DOI: 10.1534/genetics.104.031195

Genetics of Species Differences in the Wild Annual Sunflowers, *Helianthus annuus* and *H. petiolaris*

Christian Lexer,*[,1] David M. Rosenthal,[†] Olivier Raymond,[‡] Lisa A. Donovan[†] and Loren H. Rieseberg[§]

**Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3DS, United Kingdom, [†]Department of Plant Biology, University of Georgia, Athens, Georgia 30602, [‡]Laboratoire de Biologie Moleculaire et Phytochimie, Université Claude Bernard Lyon 1, F-69622 Villeurbanne, France and [§]Department of Biology, Indiana University, Bloomington, Indiana 47405*

Manuscript received May 13, 2004
Accepted for publication December 21, 2004

ABSTRACT

Much of our knowledge of speciation genetics stems from quantitative trait locus (QTL) studies. However, interpretations of the size and distribution of QTL underlying species differences are complicated by differences in the way QTL magnitudes are estimated. Also, many studies fail to exploit information about QTL directions or to compare inter- and intraspecific QTL variation. Here, we comprehensively analyze an extensive QTL data set for an interspecific backcross between two wild annual sunflowers, *Helianthus annuus* and *H. petiolaris*, interpret different estimates of QTL magnitudes, identify trait groups that have diverged through selection, and compare inter- and intraspecific QTL magnitudes. Our results indicate that even minor QTL (in terms of backcross variance) may be surprisingly large compared to levels of standing variation in the parental species or phenotypic differences between them. Morphological traits, particularly flower morphology, were more strongly or consistently selected than life history or physiological traits. Also, intraspecific QTL were generally smaller than interspecific ones, consistent with the prediction that larger QTL are more likely to spread to fixation across a subdivided population. Our results inform the genetics of species differences in Helianthus and suggest an approach for the simultaneous mapping of inter- and intraspecific QTL.

RECENT years have seen tremendous conceptual and technical advances in speciation genetics. Testable hypotheses have been formulated regarding the genetic architecture of adaptation and speciation (Orr and Coyne 1992; Orr 1998a,b; Noor *et al.* 2001; Navarro and Barton 2003), and an ever-growing pool of neutral polymorphic markers (deVienne 2003) as well as improved methods for quantitative trait locus (QTL) mapping (Zeng 1994; Kao *et al.* 1999; Mauricio 2001) now allow geneticists to map QTL underlying species differences (Orr 2001). Progress also has been made in identifying candidate "speciation genes" (Greenberg *et al.* 2003; Presgraves *et al.* 2003). These developments have stimulated renewed interest in the "nature of interspecific differences," a topic that has received little attention since the publication of Haldane's survey >60 years ago (Haldane 1938).

The phenotypic differences that separate species or divergent populations are typically categorized as those traits directly involved in reproductive isolation *vs.* "ordinary" traits that differ between populations without an obvious role in blocking gene flow (Orr 2001). Of course, this division is overly simplistic. Experimental evidence indicates that genes or QTL underlying ordinary differences between species often have correlated (presumably pleiotropic) effects on reproductive isolation (Rice and Hostert 1993; Hawthorne and Via 2001; Greenberg *et al.* 2003). Moreover, many (most?) ordinary differences among taxa appear to be maintained by divergent natural selection in the wild (reviewed by Lexer *et al.* 2003a) and must also contribute to isolation. Thus, it may be more useful to classify species differences in terms of the evolutionary forces responsible for their differentiation. Although this often was not possible in the past, Orr (1998b) developed a "QTL sign test," which exploits information about the sign or direction of QTL effects to make inferences about the history of phenotypic selection.

The rationale for the test is that traits with a history of directional selection will have QTL effects mostly in the same direction, whereas QTL with opposing effects should be common for traits diverging under neutrality. The proportion of antagonistic QTL for a given trait also informs us about the potential for transgressive segregation, *i.e.*, the release of cryptic variation in hybrids (deVincente and Tanksley 1993). Transgressive segregation is frequently observed in interspecific crosses (reviewed by Rieseberg *et al.* 1999) and is caused primarily by complementary gene action, *i.e.*, the "reshuffling" in hybrids of alleles with opposing effects

[1]*Corresponding author:* Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3DS, United Kingdom.
E-mail: c.lexer@kew.org

within each parental line (Rieseberg *et al.* 1999, 2003; Lexer *et al.* 2003b).

Another topic of interest to speciation geneticists and a second focus of the present study is the size and distribution of genetic factors fixed during a population's approach toward its fitness optimum (Fisher 1930; Wright 1931; reviewed by Barton and Keightly 2002). Theory predicts that the "adaptive walk" starts with large steps that become progressively smaller as a population nears the optimum, resulting in an exponential distribution of fixed genetic effects (Orr 1998a, 2001). The prediction that QTL with large effects may sometimes contribute to adaptive evolution has indeed been verified in some instances (*e.g.*, Bradshaw *et al.* 1998; Schemske and Bradshaw 1999; Sucena and Stern 2000); however, existing data also indicate that genetic architecture may vary greatly among taxa (True *et al.* 1997; Macdonald and Goldstein 1999; Zeng *et al.* 2000; Fishman *et al.* 2002).

Much of the confusion over effect sizes/distributions may stem from differences in how they are reported: most studies report QTL magnitudes in terms of PVE, the percentage of variation explained in the segregating population (Lynch and Walsh 1998). Others calculate QTL effect sizes in terms of the standing variation within the parental populations or the phenotypic gap between them (*e.g.*, True *et al.* 1997; Zeng *et al.* 2000; Fishman *et al.* 2002). These latter measures may be preferable in many cases. Scaling QTL for standing variation seems intuitive, since this is the size of the genetic effect that natural selection sees (Orr 2001). Scaling it for the interspecific gap particularly makes sense in speciation studies, where QTL magnitudes need to be interpreted in terms of species divergence rather than variation in the mapping population.

A third focus of the present study concerns the nature of inter- *vs.* intraspecific QTL variation. Some authors have argued that QTL underlying species differences are likely to be larger than those polymorphic within species because minor QTL are less likely to spread to fixation across a subdivided population (Rieseberg and Burke 2001; Rieseberg *et al.* 2004). Also, it is not clear whether the genes causing interspecific differences often contribute to intraspecific polymorphism as well. Unfortunately, most QTL data generated to date are not appropriate for addressing these questions because results from both intra- and interspecific studies are rarely reported from the same taxa in the same environment. Also, some fraction of the QTL detected in interspecific crosses may be restricted to those populations employed for mapping (Mauricio 2001). Ideally, QTL studies that detect QTL at both inter- and intraspecific levels within the same experiment should be designed. This is possible in wild annual sunflowers because they are self-incompatible outcrossers and therefore possess ample genetic variability that can be captured in experimental pedigrees.

The two annual sunflower species studied here, *Helianthus annuus* and *H. petiolaris*, share a wide sympatric distribution across the central and western United States. They are easily distinguished by several morphological and chromosomal features and by divergent linkage relationships caused by a minimum of 20 chromosomal breakages and fusions (Burke *et al.* 2004). They also occur in divergent clades based on plastid DNA (Rieseberg *et al.* 1991) and nuclear ribosomal DNA variation (Rieseberg 1991) and have different ecological requirements. In general, *H. annuus* is restricted to heavy clay soils and *H. petiolaris* to dry sandy soils. Nonetheless, these two habitats are often found in close proximity throughout the central and western United States, resulting in the production of innumerable hybrid swarms or "mosaic" hybrid zones. In addition, *H. annuus* and *H. petiolaris* have given rise to at least three stabilized diploid hybrid species that are strongly divergent from their parents with respect to their habitat preferences [sand dune, desert floor, and salt marsh habitats (Rosenthal *et al.* 2002)]. This makes it feasible to use interspecific crosses between *H. annuus* and *H. petiolaris* to study the adaptive potential of parental species QTL when recombined in hybrids (Lexer *et al.* 2003b; Rieseberg *et al.* 2003).

Here, we ask the following questions related to the genetic architecture of species differences in wild Helianthus:

1. What are the distributions of interspecific QTL magnitudes when expressed relative to the interspecific phenotypic gap, to the standing variation in *H. annuus*, or as PVE in the mapping population, and how do these different estimates affect interpretations of the genetic architecture of species differences?
2. What are the proportions of opposing QTL for different classes of phenotypic traits, and what do these ratios tell us about the role of selection *vs.* neutral processes in the origin of species differences?
3. Do intra- and interspecific QTL differ in magnitude and does the same genomic interval often affect both intra- and interspecific differences?

In addition, we explore the role of epistasis, because the QTL sign tests that we performed assume that QTL effects are largely additive. To our knowledge, ours is the first QTL study that directly compares inter- and intraspecific QTL variation in the same mapping population. It is also the most extensive QTL data set to date reporting alternative, biologically meaningful estimates of QTL effect sizes.

