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Molecular and Quantitative Genetic Differentiation in European Populations of *Silene latifolia* (Caryophyllaceae)

CÉLINE JOLIVET[1,2] and GIORGINA BERNASCONI[1,2,*]

[1]*University of Lausanne, Department of Ecology and Evolution, CH-1015 Lausanne, Switzerland and* [2]*University of Zurich, Institute of Environmental Sciences, CH-8057 Zürich, Switzerland*

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• *Background and Aims* Among-population differentiation in phenotypic traits and allelic variation is expected as a consequence of isolation, drift, founder effects and local selection. Therefore, investigating molecular and quantitative genetic divergence is a pre-requisite for studies of local adaptation in response to selection under variable environmental conditions.
• *Methods* Among- and within-population variation were investigated in six geographically separated European populations of the white campion, *Silene latifolia*, both for molecular variation at six newly developed microsatellite loci and for quantitative variation in morphological and life-history traits. To avoid confounding effects of the maternal environment, phenotypic traits were measured on greenhouse-reared F_1 offspring. Tests were made for clinal variation, and the correlations among molecular, geographic and phenotypic distances were compared with Mantel tests.
• *Key Results* The six populations of *Silene latifolia* investigated showed significant molecular and quantitative genetic differentiation. Geographic and phenotypic distances were significantly associated. Age at first flowering increased significantly with latitude and exhibited a Q_{st} value of 0·17 in females and 0·10 in males, consistent with adaptation to local environmental conditions. By contrast, no evidence of isolation-by-distance and no significant association between molecular and phenotypic distances were found.
• *Conclusions* Significant molecular genetic divergence among populations of *Silene latifolia*, from the European native range is consistent with known limited seed and pollen flow distances, while significant quantitative genetic divergence among populations and clinal variation for age at first flowering suggest local adaptation.

Key words: *Silene alba*, white campion, microsatellite DNA, population differentiation, population structure, clinal variation, life-history traits.

INTRODUCTION

Population differentiation results from interactions between several mechanisms including colonization history, gene flow, drift, mutation and selection under variable environmental conditions that can lead to local adaptation (Slatkin, 1985; Hagen and Hamrick, 1998; Richards *et al.*, 1999; Chan and Arcese, 2003; Kawecki and Ebert, 2004). Assessing the contribution of each of those mechanisms can prove difficult; however, estimating variation among extant populations at neutral markers and at phenotypic traits potentially under local selection is an important step to understand micro-evolutionary processes and local adaptation in particular (Kawecki and Ebert, 2004). Importantly, phenotyping individuals reared for one or more generations under homogenous laboratory conditions allows direct comparisons of quantitative traits, because it reduces the impact of maternal effects, and allows a better estimate of heritable variation. It has been suggested that differentiation can be explained by directional selection, if divergence at such quantitative traits is relatively higher compared with divergence at neutral markers, as evaluated by comparing Wright's F_{ST} (Wright, 1931) to Q_{ST} (analogous to F_{ST} for quantitative traits; Spitze, 1993; Merila and Crnokrak, 2001).

Silene latifolia occurs throughout Europe and the Mediterranean region (Baker, 1947), and nine European races have been described previously based on morphological and chemical traits (Mastenbroek and Vanbrederode, 1986). This also showed evidence that *Silene latifolia* likely found refuge in Northern Africa during the last glaciations from where it colonized its current range with the spread of agriculture (Mastenbroek and Vanbrederode, 1986; Vellekoop *et al.*, 1996). There is also strong structure in the Y-chromosome sequence in the European range (Ironside and Filatov, 2005). However, it is not known whether populations are structured at neutral markers that reflect more recent evolutionary history compared with Y-chromosome variation. There is further evidence that within the European range, *S. latifolia* populations are differentiated for herbivore attack rates (Wolfe *et al.*, 2004), sexually dimorphic traits (Delph *et al.*, 2002), and infection success of the fungal pathogen *Mycrobotrium violaceum* (Kaltz *et al.*, 1999). However, as far as is known, no study to date simultaneously has addressed phenotypic and molecular divergence in natural populations.

The aims of the present study were (*a*) to characterize among-population differentiation at molecular traits (assessed using six newly developed, highly polymorphic microsatellite DNA markers; S. Teixeira and G. Bernasconi, unpubl. res.) and at phenotypic traits (measured under standardized environmental conditions) in *S. latifolia*, (*b*) to

* For correspondence. E-mail giorgina.bernasconi@unil.ch

compare molecular and quantitative genetic differentiation with geographical distance. Ultimately, this should help to identify traits that potentially reveal local adaptation.

