QTL Analysis of Intraspecific Differences between Two *Silene vulgaris* Ecotypes

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**Background and Aims** Serpentine soils provide a highly selective substrate for plant colonization and growth and represent an ideal system for studying the evolution of plant-ecotypes. In the present study the aim was to identify the genetic architecture of morphological traits distinguishing serpentine and non-serpentine ecotypes of *Silene vulgaris*.

**Methods** Using an *F₂* mapping population derived from an intraspecific cross between a serpentine and a non-serpentine ecotype of *S. vulgaris*, the genetic architecture of 12 morphological traits was explored using a quantitative trait locus (QTL) analysis.

**Key Results** The QTL analysis identified a total of 49 QTLs, of which 24 were classified as major QTLs. The mean number of QTLs per trait category was found to correspond well with numbers reported in the literature for similar crosses. Clustering of QTLs for different traits was found on several linkage groups.

**Conclusions** Morphological traits that differentiate the two ecotypes are strongly correlated, presumably as a consequence of the joint effects of extensive linkage of QTLs for different traits and directional selection. The signature of consistent directional selection was found for leaf and shoot trait divergence. Intraspecific ecotype differences in *S. vulgaris* were found to be distributed across the entire genome. The study shows that QTL analyses on non-model organisms can provide novel insights into the genetic basis of plant diversification.

**Key words:** AFLP, directional selection, ecological divergence, ecotype, habitat adaptation, intraspecific differences, linkage map, QTL, serpentine, *Silene vulgaris*.

**INTRODUCTION**

The genus *Silene* comprises about 700 species worldwide, of which 194 species have been reported for Europe (Chater *et al.*, 1993). The centre of diversification of *Silene* is located in the eastern Mediterranean region (Greuter, 1997). The bladder campion, *Silene vulgaris* s.l. (Moench) Garcke, a member of the section Inflatae, is subdivided into five subspecies (Chater *et al.*, 1993), three of which occur in Switzerland (Aeschimann and Bocquet, 1983; Aeschimann, 1985); *S. v. ssp. vulgaris*, *S. v. ssp. glareosa* (Jordan) Marsden-Jones & Turill and *S. v. ssp. prostrata* (Gaudin) Chater & Walters. These taxa differ in their habitat preferences and are characterized by morphological differences, most notably leaf and flower characters as well as shoot attributes. Some of these characters also differentiate two ecotypes of *S. vulgaris* s.l. that grow parapatrically in the vicinity of Davos (Switzerland): one on serpentine soil, the other on nearby montane meadows off serpentine. While the latter ecotype corresponds to *ssp. vulgaris*, the taxonomic status of the serpentine population is unclear. Some morphological characters are typical for *ssp. glareosa*, others for *ssp. prostrata*. The difficulties associated with assigning individual populations, in the present case the serpentine population, to intraspecific taxa indicates that *S. vulgaris* s.l. is a morphologically and ecologically highly variable species. This makes it an ideal study organism to investigate intraspecific morphological differences that may be caused by ecological adaptation.

Serpentine soils are characterized by high, potentially toxic, concentrations of Ni and Mg, low concentrations of plant nutritional elements and by having a low Ca:Mg ratio. Because of their granular texture, serpentine soils are also very dry. Thus, a range of chemical and physical factors influence plant growth, but the high Ni concentrations and the abnormal Ca:Mg ratio are considered to be crucial for plant survival (Proctor and Woodell, 1975; Kruckeberg, 1984; Brady *et al.*, 2005).

As a consequence of adaptations to these specific edaphic conditions, a specialized serpentine flora has evolved in many serpentine areas. Kruckeberg *et al.* (1990) listed the specific morphological adaptations characterizing the flora on serpentine. A dwarf stature, large root systems and xeromorphy, i.e. smaller, leathery leaves, as well as shorter internodes, are considered typical for plants growing on serpentine soils. Such morphological characteristics are manifested in the *S. vulgaris* serpentine ecotype investigated in this study.

