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Genetic analysis of floral anthocyanin pigmentation traits in Asiatic hybrid lily using molecular linkage maps

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Abstract To understand the genetic background of two floral anthocyanin pigmentation traits, anthocyanin pigmentation in the flower tepals and spot formation, in the Asiatic hybrid lily (2n = 24), segregation of the two traits among 96 F_1 plants derived from a cross between commercial cultivars 'Montreux' and 'Connecticut King' were investigated. 'Montreux' has anthocyanin pigmentation in the tepals with many spots, and 'Connecticut King' has flowers with carotenoid pigmentation without spots. The F_1 plants with or without anthocyanin pigment in the tepals segregated with a 1:1 segregation ratio, indicating that a single gene controls anthocyanin pigmentation in the tepals. The number of spots per square centimeter of all tepals showed continuous distribution in the F_1 plants. To map the loci for the two anthocyanin pigmentation traits, molecular linkage maps in the Asiatic hybrid lily were constructed using a double pseudo-testcross strategy, with the same F_1 plants used for phenotypic evaluation, and 212 PCR-based DNA markers. The trait for anthocyanin pigmentation in tepals was used as a trait marker. The map of 'Montreux' comprised 95 markers in 26 linkage groups, and the map of 'Connecticut King' used 119 markers in 24 linkage groups. The total map lengths were 867.5 and 1,114.8 cM, respectively. The trait locus for anthocyanin pigmentation in the tepals was between markers ASR35-180 and P506-40 in linkage group 1 of the 'Montreux' map with a map distance of 1.2 cM and 2.6 cM, respectively. A single-point analysis of quantitative trait loci (QTLs) for tepal spot number identified two putative QTLs in linkage groups 1 and 19 of the 'Connecticut King' map. One putative QTL in linkage group 19 explained 64% of the total phenotypic variation. Because both putative QTLs were mapped on the linkage map of 'Connecticut King' that has no spots, dominant alleles of them might suppress spot formation.

Keywords *Lilium* sp. · Double pseudo-testcross · Flower color · Quantitative trait loci · Tepal spots

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Introduction

The genus *Lilium* comprises more than 90 species (Asano 1989) and is classified into six sections (Smyth et al. 1989). Most species of the section Sinomartagon, such as *Lilium dauricum*, *Lilium maculatum*, *Lilium concolor*, *Lilium leichtlinii*, *Lilium davidii* and *Lilium cernuum*, are distributed in East Asia. The Asiatic hybrid lily, one of the most important ornamental plants worldwide, is derived from inter-specific crosses of the section Sinomartagon (Leslie 1982). Most Asiatic hybrid lily cultivars have flowers with carotenoid pigmentation (yellow or orange) and the flowers of some modern cultivars (ex. 'Montreux') have anthocyanin pigmentation (pink). Most species in the section Sinomartagon have spots on their tepals and many cultivars have brown or black spots on the tepals. These spots are anthocyanin (Banba 1967). As cultivars with no or fewer spots are in demand, they have been bred (ex. 'Connecticut King'). However, the genetic bases of flower color and spot formation are little understood because of the heterozygous genome structure in Asiatic hybrid lily.

Constructing molecular linkage maps and mapping the loci for qualitative and quantitative traits are important to understand the genetic background of the agronomically important traits (Tanksley 1993; Yano and Sasaki 1997). Molecular linkage maps of allogamous

and vegetatively reproducing species, such as forest trees (Grattapaglia and Sederoff 1994; Nikaido et al. 2000), fruit trees (Maliepaard et al. 1998; Garcia et al. 1999), industrial crop trees (Herran et al. 2000; Lespinasse et al. 2000; Risterucci et al. 2000) and ornamental plants (Debener and Mattiesch 1999; Dunemann et al. 1999), have been constructed using a double pseudo-testcross strategy. Linkage maps have enabled the evaluation of complicated traits in these species (Conner et al. 1998; Dunemann et al. 1999; Garcia et al. 1999, 2000; King et al. 2000; Ling et al. 2000). Thus, constructing molecular linkage maps of the Asiatic hybrid lily is important to understand the genetic basis of its useful traits.

