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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 F_2 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 (Aeschimannn and Bocquet, 1983; Aeschimannn, 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 infraspecific 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), *Mimulus* (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

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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 intraspecific 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 F_1 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 F_1 plants was identified as described above and was manually self-pollinated to obtain the F_2 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 F_1 and the F_2 populations. Ninety days after germination, flower number (fln), 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 JMP V 5.1 software package for Macintosh (SAS Institute, Cary, NC, USA).

Genotyping

Leaves from parental, F_1 and F_2 plants were lyophilized and stored at −80 °C. To extract genomic DNA, 20 mg of dry material was ground in 2-ml Eppendorf tubes. DNA extraction was performed using the DNeasy plant mini kit (Qiagen, Hilden, Germany). Amplified fragment length polymorphisms (AFLP) were resolved following Vos *et al.* (1995) with minor modifications described in detail in Bratteler *et al.* (2006*b*). To achieve a better marker distribution throughout the genome, two different restriction enzyme combinations, *Eco*RI/*Mse*I (EM) and *Eco*RI/*Taq*I (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 F_1 individual were scored as dominant markers with Genotyper 3.7. This led to an expected segregation ratio of 3 : 1 in the F_2 population. Monomorphic or near monomorphic loci in the F_2 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 ($\alpha = 0{\cdot}05$, $\chi^2 < 3{\cdot}84$).

Linkage map construction

Initial framework maps were constructed with 300 AFLP markers and 80 F_2 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 Ooijen 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*

Cat.	Trait	Trait abbrev.	Unit of measurement	Paternal (serpentine) $n = 39$	Maternal (non-serpentine) $n = 60$	F_1 $n = 51$	F_2 $n = 263$	F_2 phenotype
1	Calyx diameter	cad	cm	1·07 ± 0·14[a]	1·15 ± 0·12[a]	1·22 ± 0·15	1·00 ± 0·16[b]	Neg. transgr.
1	Calyx length	cal	cm	1·37 ± 0·12[a]	1·43 ± 0·17[a]	1·43 ± 0·13	1·22 ± 0·15[b]	Neg. transgr.
1	Petal tip length	ptl	cm	0·61 ± 0·08[a]	0·62 ± 0·08[a]	0·62 ± 0·08	0·57 ± 0·07[b]	Neg. transgr.
1	No. of flowers	fln	count	46·23 ± 23·70[a]	37·63 ± 18·46[a]	62·98 ± 31·08	29·30 ± 23·41[b]	Neg. transgr.
2	Leaf dry weight per cm^2	drw	mg cm^{-2}	5·95 ± 0·94[a]	6·53 ± 1·41[a]	5·00 ± 0·71	4·39 ± 1·22[b]	Neg. transgr.
2	Leaf wet weight per cm^2	wew	mg cm^{-2}	40·07 ± 7·65[a]	29·83 ± 5·44[b]	30·05 ± 3·47	30·10 ± 4·88[b]	Maternal-like
2	Leaf length	lel	cm	3·58 ± 0·69[a]	5·37 ± 1·18[b]	4·53 ± 0·77	4·63 ± 1·13[c]	Intermediate
2	Leaf width	lew	cm	1·09 ± 0·26[a]	1·78 ± 0·44[b]	1·40 ± 0·30	1·89 ± 0·65[b]	Maternal-like
2	Leaf area	lea	cm^2	2·72 ± 1·06[a]	6·97 ± 3·07[b]	4·56 ± 1·74	6·30 ± 3·08[b]	Maternal-like
3	Internode length	inl	cm	6·56 ± 1·77[a]	7·4 ± 1·77[b]	8·37 ± 1·30	6·99 ± 1·65[ab]	Intermediate
3	No. of shoots	shn	count	6·82 ± 2·61[a]	4·43 ± 2·45[b]	5·58 ± 2·76	3·71 ± 2·83[b]	Maternal-like
3	Height	hei	cm	28·52 ± 8·24[a]	32·14 ± 7·06[a]	35·88 ± 6·21	29·67 ± 9·53[a]	Paternal-like

Morphological character expression in units of measurements in paternal, maternal, F_1 and F_2 populations (mean ± s.d.).
Different letters following mean values indicate significant difference of the paternal, maternal and F_2 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 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 F_2 individuals were then genotyped for these markers and the maps were recalculated using the genotype information of the entire F_2 mapping population. The independent linkage groups within the two separate parental maps obtained are arbitrarily numbered and presented in Fig. 1.