MATERIALS AND METHODS

Mapping population and experimental design: The present study is based on an interspecific second-generation backcross population (BC_2) between *H. annuus* and *H. petiolaris*, described in detail by Lexer *et al.* (2003b,c) and Rieseberg

et al. (2003). Briefly, a single F_1 hybrid between *H. annuus* (population ANN1295) and *H. petiolaris* (population PET1277) was backcrossed to a second individual from PET1277, resulting in 38 BC_1 progeny. This was followed by a second round of backcrossing of the 38 BC_1 plants toward a single, third individual of PET1277. The latter step was necessary to increase achene numbers, since early generation hybrids from this cross are semisterile. Approximately 400 BC_2 seedlings and 20 plants each of *H. annuus* and *H. petiolaris* were propagated in the greenhouses of the University of Georgia, Athens, Georgia, as described by Rieseberg *et al.* (2003, supplementary material). A final total of 384 BC_2 plants, 20 *H. annuus*, and 17 *H. petiolaris* were subjected to the phenotypic trait measurements described below.

The 384 BC_2 plants and parental species samples employed here formed part of a comparative genomic study on the hybrid origin of ecological differences in wild sunflowers (Rieseberg *et al.* 2003). In that study, interspecific QTL were mapped by studying marker alleles segregating from *H. annuus*, the donor parent, against a *H. petiolaris* background, and the resulting QTL data were used for genomic comparisons of experimental BC_2 hybrids with three wild sunflower hybrid species. Here, we extend the interspecific backcross model to an analysis of *intra*-specific QTL segregating in the same backcross population, and we use the combined inter- and intraspecific QTL data to study the genetics of species differences between *H. annuus* and *H. petiolaris*. We also extend the original analyses, which focused on transgressive segregation, to estimate QTL effect sizes, test for directional selection during phenotypic divergence, and calculate the magnitude of interaction effects for QTL with significant epistatic interactions.

Phenotypic measurements and genetic map construction: The phenotypic measurements of BC_2 plants and parental species samples were described in detail by Rieseberg *et al.* (2003, supplementary material). For the sake of clarity, the trait category, abbreviation, and complete name of each trait, as well as units of measurement, phenotypic gaps between species, and standing variation in *H. annuus* are summarized in Table 1. Assaying all plants within a randomized block design allowed us to test for environmental effects on phenotypic trait expression prior to QTL analyses (below).

Molecular marker genotypes were collected as described in Lexer *et al.* (2003b), using the protocols of Burke *et al.* (2002) in the case of microsatellites, and in Rieseberg *et al.* (2003) for AFLPs. An interspecific genetic linkage map was constructed on the basis of recombination estimates in a BC_2 model using Mapmanager QTX version b16 (Manly *et al.* 2001), as described in detail by Lexer *et al.* (2003b). The map shows several cases of pseudolinkage and/or fragmentation, two phenomena that are often observed in interspecific hybrid crosses and were expected, given the degree of karyotypic divergence between *H. annuus* and *H. petiolaris* (Rieseberg *et al.* 1995; Burke *et al.* 2004).

Interspecific QTL analyses: QTL analyses were performed on transformed data whenever traits deviated from normality and on residuals from a one-way ANOVA with block as the main factor for traits with significant environmental effects. QTL were detected by composite interval mapping (CIM; Zeng 1994) using Mapmanager QTX version b16 (Manly *et al.* 2001), as described by Rieseberg *et al.* (2003, supplementary material). Genomewide threshold levels for declaring the presence of a QTL were determined by 1000 permutations for each trait, and one-LOD support intervals were calculated from the CIM results. Composite interval mapping was also employed to estimate the PVE and the heterozygous additive effect (a) of each QTL. Within the present study, a was recalculated from phenotypic raw data for each trait (as opposed to Box-Cox transformed data or residuals from ANOVA used for QTL detection), thereby providing estimates of a in the original units of measurement for each character (appendix).

For the purpose of the present study, QTL effect sizes were reported in three ways: (1) PVE in the mapping population; (2) relative to the difference in phenotypic line means (d) between *H. annuus* and *H. petiolaris* grown in the greenhouse [percentage species difference = $(a/d) \times 100$]; and (3) relative to the level of standing variation (the phenotypic standard deviation) in greenhouse-grown *H. annuus* [percentage standing variation = (a/phenotypic sd) $\times$ 100]. Differences in QTL effect sizes across the trait categories "complementary genes present/absent" were tested by t-tests for independent samples on natural log-transformed data, equal variances not assumed. This analysis was motivated by the fact that traits with only small interspecific differences may sometimes be controlled by complementary genes. These opposing QTL may have large phenotypic effects, but nevertheless may not result in recognizable interspecific differences because they mask each other. An analysis of QTL magnitudes across traits *with* or *without* complementary genes should therefore allow us to visualize this "cryptic variation." Differences across the trait categories "morphology/life history/physiology" were tested on transformed data as well, using nonparametric Kruskal-Wallis tests (Levene's test for equality of error variances: $F = 11.325$, $P < 0.001$). All of the tests were carried out with SPSS (Chicago).

Numerous epistatic interactions were reported in this population previously (Rieseberg *et al.* 2003); however, in that study the sizes of interaction effects were not estimated. To explore the role of epistasis in wild sunflowers more thoroughly, we considered it critical to ask whether the interaction effects exceeded the main effects for the QTL combinations in question. In particular, the absence of a major role for gene interactions is a prerequisite for QTL sign tests (below), which assume additivity of QTL effects. Within the present study, effect sizes of previously detected interactions were estimated by linear modeling in SPSS.

QTL sign tests: To test for the relative importance of natural selection *vs.* genetic drift in the evolution of phenotypic differences between *H. annuus* and *H. petiolaris*, we performed Orr's (1998) QTL sign test. This test compares the observed number of plus-and-minus alleles in the "high line" with that expected under neutrality. If the ratio of plus-to-minus alleles in the high line (or the proportion of antagonistic QTL) is more extreme than that expected under neutrality, then a causative role for directional selection in the origin of the observed trait differences is inferred. We did not perform the equal-effects test described by Orr (1998b), because it has been shown that it may be affected by ascertainment bias (*i.e.*, the traits to which the test is applied may be more divergent than average) (Anderson and Slatkin 2003). Instead, we used the second, alternative version of the test as recommended by Anderson and Slatkin (2003). This version is conditioned on the distribution of QTL effects, which is assumed to be known without error. Sign tests were performed for three different main categories of traits (morphology, life history, and physiology) and, in addition, for three subcategories of morphological traits (root/shoot morphology, flower morphology, and leaf morphology), and two subcategories of physiological traits (mineral ion content *vs.* photosynthesis). Seed weight (SEEDW) was omitted from the analyses because it was difficult to categorize. Tests were carried out with a computer program provided by Allan Orr, which employs a Monte Carlo approach for calculating probabilities. QTL were assumed to be exponentially distributed (gamma distributions with shape parameter $\beta = 1$). Heterozygous QTL effects and the phenotypic line difference R were calculated as averages over traits within each category. Note that the scale parameters (α) of QTL distributions may also depend on the QTL detection

TABLE 1

Phenotypic traits analyzed in this study

Trait category	Trait abbreviation	Complete name	Unit of measurement	Interspecific phenotypic gap	Standing variation in *H. annuus*[b]
Morphology	BRHGT[a]	Height at first branch	cm	33.6	14.8
	BRNUM[a]	Branch no. at harvest	count	5.4	5.4
	DISKDIA[a]	Disk diameter	mm	11.2	5.1
	HARGHT[a]	Height at final harvest	cm	83.0	34.2
	HGT1[a]	Height 7 days after planting	cm	26.9	8.0
	HYPOLG	Hypocotyl length at 96 hr	mm	3.0	4.3
	LFAREA[a]	Leaf area	cm^2	38.3	36.8
	LFLGTH[a]	Leaf length	mm	31.8	27.4
	LFSHAP[a]	Leaf shape	mm/mm	0.64	0.27
	LFTOUGH[a]	Average leaf toughness	Relative scale	50.6	28.
	LFWDTH[a]	Leaf width	mm	33.8	2.4
	LIGWDTH[a]	Ligule width	mm	1.7	2.1
	LIGLGTH[a]	Ligule length	mm	6.4	6.0
	LIGNUM[a]	Ligule number	count	6.7	3.1
	PETLEN[a]	Petiole length	mm	32.3	22.1
	PHYLGTH[a]	Phyllary length	mm	8.3	21.3
	PHYNUM[a]	Phyllary number	count	8.2	4.6
	PHYSHAP[a]	Phyllary shape	mm/mm	2.1	0.61
	PHYWDTH[a]	Phyllary width	mm	5.3	1.1
	RTLG96	Root length after 96 hr	mm	6.8	6.0
	SEEDW[a]	Seed weight	mg	3.9	0.37
	STEMDIA[a]	Stem diameter 2 cm above ground	mm	7.0	1.6
Life history	BUDDAY	Days until budding	day	3.5	11.1
	FLBIO[a]	Flower biomass	g	45.7	10.4
	FLODAY[a]	Days until first floret	day	10.6	13.9
	FLRNUM[a]	Flower no. at 65 days after planting	count	8.6	0.76
	RGR	Growth rate	cm/day	0.19	0.40
	SHBIO[a]	Shoot biomass	g	161.9	40.7
Physiology	B	Leaf boron concentration	ppm	0.05	19.2
	CA	Leaf calcium concentration	ppm	2758	7064
	COND	Leaf stomatal conductance	$mmol/m^2 \cdot s$	0.44	0.88
	CI[a]	Leaf intercellular CO_2 concentration	ppm	5.5	15.3
	K[a]	Leaf potassium concentration	ppm	12481	6831
	LFSUC	Leaf succulence	(wet wt-dry wt)/ leaf area in cm^2	0.20	4.5
	MG[a]	Leaf magnesium concentration	ppm	930	582.8
	MN	Leaf manganese concentration	ppm	19.7	32.8
	NA	Leaf sodium concentration	ppm	83.6	279.4
	P[a]	Leaf phosphorus concentration	ppm	856.9	832.8
	PHOTO[a]	Leaf photosynthesis	$\mu mol/m^2 \cdot s$	4.0	4.03
	SLA[a]	Specific leaf area	cm^2/g	112.3	50.6