Morphological differences between ecotypes are quantitative. To estimate the number of loci controlling these quantitative trait differences, the locations of these loci in the genome and their individual effect sizes, quantitative trait locus (QTL) studies can be used. Such studies have been used to unravel the genetic architecture of trait differences in various taxa, e.g. *Helianthus* (Lexer *et al.*, 2005), *Lycopersicon* (Grandillo and Tanksley, 1996), *Mimus* (Bradshaw *et al.*, 1998), *Quercus* (Saintagne *et al.*, 2004) or *Zea* (Westerbergh and Doebley, 2002).

In the present study, the genetic architecture underlying intraspecific ecotype differences separating two parapatric ecotypes of *Silene vulgaris* is investigated. The goals of...
the study were to characterize the genetic architecture of
phenotypic differences that distinguish serpentine and
non-serpentine *S. vulgaris* populations, and to compare
numbers and magnitudes of QTLs detected in this
intraspecific cross with results from similar studies on
other plant species.

**MATERIALS AND METHODS**

**Study sites**

The serpentine study site is located in the subalpine zone
in the vicinity of Davos (Switzerland) and is the result of
a rockfall after the retreat of the glaciers approx. 12 000
years ago. The non-serpentine site is a nutrient-rich,
montane meadow located near Klosters.

**Mapping population**

The mapping population was derived from an intraspe-
cific cross between a serpentine and a non-serpentine
ecotype of *Silene vulgaris*. The plant representing the
serpentine ecotype was raised from seeds sampled from
plants growing on the serpentine area (Davos) and was
used as pollen donor in the cross. The non-serpentine
ecotype was raised from seeds collected from plants
growing on the meadow near Klosters. This plant was
used as seed parent. The parental plants for the cross
were selected as follows. Seeds from ten open-pollinated seed
capsules per ecotype were collected in the field. Twenty
seeds from each capsule were then germinated and grown
in the greenhouse. After 6 weeks, two offspring per seed
family were subjected to a multiple-concentrations test
(Schat and Ten Bookum, 1992) to identify the most
Ni-sensitive and the most Ni-tolerant plants. The two
plants with maximal and minimal Ni tolerance were
chosen as parental plants and were crossed experimentally
to get the F1 population. As expected, the most Ni-tolerant
plant identified came from the serpentine ecotype, and the
most sensitive plant from the non-serpentine ecotype.

Subsequently, the most Ni-tolerant individual out of 25 F1
plants was identified as described above and was manually
self-pollinated to obtain the F2 generation. All plants were
grown in 12-cm pots filled with a mixture of silica sand
and standard potting soil (1:5) in a greenhouse at
Eschikon, Zürich, Switzerland. Plants were placed on
movable tables and the tables were randomly shuffled
once a week. Light conditions were a mix of sunlight and
mercury vapour lamps. Plants were watered every third
day and fertilized when necessary.

**Phenotypic traits**

Twelve traits that are either potentially involved in
adaptation to serpentine or characterize phenotypic
differences between the serpentine and the non-serpentine
ecotype were measured (Table 1). Where more than one
sample was taken for measurements, the mean over all
samples was used for data analysis. All measurements
were made at the same time for plants of the paternal, the
maternal, the F1 and the F2 populations. Ninety days after
germination, flower number (fn), plant height (hei), leaf
area (lea), leaf dry weight (drw), leaf wet weight (wew),
leaf length (lel), leaf width (lew), internode length (inl)
and numbers of shoots (shn) were recorded. In addition,
calyx length (cal), calyx diameter (cad) and petal length
(ptl), were measured. Fln was counted including buds
larger than 0.5 cm. Lea, drw, wew, lel and lew were
measured at the last two pairs of leaves before the
inflorescence. Lea was determined using a leaf area meter
LICOR 3000A (LiCor, Lincoln, NE, USA). Cal, cad and
ptl were measured from digital pictures of the three first
opening flowers per plant using the public domain
NIH Image software (US National Institutes of Health).
Trait correlations were calculated with Vos et al.
(1995) to achieve a better marker distribution throughout the
genome, two different restriction enzyme combinations,
*EcoRI/MseI* (EM) and *EcoRI/TaqI* (ET) were chosen. Selective amplifications were
done using various combinations of E primers with three
selective nucleotides and M and T primers with two, three
or four selective nucleotides. The genotypes were resolved
on an ABI PRISM 3100-Avant Genetic Analyzer and runs
were analysed with the Genescan 3.7 and Genotyper 3.7
software (all Applied Biosystems, Foster City, CA, USA).