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).

Genome-wide threshold LOD values to declare a QTL to be significant were determined with 1000 permutations (Churchill and Doerge, 1994) for each trait as implemented in MapQTL.

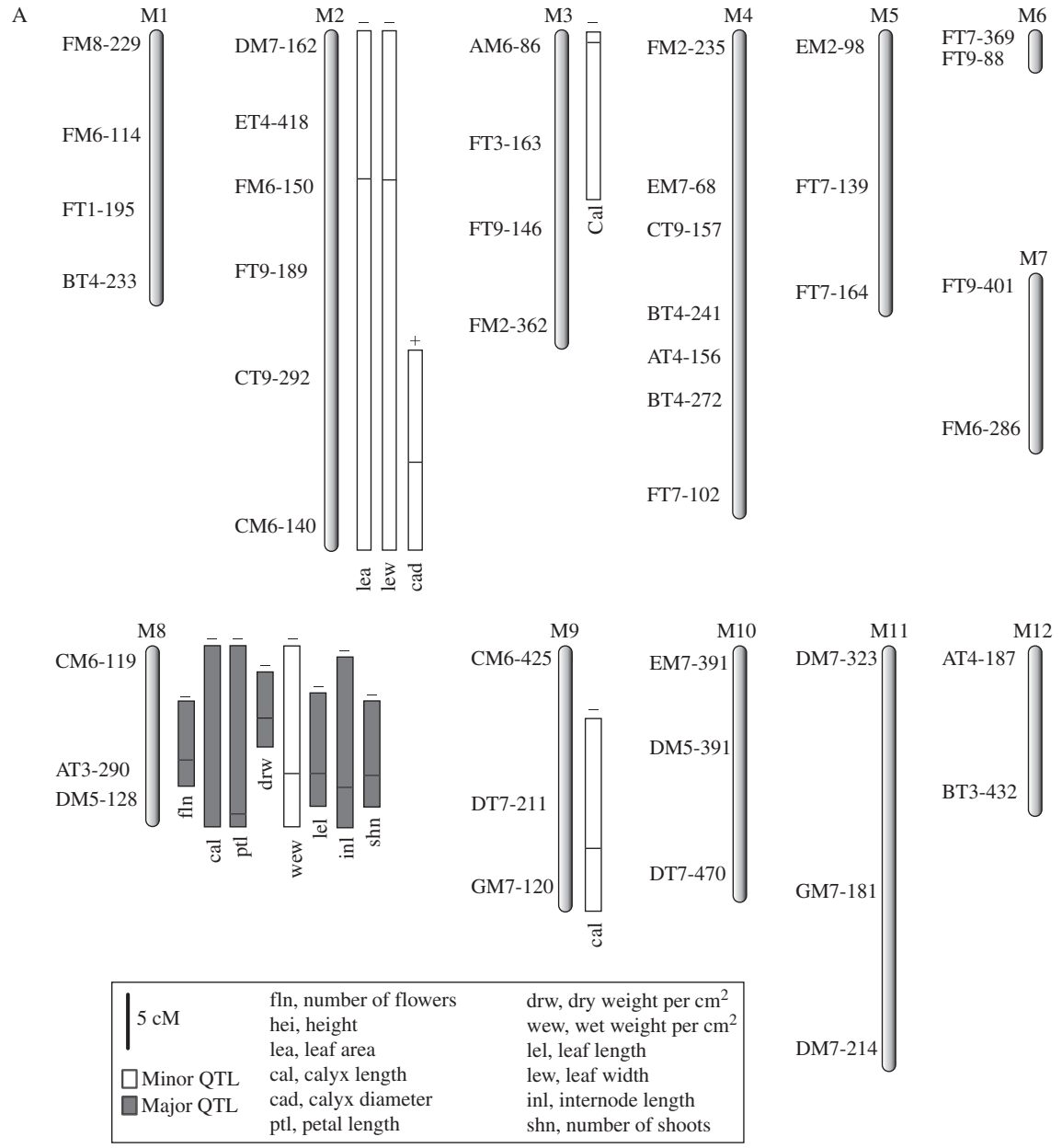
Additional statistical analyses

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**. Numbers of QTLs for each trait were Box-Cox-transformed. The trait categories 'flower', 'leaf' and 'shoot' were added to the existing trait category 'morphology' for further analyses. Only studies on plants were taken into account. All statistical analyses were performed using JMP V 5.1 (SAS Institute, Cary, NC, USA). Significance levels were corrected for multiple testing by sequential Bonferroni where appropriate (Rice, 1989).