[a] Traits differ significantly between *H. annuus* and *H. petiolaris* (Rosenthal *et al.* 2002).

[b] Standing variation in *H. annuus* is the phenotypic standard deviation of each trait under greenhouse conditions. For details see text.

limit. Preliminary runs revealed that the test results were insensitive to the exact detection limit specified, and the final runs were performed by specifying a limit of 2%.

Intraspecific QTL analyses: Multi-allelic codominant microsatellite markers were rescored for intraspecific polymorphisms segregating from two different *H. petiolaris* genotypes: the original *H. petiolaris* parent used to generate the F_1, and the *H. petiolaris* backcross parent used to generate the BC_1. Because many of the microsatellite loci displayed just a single band segregating from *H. annuus* (the species from which the PCR primers were isolated), the number of multi-allelic markers segregating within *H. petiolaris* was not sufficient to permit QTL detection by interval mapping methods. Therefore, we combined the markers segregating from both *H. petiolaris* parental genotypes and detected intraspecific QTL by single-marker regression, using Mapmanager QTX version b16 (Manly *et al.* 2001). This allowed us to study the segregation of marker alleles and linked QTL from both *H. petiolaris* genotypes against the third *H. petiolaris* parent of the pedigree.

Our goal was to test for the presence of intraspecific QTL

at genomic locations where interspecific QTL were already known to exist and to estimate their magnitudes. Intraspecific QTL variation present at such positions was considered to be allelic with the interspecific QTL. This was considered appropriate, until the possible presence of multiple-linked QTL can be tested by fine mapping in later backcross generations. Since QTL positions were fixed prior to testing (defined by the markers nearest to interspecific QTL), likelihood ratio and χ^2 statistics for single-marker regressions are applicable (Zeng 1994; Fishman *et al.* 2002). However, declaring significance thresholds in QTL studies is subject of ongoing debate; hence significance thresholds were also reported after correction for the number of markers tested using sequential Bonferroni (Rice 1989), and the results are presented in both ways. Additive effects (a) for intraspecific QTL were obtained from linear marker regressions in Mapmanager QTX, and the PVE of each QTL was calculated from forward multiple regression in SPSS; PVE was calculated as the change in R^2 when a *H. petiolaris* marker allele was added to a regression model that already contained the *H. annuus* QTL.

RESULTS

Effect sizes of interspecific QTL: The additive effects of 185 interspecific QTL (represented in Figure 1) were scaled for the phenotypic gap between *H. annuus* and *H. petiolaris* and for the level of standing variation within the donor species *H annuus*. The complete data set including all three types of QTL size estimates (PVEs, percentage of species difference, and percentage of standing variation) is provided in the appendix, along with heterozygous additive effects (a) in the original units of measurement, as well as trait names, trait categories, linkage group numbers, and map positions as identifiers for each QTL. The QTL detected in this study were generally minor when QTL sizes were expressed in the form of traditional PVEs (mean 6.6%, mode 6.0%, range 4–17%). However, QTL were often surprisingly large when expressed as percentage of species difference between *H. annuus* and *H. petiolaris* (mean 162%, mode 95%, range 0.9–4498%) or as percentage of standing variation in *H. annuus* (mean 53%, mode 45%, range 3.5–362%).

Comparisons of QTL sizes across different trait categories are depicted in Figure 2. QTL sizes did not differ among traits with or without opposing or "complementary" QTL as long as they were expressed in terms of PVE or percentage of standing variation (Figure 2A). However, differences were dramatic when QTL sizes were expressed as percentage of species difference (Figure 2A; $t = 2.829$, $P < 0.01$). Large QTL magnitudes relative to interspecific phenotypic gaps indicate the presence of complementary genes (cryptic genetic variation), as discussed below.

Figure 2B shows differences in QTL size across three major groups of phenotypic characters: morphology, life history, and physiology. Again, differences were significant when QTL sizes were expressed as percentage of species difference, with QTL for physiological traits being on average much larger (compared to the phenotypic gap) than QTL controlling morphological or life history characters (Figure 2A; Kruskal-Wallis test: $\chi^2 = 86.127$, $P < 0.001$). This makes sense, since physiological characters in this BC_2 population had the highest proportion of traits with more than one antagonistic QTL (10 of 12 traits), indicating the greatest potential for complementary gene action.

Epistasis: Although 63 significant epistatic interactions for 18 different traits were previously observed in this interspecific BC_2 (Rieseberg *et al.* 2003), the analysis of effect sizes conducted here reveals that interaction effects were generally small compared to the main effects (Table 2; average interaction effect 1.2%, range 0.1–8.3%). The interaction effects exceeded the main effects for only two QTL combinations: for QTL controlling BRHGT on linkage groups 4 and 8b (interaction PVE is 16.2%) and for QTL controlling LIGWDTH on linkage groups 6a and 15 (interaction PVE is 6.1%). Hence, gene interactions played a minor role in this experiment compared to additive QTL main effects, which is a requirement for studying the evolutionary history of QTL through sign tests.

QTL sign tests: QTL sign tests for different trait categories allowed us to test for the role of natural selection *vs.* neutral processes in the origin of phenotypic differences between *H. annuus* and *H. petiolaris*. Table 3 shows the number of traits included in each category, the number of antagonistic QTL, total number of QTL, plus-and-minus QTL ratios, and the shape and scale of QTL distributions assumed for each group of traits. The results indicate a likely history of directional selection for morphological characters, but not for traits involved in life history or physiology (Table 3). Sign tests for different groups of morphological characters suggest that a history of directional selection is most likely for traits involved in flower morphology. Dividing physiological characters into two subcategories revealed a marginally significant test result for traits involved in photosynthesis, but suggested that too few factors of the same direction were driven to fixation to allow rejection of the null hypothesis of neutrality for mineral ion content traits.

Intraspecific QTL analyses: The genomic positions of 72 interspecific QTL were tested for the presence of intraspecific QTL segregating for the same traits within *H. petiolaris*. For 28 of these, intraspecific QTL were indeed found to be segregating within *H. petiolaris* when significance thresholds of single tests were considered ("fixed testing positions"), and 10 of these were significant after adjusting for multiple tests (Figure 1; Table 4). These figures underestimate the actual number of intraspecific QTL present in the BC_2, because tests could be performed only for QTL flanked by multi-allelic markers. The PVEs of intraspecific QTL were generally smaller than those of interspecific ones (mean is 2.2% for intraspecific QTL *vs.* 6.9% for interspecific QTL present at the same positions). However, the compari-

