.1	**< 0.0001**	6.98	**< 0.0001**	6.6	**< 0.0001**

	T Style-Stigma	
Sources of Variation	Z value	*P* value
Block	1.37	0.086
Line	6.43	**< 0.0001**

Variation in each trait is partitioned among spatial blocks and line (*i.e.*, RIL) designated as random factors. The letter "T" precedes names of traits that were Box-Cox transformed.

TABLE S4

ANOVA of floral and non-floral traits measured in a population of *B. rapa* RILs raised under field and greenhouse conditions (Environment).

	Hypocotyl Ln			Days to Flower			Leaf Ln			Primary Infl. Ht		
Sources of Variation	df	F value	P value	df	F value	P value	df	F value	P value	df	F value	P value
Environment	1, 136	0.07	0.79	1, 272	0.06	0.81	1, 135	0.04	0.85	1, 126	1.53	0.22
Random Effects		Z value	P value		Z value	P value		Z value	P value		Z value	P value
Line		3.46	**0.0003**		.	.		6.26	< **0.0001**		5.91	< **0.0001**
Line × Environ		4.57	< **0.0001**		8.95	< **0.0001**		2.96	**0.0016**		3.45	**0.0003**
Sources of Variation		Petal Ln			Midpoint Ln			Petal Wd			Filament Ln	
	df	F value	P value	df	F value	P value	df	F value	P value	df	F value	P value
Environment	1, 133	0.09	0.76	1, 138	0.01	0.94	1, 115	0.44	0.51	1, 143	0.11	0.74
Random Effects		Z value	P value		Z value	P value		Z value	P value		Z value	P value
Line		4.67	< **0.0001**		3.76	< **0.0001**		5.87	< **0.0001**		3.73	< **0.0001**
Line × Environ		3.12	**0.0009**		3.32	**0.0005**		3.04	**0.0012**		4.04	< **0.0001**
Sources of Variation		Anther Ln			Ovary Ln			Style-Stigma Ln				
	df	F value	P value	df	F value	P value	df	F value	P value			
Environment	1, 128	0.63	0.43	1, 133	0.01	0.9	1, 133	0.2	0.66			
Random Effects		Z value	P value		Z value	P value		Z value	P value			
Line		5.62	< **0.0001**		3.76	< **0.0001**		4.1	< **0.0001**			
Line × Environ		3.73	< **0.0001**		3.89	< **0.0001**		3.36	**0.0004**			

Variation in each trait was partitioned among environment (fixed factor), line (random factor), and the line × environment interaction (random factor). Analysis was performed on trait residuals of each replicate plant after accounting for variation in blocking factors.

TABLE S5

Markers closest to QTL positions (and 2-LOD support limits) identified in CIM analysis of floral and non-floral traits of *B. rapa* RILs raised in field and greenhouse environments.