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 ($P < 0{\cdot}05$): 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 ($P < 0{\cdot}05$) 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

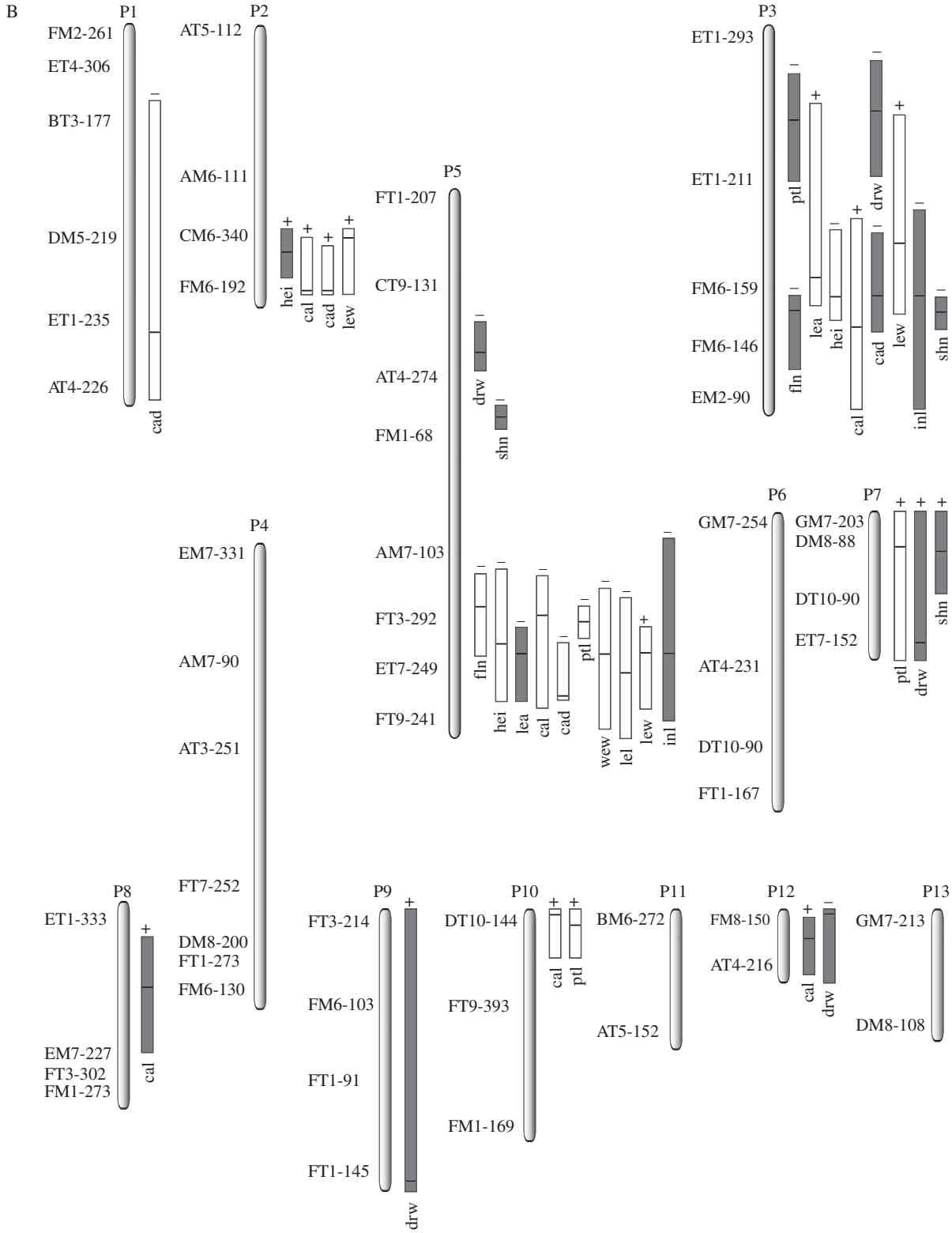


FIG. 1. (A) Maternal linkage map derived from an F_2 cross between a serpentine tolerant and a non-tolerant ecotype of *Silene vulgaris*. Linkage groups M1–M12 have marker names on the left while boxes on the right of each linkage group indicate QTL magnitudes and positions within 2-LOD confidence limits. 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.