MATERIALS AND METHODS

Study species

White campion *Silene latifolia* (Poiret) [= *S. alba* (Miller) Krause, = *Melandrium album* (Miller) Garcke, Caryophyllaceae] is a dioecious short-lived perennial plant native from Europe. *Silene latifolia* is patchily distributed along disturbed roadside and agricultural habitats, on calcareous and sandy soils (Baker, 1947). In Switzerland, the plant emerges in early spring, flowers from May to October and overwinters as a rosette. It builds up to a maximum of 40 fruits per season with 48–359 seeds/fruit (Baker, 1947). Sex determination is chromosomal, males being the heterogametic sex. The species is sexually dimorphic for numerous floral and vegetative traits (Meagher, 1992, 1994; Delph and Meagher, 1995; Delph *et al.*, 2002, 2005). *Silene latifolia* is animal-pollinated, mainly by nocturnal moths, including *Hadena bicruris*, which also acts as seed predator (Shykoff and Bucheli, 1995; Young, 2002; Wolfe *et al.*, 2004; Jolivet and Bernasconi, 2006). *Silene latifolia* was introduced in North America in the mid-1800s where it has become invasive and is now considered as a pest species (McNeill, 1977; Wolfe, 2002).

Analysis of genetic structure

Seeds and leaves were sampled from 25–50 females from six populations in central Europe (Fig. 1), one population from Switzerland [Cottendart (CT)], three populations from France [Gagny (PA), Village-Neuf (VN) and Saint-Martin d'Uriage (GR)], one population from Northern Italy [Sesto-Calende (SC)] and one population from the Netherlands [Millingerwaard (HO)]. Samples were collected from plants at least 2 m apart from each other, except in two small populations (GR and VN) where all flowering females were sampled. The leaves were dried and stored in silica gel.

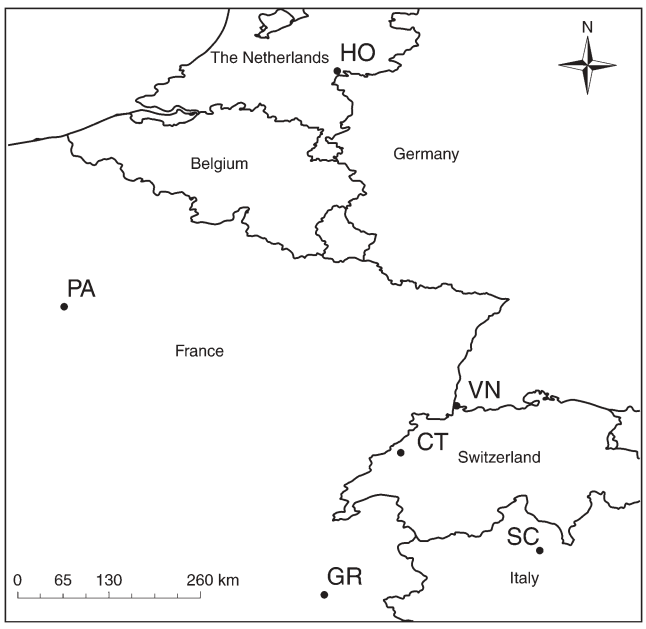

FIG. 1. Geographic location of *Silene latifolia* populations studied for molecular and phenotypic differentiation. GPS co-ordinates and estimated number of individuals (*n*) were: VN, 47°36′25″N/7°33′31″E (80); CT, 46°58′30″N/6°50′50″E (1000); HO, 51°52′45″N/6°00′55″E (2000); GR, 45°09′51″N/5°51′34″E (60); PA, 48°53′11″N/2°32′36″E (400); SC, 45°44′08″N/8°37′00″E (>100).

To study molecular genetic differentiation among populations, six newly developed microsatellite DNA primers from European *S. latifolia* plants were used (S. Teixeira and G. Bernasconi, unpubl. res.). DNA was extracted from dried leaves using the cetyltrimethyl ammonium bromide (CTAB) DNA extraction protocol (Doyle and Doyle, 1987). The DNA was quantified using λ*Hin*dIII marker and diluted to standardize DNA concentration to 2 ng μL^{-1}. For the duplex SL6 (FAM) and SL8 (HEX) and the duplex SL14 (FAM) and SL15 (HEX), polymerase chain reaction (PCR) was performed using 1× Qiagen Multiplex PCR master Mix, 2 μM of each primer and 10 ng of genomic DNA. For the primers SL1 (FAM) and SL4 (HEX), the Qiagen HotStartTaq Mix was used. The PCR amplification was conducted in a Biometra thermocycler with the following programme: 15 min at 95 °C; 30 cycles composed of 30 s denaturation at 94 °C, 90 s at $T_m = 60$ °C (SL6, SL8, SL14, SL15 and SL1) $T_m = 50$ °C (SL4) and 60 s elongation at 72 °C, followed by a final elongation step of 30 min at 60 °C. Fragments were analysed on an ABI 3100 genetic analyser (Applied Biosystems) with the internal size standard Genescan 350 and scored with the software GeneMapper v3·7 (Applied Biosystems). Two loci (SL14 and SL15) are X-linked (S. Teixeira and G. Bernasconi, unpubl. res.). However, this does not affect the present analysis because only females, which have two alleles at all loci, were genotyped. Individuals were scored according to the size and pattern observed for each allele.