QTL	Trait	Chr	Position	Marker	2-LOD Range	2-LOD Markers
Field	Anther Ln	1	20.2	fito019d	(15.2, 28.3)	(pX151aH, pX136bE)
Field	Petal Wd	1	21.7	fito083	(16.5, 26.3)	(pX151aH, pX136bE)
Field	Days To Flower	1	32.8	pW179aH	(26.3, 46.3)	(pX136bE, fito389)
Field	Leaf Ln	2	2 †	BRMS046	(0, 4)	(BRMS046, pX123bH)
Field	Leaf Ln	2	52.9	pW208aH	(47.4, 66.4)	(pW227cE, pW205bH)
Field	Ovary Ln	3	19.2	pX144bE	(0, 25.5)	(pX142bH, pW174cX)
Field	Primary Infl. Ht	3	33.3	pW214aX	(25.5, 37.8)	(pW174cX, pX141cE)
Field	Anther Ln	3	33.3	pW214aX	(27.1, 39.8)	(pW152cH, pW147bH)
Field	Anther Ln	3	77.1	pW179b	(72.6, 79.4)	(fito024b, pW166aH)
Field	Petal Ln	3	77.1	pW179b	(72.6, 85)	(fito024b, pW244bH)
Field	Filament Ln	3	79.1	pW166aH	(66.9, 85)	(pW127aE, pW244bH)
Field	Midpoint Ln	3	86.5	pW177aH	(79.1, 93.7)	(pW166aH, fito019c)
Field	Petal Wd	3	91	pW169aX	(86.5, 101.7)	(pW177aH, fito506)
Field	Style-Stigma Ln	5	0	pX101dH	(0, 12)	(pX101dH, fito130c)
Field	Petal Wd	5	21.4	pW169bX	(14, 28.4)	(fito130c, pX149cX)
Field	Filament Ln	6	22.8	pW145aH	(17.1, 31.6)	(pW160bH, pW127aE)
Field	Branch Ln	6	22.8	pW145aH	(17.1, 27.6)	(pW160bH, pW138bX)
Field	Petal Wd	6	24.3	pW160cH	(15.4, 32.1)	(pW160bH, pW127aE)
Field	Petal Ln	6	24.3	pW160cH	(15.4, 32.1)	(pW160bH, pW127aE)
Field	Primary Infl. Ht	6	44.8	fito227	(39.5, 61)	(pX119dX, fito041)
Field	Anther Ln	6	46.8	BRMS026b	(40.6, 69)	(fito296, fito041)
Field	Branch Ln	7	0	BRMS040	(0, 10)	(BRMS040, pW222bE)
Field	Ovary Ln	7	23.5	fito066a	(15.4, 29.2)	(pW200cX, fito348)
Field	Filament Ln	7	29.2	fito348	(23.5, 37.8)	(fito066a, pW130cE)
Field	Midpoint Ln	7	29.2	fito348	(17.7, 37.8)	(pW108aH, pW130cE)
Field	Petal Ln	7	39.8	pX130bD	(37.2, 43.5)	(pW130cE, pW164cH)

Field	Hypocotyl Ln	7	58.5 †	pW134aH	(51.8, 59.3)	(fito101b, pW150cH)
Field	Ovary Ln	8	10.4	pW245aE	(0.4, 26.8)	(pW177aE, fito429)
Field	Style-Stigma Ln	8	34.5	pW120cX	(28, 34.5)	(fito429, pW120cX)
Field	Petal Ln	9	7.6	pX123dH	(0, 12.5)	(fito097b, fito033b)
Field	Midpoint Ln	9	18.5	fito514	(11.6, 23.1)	(fito033b, pW130cX)
Field	Primary Infl. Ht	9	22.7	pW188cE	(14.5, 28.6)	(fito033b, fito549)
Field	Hypocotyl Ln	9	37.3	fito367b	(31.5, 44.8)	(pX126aD, fito118a)
Field	Leaf Ln	9	38.7	fito555	(35.3, 44.8)	(fito410, fito118a)
Field	Ovary Ln	9	61.8	pW246cX	(52.3, 66.4)	(fito151a, isgpM)
Field	Midpoint Ln	9	61.8	pW246cX	(52.3, 66.4)	(fito151a, isgpM)
Field	Filament Ln	9	63.3	pW246cX	(52.3, 66.4)	(fito151a, isgpM)
Field	Hypocotyl Ln	10	0	fito424	(0, 11.2)	(fito424, pX139cH)
Field	Style-Stigma Ln	10	29.4	pW255aE	(21.9, 34.7)	(pW155cX, pW129dH)
Greenhouse	Petal Wd	1	13.2	fito433	(8.3, 18.4)	(pX147hE, fito516)
Greenhouse	Anther Ln	1	18.4	fito516	(13.2, 26.3)	(fito433, pX136bE)
Greenhouse	Primary Infl. Ht	1	30.3	fito222	(24.9, 36.3)	(pX136bE, pX122aH)
Greenhouse	Leaf Wd	1	32.1 †	fito222	(24.9, 48.7)	(pX136bE, fito389)
Greenhouse	Anther Ln	2	26.4	BRMS008	(19.2, 35.6)	(fito338a, BRMS001)
Greenhouse	Midpoint Ln	2	27.5	pW241bH	(20, 35.6)	(fito338a, BRMS001)
Greenhouse	Style-Stigma Ln	2	43.8	BRMS026a	(36.9, 55.1)	(fito118b, pW208aH)
Greenhouse	Leaf Wd	2	55.4	pX110cX	(42, 65.1)	(BRMS026a, pW205bH)
Greenhouse	Hypocotyl Ln	2	76.7 †	pW249aX	(63.1, 87.3)	(pW205bH, pX124bX)
Greenhouse	Petal Ln	3	19.2	pX144bE	(6, 25.5)	(pX142bH, pW174cX)
Greenhouse	Anther Ln	3	23.2	pW189aH	(8, 35.8)	(pX142bH, pX141cE)
Greenhouse	Primary Infl. Ht	3	40	pW147bH	(33.8, 47.6)	(pW129aH, fito109b)
Greenhouse	Anther Ln	3	66.1	pX151eH	(55.2, 72.6)	(BRMS031a, fito024b)
Greenhouse	Petal Ln	3	79.4	pW166aH	(74, 105.7)	(fito413, fito506)
Greenhouse	Midpoint Ln	3	91.0	pW169aX	(83.4, 97.7)	(pW244bH, fito019c)
Greenhouse	Petal Wd	3	91.0	pW169aX	(86.5, 103.7)	(pW177aH, fito506)
Greenhouse	Midpoint Ln	4	31.7	pW120dX	(24.3, 36.5)	(pX130eD, pX129dX)
Greenhouse	Hypocotyl Ln	4	57.4	pW178bE	(47.3, 65.7)	(pX111eD, fito495)
Greenhouse	Petal Wd	6	22.4	fito068	(15.4, 32.1)	(pW160bH, pW127aE)