Fragments present in one parent, absent in the other
parent and present in the *F1* individual were scored as
dominant markers with Genotyper 3.7. This led to an
expected segregation ratio of 3:1 in the *F2* population.
Monomorphic or near monomorphic loci in the
*F2* population were not used for mapping. Additionally,
markers with >20% missing data were omitted from
further analysis. Deviations from expected Mendelian
marker segregation were tested with chi-square tests
(α = 0.05, χ² < 3.84).

**Linkage map construction**

Initial framework maps were constructed with
300 AFLP markers and 80 *F2* individuals, mostly
consisting of the most Ni-tolerant and the most
Ni-sensitive plant individuals. In a two-step procedure,
MapMaker 3.0 (Lincoln et al., 1992) and JoinMap 3.0
(Van Oojen and Voorrips, 2001) were used to calculate
two separate, maternal (i.e. non-serpentine *S. vulgaris*)
and paternal (i.e. serpentine *S. vulgaris*) maps. First, determination of linkage groups with markers segregating in the
### Table 1. Phenotypic traits analysed in this study including major trait categories (1 = flower, 2 = leaf, 3 = shoot), complete trait names, abbreviations and units of measurements

<table>
<thead>
<tr>
<th>Cat.</th>
<th>Trait</th>
<th>Trait abbrev.</th>
<th>Unit of measurement</th>
<th>Paternal (serpentine) n = 39</th>
<th>Maternal (non-serpentine) n = 60</th>
<th>F₁ n = 51</th>
<th>F₂ n = 263</th>
<th>F₂ phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Calyx diameter</td>
<td>cad</td>
<td>cm</td>
<td>1.07 ± 0.14a</td>
<td>1.15 ± 0.12a</td>
<td>1.22 ± 0.15</td>
<td>1.00 ± 0.16b</td>
<td>Neg. transgr.</td>
</tr>
<tr>
<td>1</td>
<td>Calyx length</td>
<td>cal</td>
<td>cm</td>
<td>1.37 ± 0.12a</td>
<td>1.43 ± 0.17a</td>
<td>1.43 ± 0.13</td>
<td>1.22 ± 0.15b</td>
<td>Neg. transgr.</td>
</tr>
<tr>
<td>1</td>
<td>Petal tip length</td>
<td>ptl</td>
<td>cm</td>
<td>0.61 ± 0.08a</td>
<td>0.62 ± 0.08a</td>
<td>0.62 ± 0.08</td>
<td>0.57 ± 0.07b</td>
<td>Neg. transgr.</td>
</tr>
<tr>
<td>1</td>
<td>No. of flowers</td>
<td>fln</td>
<td>count</td>
<td>46.23 ± 23.70a</td>
<td>37.63 ± 18.46a</td>
<td>62.98 ± 31.08</td>
<td>29.30 ± 23.41b</td>
<td>Neg. transgr.</td>
</tr>
<tr>
<td>2</td>
<td>Leaf dry weight per cm²</td>
<td>drw</td>
<td>mg cm⁻²</td>
<td>5.95 ± 0.94a</td>
<td>6.53 ± 1.41a</td>
<td>5.00 ± 0.71</td>
<td>4.39 ± 1.22b</td>
<td>Neg. transgr.</td>
</tr>
<tr>
<td>2</td>
<td>Leaf wet weight per cm²</td>
<td>wew</td>
<td>mg cm⁻²</td>
<td>40.07 ± 7.65a</td>
<td>29.83 ± 5.44b</td>
<td>30.05 ± 3.47</td>
<td>30.10 ± 4.88b</td>
<td>Maternal-like</td>
</tr>
<tr>
<td>2</td>
<td>Leaf length</td>
<td>lel</td>