Many molecular markers are necessary to construct linkage maps. In *Lilium*, PCR-based markers have been developed, such as randomly amplified polymorphic DNA (RAPD) markers (Yamagishi 1995; Yamagishi et al. 2002) and inter-simple sequence repeat (ISSR) markers (Yamagishi et al. 2002).

The Asiatic hybrid lily is a vegetatively propagated bulbous plant and needs more than 2 years from sowing to anthesis. Thus, marker-aided selection (MAS) will be useful in breeding programs of this lily because MAS can select plants with traits in demand at the seedling stage. MAS is also valuable for gene pyramiding (Hittalmani et al. 2000). Identification of markers linked to useful traits is a first step for MAS. DNA markers linked to the trait loci for the number of petals in the carnation (Scovel et al. 1998), the petal number and flower color in diploid rose (Debener and Mattiesch 1999) and leaf chlorosis and flower color in *Rhododendron* (Dunemann et al. 1999) have been identified, but no reports exists of the mapping of useful trait loci in other ornamental crops.

We believe this study is the first to construct molecular linkage maps of the Asiatic hybrid lily using PCR-based molecular markers and to map the floral trait loci associated with anthocyanin pigmentation. Our results are important to understand the genetic basis of the traits and to apply molecular markers linked to mapped loci for MAS.

Materials and methods

Plant materials

Two commercial Asiatic hybrid lily cultivars, 'Montreux' and 'Connecticut King' (2n = 24), were used. 'Montreux' has pink tepals with many spots, and 'Connecticut King' has yellow tepals with no spots (Fig. 1). 'Montreux' was crossed with 'Connecticut King' in 1997 and their F_1 plants were individually grown in pots. Ninety six F_1 plants were randomly selected and were used to evaluate the phenotype and to construct linkage maps.

Phenotypic evaluation

One flower in each of the F_1 plants and their parents was collected at anthesis. To evaluate the anthocyanin in the tepals, one segment of 7 × 20 mm was cut from the upper half of each of the six tepals excluding brown spots. A total of six segments from one flower were immersed in 2 ml of 5% formic acid solution overnight, and then were rinsed twice with 1 ml of 5% formic acid solution for 2 h. After filtration using a cellulose acetate filter (0.45 μm, Toyo filter paper, Japan), the anthocyanin concentration in a total of 4 ml of 5% formic acid solution was measured using a spectrophotometer at A_{515}.

Thin-layer chromatography was carried out to identify the kinds of anthocyanin pigments, as *Lilium* species contain cyanidin 3-rutinoside as a major anthocyanin and cyanidin 3-rutinoside-7-glycoside as a minor anthocyanin (Nørbæk and Kondo 1999). Tepal pigments were extracted from 'Montreux' and 11 F_1 plants having pink tepals, using 5% formic acid solution as described above. Pigments in tepal spots were also extracted from an F_1 plant having yellow tepals with many spots by the same methods described above. These pigments were analyzed by thin-layer chromatography (Funacell FC-2020, Funakoshi, Japan) with n-butanol – acetic acid – H_2O (4:1:2). Cyanidin 3-rutinoside and cyanidin 3-rutinoside-7-glucoside purified by high performance liquid chromatography (HPLC) were used as control samples.

All spots on one flower (six tepals) were counted, and the total number of spots per square centimeter of all six tepals was used for data analysis. The tepal area was measured using a leaf-area meter.