Greenhouse	Anther Ln	6	36.1	fito423	(31.6, 43)	(pW127aE, pX136dE)
Greenhouse	Ovary Ln	6	37.0	fito423	(31.6, 42.3)	(pW127aE, pW219aE)
Greenhouse	Anther Ln	6	40.3	fito016	(39.6, 43)	(pW200bX, pX136dE)
Greenhouse	Leaf Wd	6	42.3	pW219aE	(37.5, 47)	(fito378b, BRMS026b)
Greenhouse	Primary Infl. Ht	6	42.3	pW219aE	(39.5, 57.0)	(pX119dX, BRMS026b)
Greenhouse	Leaf Ln	6	46.8	BRMS026b	(40.3, 55)	(fito016, BRMS026b)
Greenhouse	Petal Wd	7	15.4	pW200cX	(6, 23)	(BRMS040, fito057)
Greenhouse	Midpoint Ln	7	41.5	pX130bD	(29.2, 44.9)	(fito348, pW164cH)
Greenhouse	Midpoint Ln	7	58.5	pW134aH	(51.8, 59.3)	(fito101b, pW150cH)
Greenhouse	Petal Wd	8	10.4 †	pW245aE	(0, 18.8)	(BRMS006, pW138aX)
Greenhouse	Style-Stigma Ln	8	30.5	pW245bX	(18.8, 34.5)	(pW138aX, pW120cX)
Greenhouse	Days To Flower	9	2 †	fito097b	(0, 5.6)	(fito097b, pX123dH)
Greenhouse	Primary Infl. Ht	9	22	BRMS016	(12.5, 27.1)	(fito033b, fito549)
Greenhouse	Petal Wd	9	33.3	fito518	(23.1, 38)	(pW130cX, fito448)
Greenhouse	Midpoint Ln	9	37.3	fito367b	(35.3, 44.7)	(fito410, fito118a)
Greenhouse	Ovary Ln	9	46.0	pW233bE	(38.7, 53.8)	(fito555, fito151a)
Greenhouse	Filament Ln	9	52.3	fito151a	(38.7, 66.4)	(fito555, isgpM)
Greenhouse	Midpoint Ln	10	2 †	fito424	(0, 11.2)	(fito424, pX139cH)
Greenhouse	Days To Flower	10	25.4	pW155cX	(17.9, 29.4)	(pW155cX, pW255aE)
Greenhouse	Leaf Ln	10	29.4	pW255aE	(13.9, 29.7)	(FLC1aE, pW256aE)
Greenhouse	Primary Infl. Ht	10	29.4	pW255aE	(27.1, 35.8)	(fito549, pW129dH)
Greenhouse	Hypocotyl Ln	10	30.4	pW240aE	(17.9, 34.7)	(pW155cX, pW129dH)