Genetic data were analysed using Fstat 2·9·3·2 (Goudet, 1995). Hardy–Weinberg equilibrium (null hypothesis: F_{IS} and F_{IT} equal to zero) was tested for within populations and over the entire sample with 2000 randomizations. Because F_{IS} is the inbreeding coefficient of an individual relative to its population, a significant deviation from zero would indicate homozygote excess within populations. F_{IT} is the overall inbreeding coefficient of an individual relative to the total sample, and a significant deviation from zero would indicate homozygote excess over the total dataset.

Based on genetic data for a large number of controlled crosses (S. Teixeira *et al*, University of Lausanne, Switzerland, unpub. res.) suggesting that null alleles may occur, the present data set for presence of null alleles was examined. Null allele frequencies, adjusted allele frequencies and adjusted genotypes were estimated using the Brookfield1 algorithm in Micro-checker 2·2·3 (Brookfield, 1996; Van Oosterhout *et al.*, 2004). The Brookfield estimator calculates null allele frequency by comparing expected and observed heterozygosity and taking into account that there is no missing value in the sample. All the following

analysis is reported for both the sample without (N_1) and after (N_2) null allele correction.

Expected total heterozygosity (H_T) and average heterozygosity within populations (H_S) were estimated. For highly polymorphic loci, values of H_S and H_T can approach unity. For this case, Hedrick (2005) suggested that among-population differentiation should be assessed by comparing G_{ST} values [$G_{ST} = (H_T - H_S)/H_T$] to the maximum possible G_{ST} [$G_{STmax} = (1 - H_S)/(1 + H_S)$; Nei, 1987], and defined a standardized measure of differentiation ($G'_{ST} = G_{ST}/G_{STmax}$). This standardized measure accounts for differences in mutation rate when several molecular markers are compared. On the other hand, F_{ST} measures the relative difference of heterozygosity of within population compared with the total sample of populations, thus addressing the extent of population differentiation [$F_{ST} = A/(A + B + C)$, whereby A = variance of allelic frequencies between populations, B = variance of allelic frequencies between individuals within population, C = variance of allelic frequencies between gametes within individual; Weir and Cockerham 1984]. A 95 % confidence interval of the F_{ST} value obtained was calculated with a bootstrap procedure. Population differentiation (null hypothesis: F_{ST} equal to zero) was tested for in the N_1 dataset using log-likelihood G statistics, which does not require that data fulfil Hardy–Weinberg equilibrium (Goudet *et al.*, 1996), and pairwise tests of differentiation for each pair of populations were conducted, calculating confidence levels at 5% after Bonferroni corrections.

Plant rearing and phenotyping

To assess morphological and life-history traits differentiation, plants from field-collected seeds (parental generation, P) were reared under homogeneous conditions in the greenhouse and were crossed to obtain an F_1 generation. This was done to minimize maternal environmental effects on phenotypic measures, which were taken on F_1 individuals. To obtain the F_1, 20 seeds/fruit from 15 fruits/population (each fruit from a different plant) from six populations (SC, GR, VN, CT, PA and HO; Fig. 1) were germinated. Seeds from the same fruit were germinated together in Petri dishes on cotton wool and filter paper and watered with 10^{-3} mol L^{-1} gibberellic acid (GR, VN, SC) in a germination cabinet [temperature (T) = 21 °C, relative humidity (RH) = 80 %, day/night length (D/N) = 16/8 h]. For the CT, PA and HO populations, the seeds were germinated in Jiffy peat pellets (Jiffy7, 703) in the greenhouse (see below). After 15 d the seedlings were placed in 10-cm pots containing a 3 : 1 mixture of soil:sand (Tref BF4 from GVZ-Bolltec, Zurich, Switzerland; sand particles 0·5–3 mm). Due to greenhouse space limitations and the necessity to have a large enough sample to conduct crosses, two different greenhouses were used to rear the P generation. The populations were reared in two distinct greenhouses (blocks) as follows: GR, VN, SC [23/19 °C D/N, RH = 30 %, D/N = 16/8 h, artificial light only (lamps EYE Clean-Ace, 6500 K, 400 W, Iwasaki Electronics Co., Japan)] at the University of Zurich and CT, PA, HO (23/18 °C D/N, RH = 55 %, D/N = 16/8 h; artificial light as in the other greenhouse and natural light) at the University of Lausanne. Plants were placed at randomized positions in the greenhouse. Potential differences between blocks were accounted for in the analysis and in crossings by using each block separately or by using residuals of a model with block as an explanatory variable. F_1 individuals were obtained by crossing one female from each field-collected seed family with a male from the same population but a different family. For the F_1, the same rearing conditions were used as those described above for the block CT, PA, HO.

Recorded were the proportion of seeds that germinated, germination time (number of days between sowing and germination), the proportion of individuals flowering (within 115 d from sowing), age at first flowering (number of days between germination and flowering) and offspring gender. In populations CT, HO and PA, additionally size at first flowering (number of leaves, length of the longest leaf, height of the stem and number of stems) was measured. The coefficient of variation [CV = (s.d × 100/mean) × (1 + 1/4n] was calculated following Sokal and Rohlf (1981) for each trait to address the amount of variation within population.