Analysis of PCR-based markers

Total DNA isolation from fresh leaves of the F_1 plants and their parents, and their RAPD and ISSR analyses, were made as described previously (Yamagishi 1995; Yamagishi et al. 2002). For

Fig. 1 Asiatic hybrid lily cultivars 'Montreux' (left) and 'Connecticut King' (right) used as parents. 'Montreux' has many spots on the tepals, and 'Connecticut King' has no spots

Table 1 Originally designed 15-base and 3′ASSR primers

Primer	Sequence (5′ to 3′)	Primer	Sequence (5′ to 3′)
15-base random primer		3ASSR08	$(TC)_7GGA$
P617	CCCGACACCAGGTGA	3ASSR09	$(CA)_7GTT$
P620	GGCTATTCAGCTGGC	3ASSR11	$(CT)_7ACA$
P621	GGCGCAATTCATGGC	3ASSR13	$(CA)_7GTC$
P622	GCGATGACACAGGAC	3ASSR15	$(CT)_7ATG$
P623	ACGGGGTTTACCGCT	3ASSR19	$(CT)_7AAA$
P626	GTCGCTTCTGCAGCA	3ASSR20	$(CT)_7AGT$
P627	GCCTGCCTGCTGACG	3ASSR21	$(CA)_7GTA$
P628	GGAACCCAAGAGGAG	3ASSR25	$(CT)_7GAC$
P629	GTGTGCCGGTGTAGG	3ASSR26	$(CT)_7GCA$
P630	CCTGCAGCTCACGGA	3ASSR27	$(CT)_7GAG$
P635	CACGAGCGCAGTCCG	3ASSR28	$(CT)_7AGA$
P640	ATAGCGGCGTGCCAG	3ASSR29	$(CT)_7GTA$
P641	AGCCTGTCGTACGTG	3ASSR30	$(CT)_7GAA$
P642	GGACCACCGTAAGCC	3ASSR31	$(CT)_7TAG$
P644	GCAGATGGCACGGAG	3ASSR35	$(CT)_7TGA$
P645	GCTCTGGCGCACCGA	3ASSR37	$(CA)_7TGA$
P646	CACCCGTAGCGTGAG	3ASSR39	$(AG)_7CTT$
P647	CAGCGCACACATACC	3ASSR40	$(TCT)_5AG$
P649	CTCGCCCATCCAGCC	3ASSR42	$(GACA)_4C$
P650	GACACGGCCCGATAG	3ASSR46	$(AG)_8TT$
P651	CTCCCGGCGAGTGGA	3ASSR50	$(CA)_7AGT$
P652	CTTCGCTCGAACGCG	3ASSR52	$(CT)_7TGG$
P653	CAGTACCAGGTGGCG	3ASSR53	$(AG)_8CA$
P657	TGTCCCAACCCGGCA	3ASSR54	$(AG)_8GT$
3′ ASSR primer		3ASSR56	$(TG)_7ACG$
3ASSR01	$(GA)_8TC$	3ASSR61	$(CT)_7TGT$
3ASSR02	$(CT)_7ATC$	3ASSR62	$(TG)_7ACT$
3ASSR06	$(AG)_7TC$	3ASSR63	$(TG)_7AAC$
3ASSR07	$(TG)_7ACC$		

RAPD analysis, 17 10-base primers (15 primers were purchased from Operon, Alameda, USA, and two primers, Y24 and Y29, were originally designed, Yamagishi 1995), four 12-base primers (purchased from Wako, Japan), 37 15-base primers and 14 20-base primers were used. Of thirty seven 15-base primers, 24 were originally designed (Table 1). Sequences of the other 15-base primers and all 20-base primers were shown in Debener and Mattiesch (1999). Four semi-random primers (Y07, Y37, Y38 and Y45, Yamagishi 1995) were also used for RAPD analysis. For ISSR analysis, 33 3′-anchored simple sequence repeat (3′ASSR) primers that were originally designed (Table 1), and six UBC primers (UBC835, UBC841, UBC844, UBC857, UBC868 and UBC873, at the University of British Colombia, Vancouver, Canada, http://www.biotech.ubc.ca/frameset.html) were used.