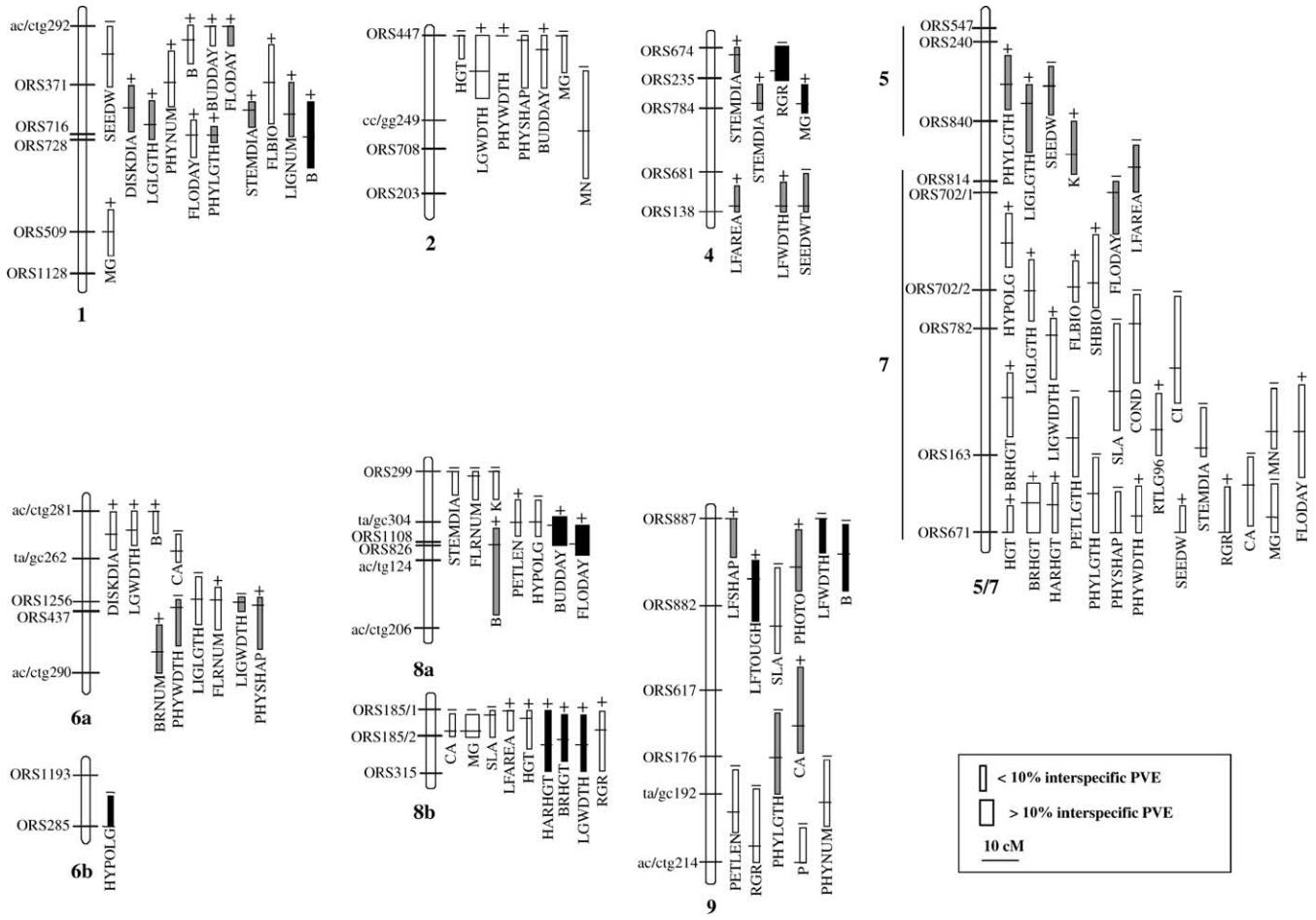

FIGURE 1.—Representation of inter- and intraspecific QTL analyses in a second generation backcross (BC_2) population between *H. annuus* and *H. petiolaris*. Marker names are listed to the left of each linkage group, and boxes to the right of each group indicate QTL positions, PVE, and QTL directions (+/−). Horizontal bars mark QTL likelihood peaks within 1-LOD support limits. Linkage groups were assigned according to genetic maps for *H. annuus* (BURKE *et al.* 2004). Marker groupings that differed between the inter- and intraspecific maps due to fragmentation or pseudolinkage in the interspecific BC_2 are indicated by *a* and *b* or by thick black lines to the left of a group, respectively. Interspecific QTL whose positions were tested for the presence of intraspecific QTL variation are indicated by shading, and positions at which an intraspecific QTL was found are solid; *i.e.*, solid and shaded QTL positions together indicate the trait/locus combinations tested. Trait abbreviations are explained in Table 1.

son of intra- *vs.* interspecific PVEs for each QTL in Figure 3 also reveals two outliers: a QTL for boron uptake (B) and one for magnesium uptake (MG) that had markedly higher intraspecific PVEs. In fact, intraspecific PVEs for these QTL were roughly comparable to the interspecific ones (Figure 3). These traits/QTL have been shown to be under strong directional selection in the same interspecific hybrid population when planted in the habitats of two wild sunflower hybrid species (LEXER *et al.* 2003b,c; GROSS *et al.* 2004). Thus, our results indicate the presence of potentially adaptive QTL variation *within* a wild sunflower population.

DISCUSSION

Interspecific QTL magnitudes relative to backcross variance, standing variation, and phenotypic gaps: QTL effect sizes or magnitudes in this study were estimated in three different biologically meaningful ways: relative to the phenotypic gap between the two sunflower species studied, relative to the standing variation in the donor species *H. annuus*, and in terms of PVE in the interspecific backcross (BC_2) mapping population. This allowed us to compare all three QTL size estimates for a data set of 185 QTL for 40 phenotypic traits (Figure 1; APPENDIX), most of which exhibited significant interspecific differences between *H. annuus* and *H. petiolaris* (Table 1). The differences among the three types of QTL size estimates were striking (Figure 2). Most of the QTL detected were minor when expressed in terms of PVE but were suprisingly large when expressed in terms of standing variation or species differences (Figure 2; APPENDIX). How can these contrasting results be reconciled and what are their implications for the genetic architecture of species differences?

Comparisons with the QTL literature are only partially explanatory. MORJAN and RIESEBERG (2004) reviewed QTL studies on undomesticated animals and

