et al. (1996) study was derived from an interspecific backcross [(C57BL/6J × Mus spreitus)F1 × C57BL/6J], while the X-linked QTL in this study was discovered from an outbred base ([JU × CBA]F1 × CFLP).

The QTL also has significant effects on all of the organ weights at 10 weeks, except for spleen weight in females (Table 1). The percentage increase is generally higher in females than in males (Figure 1). Empty carcass weight mainly reflects features of skeletal muscles and bones because the QTL effects on growth are not accompanied by changes in fatness (Rance et al. 1997b). Taken together, the data suggest that the QTL has somewhat greater effects on organ weights that body weight (or organ weights as a fraction of body weight), although the organ weight measures are subject to greater noise. Total food intake and daily food intake in H mice are significantly higher than those in L mice in both sexes (P < .05). In terms of feeding efficiency, food intake per gram gain implies higher energy consumption per gram gain in L mice than in H mice, but the differences are nonsignificant (P > .05) (Table 1, Figure 1).

In summary, H mice have higher body weight, organ weight, and food intake, and longer body size and tail length than L mice. Although the QTL can be detected through its effects on several traits, the data suggest that body weight at 10 weeks is close to the optimal single trait for testing the QTL effect: it is easy to measure and subject to low environmental variance. All of the traits affected by the QTL are controlled by highly complex processes. The most promising approach to further the understanding of the QTL would seem to be identification of the underlying locus.

References


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Non-Sex-Linked, Nuclear Cleaved Amplified Polymorphic Sequences in Silene latifolia

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Cleaved amplified polymorphic sequences (CAPS) were identified for five nuclear genes in Silene latifolia. By using published cDNA sequences of S. latifolia, pairs of primers were designed to amplify small regions of six nuclear genes. Targeted regions were successfully amplified, two of which included introns. By using direct sequencing of diploid individuals, suitable polymorphic sites for CAPS markers were rapidly detected in five of six of these gene regions, thus avoiding the tedious screening of a large panel of restriction enzymes. Using controlled progenies, we have also shown that all these CAPS markers segregated independently of the sex phenotype, thus demonstrating that the genes analyzed here are not located in the nonrecombining region of the sex chromosomes.

Silene latifolia is a diploid (2n = 24), dioecious species with an X/Y chromosomal sex determination system (Westergaard 1958), widely distributed in Europe, parts of Asia, and in the eastern United States (McNeill 1978; Prentice 1979). It is a model organism in diverse fields, including the study of the sex determination system (e.g., Farbos et al., 1999), the evolution of sex chromosomes (e.g., Guttman and Charlesworth 1998), species hybridization (Goulson and Jerrim 1997), sex-ratio distortion (Taylor 1999), host parasite interactions (e.g., Kaltz et al., 1999), and population structure (e.g., McCauley 1994). Apart from the sequence data recently obtained for an X/Y-linked gene (Filatov et al., 2000), thus far only allozymes have been used for studying diversity of nuclear genes.

Cleaved amplified polymorphic sequence (CAPS; Konieczny and Ausubel, 1993) genetic markers present several advantages: they are codominant and provide a relatively cheap, fast, and robust way to score variants. Extensively exploited in Arabidopsis thaliana (e.g., McKinney and Meagher 1998), they have also been developed recently for other plant species, mainly for the purposes of developing genetic maps and marker-assisted selection (e.g., Graner et al., 1999; Nikaido et al., 2000; Zheng et al., 1999). So far, very few plant population genetics studies have exploited CAPS markers (e.g., Bergelson et al., 1998; Tsumura et al. 1999). They are, however, expected to be very useful for such studies since they target either indel or substitution DNA sequence variation, and are expected to display less homology than other markers, such as allozymes (Ouborg et al. 2000).

Here we describe the acquisition of CAPS genetic markers for five nuclear genes in Silene latifolia. To obtain these markers, we amplified small gene regions using published cDNA sequences of Silene latifolia (Barbacar et al. 1997) to design pairs of oligonucleotide primers. Although heterozygous individuals are expected in this outcrossing species, and indel polymorphism may prevent analysis by direct sequencing, we have successfully used direct sequencing to identify polymorphic restriction sites in five genes. Amplification of exonic regions facilitated this step, since indel polymorphism within exon regions was observed in only one gene. Finally, we have also analyzed the segregation of these markers in relation to the sex phenotype in controlled progenies, in order to test for their location in the non-
recombining region of the sex-chromosomes.

All five markers described here are readily exploitable for population genetic studies of *S. latifolia*, including analyses of population structure and population dynamics or species hybridization studies. They may also be useful for the development of genetic maps of *S. latifolia*. Moreover, since we have demonstrated that these markers are not in the nonrecombining region of the sex chromosomes, the genes can be useful for studies of nucleotide polymorphism. In particular, data on nucleotide diversity in recombining chromosomes are required to test for the reduction of nucleotide diversity the Y chromosome and to analyze the genetic processes responsible for this reduced diversity.