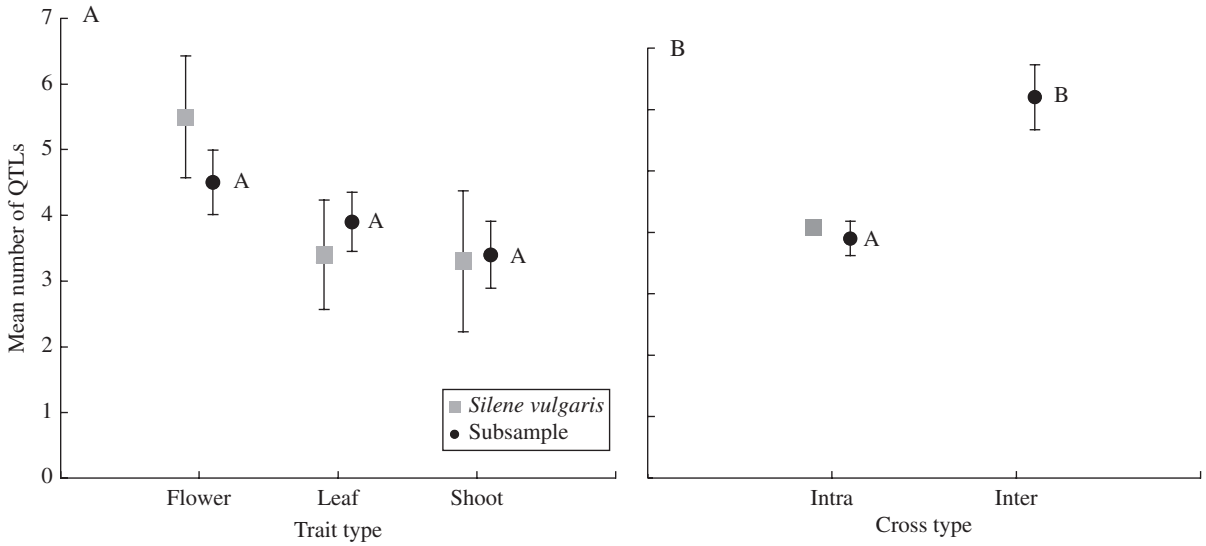


FIG. 2. Comparison of the mean number of QTL of the subsample (see Materials and Methods) and the results reported for *Silene vulgaris*. Numbers are presented for intraspecific morphological trait types (A) and type of cross (B) separately. Different letters beside mean values indicate significant difference of traits calculated for the subsample (Tukey–Kramer HSD test, $P < 0{\cdot}05$).

TABLE 2. *Correlations among traits in the entire* F_2 *population*

	cal	cad	ptl	fln	lea	drw	wew	lel	lew	inl	hei	shn
cal	–											
cad	0·36***	–										
ptl	0·43***	0·44***	–									
fln	0·15	0·29***	0·32***	–								
lea	0·27***	0·23*	0·32*	0·49***	–							
drw	0·14	0·13	0·35***	0·51***	0·21	–						
wew	0·14	0·22*	0·41***	0·40***	0·50***	0·47***	–					
lel	0·32***	0·33***	0·46***	0·58***	0·87***	0·29	0·54***	–				
lew	0·16***	0·16	0·19	0·37***	0·91***	0·14	0·39***	0·63***	–			
inl	0·22*	0·44***	0·37***	0·49***	0·34***	0·40***	0·23	0·40***	0·29***	–		
hei	0·12	0·32***	0·17	0·34***	0·16*	0·26	0·01	0·19***	0·18*	0·84***	–	
shn	0·09	0·21*	0·27*	0·66***	0·39***	0·37***	0·32***	0·49***	0·29	0·47***	0·35***	–

* $P < 0{\cdot}05$, *** $P < 0{\cdot}001$; corrected for multiple tests by sequential Bonferroni (Rice, 1989).

(Knapp *et al*., 1995), but it was not possible to determine homologies among linkage groups.