Segregating markers in F_1 plants were scored for presence (1) or absence (2) of the amplified fragments. In total, 345 markers amplified by 115 primers were used to construct the maps. The primer code and one-tenth of the amplified fragment-size indicated the marker names. The primer code starting with letters 'OP', 'W', 'P', 'Y', 'ASR' and 'UBC' designated 10-base Operon RAPD primers, 12-base RAPD primers, 15- or 20-base RAPD primers, semi-random RAPD primers or originally designed 10-base RAPD primers, 3′ASSR primers and UBC primers, respectively. The segregation ratios in all markers were compared with the expected segregation ratios of 1:1 (if markers were present in either parent) or 3:1 (if markers appeared in both parents) using the chi-square test at 5% and 1% significance levels.

Map construction and mapping of quantitative trait loci (QTLs)

Two separate data sets were obtained, one for each parent. To detect the linkage of markers in the repulsion phase, marker genotypes in the duplicated data sets were reversed and used to construct maps (Grattapaglia and Sederoff 1994). Linkage maps were constructed for each parent using a double pseudo-testcross strategy. A threshold of LOD value > 4.0 was employed to make the linkage maps. The Kosambi mapping function was used to transform the recombination frequency to genetic distances. A three-step procedure was used to construct the maps. (1) Only markers present in either parent that fitted the 1:1 expected segregation ratio were initially used to construct the core linkage groups using Map Manager XP software (Manly and Cudmore 1998). (2) Subsequently, skewed markers inherited from either parent were placed in the core linkage groups using the same software. If the latter markers disturbed the already established marker order in the core linkage groups, they were discarded. (3) Lastly, linkage between 3:1 segregating markers (inherited from both parents) and the already mapped markers were analyzed using LINKEM software (Vowden and Ridout, http://www.ukc.ac.uk/IMS/statistics/people/M.S.Ridout/link1.html). Positions of the linked 3:1 markers were additionally indicated on the linkage maps.

To detect markers linked to quantitative trait loci (QTLs) for spot formation, the spot number per tepal area was analyzed using a single-point analysis of the QGene program (Nelson 1997). A threshold of *F*-value > 15 was used to detect putative QTLs, which were described by the code of the linkage group designated after *qST* (QTL for spots on the tepal).

Results

Evaluation of anthocyanin pigmentation traits in flowers

To assess if F_1 plants had either, or both of, cyanidin 3-rutinoside and cyanidin 3-rutinoside-7-glycoside in this study, the pigments in tepals extracted from 'Montreux' and 11 F_1 plants, and the pigments in tepal spots extracted from one F_1 plant, were separated using thin-layer

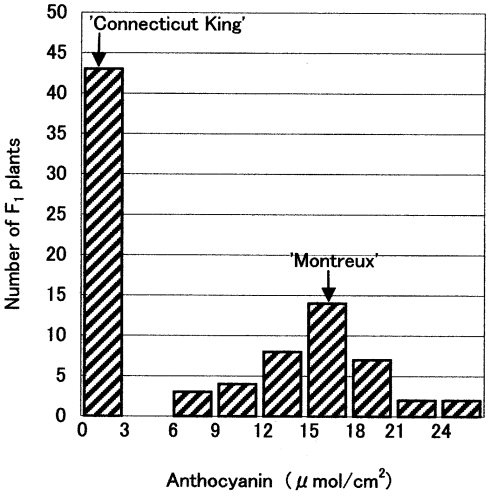

Fig. 2 Frequency distribution of anthocyanin in the tepals of F_1 plants derived from 'Montreux' × 'Connecticut King'. *Arrows* indicate mean values in the parents