<td>cm</td>
<td>3.58 ± 0.69a</td>
<td>5.37 ± 1.18a</td>
<td>4.53 ± 0.77</td>
<td>4.63 ± 1.13c</td>
<td>Intermediate</td>
</tr>
<tr>
<td>2</td>
<td>Leaf width</td>
<td>lew</td>
<td>cm</td>
<td>1.09 ± 0.26a</td>
<td>1.78 ± 0.44a</td>
<td>1.40 ± 0.30</td>
<td>1.89 ± 0.65c</td>
<td>Maternal-like</td>
</tr>
<tr>
<td>2</td>
<td>Leaf area</td>
<td>lea</td>
<td>cm²</td>
<td>2.72 ± 1.06a</td>
<td>6.97 ± 3.07a</td>
<td>4.56 ± 1.74</td>
<td>6.30 ± 3.08b</td>
<td>Maternal-like</td>
</tr>
<tr>
<td>3</td>
<td>Internode length</td>
<td>inl</td>
<td>cm</td>
<td>6.56 ± 1.77a</td>
<td>7.4 ± 1.77b</td>
<td>8.37 ± 1.30</td>
<td>6.99 ± 1.65b</td>
<td>Intermediate</td>
</tr>
<tr>
<td>3</td>
<td>No. of shoots</td>
<td>shn</td>
<td>count</td>
<td>6.82 ± 2.61a</td>
<td>4.43 ± 2.45b</td>
<td>5.58 ± 2.76</td>
<td>3.71 ± 2.83</td>
<td>Maternal-like</td>
</tr>
<tr>
<td>3</td>
<td>Height</td>
<td>hei</td>
<td>cm</td>
<td>28.52 ± 8.24a</td>
<td>32.14 ± 7.06a</td>
<td>35.88 ± 6.21</td>
<td>29.67 ± 9.53a</td>
<td>Paternal-like</td>
</tr>
</tbody>
</table>

Morphological character expression in units of measurements in paternal, maternal, F₁ and F₂ populations (mean ± s.d.). Different letters following mean values indicate significant difference of the paternal, maternal and F₂ populations (Tukey–Kramer HSD test, P < 0.05).

Expected 3:1 ratio started with a minimum LOD threshold of 4.0 and a recombination threshold of r = 0.4 for initial grouping. For each linkage group, a subset of the most reliable markers with a relative likelihood ratio greater than LOD = 2 was calculated to reach a consistent linear local marker order. In a second step, the resulting local orders were implemented in JoinMap as ‘fixed order’ and all remaining markers, including the distorted ones, were placed as accessory markers. After that, 97 markers that were approximately evenly spaced across the linkage groups at 10-cM intervals were selected out of the 300 initially used markers. These markers grouped with a minimum LOD threshold of 4.0. All the 263 F₂ individuals were then genotyped for these markers and the maps were recalculated using the genotype information of the entire F₂ mapping population. The independent linkage groups within the two separate parental maps were placed as accessory markers. After that, 97 markers within six species with 55 traits and were used as a basis for Fig. 2A. For the comparison of cross types (Fig. 2B), all of the 68 traits were used. These data, combining results of 22 studies (henceforth called ‘subsample’) were extracted from the appendix of Rieseberg et al. (2002) and are available as Supplementary Information.