For time to germination and age and size at first flowering, Q_{ST}[$Q_{ST} = V_b/(V_b + 4 \times V_w)$] and broad-sense heritabilities H^2[$H^2 = 2V_w/(V_w + V_i)$], whereby V_b is the variance component between populations, V_w is the variance component between families within population, and V_i is the variance component between individuals, were estimated by the moments method for a full-sib design as described by Evanno *et al.* (2006) (see also Spitze, 1993). For the traits measured in only one block, variance components were calculated directly. For traits measured in both blocks, variance components were calculated using the residuals of the trait studied (where applicable, with adequate transformation as determined with a box–cox procedure) after accounting in ANOVA for block effect. This was calculated separately for each sex. Then variance components between populations and between families within populations were estimated (Evanno *et al.*, 2006). As discussed by O'Hara and Merilä (2005), a sample of six populations may not grant sufficient precision for estimating Q_{ST}. With this sample size, variance of Q_{ST} estimates may be very large (Goudet and Büchi, 2006). However, a jackknife method was used to estimate 95 % confidence intervals for Q_{ST} values to quantify this variation although, because of the above caveats, a formal comparison of Q_{ST} and F_{ST} was not done.

Comparison of molecular, geographic and phenotypic distance matrices

To compare the extent of differentiation at molecular markers and quantitative traits molecular, phenotypic and geographical distance matrices were calculated. For the phenotypic distance matrix, the proportion of seeds that germinated, the proportion of seedlings which flowered, time to germination and age at first flowering were included. From this analysis measures of size at first flowering were excluded because they were recorded in only one block;

also excluded was the sex ratio because it had low inter-population variation (see Results and Table 2A). A Euclidian distance matrix was calculated from centred and scaled phenotypic data. For the molecular distance matrix, the pairwise F_{ST} matrix was used on the N_1 data set calculated with Fstat. The geographical distance matrix was determined using aerial pairwise distances between populations (kilometres). Mantel tests between each pair of distance matrices were performed using the R package ade4 for Mantel tests and 2000 permutations (Chessel *et al.*, 2004). For all other statistical analyses, the R software v. 2·0·0 (Ihaka and Gentleman, 1996; R Development Core Team, 2004) was used.

RESULTS

Molecular genetic differentiation

Between 34 and 61 alleles per locus were found over all six populations (Table 1A). Several alleles were separated by only two base pairs. Controlled crosses and paternity analysis (with 20 offspring/cross, and a total of over 1000 offspring scored) using plants from these populations revealed that offspring display allele sizes consistent with the genotype of their parents, i.e. scored allele sizes are stable (Jolivet and Bernasconi, 2007). Total expected heterozygosity (H_T) and average expected heterozygosity within populations (H_S) were, respectively, 0·962 and 0·927 in the N_1 data set (0·964 and 0·934 in the N_2 data set; Table 1B). This indicates that the loci are highly polymorphic and that homozygotes were extremely rare. Observed heterozygosity (H_o) was 0·686, indicating heterozygote deficit. Overall, F_{IS} (0·26) and F_{IT} (0·29) estimates were significantly different from zero ($P < 0·0005$), indicating deviation from Hardy–Weinberg equilibrium both within populations and in the total dataset. This was accounted for in the test for population differentiation. The standardized measure of population differentiation (G'_{ST}) over all loci was 0·94 (Table 1B). Overall F_{ST} was 0·041 (95 % confidence interval 0·031–0·050) in N_1 (0·04 in N_2), and significantly greater than zero ($P < 0·0005$). Pairwise tests of differentiation were all significant (P-values all significant at the 5 % confidence level after Bonferroni corrections). Altogether these results indicate significant molecular genetic differentiation among the present study populations.

Quantitative genetic differentiation

The coefficients of variation for phenotypic traits (calculated using means of F_1 seed families as observations) ranged from 2 % to 44 % (Table 2), indicating high variation for many traits. Test were carried out to find out if there was significant quantitative genetic differentiation on the traits measured and if there was variation at the level of population, and of family within population. Stem height and length of the longest leaf were not significantly different between genders, but differed significantly among populations and families (Table 3). Number of leaves and number of stems were, respectively, significantly and marginally significantly sexually dimorphic, and differed significantly among families, but not between populations (Table 3). Sexual dimorphism in the number of stems was also marginally significantly different among families within populations. Males had a marginally significant larger number of stems (Table 2C), but this probably depended on one relatively extreme population (PA). Time to germination and age at first flowering (residuals accounting for block) were significantly sexually dimorphic. Moreover, they varied significantly between families within populations and between populations. Females germinated later, but flowered earlier than males (Table 2B, C). Since these traits were measured on F_1 plants, it can be assumed that significant between-family and within-population variation in all the traits tested above indicates heritable variation.