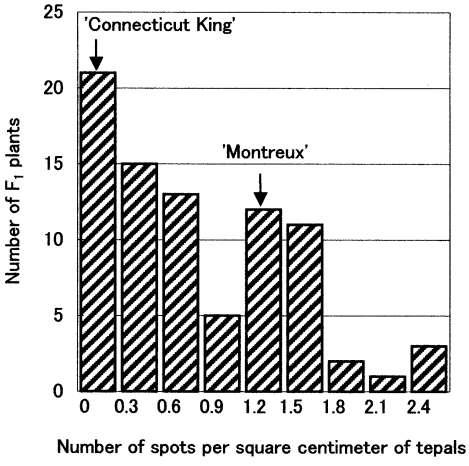

Fig. 3 Frequency distribution of the number of spots per square centimeter of tepals in F_1 plants derived from 'Montreux' × 'Connecticut King'. *Arrows* indicate mean values in the parents

chromatography. Only cyanidin 3-rutinoside was detected in the tepals of each plant by comparison of the Rf value with control samples. In a similar manner, anthocyanin of the tepal spots was identified as cyanidin 3-rutinoside (data not shown), indicating that 'Montreux' and the F_1 plants contain only cyanidin 3-rutinoside and that tepals and tepal spots contain the same pigment. In the experiment described below, we measured the anthocyanin in tepals of the F_1 plants using a spectrophotometor at A_{515}, as a wavelength scan of cyanidin 3-rhutinoside dissolved in 5% formic acid solution showed maximum absorbance at 515 nm (data not shown).

Cyanidin 3-rutinoside in the tepals of F_1 plants and in the two parents was measured; 'Montreux' had the pigment (17.26 ± 2.11 μmol/cm^2, mean ± SD), whereas 'Connecticut King' had little or no anthocyanin pigment (0.11 ± 0.03 μmol/cm^2). The frequency distribution of the anthocyanin in F_1 plants showed that they can be separated into two groups; (1) having little or no anthocyanin pigment (<3 μmol/cm^2), and (2) having cyanidin 3-rutinoside (>6 μmol/cm^2) (Fig. 2). The segregation ratio was 43:40, which fitted the theoretical segregation ratio of 1:1 (chi-square value 0.06^{ns}), indicating that a single gene controls the presence or absence of anthocyanin. The trait for anthocyanin pigmentation on tepals was used as a trait marker when the genetic linkage map was constructed.

The number of spots per square centimeter of tepals was 1.49 ± 0.13 (mean ± SD) in 'Montreux' and less than 0.01 in 'Connecticut King'. Frequency histograms in F_1 plants showed a continuous distribution from 0 to 2.7 (Fig. 3), indicating that several genes are associated with the number of spots.

Linkage map construction and mapping

Of the 345 markers amplified using 115 primers, 139 and 154 markers were present only in 'Montreux' and 'Connecticut King', respectively, and 52 markers appeared in both parents and segregated in the F_1 plants. Seven (5%) and 11 (7%) markers detected in either parent did not segregate among F_1 plants (Table 2), indicating that these markers were homozygous in the parents or were derived from organelle genomes. All PCR-based markers that segregated in F_1 plants were tested for expected segregation ratios using the chi-square test. The proportion of 1:1 markers showing significant segregation distortions ($P < 5\%$) was 23.5% and 24.5% in 'Montreux' and 'Connecticut King', respectively, and 28.8% of the 3:1 markers (Table 2). The

Table 2 Analysis of marker segregation for three different marker types in the mapping population

Item	From 'Montreux'	From 'Connecticut King'	From both parents	Total
Markers analyzed in F_1 plants	139	154	52	345
Bands present in all F_1 plants	7	11	–	18
Bands segregating in F_1 plants	132	143	52	327
Expected segregation ratios	1:1	1:1	3:1	
Markers showing segregation distortion at the 5% significance level (%)	31 (23.5)	35 (24.5)	15 (28.8)	81(24.8)