**QTL analysis**

All analyses were performed on Box-Cox transformed data whenever traits deviated from normality. Mapping of all traits was done with interval mapping (IM) followed by composite interval mapping [CIM (Zeng, 1994), referred to as MQM mapping in MapQTL (Van Ooijen, 2004)]. CIM expands interval mapping to include markers elsewhere in the genome as cofactors. This increases the power and precision of interval mapping by identifying and removing from the error the residual variation caused by other QTLs. Unless noted otherwise, a set of two cofactors was selected for each QTL following Van Ooijen (2004). 2-LOD support intervals were calculated from the CIM results (see Table 3). A QTL was defined as major when the percentage of variance explained (PVE) was over 25%.

Directions of QTL effects (plus or minus) for each parental map were based on the homozygote mean value in comparison to the heterozygous genotype class for each QTL. This means that the paternal map shows the effects of the maternal QTL alleles in the homozygous state and vice versa. The direction of each QTL was checked with a marker regression using SPSS (SPSS Inc., Chicago, IL, USA). Significance levels were corrected for multiple testing by sequential Bonferroni where appropriate (Rice, 1989).

**Additional statistical analyses**

For comparison of the data found for *S. vulgaris*, the number of QTLs from 64 traits (Fig. 2) of nine different plant species was examined. Of these, intraspecific crosses included six species with 55 traits and were used as a basis for Fig. 2A. For the comparison of cross types (Fig. 2B), all of the 68 traits were used. These data, combining results of 22 studies (henceforth called ‘subsample’) were extracted from the appendix of Rieseberg et al. (2002) and are available as Supplementary Information.

**RESULTS**

**Phenotypic traits**

The morphological traits measured were assigned to three classes: flower, shoot and leaf (Table 1). Serpentine-tolerant
and non-tolerant populations differed significantly in six of 12 morphological traits ($P < 0.05$): wew, lel, lew, inl, shn and lea. Mean values for the parental populations, the $F_1$ hybrids and the $F_2$ individuals are summarized in Table 1. Significant ($P < 0.05$) correlations were observed between 50 out of 66 trait-pairs (Table 2). Regarding the joint results of traits that are not different between the parental populations (cal, cad, ptl, fln, drw and hei; Table 1), 30% of the correlations of these traits are not significantly different with 20 non-significant of 66 correlations. This amount drops to 18% with traits differing between the parental populations with 12 non-significant correlations of a total of 66 correlations.

**Linkage maps**

Forty-two AFLP loci were placed on the maternal map and 55 AFLP loci on the paternal map, resulting in 12 and 13 linkage groups, respectively. The haploid chromosome number of *S. vulgaris* is 12, thus at least one chromosome is represented by more than one linkage group. Total map length $L$ is 704-8 cM for the paternal and 435-3 cM for the maternal map. The average intermarker distance is 6-1 cM for the maternal and 11-7 cM for the paternal map. In the absence of codominant markers, two separate, a paternal and a maternal, maps are presented. As the main goal of this study lay in the identification of major QTLs, two separate coupling-phase genetic maps are appropriate.
Fig. 1. (A) Maternal linkage map derived from an $F_2$ cross between a serpentine tolerant and a non-tolerant ecotype of *Silene vulgaris*. Linkage groups M1–M12 have marker names on the left while boxes on the right of each linkage group indicate QTL magnitudes and positions within 2-LOD confidence limits. QTL signs marked with + and - show the effect of the paternal QTL alleles in the homozygous state. (B) Paternal linkage map derived from an $F_2$ cross between a serpentine tolerant and a non-tolerant ecotype of *Silene vulgaris*. Linkage groups P1–P13 have marker names on the left while boxes on the right of each linkage group indicate QTL magnitudes and positions within 2-LOD confidence limits. QTL signs marked with + and - show the effect of the maternal QTL alleles in the homozygous state. For an explanation of abbreviations, etc. see part A.
QTL analysis

Four linkage groups of the maternal and nine linkage groups of the paternal maps harboured QTLs of the 12 traits analysed (Table 3). Two to nine QTLs were detected for each trait. The magnitudes of the QTLs ranged from 4 PVE up to 65.3 PVE. Of a total of 49 QTLs found here in *Silene vulgaris*, 24 were ‘major’ ones. Consequently, the distribution of QTL sizes shows a strong bias towards large QTLs (Table 3 and Fig. 1).