Latitude was significantly, positively correlated with the age at first flowering (family means) and marginally significantly affected by gender. Block had no significant effect and therefore analysis was conducted on raw data rather than residuals (ANCOVA: latitude, $1·71 \pm 0·25$, $t_9 = 6·7$, $P < 0·0001$; gender, $t_9 = 2·2$, $P = 0·053$; Fig. 2). Plants from more southern latitudes flowered earlier than plants originating from populations further north.

Q_{ST} values for time to germination, age at first flowering (both blocks) and size at first flowering (block HO, CT, PA only) varied between 0 and 0·17 and broad-sense heritabilities varied between 0·28 and 0·73 (Table 4). Interestingly, females exhibited higher Q_{ST} and broad-sense heritability values than males, and confidence intervals of Q_{ST} values of age at flowering and time at germination were not overlapping with the confidence interval of F_{ST} (0·031–0·050).

Comparison of molecular, geographic and phenotypic distance matrices

The phenotypic and geographical distances matrices were significantly correlated (Mantel test, $r = 0·62$, $P = 0·03$). By contrast, there was no significant association either between geographic and molecular distance matrices (Mantel test, $r = 0·08$, $P = 0·4$) nor between phenotypic and molecular distance matrices (Mantel test, $r = -0·06$, $P = 0·6$). Thus, the differentiation shown by variation at microsatellite DNA loci was not directly associated with the variation observed in phenotypic traits, and did not show isolation-by-distance at this scale. By contrast, the significant correlation between quantitative trait variation and geography confirms the above-reported clinal variation in the onset of flowering.

DISCUSSION

Significant population differentiation and very high polymorphism were observed at six microsatellite DNA loci, as was also observed within the same genus in *Silene vulgaris* (9–40 alleles per loci in one population; Juillet *et al.*, 2003). High molecular genetic variation within populations might reflect the obligate 'outcrossing' mode of reproduction of the dioecious *Silene latifolia* (Hagen and Hamrick, 1998), although *S. vulgaris*, which harbours

TABLE 1. *(A) Number of alleles observed in six European* Silene latifolia *populations at six microsatellite loci; (B) observed heterozygosity (H_o), Nei's estimation of heterozygosity (H_S, H_T), G_{ST} and maximum possible G_{ST} (see Hedrick, 2005)*

(A) Number of alleles

Locus	Population						
	VN	CT	HO	GR	PA	SC	All populations
	Sample size						
	27/22	35/27	30/23	25/22	30/23	30/26	177/143
SL1	23/22	30/29	16/16	24/23	22/17	22/21	54/55
SL4	14/15	24/23	17/15	10/11	20/21	16/15	34/34
SL6	23/22	35/29	28/25	15/16	26/25	29/28	61/61
SL8	13/14	19/19	13/13	12/12	21/20	21/19	48/46
SL14	22/22	28/25	16/16	14/14	23/18	20/20	47/46
SL15	16/15	24/22	16/12	11/11	17/14	17/16	34/30

(B) Observed heterozygosity

Locus	H_o	H_S	H_T	G_{ST}	G_{ST} (max)	G'_{ST}
SL1	0·679/0·704	0·952/0·953	0·973/0·974	0·022/0·021	0·025/0·024	0·89/0·87
SL4	0·518/0·665	0·913/0·931	0·955/0·959	0·044/0·030	0·045/0·036	0·98/0·83
SL6	0·828/0·826	0·958/0·962	0·978/0·980	0·021/0·018	0·021/0·019	0·98/0·95
SL8	0·526/0·686	0·893/0·918	0·938/0·949	0·048/0·033	0·056/0·043	0·85/0·77
SL14	0·774/0·791	0·936/0·939	0·971/0·971	0·035/0·032	0·033/0·031	1·00/1·00
SL15	0·789/0·796	0·911/0·903	0·954/0·952	0·045/0·051	0·047/0·051	0·97/1·00
All loci	0·686/0·745	0·927/0·934	0·962/0·964	0·036/0·031	0·0.38/0·034	0·94/0·90

The ratio of observed to maximum possible G_{ST} indicates the extent of population differentiation (G'_{ST}).

For each locus, the values (x/y) for two data sets are given: the one including all N_1 females genotyped, and the subset of N_2 females after null allele correction.

Sample sizes are given as N_1/N_2.

similar variability at microsatellite loci, is gynodioecious (Glaettli *et al.*, 2006). Significant departures from Hardy–Weinberg equilibrium were observed. However, as is often the case, it is not possible to infer which mechanism (selection, non-random mating, Wahlund effect, null alleles) may have caused this departure. Null alleles were observed at all loci considered. Null alleles result in false homozygotes, thus inflating F_{IS} and deflating H_o. Another probable explanation for this deviation from Hardy–Weinberg equilibrium is subpopulation structure (Wahlund effect). Such a substructuring is possible in *S. latifolia*, which has a metapopulation dynamics and patchy distribution of individuals within demes (Richards *et al.*, 2003). Consistent with the idea of population substructuring, in experimental arrays, pollen flow was low between individuals separated by >100 m (Richards *et al.*, 1999) and in invasive populations both allozymes and cpDNA revealed high molecular genetic structure at fine scale (McCauley, 1994, 1997). Therefore, the present study populations might show subpopulation structure, and only studies of fine-scale genetic structure (e.g. Hardy *et al.*, 2004) may resolve whether samples such as those collected for the present study in fact represent different subpopulations. Furthermore, the occurrence of null alleles and inbreeding within populations may provide additional explanations for this deviation from Hardy–Weinberg equilibrium. It must be noted that two of the investigated loci are X-linked (S. Teixeira and G. Bernasconi, unpubl. res.), and should thus be expected to have a different effective population size than autosomal loci.