TABLE S6

Markers closest to genomic positions identified in epistatic analyses of *B. rapa* RILs raised in A) the field and B) the greenhouse.

Trait	Chr	Position (cM)	Marker A	Chr	Position (cM)	Marker B
A) Field						
Leaf Ln	5	34	pW125cE	7	8	pW222bE
Branch LN	2	20	fito338a	5	8	fito130c
Branch LN	2	36	BRMS001	8	28	fito429
Branch LN	3	8 †	pX142bH	6	72 †	pX110aX
Primary Infl. Ht	3	4	pX142bH	6	72	pX110aX
Style-Stigma Ln	4	48 †	pX111eD	9	66 †	isgpM
B) Greenhouse						
Leaf Ln	4	4	fito066c	8	34	pW120cX
Leaf Wd	3	34	pW129aH	9	56	fito151a
Petal LN	2	78	pW249aX	10	46	pW120aX
Petal LN	4	56	pW133bH	5	50	fito130b
Midpoint LN	2	76	pW249aX	10	42	pW120aX
Filament LN	2	78	pW249aX	10	48	pW120aX
Anther LN	3	86	pW177aH	4	64	fito495
Ovary LN	2	78	pW249aX	10	42	pW120aX

† *P*-value < 0.07

TABLE S7

ANOVA of phenotypic traits measured in cultivated and wild accessions of *B. rapa* raised in a field experiment at the University of Minnesota.

	Petal Ln			Midpoint Ln			Petal Wd		
Factors	Var. Est.	Z value	P value	Var. Est.	Z value	P value	Var. Est.	Z value	P value
Accession	0.68	1.72	**0.0431**	0.19	2.0	**0.023**	0.28	1.64	**0.05**
Residual	1.16	6.35	< **0.0001**	0.20	6.42	< **0.0001**	0.63	6.37	< **0.0001**

	Filament Ln			Anther Ln			Ovary Ln			Style-Stigma Ln		
Factors	Var. Est.	Z value	P value	Var. Est.	Z value	P value	Var. Est.	Z value	P value	Var. Est.	Z value	P value
Accession	0.18	1.6	**0.0543**	0.06	2.02	**0.0219**	0.13	1.69	**0.0453**	0.24	2.17	**0.015**
Residual	0.50	6.41	< **0.0001**	0.07	6.44	< **0.0001**	0.36	6.45	< **0.0001**	0.17	6.44	< **0.0001**

See Table S2 for the National Genetic Resources Program accessions surveyed.

TABLE S8

Genotypic correlations of floral trait BLUPs for wild and cultivated accessions of *B. rapa* raised in the agricultural field at the University of Minnesota in the summer of 2004.

r_G		Pet L	Mid L	Pet W	Fil L	An L	Ov L	Sty L
Petal Ln	(Pet L)		0.89**	0.80*	0.80*	0.47	0.40	0.69*
Midpoint Ln	(Mid L)			0.68*	0.83**	0.24	0.29	0.85**
Petal Wd	(Pet W)				0.44	0.39	0.31	0.46
Filament Ln	(Fil L)					0.44	0.05	0.64*
Anther Ln	(An L)						0.45	-0.02
Ovary Ln	(Ov L)							0.13
Style-Stigma Ln	(Sty L)							

* $P < 0.05$, ** $P < 0.001$