The number of QTLs detected per trait was smallest for wew and lel (two) and largest for cad (nine). Of the eight linkage groups carrying multiple QTLs, all show a clear overlap of QTLs for different traits, suggesting either pleiotropy or linkage of multiple QTLs (based on LOD-2 support intervals). Only five QTLs out of 49 were not associated with any other QTL (Fig. 1).

The comparison of the data reported in the literature with this study revealed that the two datasets are comparable (Fig. 2). The numbers of QTLs found in *S. vulgaris* per category (i.e. flower, leaf and shoot) were not significantly different from those reported for other intraspecific crosses (Fig. 2A). The mean QTL number of 4.1 per trait detected in this study, an intraspecific cross, was similar to the 3.7 QTLs per trait reported on average in the literature (Fig. 2B). Likewise, the mean number of antagonistic QTLs per trait detected for *S. vulgaris* was 1.2 compared with 1.1 found in other species.

DISCUSSION

The objective of this study was to assess the genetic basis of morphological differences between serpentine and
non-serpentine ecotypes of *Silene vulgaris*. Serpentine soils provide a hostile habitat for non-adapted plant populations, and evidence suggests that differences in traits potentially involved in serpentine adaptation, such as Ni tolerance and leaf succulence, have diverged between serpentine and non-serpentine ecotypes as a consequence of consistent directional selection (Bratteler et al., 2006a). Thus, directional selection may act on at least some genomic segments that harbour QTLs for traits involved in habitat adaptation.

The numbers of QTLs found for different morphological traits were similar to those reported for other intraspecific plant crosses and suggest that most trait differences between the two *S. vulgaris* ecotypes are controlled by a small number of loci. A more conspicuous feature of the cross investigated between the two ecotypes was that strong correlations were observed among most traits.

In the present study, strong clustering of QTLs for different traits was observed. On paternal linkage group 5, for example, QTLs for all 12 traits investigated were found, and ten of 12 traits mapped to paternal linkage group 3. Such a pattern could be a consequence of linkage of QTLs for different traits, or could be due to pleiotropy, where a single gene affects multiple traits. It is presently not possible to distinguish between these two scenarios, because high-resolution linkage maps would be required (Lynch and Walsh, 1998) that are not available for the study species at the moment. However, the observed clustering of QTLs for different traits sets the stage for extensive trait correlations.

In principle, trait correlations can evolve as a consequence of either ecological or genetic factors. Directional selection on one or few traits, e.g. as a consequence of habitat adaptation, may lead to indirect selection on other traits (Falconer, 1989) which leads to trait correlations even if traits are not linked. Alternatively, trait correlations can be due to physical linkage between traits, as indicated by the observation that QTLs for different traits map to the same genomic segment. Evidence for directional selection acting on nickel tolerance and succulence, two traits potentially involved in serpentine adaptation, has been reported (Bratteler et al., 2006a). Together with the observed clustering of QTLs, this leads to the proposal that the extensive trait correlations observed here are the result of both ecological and genetic factors that affected trait evolution concordantly.