No evidence for isolation-by-distance was found, and in fact the genetically most similar populations were geographically distant (PA, central France, and SC, northern Italy). This result differs from other studies, where allozyme variation indicated isolation-by-distance in *Silene latifolia* populations along the Rhine Valley (Delmotte *et al.*, 1999). Vellekoop *et al.* (1996) also found an east–west pattern with RAPD markers, but on a much broader scale. The present populations are mostly distributed in the transition zone between the east and west races (Mastenbroek *et al.*, 1984; Prentice *et al.*, 1984), and this may explain the lack of association between molecular and geographic distance matrices. The present microsatellite DNA loci may also be so polymorphic that detecting geographical patterns becomes difficult. Because distinct alleles sometimes had very similar sizes, backward mutations may have occurred and some alleles of the same size in different populations might thus have different histories (homoplasy). Future studies investigating variation at additional molecular markers could increase resolution and help to clarify geographical patterns in this intermediate zone.

There was significant among-population differentiation in phenotypic traits, including components of size at first flowering (height of the stem, length of the longest leaf)

TABLE 2. *Phenotypic traits measured on greenhouse-reared,* F_1 *offspring obtained from within-population crosses among field-collected* Silene latifolia *seed families (*n = *8 seed families/population;* n = *8 offspring/family, i.e. a total of 384 offspring measured)*

(A)

Population	HO	CT	PA	SC	VN	GR
Proportion germinated	0·93 ± 0·06/6·7	0·96 ± 0·05/5·4	0·81 ± 0·23/29·3	0·99 ± 0·02/2·1	0·93 ± 0·08/8·9	0·96 ± 0·06/6·4
Proportion flowering	0·90 ± 0·14/16	0·98 ± 0·04/4·2	0·86 ± 0·15/18·0	0·99 ± 0·02/2·1	0·97 ± 0·04/4·3	0·98 ± 0·04/4·2
Sex ratio (F/F + M)	0·51 ± 0·14/28·3	0·53 ± 0·14/27·2	0·62 ± 0·19/31·6	0·55 ± 0·10/18·8	0·58 ± 0·14/24·9	0·57 ± 0·13/23·5

(B)

	Population					
	SC		VN		GR	
	Females	Males	Females	Males	Females	Males
Germination time (d)	4·9 ± 0·7/14·7	4·5 ± 0·6/13·8	6·8 ± 2·2/33·4	5·9 ± 1·8/31·5	7·0 ± 1·7/25·0	6·5 ± 2·3/36·5
Age at first flowering (d)	29·4 ± 2·0/7·0	32·7 ± 4·1/12·9	36·4 ± 2·9/8·2	38·8 ± 2·8/7·4	33·7 ± 4·1/12·5	34·5 ± 5·6/16·7

(C)

	Population					
	HO		CT		PA	
	Females	Males	Females	Males	Females	Males
Germination time (days)	8·4 ± 3·2/9·3	7·3 ± 3·6/44·2	8·1 ± 2·8/35·6	8·4 ± 3·6/44·2	12·6 ± 3·4/27·8	12·4 ± 3·8/31·6
Age at first flowering (d)	41·1 ± 4·3/10·8	45·4 ± 11·4/25·9	33·8 ± 4·5/13·7	37·6 ± 3·9/10·7	40·8 ± 7·2/18·2	41·3 ± 4·5/11·2
Stem height (cm)	53·2 ± 7·6/14·7	55·3 ± 7·0/13·1	44·4 ± 3·9/9·1	44·5 ± 3·9/9·0	50·1 ± 4·2/8·6	49·5 ± 5·1/10·6
Longest leaf (cm)	15·4 ± 1·7/11·4	15·5 ± 2·8/18·6	11·4 ± 2·7/24·4	12·0 ± 2·8/24·1	14·2 ± 3·3/24·0	14·3 ± 2·7/19·5
No. of leaves	73·0 ± 10·7/15·1	104·4 ± 12·3/12·1	74·2 ± 14·5/20·2	99·8 ± 22·6/23·4	87·8 ± 22·8/26·8	119·6 ± 21·3/18·4
No. of stems	2·6 ± 0·7/27·8	2·9 ± 0·9/32·0	2·4 ± 1·0/43·0	2·8 ± 1·1/40·5	2·9 ± 1·1/ 39·1	3·7 ± 1·6/44·6

Data are given as mean ± s.d./coefficient of variation (CV), and were calculated as family means and, where applicable, separately by gender. (A) Proportion of the offspring which germinated, proportion flowering and offspring sex ratio. (B) Phenological traits for populations SC, VN and GR. (C) Phenological traits and size at first flowering for populations HO, CT and PA.