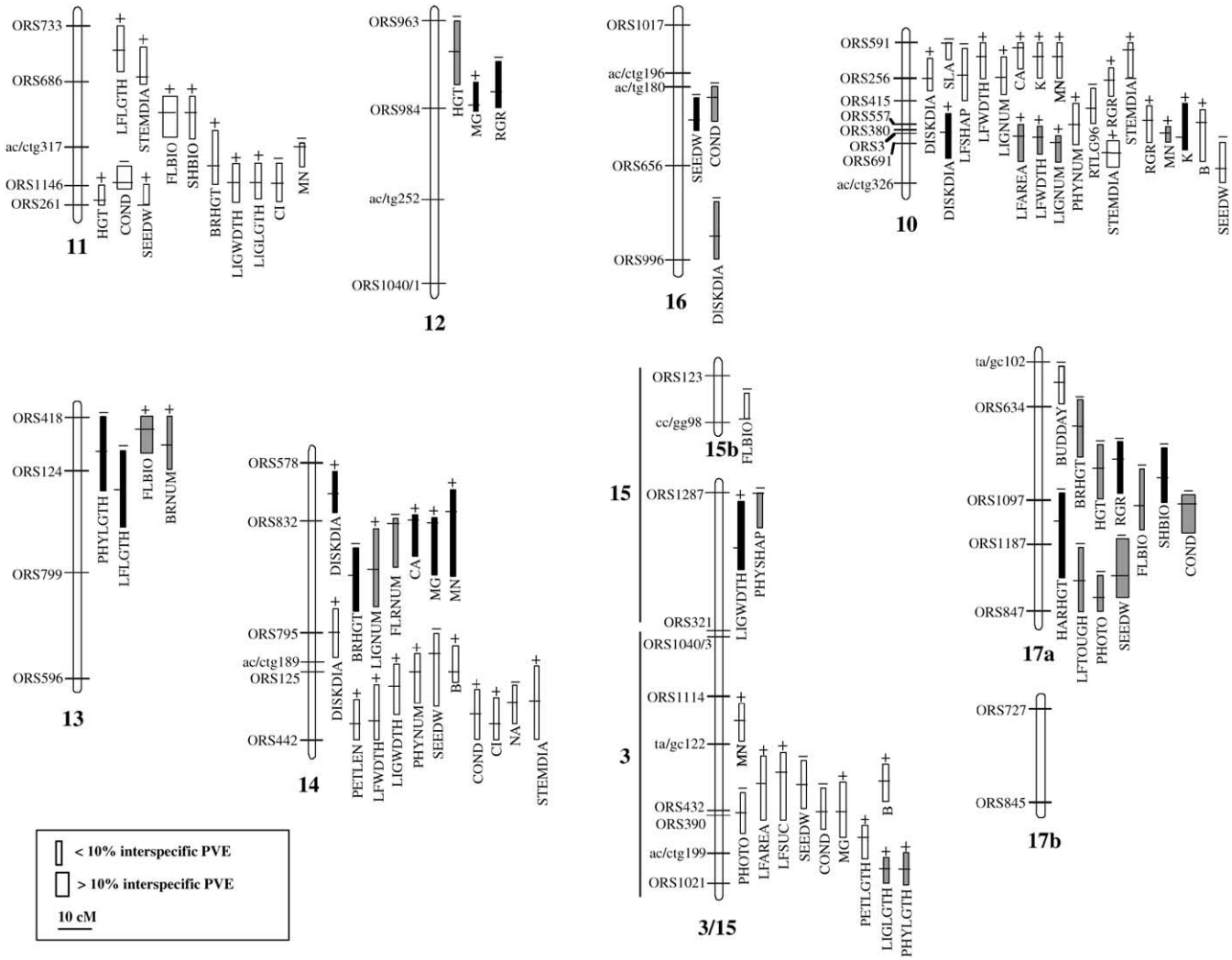

FIGURE 1.—*Continued.*

reported QTL sizes in terms of PVE, standing variation, and parental line differences for the leading QTL for data sets of 79, 20, and 26 traits, respectively. For ease of comparison, we report averages over *all* QTL for each trait here (leading and minor QTL combined). Consistent with the present data on Helianthus, average QTL sizes in the literature were relatively small when measured as PVE (8.0 ± 0.7%) compared to estimates in terms of standing variation (74.2 ± 14.3%) or parental line difference (30.0 ± 4.6%), although the ranking of the latter two types of estimates was reversed compared to the present study. However, existing QTL studies generally did not measure QTL sizes in all three ways in parallel; hence differences among estimation methods may be confounded with differences among taxa, trait types, or mating systems. Indeed, all three factors had a significant influence on average QTL size in the literature ($P < 0.005$; *F*-test of one-way ANOVAs). Comparisons with the QTL literature on wild plants (reviewed by RIESEBERG and BURKE 2001) are even less conclusive because QTL sizes were rarely reported in ways other than PVE, a notable exception being an interspecific QTL analysis of floral traits in Mimulus (FISHMAN *et al.* 2002). In that study, QTL effects were generally small regardless of how they were estimated.

The finding that most QTL detected in our study are minor when expressed in terms of PVE is not surprising considering that our mapping population segregated for both intra- and interspecific variation. However, in wild species it may be more useful to estimate QTL sizes relative to levels of standing variation or interspecific phenotypic gaps, particularly if the focus is on species divergence and genetic effects that are "visible" to natural selection. Our finding that QTL explain on average 53% of the standing variation in the donor species *H. annuus* is remarkable. Comparing QTL to the phenotypic standard deviation in *H. annuus* is conservative because phenotypic variation in wild populations of this species is high as demonstrated by greenhouse studies (ROSENTHAL *et al.* 2002; WELCH and RIESEBERG 2002a) and field experiments (LEXER *et al.* 2003c; GROSS *et al.* 2004). In the most comprehensive of these studies (ROSENTHAL *et al.* 2002), for example, phenotypic standard deviations in *H. annuus* averaged 1.6 times that of

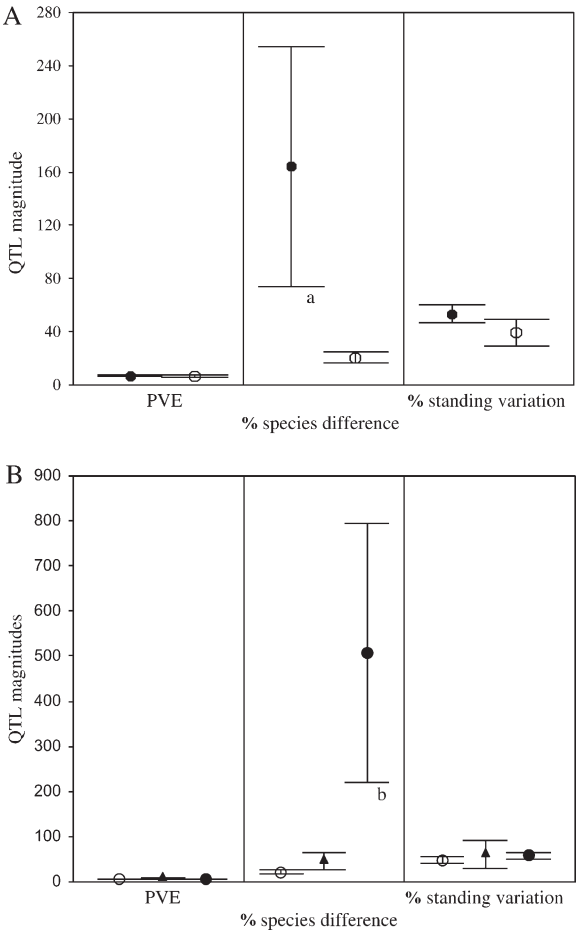

FIGURE 2.—Interspecific QTL sizes measured as PVE, percentage of species difference, and percentage of standing variation in *H. annuus*, compared across different trait categories. (A) Traits with complementary genes (solid circles) and traits without complementary genes (open circles). (B) Morphological traits (open circles), life history traits (solid triangles), and physiological traits (solid circles). "a" indicates the difference between trait categories: $P < 0.01$, *t*-test with equal variances not assumed. "b" indicates the difference between categories $P < 0.001$, nonparametric Kruskal-Wallis test.

TABLE 2

Summary of epistatic interaction effects for each trait

Trait[a]	No. of interactions ($P \leq 1.0 \times 10^{-05}$)[b]	Average PVE of interactions[c]
BRHGT	2	8.3
DISKDIA	4	0.9
HARGHT	6	0.2
HGT1	3	0.4
LFAREA	1	0.3
LIGWDTH	5	1.4
LGLGTH	1	1.0
LIGNUM	1	0.4
PHYNUM	2	1.7
STEMDIA	1	0.6
BUDDAY	5	0.9
FLODAY	2	1.2
RGR	2	0.3
SHBIO	1	0.1
B	6	0.6
Ca	8	0.4
Mg	5	1.6
Mn	7	1.7
PHOTO	1	0.1

[a] For definitions of trait abbreviations, see Table 1.
[b] Detection of epistatic interactions described by RIESEBERG *et al.* (2003).
[c] Traits not involved in significant interactions are not listed.

H. petiolaris. Thus, these minor QTL, when defined in terms of PVE, do represent formidable genetic effects "in the eyes of natural selection."

An even more striking finding is that, on average, QTL explained 162% of the species difference between *H. annuus* and *H. petiolaris*. This extraordinary result apparently is possible because of the frequent presence of QTL with opposing effects (*i.e.*, complementary genes), which allows the cumulative effect of all plus QTL or all minus QTL for a given trait to be considerably larger than the interspecific gap. Consistent with this hypothesis, QTL magnitudes for traits with complementary genes are much larger than those for traits lacking complementary genes (Figure 2A). Likewise, QTL sizes in terms of percentage of species difference were larger for physiological traits compared to morphological or life history traits (Figure 2B). Again, physiology was the trait category that exhibited the largest proportion of traits with complementary genes.

Signature of selection *vs.* neutral processes in wild Helianthus: The signature of selection on interspecific character differences in sunflowers differed markedly from a previous literature survey of 86 QTL studies involving 572 traits in animals and plants (RIESEBERG *et al.* 2002). That study showed that selection was the primary cause of interspecific differences for all trait categories and that physiological and life history traits were more strongly selected on average than morphological traits. In this study, however, significant selection was detected for flower morphology (QTL ratios for this trait category were similar to those reported by RIESEBERG *et al.* 2002), but *not* for life history or physiology (Table 3).

The elevated QTL ratios (*i.e.*, high proportions of opposing QTL) for life history and physiological traits in Helianthus are puzzling. *H. annuus* and *H. petiolaris* differ substantially for most of these traits (Table 1), so high QTL ratios cannot be attributed to a lack of differentiation. Possibly, these trait differences arose very early in the divergence of the two species and have been maintained by stabilizing selection since then, which might permit the accumulation of opposing QTL. Regardless of the explanation, the high proportion of opposing QTL appears to have facilitated ecological divergence of the three homoploid hybrid species arising from this cross (RIESEBERG *et al.* 2003). For example,

TABLE 3

QTL sign tests for different trait categories

Trait category	No. of traits included	No. of antagonistic QTL	Total QTL	QTL ratio	Gamma (shape/scale)	*P*
Morphology	21	27	94	0.287	1/22.7	0.000****
Root/shoot morphology	7	10	31	0.322	1/20.83	0.110
Flower morphology	8	9	42	0.214	1/23.26	0.000****
Leaf morphology	6	7	21	0.333	1/24.39	0.330
Life history	6	10	29	0.345	1/17.86	0.204
Physiology	9	19	49	0.388	1/22.22	0.167
Mineral ion content	5	15	33	0.455	1/24.15	0.708
Photosynthesis	4	4	16	0.250	1/20.00	0.091

**** Significance levels for each test $P < 0.001$.

selection studies indicate that regulation of mineral ion uptake played a central role in salt mash adaptation by *H. paradoxus* (Lexer *et al.* 2003b,c). Most of the mineral ion content characters studied here exhibit large proportions of complementary (opposing) QTL (see appendix). This cryptic variation is released in hybrids (Lexer *et al.* 2003b; Rieseberg *et al.* 2003), resulting in transgressive character expression and increased oppor-