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

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

Trait	Linkage group	Corresponding marker	PVE (%)	QTL direction	Support interval	Position (cM)	LOD
(A) Maternal map (Fig. 1A)							
cal	3	AM6-86	9·7	–	0–16	1	3·39
	8	AT3-290	**31·2**	–	0–17	9	3·54
	9	DT7-211	22·8	–	7–25	19	4·38
cad	2	CT9-292	20·0	+	20–49	41	5·51
ptl	8	DM5-128	**36·3**	–	0–17	17	4·64
fln	8	AT3-290	**45·5**	–	5–13	11	8·25
lea	2	FM6-150	24·5	–	0–49	14	3·36
drw	8	AT3-290	**62·0**	–	3–10	7	20·97
wew	8	AT3-290	19·6	–	0–17	12	3·87
lel	8	AT3-290	**30·6**	–	5–15	12	3·80
lew	2	FM6-150	20·5	–	0–49	14	5·26
inl	8	AT3-290	**27·5**	–	1–17	13	3·42
shn	8	AT3-290	**45·2**	–	5–15	12	8·96
hei	–						
(B) Paternal map (Fig. 1B)							
cal	2	FM6-192	8·0	+	27–34	34	2·28
	3	FM6-146	4·0	+	19–47	37	2·24
	5	FT3-292	12·8	–	46–62	51	5·63
	8	EM7-227	**25·0**	+	3–17	9	3·57
	10	DT10-144	11·0	+	0–6	0	4·80
	12	FM8-150	**40·7**	+	1–7	4	6·47
cad	1	ET1-235	10·0	–	12–46	38	6·40
	2	FM6-192	10·5	+	28–34	34	3·63
	3	FM6-159	**39·9**	–	25–37	33	12·54
	5	ET7-249	24·9	–	50–57	57	9·05
ptl	3	ET1-211	**40·3**	–	5–18	11	3·91
	5	FT3-292	10·2	–	50–54	25	5·62
	7	DM8-88	16·5	+	0–18	4	3·32
	10	DT10-144	12·6	+	0–6	2	2·75
fln	3	FM6-159	**39·5**	–	33–42	35	5·92
	5	FT3-292	22·5	–	46–56	50	13·50
lea	3	ET1-211	9·3	+	9–35	23	6·31
	5	ET7-249	**26·6**	–	52–61	56	7·69
drw	3	ET1-211	**45·4**	–	3–17	10	3·80
	5	AT4-274	**57·8**	–	15–21	19	3·32
	7	ET7-152	**46·7**	+	0–18	16	7·09
	9	FT1-145	**38·0**	+	0–34	34	4·27
	12	FM8-150	**26·6**	–	0–9	0	3·03
wew	5	ET7-249	15·4	–	48–65	56	3·43
lel	5	ET7-249	24·4	–	49–66	58	6·24
lew	2	CM6-340	18·9	+	26–34	27	3·70
	3	ET1-211	17·6	+	10–34	25	10·21
	5	ET7-249	16·1	+	53–63	56	7·50
inl	3	FM6-159	**30·5**	–	23–47	33	4·28
	5	ET7-249	**26·1**	–	42–64	56	4·53
shn	3	FM6-159	**50·9**	–	33–37	35	7·30
	5	FM1-68	**65·3**	–	25–28	27	7·44
	7	DM8-88	**60·4**	+	0–10	5	7·44
hei	2	CM6-340	**28·2**	+	24–30	27	3·51
	3	FM6-159	23·7	–	26–37	33	4·49
	5	FT3-292	24·3	–	46–62	55	9·06

Support intervals are calculated with 2-LOD.

PVE is the percent of F_2 phenotypic variance explained, calculated by interval mapping in MapQTL. PVE numbers in bold indicate major QTLs.

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*., 2006*a*). 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*., 2006*a*). 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 concertedly.

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 F_2 population are not expected to be the consequence of directional selection (e.g. Albertson and Kocher, 2005). All flower traits of the F_2 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

varying between the two ecotypes. This may indicate that traits differing between the parental populations have a history of 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, 1998*b*; 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 (1998*a*, 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 F_1 generation, and substantial segregation distortion of AFLP markers in the F_2 generation (Bratteler *et al*., 2006*b*). In addition, the smaller and fewer flowers observed in the F_2 generation could be a consequence of hybrid breakdown. Alternatively, inbreeding depression due to selfing in the F_1 generation could account for this phenomenon, because inbreeding depression is known to occur in *S. vulgaris* (McCauley and Brock, 1998; Emery and McCauley, 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://aob.oxfordjournals.org.

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