TABLE 3. *ANOVA for components of size at first flowering (stem height, length of the longest leaf, number of leaves, number of stems) in populations HO, CT and PA, and for time to germination and age at first flowering in populations HO, CT, PA, SC, VN and GR*

Source of variation	d.f.	MS	*F*	*P*
Stem height				
Gender	1	3·1	0·13	0·75
Population	2	2823·1	6·81	0·005
Family (population)	21	414·6	4·44	<0·0001
Gender × population	2	24·2	0·19	0·83
Gender × family (population)	20	127·9	1·37	0·13
Error	335	93·4		
Length of the longest leaf				
Gender	1	2·4	0·14	0·74
Population	2	422·5	3·97	0·03
Family (population)	21	106·4	7·74	<0·0001
Gender × population	2	16·5	0·86	0·3
Gender × family (population)	20	19·1	1·39	0·44
Error	335	13·8		
Number of leaves				
Gender	1	58456	1885·7	0·0005
Population	2	5591	1·55	0·23
Family (population)	21	3616	5·45	<0·0001
Gender × population	2	31	0·06	0·95
Gender × family (population)	20	503	0·76	0·44
Error	335	664		
Number of stems				
Gender	1	5·3	11·18	0·08
Population	2	3·67	0·35	0·71
Family (population)	21	10·45	5	<0·0001
Gender × population	2	0·45	0·14	0·87
Gender × family (population)	20	3·18	1·52	0·07
Error	335	2·09		
Time to germination				
Gender	1	0·047	23·87	0·005
Population	5	0·093	5·86	0·0003
Family (population)	42	0·016	7·57	<0·0001
Gender × population	5	0·002	0·64	0·67
Gender × family (population)	41	0·003	1·46	0·03
Error	757	0·002		
Age at first flowering				
Gender	1	0·00058	24·38	0·004
Population	5	0·00094	5·9	0·0003
Family (population)	42	0·00016	7·13	<0·0001
Gender × population	5	0·00002	0·75	0·59
Gender × family (population)	41	0·00003	1·42	0·045
Error	757	0·00002		

and phenological traits (time to germination, age at first flowering). Because F_1 individuals were phenotyped, this is not (or at least not strongly) affected by maternal environmental variation and thus probably reflects genetic divergence among populations. Variation within populations for these traits was also significant and substantial, and these traits exhibited high broad-sense heritabilities. However, these results are only indicative because a full-sib design was used and thus non-additive genetic effects (dominance and epistatic genetic variances) could affect the estimation. These results are concordant with

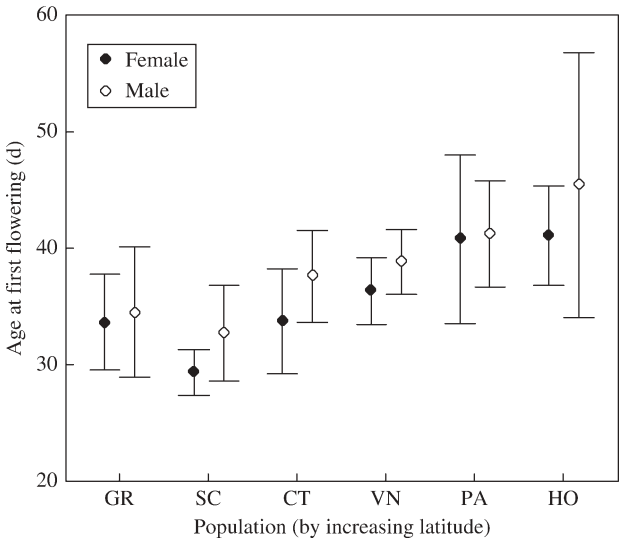

FIG. 2. Clinal variation in age at first flowering in *Silene latifolia* originating from six geographically separated populations. Age at first flowering is defined as the number of days from germination to opening of the first flower; the graph shows observed family means (± s.d.) for male and female F_1 plants ($n = 8$ families/population).

other studies that have found significant among-population divergence for traits including the morphology of pollen, flowers, fruits and leaves (Mastenbroek *et al.*, 1984; Prentice *et al.*, 1984; Mastenbroek and Vanbrederode, 1986; Delph *et al.*, 2002).