Genetic correlations between floral and vegetative traits have been reported previously in various studies (Schwaegerle and Levin, 1991; Campbell et al., 1994; Armbruster, 2002). Based on observed trait correlations alone, it is often difficult to distinguish between traits that are directly affected by selection and those that are influenced by indirect selection. In *S. vulgaris*, two lines of evidence show that floral differences between the two ecotypes are unlikely to have a history of divergent selection, in contrast to leaf and shoot characters. First, trait differences leading to transgressive segregation in the *F₂* population are not expected to be the consequence of directional selection (e.g. Albertson and Kocher, 2005). All flower traits of the *F₂* population are negatively transgressive, which is not in line with a history of consistent directional selection. Secondly, traits not differing between the parental populations have more non-significant correlations (Tables 1 and 2) than traits

### Table 3. Results of QTL analyses including trait, linkage group (see Fig. 1), corresponding markers, PVE, QTL direction, support intervals, QTL positions and LODs

<table>
<thead>
<tr>
<th>Trait group</th>
<th>Corresponding marker</th>
<th>PVE (%)</th>
<th>QTL direction</th>
<th>Support interval (cM)</th>
<th>LOD</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) Maternal map (Fig. 1A)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cal</td>
<td>AM6-86</td>
<td>9.7</td>
<td>–</td>
<td>0–16</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>AT3-290</td>
<td>31.2</td>
<td>–</td>
<td>0–17</td>
<td>9</td>
</tr>
<tr>
<td>9</td>
<td>DT7-211</td>
<td>22.8</td>
<td>–</td>
<td>7–25</td>
<td>19</td>
</tr>
<tr>
<td>cad</td>
<td>CT9-292</td>
<td>20.0</td>
<td>+</td>
<td>20–49</td>
<td>41</td>
</tr>
<tr>
<td>ptl</td>
<td>DMS-128</td>
<td>36.3</td>
<td>–</td>
<td>0–17</td>
<td>17</td>
</tr>
<tr>
<td>fla</td>
<td>AT3-290</td>
<td>45.5</td>
<td>–</td>
<td>5–13</td>
<td>11</td>
</tr>
<tr>
<td>lea</td>
<td>FM6-150</td>
<td>24.5</td>
<td>–</td>
<td>0–49</td>
<td>14</td>
</tr>
<tr>
<td>drw</td>
<td>AT3-290</td>
<td>62.0</td>
<td>–</td>
<td>3–10</td>
<td>7</td>
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<tr>
<td>wew</td>
<td>AT3-290</td>
<td>20.6</td>
<td>–</td>
<td>0–17</td>
<td>12</td>
</tr>
<tr>
<td>lel</td>
<td>AT3-290</td>
<td>30.6</td>
<td>–</td>
<td>5–15</td>
<td>12</td>
</tr>
<tr>
<td>lew</td>
<td>FM6-150</td>
<td>20.5</td>
<td>–</td>
<td>0–49</td>
<td>14</td>
</tr>
<tr>
<td>inl</td>
<td>AT3-290</td>
<td>27.5</td>
<td>–</td>
<td>1–17</td>
<td>13</td>
</tr>
<tr>
<td>shn</td>
<td>AT3-290</td>
<td>45.2</td>
<td>–</td>
<td>5–15</td>
<td>12</td>
</tr>
<tr>
<td>(B) Paternal map (Fig. 1B)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cal</td>
<td>FM6-192</td>
<td>8.0</td>
<td>+</td>
<td>27–34</td>
<td>34</td>
</tr>
<tr>
<td>3</td>
<td>FM6-146</td>
<td>4.0</td>
<td>+</td>
<td>19–47</td>
<td>37</td>
</tr>
<tr>
<td>5</td>
<td>FT3-292</td>
<td>12.8</td>
<td>+</td>
<td>46–62</td>
<td>51</td>
</tr>
<tr>
<td>8</td>
<td>EM7-227</td>
<td>25.0</td>
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Support intervals are calculated with 2-LOD.
PVE is the percent of *F₂* phenotypic variance explained, calculated by interval mapping in MapQTL. PVE numbers in bold indicate major QTLs.
QTLs should be rare (Orr, 1998; consistent directional selection, QTL effects should vary in the directions of QTL effects. If traits have diverged under consistent directional selection as this may lead to stronger correlations between traits.