**Materials and Methods**

**Plant Material**

Individual plants from laboratory strains were crossed under controlled conditions in a greenhouse. Three full-sib families (F7, F8, and F19) were grown, and 19-22 plants per family were sampled to test for the cosegregation of the CAPS markers with the sexual phenotypes (see Table 1).

**Molecular Methods and Data Analyses**

Pairs of oligonucleotide primers were designed from published cDNA sequences of *S. latifolia* (Barbunar et al. 1997) to amplify small regions of six genes (Table 2). All these genes are expressed in reproductive organs of *S. latifolia*, one of them only in male flowers (Barbunar et al. 1997). DNA was extracted as described by Fila and Charlesworth (1999). Polymerase chain reactions (PCRs) were done in 50 μl of 10 mM Tris-HCl pH 9, 50 mM KCl, 0.1% Triton X-100, 2.5 mM MgCl₂, 0.25 mM each of dATP, dCTP, dTTP, and dGTP, 0.5 μM of each primer, 100 ng DNA, 2 units of *Taq* polymerase (Promega), and overlaid with mineral oil. The following PCR cycling was carried out in a programmable thermal cycler (PTC 100 MJ Research): 3 min at 94°C, 35 cycles of 1 min at 94°C, 1 min at 56°C, and 36 cycles of 1 min at 94°C, 1 min at annealing temperature (Table 2), 1–1.5 min at 72°C, followed by an additional 5 min at 72°C. The PCR products were loaded on 1% agarose gels containing ethidium bromide and electrophoresed in 1 X TBE buffer. Amplified products were isolated from one or two individuals originating from laboratory strains, purified using Qiagikit (Qiagen) and directly sequenced using the ABI PRISM BigDye Terminator Cycle Sequencing Kit according to the manufacturer’s instructions (PE Applied Biosystems). Homology with the published sequences was confirmed by BLAST searches. To obtain CAPS markers, we used the sequence data to identify polymorphic restriction sites. This was done either within individuals by the presence of double peaks in the chromatograms (Hare and Palumbi 1999) or by comparing sequences from different plants, including the published sequence. This allowed us to avoid screening a large panel of restriction enzymes (see for instance Konieczny and Ausubel 1993; Lowe et al. 1998). Restriction enzyme reactions were incubated at 37°C (or at 25°C overnight for *Scl*) and resolved in 2% agarose gels.

**Results and Discussion**

A single band was amplified for all six genes. For two genes, the band sizes were larger than predicted from the cDNA sequences, suggesting the presence of introns in the amplified regions (Table 2). Our sequences were aligned with the pub-

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**Table 1. Segregation of five CAPS markers and the sexual phenotypes in full sibships (F7, F8, and F19), all with one heterozygous paternal parent for the marker considered**

<table>
<thead>
<tr>
<th>CAPS marker</th>
<th>Family no.</th>
<th>Observed segregation (S:H:B)</th>
<th>Total</th>
<th>Female</th>
<th>Male</th>
<th>P (exact test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCLS 8/Mldll</td>
<td>F8</td>
<td>4:3:6</td>
<td>2:1:4</td>
<td>2:2:2</td>
<td>.79</td>
<td></td>
</tr>
<tr>
<td>F19</td>
<td>8:10:4</td>
<td>7:3:2</td>
<td>1:7:2</td>
<td>.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCLS 37.1/Rso</td>
<td>F7</td>
<td>2:13:3</td>
<td>1:6:1</td>
<td>1:7:2</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>CCLS 52/Scl</td>
<td>F19</td>
<td>6:6:4</td>
<td>3:2:2</td>
<td>3:4:2</td>
<td>.100</td>
<td></td>
</tr>
<tr>
<td>CCLS 62/Accl</td>
<td>F7</td>
<td>0:12:7</td>
<td>0:8:2</td>
<td>0:6:5</td>
<td>.36</td>
<td></td>
</tr>
<tr>
<td>CCLS 75.1/Rso</td>
<td>F8</td>
<td>6:11:4</td>
<td>5:8:1</td>
<td>1:3:3</td>
<td>.22</td>
<td></td>
</tr>
<tr>
<td>F19</td>
<td>3:9:7</td>
<td>1:5:3</td>
<td>2:4:4</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

S = homozygote for the presence of the restriction site, H = heterozygote, B = homozygote for the absence of the restriction site, F = female, M = male.

The exact test compares the segregation of the CAPS marker between female and male progeny.