The estimated Q_{ST} was relatively high for age at first flowering, germination time and length of stems. In females, quantitative differentiation was higher than neutral differentiation (F_{ST}) for two traits (age at first flowering and time to germination), as indicated by the confidence intervals. This difference could suggest that phenotypic traits might be under divergent selection in the present populations (Merila and Crnokrak, 2001). However, there are two reasons why formal testing was not done to find out if $Q_{ST} = F_{ST}$: (1) variance in Q_{ST} estimates is high when, as in the present study, relatively few (<20) populations are studied and when differentiation is high (O'Hara and Merilä, 2005; Goudet and Büchi, 2006); (2) due to the high number of alleles at each of the investigated loci, F_{ST} values were low, while the observed G_{ST} was 94 % of the theoretically possible maximum. This may bias the estimate of the Q_{ST}:F_{ST} ratio. It is of note that, unlike Q_{ST}, G'_{ST} accounts for maximum divergence, thus comparison between these two estimates would also not be appropriate. Thus, future studies with a larger sample of populations would be useful to further explore differentiation in this species; for instance, defence traits against natural enemies present in native but absent in invasive populations are particularly interesting candidate traits for local adaptation (Wolfe, 2002; Wolfe *et al.*, 2004). An important pre-requisite is to have functional studies to identify such putative defence traits (Jolivet and Bernasconi, 2006).

Quantitative genetic differentiation was significantly associated with geographic distance. Importantly, clinal

TABLE 4. *Quantitative trait divergence (Q_{ST}) and broad-sense heritabilities (H^2) for life-history traits and size in* Silene latifolia*, calculated separately for each gender using variance component estimates*

Trait	Females				Males			
	Q_{ST}	Lower CI	Upper CI	H^2	Q_{ST}	Lower CI	Upper CI	H^2
Age at first flowering	0·17	0·060	0·280	0·55	0·10	−0·004	0·190	0·28
Time to germination	0·17	0·070	0·270	0·44	0·10	0·030	0·180	0·73
Length of the stem	0·17	−0·070	0·410	0·41	0·16	−1·200	1·550	0·37
Length of longest leaf	0·12	−0·220	0·460	0·67	0·04	−0·090	0·180	0·56
Number of leaves	0·05	−0·110	0·210	0·52	−0·02	−0·150	0·110	0·36

Variance components for between-population and between-family within-population variation were calculated with residuals of one-way ANOVA accounting for variation among blocks (see Materials and methods).

Time to germination (number of days from sowing to radicle emergence) and age at first flowering (number of days between germination and flowering) were inverse-transformed prior to analysis.

Lower/upper CI = upper and lower bounds of 95 % confidence intervals (calculated with the jackknife method).

variation for age at first flowering was observed. Natural variation for flowering time is reported for many other species, including *Arabidopsis thaliana* (e.g. Shindo *et al.*, 2005, 2007), indicating that this is a critical trait both in annual but also short-lived perennials like *S. latifolia*. These results strongly suggest that *S. latifolia* adapted to local environments by its phenology and morphology. It would be interesting to know to what extent climate, light regimes and biotic interactions influence among-population variation in life-history traits. A particularly interesting biotic interaction occurs between *S. latifolia* and the specialist seed predator *Hadena bicruris* (Wolfe *et al.*, 2004; Jolivet and Bernasconi, 2006). Indeed, flowering phenology may relate to strategies for escaping from seed predators (Biere and Honders, 1996; J. A. Elzinga and G. Bernasconi, unpubl. res.).

Other studies found evidence suggesting local adaptation in this species. European and North American *S. latifolia* populations differ in life-history traits and size and fruit morphology, with North American plants being larger, faster developing, and less well defended against seed predation (Blair and Wolfe, 2004). Kaltz *et al.* (1999) observed local maladaptation of the pathogen *Microbotryum violaceum* on its host *S. latifolia*, suggesting that the plant quickly evolved resistance to the local pathogen strains. Moreover, clinal variation in seed morphology related to winter temperatures has been reported previously (Prentice, 1986). Altogether, this suggests strong adaptive potential and indicates a potential local adaptation in response to local environmental conditions. However, only future studies with an experimental approach like reciprocal transplants (Kawecki and Ebert, 2004) could demonstrate local adaptation. In particular, it would be interesting to address the question of the adaptive value of clinal variation in age at first flowering.

Significant sexual dimorphism was found for components of size at first flowering (number of leaves and stems), and for time to germination and age at first flowering. Sexual dimorphism has been studied extensively in *Silene latifolia*, with numerous traits, especially reproductive traits being sexually dimorphic (number and size of flowers; Meagher, 1992, 1994; Delph *et al.*, 2005). In the present study, focus was on life-history traits and the results differ slightly from other studies. Within two North American populations, there was no evidence of sexual dimorphism for the number of leaves and number of stems measured at first flowering, time to germination and age at first flowering (Meagher, 1992). In another study (Delph and Meagher, 1995), males started flowering earlier than females, while in the present study the reverse was found. These differences illustrate how the degree and even direction of sexual dimorphism might differ among populations (Delph *et al.*, 2002), and it would be highly interesting to resolve which selective pressures create this variation.

In conclusion, central European populations of *Silene latifolia* show significant differentiation at neutral molecular markers and at phenotypic traits. High within-population heritable variation for several traits and clinal variation for age at first flowering suggest that *S. latifolia* has a strong adaptive potential.

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