TABLE 4

Results of intraspecific QTL analyses

Trait[a]	Linkage group	Test position (cM)	Marker locus	Segregation	LR/*P*-value[b]	Additive effect[c]	R^2 of *H. annuus* QTL (%)	R^2 change through *H. petiolaris* QTL (%)
B	1	4	ORS716	1:1	19.0/ 0.0000[a]	+6.28	6	3
	9	4	ORS887	1:3	18.1/0.0000[a]	+11.51	6	6
BRHGT	14	36	ORS832	1:3	8.7/0.0032	+2.37	6	2
	8b	9	ORS315	1:3	5.0/0.0258	+1.95	7	1.5
BUDDAY	8a	16	ORS1108	1:1	11.3/0.0008[a]	−1.39	11	2
Ca	14	18	ORS832	1:3	7.2/0.0074	−774.74	4	1
DISKDIA	10	25	ORS380	1:3	14.5/0.0001[a]	+0.77	4	2.5
	14	9	ORS578	1:3	7.8/0.0053	+0.74	6	1
FLODAY	8a	20	ORS1108	1:1	12.2/0.0005[a]	−1.98	12	3
HARGHT	17a	46	ORS1097	1:1	10.4/0.0013[a]	+9.71	7	3
	8b	11	ORS315	1:1	8.8/0.0031	−7.85	7	2
HYPOLG	6b	15	ORS1193	1:3	8.6/0.0033	+0.93	6	1
K	10	26	ORS3	1:3	9.2/0.0025	−2139	7	1
LFLGTH	13	24	ORS799	1:3	5.8/0.0160	−3.41	6	1
LFTOUGH	9	19	ORS887	1:3	8.0/0.0047	−5.57	5	1
LFWDTH	9	0	ORS887	1:3	5.2/0.0228	+2.24	9	1.5
LIGWDTH	8b	12	ORS315	1:3	6.5/0.0108	+0.4	7	1
	15a	18	ORS1287	1:3	12.1/0.0005[a]	−0.52	5	3
Mg	4	17	ORS784	1:1	24.9/0.0000[a]	−306.94	8	7
	12	28	ORS984	1:3	6.1/0.0133	−161.47	9	2
	14	18	ORS832	1:3	4.7/0.0298	−129.9	5	1
Mn	14	14	ORS832	1:3	11.8/0.0006[a]	−20.73	7	4
PHYLGTH	13	11	ORS418	1:3	6.4/0.0116	+0.68	6	2
RGR	4	17	ORS784	1:3	8.2/0.0042	−0.15	8	2
	12	25	ORS984	1:3	5.4/0.0206	+0.09	8	1
	17a	29	ORS1097	1:1	10.9/0.0010[a]	+0.14	6	3
SEEDW	16	29	ORS656	1:3	7.0/0.0080	+0.09	6	2
SHBIO	17a	34	ORS1097	1:1	6.0/0.0141	−8.05	8	1

[a] For definitions of trait abbreviations, see Table 1.
[b] Significant at the $P < 0.05$ level after correction for multiple tests.
[c] Additive effects are given in the original unit of measurement for each trait.

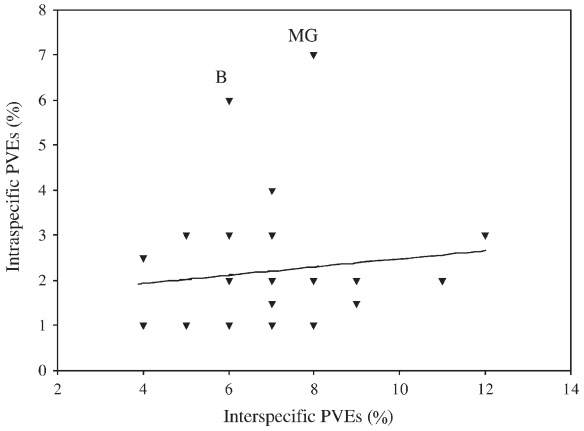

FIGURE 3.—Comparison of inter- *vs.* intraspecific QTL sizes measured as PVE in the interspecific BC_2, including best linear fit (Spearman's $r = 0.121$; $P = 0.540$). "B" and "MG" indicate QTL for leaf boron and magnesium content with unusually high intraspecific PVEs.

tunity for selection (LEXER *et al.* 2003b,c; GROSS *et al.* 2004).

Intra- *vs.* interspecific QTL variation and the spread of advantageous alleles: To our knowledge, our study represents the first attempt to map inter- and intraspecific QTL for the same taxa in the same environment. Our intraspecific QTL analysis allowed us to ask whether intraspecific QTL variation was present at genomic locations where interspecific QTL were known to exist and whether intra- and interspecific QTL differed in magnitude. Among a total of 72 genomic locations tested, we were able to detect 28 intraspecific QTL segregating among the recurrent *H. petiolaris* parental genotypes (Figure 1; Table 4), although this is certainly an underestimate of the true number of intraspecific QTL present. Nevertheless, our intraspecific QTL data allowed us to gain insights into the nature of intra- *vs.* interspecific differences in wild Helianthus.

Perhaps the most important observation is that PVEs were generally smaller for intraspecific compared to interspecific QTL (Table 4; Figure 3). This is consistent with the prediction that QTL underlying interspecific differences should be larger than those polymorphic within species (RIESEBERG and BURKE 2001). Larger QTL (or QTL under strong selection) are more likely to spread to fixation across a subdivided population, whereas minor QTL or loci with small selection coefficients may be trapped in local populations and are therefore less likely to contribute to fixed differences among species (RIESEBERG and BURKE 2001; RIESEBERG *et al.* 2004). Population subdivision is expected to magnify the differences in time to fixation for these two classes of QTL, and this is likely to be the case for *H. petiolaris*; at least two subspecies have been recognized (HEISER *et al.* 1969), and populations of *H. petiolaris* are significantly differentiated for codominant nuclear genetic markers (WELCH and RIESEBERG 2002b). However, population subdivision is not required to explain the observed differences between inter- and intraspecific QTL magnitudes. This is the case because alleles fixed among species are *not* a random sample of those present within species or populations. Any allele's chance of contributing to among-species fixed differences is a function of the selection coefficient, *s* (ORR 1998a), and this will bias fixation of interspecific differences toward larger QTL, since many/most species differences were created or are currently maintained by directional selection (ORR 2001; RIESEBERG *et al.* 2002; LEXER *et al.* 2003a).

With respect to practical implications, our intraspecific QTL results illustrate what is predicted by common sense and theory: that QTL detection in outbred pedigrees will almost always capture a certain amount of intraspecific QTL variation in addition to polymorphism between the parental lines (LYNCH and WALSH 1998). This reduction in homogeneity of the genetic background may result in underestimates of interspecific QTL magnitudes when expressed as PVE, because intraspecific polymorphism will increase the phenotypic variance in the mapping population against which interspecific QTL are compared. This is exactly what was observed in this experiment. However, we argue that the price of this deviation from an idealized line cross model (ZENG 1994; LYNCH and WALSH 1998) is outweighed by the benefits of comparing intra- and interspecific QTL architectures in the same experiment. This is particularly important for speciation genetic studies that (by definition) address the interface between intra- and interspecific variability.

Conclusions and outlook: As demonstrated by the present study on Helianthus, estimating QTL magnitudes in several biologically meaningful ways has the potential to (1) increase the amount of information that can be extracted from QTL data sets; (2) improve our understanding of the distribution of factors fixed during adaptation and speciation (FISHER 1930; WRIGHT 1931; ORR 1998a, 2001; BARTON and KEIGHTLY 2002); and (3) increase our ability to make meaningful comparisons among QTL experiments. With respect to QTL sign tests, our findings indicate that the ecological divergence of hybrid lineages is unlikely to involve traits with a history of selection during the divergence of the parental species. Rather, hybrid speciation seems to exploit the cryptic variation released at unselected traits. Finally, our results may encourage the design of QTL mapping projects that detect both inter- and intraspecific QTL within the same experiment, thereby enabling direct comparisons of the genetic basis of intra- and interspecific trait differences.

We thank Jennifer Durphy and Andrea Schwarzbach for their invaluable help during molecular and phenotypic data collection, John Burke and Kevin Livingstone for helpful discussions about various aspects of QTL analysis, and Mike Fay for helpful comments on the manuscript. This work was supported by grant R01 G59065 of the

National Institutes for Health to L.H.R. and Erwin-Schroedinger grant J2148 of the Austrian Science Foundation to C.L.

LITERATURE CITED

Anderson, E. C., and M. Slatkin, 2003 Orr's quantitative trait loci sign test under conditions of trait ascertainment. Genetics **165:** 445–446.

Barton, N. H., and P. D. Keightly, 2002 Understanding quantitative genetic variation. Nat. Rev. Genet. **3:** 11–21.

Bradshaw, H. D., K. G. Otto, E. Frewen-Barbara, K. McKay-John and D. W. Schemske, 1998 Quantitative trait loci affecting differences in floral morphology between two species of monkeyflower (Mimulus). Genetics **149:** 367–382.