Another approach to interpret QTL data is to analyse the directions of QTL effects. If traits have diverged under consistent directional selection, QTL effects should generally be in the same direction and antagonistic QTLs should be rare (Orr, 1998b; Rieseberg et al., 2002), whereas antagonistic QTLs should be common in traits that have diverged under neutrality. The mean number of antagonistic QTLs of 1-2 per S. vulgaris trait (Table 3) is only slightly higher than the average of 1-1 QTLs found in a larger sample from other plant species (see Supplementary Information). The similarity of these estimates is of interest because it suggests that ascertainment bias does not significantly distort our picture of trait architecture in plants. Rieseberg et al. (2002) have discussed that the tendency of researchers to focus on the most important or most divergent traits that differ between populations used for experimental crosses could result in a higher proportion of traits with a history of directional selection and thus in a lower number of antagonistic QTLs. In the present study, however, 50% of the traits were not significantly different between the parental populations, but this did not lead to a substantially higher estimate of antagonistic QTLs.

The number of QTLs detected for the different morphological characters in S. vulgaris corresponds well with the numbers reported in the literature (Fig. 2). Regarding the difference of the number of QTLs between intraspecific morphological categories, S. vulgaris data suits the subsample’s means (Fig. 2A). Interestingly, the number of QTLs associated with flower traits is larger than the numbers of QTLs reported for leaf and shoot traits. This difference may indicate that the genetic architecture of floral traits is more complex than that of vegetative traits.

The present finding of at least one major QTL for each trait fits the simulation model for the evolution of adaptive characters proposed by Orr (1998a, 2001), and is in line with other QTL studies (e.g. Bradshaw et al., 1998; Westerbergh and Doebley, 2002; Gailing et al., 2004). However, the present PVE values have to be interpreted with some caution for several reasons: QTL effects are biased upwards whenever the locations and phenotypic effects of QTLs are estimated from a single data set (Goring et al., 2001), and low sample sizes lead to overestimation of the magnitude of QTLs (Beavis, 1998).

Species differences occur at various sites within the genome and in different numbers and magnitudes of QTLs (Orr, 2001). Intraspecific differentiation of S. vulgaris populations presented here is not limited to a few genomic segments, but occurs at multiple sites within the genome. Additionally, approx. 30% of the mapped genome is associated with quantitative traits (at 2-LOD interval; Table 3). Thus, a large number of genomic regions that affect ecotypic differentiation exist. These findings are consistent with studies investigating interspecific differences, e.g. of tomato, oak or sunflower species (Grandillo and Tanksley, 1996; Saintagne et al., 2004; Lexer et al., 2005). The present results therefore indicate that these parapatric ecotypes have diverged genetically, despite their close geographic proximity. Further support for this interpretation comes from the observation of heterosis for floral traits in the F1 generation, and substantial segregation distortion of AFLP markers in the F2 generation (Bratteler et al., 2006b). In addition, the smaller and fewer flowers observed in the F2 generation could be a consequence of hybrid breakdown. Alternatively, inbreeding depression due to selfing in the F1 generation could account for this phenomenon, because inbreeding depression is known to occur in S. vulgaris (McCaulley and Brock, 1998; Emery and McCaulley, 2002).

In conclusion, the present results clearly indicate that ecotype differentiation of S. vulgaris is spread throughout the genome even though the different traits tend to form distinct clusters. These clusters, together with habitat-mediated directional selection on particular traits, may have led to the extensive trait correlations. The genetic architecture of ecotype differences was found to be comparable to intraspecific differences observed in other plants. Evidence for trait divergence as a consequence of consistent directional selection was found which supports the notion that directional selections play an important role in plant diversification (Rieseberg et al., 2002). In order to better understand evolution of plant biodiversity, it is essential that not only model organisms, but also non-model species are investigated, because they may provide novel insights into the genetic basis of plant diversification.

SUPPLEMENTARY INFORMATION
Supplementary information providing data sources for meta-analysis to calculate mean QTL numbers of different trait types is available online at http://aoj.oxfordjournals.org.

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LITERATURE CITED