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**Table 2. Polymorphism detected in six nuclear genes studied in *Silenia latifolia***

<table>
<thead>
<tr>
<th>Gene (accession no.)</th>
<th>PCR and sequencing primers</th>
<th>Annealing temperature (°C)</th>
<th>cDNA size (bp)</th>
<th>gDNA size (bp)</th>
<th>π (total length, n²)</th>
<th>Polymorphic restriction sites (position)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCLS 8/Z93046</td>
<td>F: 5’ CTGAGATTTTGGGTGATGATG 3’</td>
<td>56°C</td>
<td>287</td>
<td>287</td>
<td>0.0075 (186 bp, n = 5)</td>
<td>Scl (52), Ndll (131), MspI (176)</td>
</tr>
<tr>
<td>CCLS 37.1/Z93048</td>
<td>R: 5’ GCCGGCGCTTGTTGCTG 3’</td>
<td>65°C</td>
<td>371</td>
<td>1560a</td>
<td>0.0022 (1356 bp, n = 2)</td>
<td>MspI (543), RsoI (733)</td>
</tr>
<tr>
<td>CCLS 52/Y12529</td>
<td>R: 5’ AGCAACTCCCTGGCAATCC 3’</td>
<td>54°C</td>
<td>337</td>
<td>1380b</td>
<td>0.0020 (739 bp, n = 4)</td>
<td>Scl (400c)</td>
</tr>
<tr>
<td>CCLS 62/Z93055</td>
<td>BF: 5’ CTGAGGGCTTCCATTCTGC 3’</td>
<td>54°C</td>
<td>231</td>
<td>231</td>
<td>0.0115 (136 bp, n = 2)</td>
<td>AccII (95)</td>
</tr>
<tr>
<td>CCLS 65/Y12603</td>
<td>BR: 5’ GCCGTTTTCATCTCGGCAC 3’</td>
<td>56°C</td>
<td>372</td>
<td>372</td>
<td>0.0087 (487 bp, n = 2)</td>
<td></td>
</tr>
<tr>
<td>CCLS 79.1/Z93054</td>
<td>BF: 5’ CTGAGATTTTGGGTGATGATG 3’</td>
<td>56°C</td>
<td>316c</td>
<td>316c</td>
<td>0.0123 (162 bp, n = 3)</td>
<td>RsoI (260), Mael, BfI (199)</td>
</tr>
</tbody>
</table>

Restriction enzymes target nucleotide substitutions except where otherwise indicated.

a Approximate size.

b Number of alleles analyzed.

Sequences were analyzed over a reduced part of the amplified fragment due to indel polymorphism.

Indel polymorphic site.
lished sequences (see Table 2 for the GenBank accession numbers) and the presence of one and three introns was expected within the amplified regions of CCLS 37.1 and CCLS 52, respectively.

Out of the six amplified regions, only one (CCLS 65) showed no polymorphism, even though, for this gene, additional primers were designed to amplify the entire published cDNA sequence. From our sequence data, a very rough estimate of the average nucleotide diversity (Nei and Li 1979) over all amplified regions is \( \pi = 0.6\% \) (Table 2). This nucleotide diversity estimate is rather low compared to the few estimates available in plant species (e.g., Cummings and Clegg 1998; Huttley et al. 1997; Liu et al. 1998; Miyashita et al. 1998), including an X-linked gene in *S. latifolia* (Filatov et al. 2000). However, the samples analyzed here from laboratory material probably underestimate the diversity. Work is in progress to assess the diversity in natural populations for these loci. Polymorphic indels were also detected in three gene regions: CCLS 37.1 (one indel in the intron in the region analyzed), CCLS 52 (one indel in the third intron in the region analyzed), and CCLS 79.1 (four indels, all in the exon analyzed). For all five genes showing polymorphism, at least one of the polymorphic sites detected created a variable restriction site (Table 2).

Using these CAPS markers, we analyzed the cosegregation of five genes with the sexual phenotype in sibships derived from controlled pollinations using heterozygous paternal parents for each marker. None of the five genes analyzed here is sex linked (Table 1). Although in one family the segregation observed for the CCLS 8/NDfII CAPS marker differed significantly between male and female offspring, all three genotypes were found both in males and females, thus rejecting the hypothesis of location of CCLS 8 in the nonrecombining region of the sex chromosomes.

This study demonstrates the ease of getting single-copy codominant markers in this species. However, other plant species may not have such high diversity as *S. latifolia*, a dioecious obligate outcrosser (e.g., Liu et al. 1998). Here, suitable variants were found by direct sequencing of small amplified regions from only one or two individuals. In such highly outcrossing species, targeting exonic regions rather than including introns may be preferable since few indel polymorphism are expected in coding regions, thus facilitating analyses by direct sequencing. The markers described here should prove useful for population genetic studies of *S. latifolia* and for the development of a genetic map of *S. latifolia*. Moreover, since all markers demonstrated independent segregation from the sex phenotype, this study provides candidate genes for reference data in the studies of nucleotide polymorphism.

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