Burke, J. M., S. Tang, S. J. Knapp and L. H. Rieseberg, 2002 Genetic analysis of sunflower domestication. Genetics **161:** 1257–1267.

Burke, J. M., Z. Lai, M. Salmaso, T. Nakazato, S. Tang *et al.*, 2004 Comparative mapping and rapid karyotypic evolution in the genus Helianthus. Genetics **167:** 449–457.

de Vienne, D. (Editor), 2003 *Molecular Markers in Plant Genetics and Biotechnology.* Science Publishers, Plymouth, UK.

deVincente, M. C., and S. D. Tanksley, 1993 QTL analysis of transgressive segregation in an interspecific tomato cross. Genetics **134:** 585–596.

Fisher, R. A., 1930 *The Genetical Theory of Natural Selection.* Oxford University Press, Oxford, UK.

Fishman, L., A. J. Kelly and J. H. Willis, 2002 Minor quantitative trait loci underlie floral traits associated with mating system divergence in *Mimulus.* Evolution **56:** 2138–2155.

Greenberg, A. J., J. R. Moran, J. A. Coyne and C.-I. Wu, 2003 Ecological adaptation during incipient speciation revealed by precise gene replacement. Science **302:** 1754–1757.

Gross, B. L., N. C. Kane, C. Lexer, F. Ludwig, D. M. Rosenthal *et al.*, 2004 Reconstructing the origin of *Helianthus deserticola*: survival and selection on the desert floor. Am. Nat. **164:** 145–156.

Haldane, J. B. S., 1938 The nature of interspecific differences, pp. 19–94 in *Evolution*, edited by G. R. de Beer. Clarendon Press, Oxford, UK.

Hawthorne, D. J., and S. Via, 2001 Genetic linkage of ecological specialization and reproductive isolation in pea aphids. Nature **412:** 904–907.

Heiser, C. B., D. M. Smith, S. B. Clevenger and W. C. J. Martin, 1969 The North American sunflowers (*Helianthus*). Mem. Torr. Bot. Club **22:** 1–218.

Kao, C.-H., Z-B. Zeng and R. D. Teasdale, 1999 Multiple interval mapping for quantitative trait loci. Genetics **152:** 1203–1216.

Lexer, C., R. A. Randell and L. H. Rieseberg, 2003a Experimental hybridization as a tool for studying selection in the wild. Ecology **84:** 1688–1699.

Lexer, C., M. E. Welch, O. Raymond and L. H. Rieseberg, 2003b Natural selection for salt tolerance QTLs in wild sunflower hybrids: implications for the origin of *Helianthus paradoxus*, a diploid hybrid species. Mol. Ecol. **12:** 1225–1235.

Lexer, C., M. E. Welch, O. Raymond and L. H. Rieseberg, 2003c The origin of ecological divergence in *Helianthus paradoxus* (Asteraceae): selection on transgressive characters in a novel hybrid habitat. Evolution **57:** 1989–2000.

Lynch, M., and J. B. Walsh, 1998 *Genetics and Analysis of Quantitative Traits.* Sinauer Associates, Sunderland, MA.

Macdonald, S. J., and D. B. Goldstein, 1999 A quantitative genetic analysis of male sexual traits distinguishing the sibling species *Drosophila simulans* and *D. sechellia.* Genetics **153:** 1683–1699.

Manly, K. F., R. H. Cudmore, Jr. and J. M. Meer, 2001 Map Manager QTX, cross-platform software for genetic mapping. Mamm. Genome **12:** 930–932.

Mauricio, R., 2001 Mapping quantitative trait loci in plants: uses and caveats for evolutionary biology. Nat. Rev. Genet. **2:** 370–381.

Morjan, C. L., and L. H. Rieseberg, 2004 How species evolve collectively: implications of gene flow and selection for the spread of advantageous alleles. Mol. Ecol. **13:** 1341–1356.

Navarro, A., and N. H. Barton, 2003 Accumulating postzygotic isolation genes in parapatry: a new twist on chromosomal speciation. Evolution **57:** 447–459.

Noor, M. A. F., K. L. Grams, L. A. Bertucci and J. Reiland, 2001 Chromosomal inversions and the persistence of species. Proc. Natl. Acad. Sci. USA **98:** 12084–12088.

Orr, H. A., 1998a The population genetics of adaptation: the distribution of factors fixed during adaptive evolution. Evolution **52:** 935–949.

Orr, H. A., 1998b Testing natural selection *vs.* genetic drift in phenotypic evolution using quantitative trait locus data. Genetics **149:** 2099–2104.

Orr, H. A., 2001 The genetics of species differences. Trends Ecol. Evol. **16:** 343–350.

Orr, H. A., and J. A. Coyne, 1992 The genetics of adaptation revisited. Am. Nat. **140:** 725–742.

Presgraves, D. C., L. Balagopalan, S. M. Abmayr and H. A. Orr, 2003 Adaptive evolution drives divergence of a hybrid inviability gene between two species of *Drosophila.* Nature **423:** 715–719.

Rice, W. R., 1989 Analyzing tables of statistical tests. Evolution **43:** 223–225.

Rice, W. R., and E. E. Hostert, 1993 Laboratory experiments on speciation: What have we learned in 40 years? Evolution **47:** 1637–1653.

Rieseberg, L. H., 1991 Homoploid reticulate evolution in *Helianthus*: evidence from ribosomal genes. Am. J. Bot. **78:** 1218–1237.

Rieseberg, L. H., and J. M. Burke, 2001 The biological reality of species: gene flow, selection, and collective evolution. Taxon **50:** 235–255.

Rieseberg, L. H., S. Beckstrom-Sternberg, A. Liston and D. Arias, 1991 Phylogenetic and systematic inferences from chloroplast DNA and isozyme variation in *Helianthus* sect. *Helianthus.* Syst. Bot. **16:** 50–76.

Rieseberg, L. H., C. Van Fossen, A. Desrochers, 1995 Hybrid speciation accompanied by genomic reorganization in wild sunflowers. Nature **375:** 313–316.

Rieseberg, L. H., M. A. Archer and R. K. Wayne, 1999 Transgressive segregation, adaptation and speciation. Heredity **83:** 363–372.

Rieseberg, L. H., A. Widmer, M. A. Arntz and J. M. Burke, 2002 Directional selection is the primary cause of phenotypic diversification. Proc. Natl. Acad. Sci. USA **99:** 12242–12245.

Rieseberg, L. H., O. Raymond, D. M. Rosenthal, Z. Lai, K. Livingstone *et al.*, 2003 Major ecological transitions in wild sunflowers facilitated by hybridization. Science **301:** 1211–1216.

Rieseberg, L. H., S. A. Church and C. L. Morjan, 2004 Integration of populations and differentiation of species. New Phytol. **161:** 59–69.

Rosenthal, D., A. E. Schwarzbach, L. A. Donovan, O. Raymond and L. H. Rieseberg, 2002 Phenotypic differentiation between three ancient hybrid taxa and their parental species. Int. J. Plant Sci. **163:** 387–398.

Schemske, D. W., and H. D. Bradshaw, Jr., 1999 Pollinator preference and the evolution of floral traits in monkeyflowers (*Mimulus*). Proc. Natl. Acad. Sci. USA **96:** 11910–11915.

Sucena, E., and D. L. Stern, 2000 Divergence of larval morphology between *Drosophila sechellia* and its sibling species caused by cis-regulatory evolution of *ovo/shaven-baby.* Proc. Natl. Acad. Sci. USA **97:** 4530–4534.

True, J. R., J. Liu, L. F. Stam, Z-B. Zeng and C. C. Laurie, 1997 Quantitative genetic analysis of divergence in male secondary sexual traits between *Drosophila simulans* and *Drosophila mauritiana.* Evolution **51:** 816–832.

Welch, M. E., and L. H. Rieseberg, 2002a Habitat divergence between a homoploid hybrid sunflower species, *Helianthus paradoxus* (Asteraceae), and its progenitors. Am. J. Bot. **89:** 472–479.

Welch, M. E., and L. H. Rieseberg, 2002b Patterns of genetic variation suggest a single, ancient origin for the diploid hybrid species *Helianthus paradoxus.* Evolution **56:** 2126–2137.

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

Zeng, Z-B., 1994 Precision mapping of quantitative trait loci. Genetics **136:** 1457–1468.

Zeng, Z-B., J. Liu, L. F. Stam, C.-H. Kao, J. M. Mercer *et al.*, 2000 Genetic architecture of a morphological shape difference between two Drosophila species. Genetics **154:** 299–310.

Communicating editor: M. Feldman

APPENDIX

Complete interspecific QTL data set discussed in this study

Trait[a]	Category	Linkage group	Position (cM)[b]	Additive effect	QTL size		
					PVE	% species difference	% standing variation
BRHGT	Morphology	7	105	5.35	8	16	36
		7	133	4.1	10	12	28
		11	38	6.05	7	18	41
		14	36	−4.24	6	13	29
		17a	18	−4.89	5	15	33
		8b	9	4.68	7	14	32
BRNUM	Morphology	13	5	0.91	8	17	17
		6a	40	1.54	5	28	28
DISKDIA	Morphology	1	24	1.13	5	10	30
		10	11	1.46	7	13	28
		10	25	1.23	4	11	24
		14	9	1.11	6	10	22
		14	53	1.05	6	9	20
		16	64	−0.68	6	6	13
		6a	6	2.02	9	18	39
HARGHT	Morphology	17a	46	−18.15	7	22	53
		7	134	17.84	8	22	52
		8b	11	16.03	7	19	47
HGT1	Morphology	11	47	2.99	7	11	38
		12	9	−2.73	7	10	34
		17a	31	−2.44	5	9	31
		2	0	−1.93	6	7	24
		7	139	1.33	7	5	17
		8b	2	1.62	7	6	20
HYPOLG	Morphology	6b	15	−1.38	6	46	32
		7	60	3.1	6	104	72
		8a	14	−1.21	8	41	28
LFAREA	Morphology	10	27	3.08	7	8	8
		3	93	3.1	6	8	8
		4	49	2.73	6	7	7
		7	40	−2.65	6	7	7
		8b	0	1.74	5	5	5
LFLGTH	Morphology	11	7	13.25	6	42	48
		13	24	−7.99	6	25	29
LFSHAP	Morphology	10	8	−0.27	7	42	100
		9	0	0.22	9	34	82
LFTOUGH	Morphology	17a	65	−8.2	5	16	29
		9	19	16.47	5	33	59
LFWDTH	Morphology	10	4	3.78	4	11	158
		10	26	4.25	6	13	177
		14	79	2.94	5	9	123
		4	48	3.9	6	12	163
		9	0	−3.9	9	12	163
LIGWDTH	Morphology	11	44	0.59	6	34	28
		14	70	1.08	8	63	51
		15a	18	1.15	5	67	55
		2	10	0.99	10	58	47
		6a	4	1.46	7	85	70
		6a	25	−0.58	5	34	28
		7	85	0.78	6	45	37
		8b	12	0.71	7	41	34
LIGLGTH	Morphology	1	31	1.63	5	25	27
		11	44	1.83	4	29	31
		3	122	4.25	4	66	71
		5	21	3.33	5	52	56

(*continued*)

APPENDIX

(Continued)

Trait[a]	Category	Linkage group	Position (cM)[b]	Additive effect	QTL size		
					PVE	% species difference	% standing variation
		6a	25	−4.47	6	70	75
		7	72	3.05	4	48	51
LIGNUM	Morphology	1	25	1.06	8	16	35
		10	10	1.03	8	15	34
		10	28	1.16	6	17	38
		14	34	1.63	7	24	53
PETLEN	Morphology	14	80	6.67	4	21	30
		3	112	12.36	6	38	56
		7	113	−3.96	7	12	18
		8a	14	5.44	6	17	25
		9	89	−6.87	7	21	31
PHYLGTH	Morphology	1	34	0.95	7	11	24
		13	11	−1	6	12	26
		3	120	2.13	6	26	55
		5	15	1.3	5	16	33
		7	130	−0.81	9	10	21
		9	74	−0.74	7	9	19
PHYNUM	Morphology	1	19	1.28	9	16	28
		10	23	0.93	7	11	20
		14	66	1.41	7	17	31
		9	88	−0.86	6	11	19
PHYSHAP	Morphology	15a	0	−0.14	5	7	23
		2	1	−0.46	5	22	75
		6a	26	0.79	6	39	130
		7	139	−1.01	8	49	166
PHYWDT	Morphology	2	0	0.26	7	5	24
		6a	26	−0.28	5	5	26
		7	135	0.22	6	4	20
RTLG96	Morphology	10	19	−3.05	5	45	51
		7	112	3.93	5	58	66
SEEDW	Morphology	1	10	−0.16	5	4	43
		10	37	−0.16	5	4	43
		11	49	0.15	5	4	41
		14	59	−0.14	5	4	38
		16	29	−0.33	6	8	89
		17a	62	−0.21	17	5	57
		3	94	−0.35	5	9	95
		4	45	−0.2	4	5	54
		5	16	−0.34	6	9	92
		7	139	0.15	7	4	41
STEMDIA	Morphology	1	23	0.67	7	10	41
		10	3	0.89	8	13	55
		10	32	1.29	11	19	79
		11	15	1.27	6	18	78
		14	75	0.72	4	10	44
		4	2	0.98	7	14	60
		4	17	0.8	5	11	49
		7	116	−0.97	6	14	60
		8a	0	−0.81	9	12	50
BUDDAY	Life history	1	0	2.33	7	67	21
		17a	6	−2.81	9	8	25
		2	5	1.42	7	41	13
		8a	16	2.09	11	60	19
FLBIO	Life history	1	19	2.63	7	6	25
		11	25	7.34	10	16	71

(*continued*)

APPENDIX

(Continued)

Trait[a]	Category	Linkage group	Position (cM)[b]	Additive effect	QTL size		
					PVE	% species difference	% standing variation
		13	3	2.39	10	5	23
		15b	12	−3.92	8	9	38
		17a	43	−2.17	8	5	21
		7	71	4.05	6	9	39
FLODAY	Life history	1	0	2.53	6	24	18
		1	34	1.72	5	16	12
		7	46	−2.77	7	26	20
		7	115	2.15	5	20	15
		8a	20	2.44	12	23	18
FLRNUM	Life history	14	19	−1.51	5	18	199
		6a	25	2.75	7	32	362
		8a	2	−2.02	7	24	266
RGR	Life history	10	11	0.18	6	95	45
		10	23	0.18	6	95	45
		12	25	−0.22	8	116	55
		17a	29	−0.39	6	205	98
		4	5	−0.2	11	105	50
		7	139	0.19	7	100	48
		8b	6	0.18	8	95	45
		9	101	−0.18	9	95	45
SHBIO	Life history	11	25	22.97	6	14	56
		17a	34	−1.44	8	1	4
		7	70	20.61	7	13	51
B	Physiology	1	4	13.15	6	2630	69
		1	35	14.27	4	2854	74
		10	22	13.07	6	2614	68
		14	63	10.69	9	2138	56
		3	85	16.92	5	3384	88
		6a	0	22.49	7	4498	117
		8a	21	8.26	6	1652	43
		9	13	−10.84	5	2168	57
Ca	Physiology	10	1	2256	5	82	32
		14	18	1682	4	61	24
		6a	10	−2411	9	87	34
		7	126	−1760	5	64	25
		8b	6	−1738	7	63	25
		9	60	1443	6	52	20
COND	Physiology	11	44	−0.33	10	75	38
		14	76	0.23	6	52	26
		16	21	−0.36	6	82	41
		17a	42	−0.27	10	61	31
		3	102	−0.26	8	59	68
		7	82	−0.35	6	80	40
CI	Physiology	11	44	−4.24	8	77	28
		14	79	4.19	8	76	28
		7	94	−5.83	8	106	38
K	Physiology	10	5	5338	7	43	78
		10	28	4565	5	37	67
		5	35	4761	4	38	70
		8a	0	−3314	6	27	49
LFSUC	Physiology	3	91	2.73	5	1365	61
Mg	Physiology	1	66	322.9	8	35	55
		12	28	268.47	9	29	46
		14	18	260.92	5	28	45
		2	0	−233.17	5	25	40

(*continued*)

APPENDIX

(Continued)

Trait[a]	Category	Linkage group	Position (cM)[b]	Additive effect	QTL size		
					PVE	% species difference	% standing variation
		3	103	331.78	5	36	57
		4	17	405.72	8	44	70
		7	137	−290.13	7	31	50
		8b	6	−324.01	10	35	56
Mn	Physiology	10	5	46.56	7	236	142
		10	26	42.68	7	216	130
		11	36	−14.57	5	74	44
		14	14	36.99	7	188	113
		2	31	−21.8	5	111	67
		3	74	40	7	203	122
		7	116	−24.14	5	122	74
Na	Physiology	14	72	−138.21	6	165	50
P	Physiology	9	105	−285.71	6	33	34
PHOTO	Physiology	17a	70	−2.22	8	56	55
		3	102	−2.1	8	53	52
		9	16	4.27	5	107	106
SLA	Physiology	10	0	−26.05	5	23	52
		7	100	−19.27	6	17	38
		8b	1	−16.03	5	14	32
		9	35	−33.37	4	30	66

PVE, percentage of variation explained in the interspecific backcross.

[a] For definitions of trait abbreviations, see Table 1.

[b] QTL were detected at an experiment-wide significance threshold of $P < 0.05$ (Rieseberg *et al.* 2003).