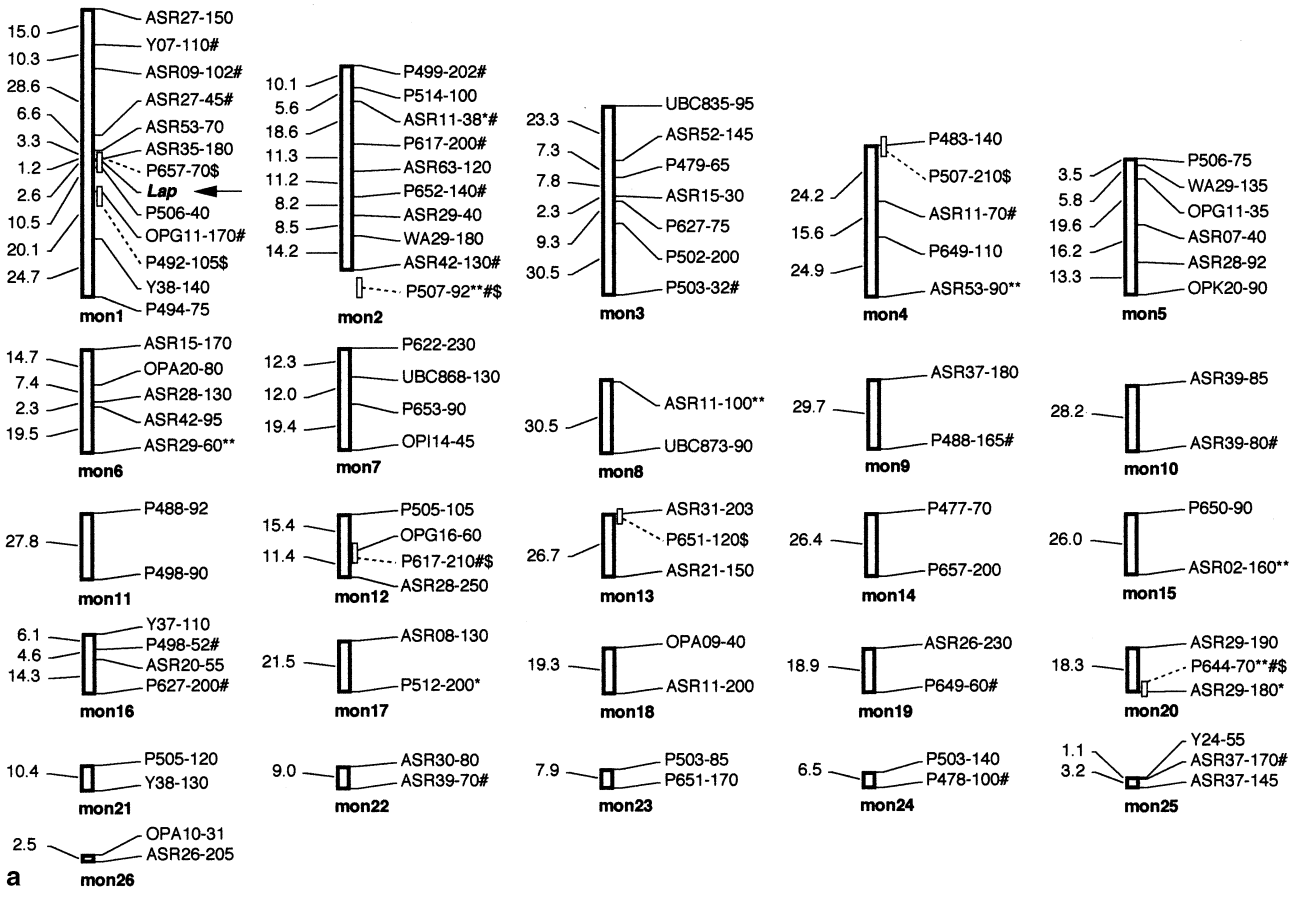

Fig. 4 Molecular linkage maps of Asiatic hybrid lily cultivars, 'Montreux' (*a*) and 'Connecticut King' (*b*), and the positions of mapped loci associated with floral anthocyanin pigmentation. The linkage maps were constructed using F_1 plants derived from a cross between 'Montreux' and 'Connecticut King' using a double pseudo-testcross strategy. Markers are shown on the right of the linkage groups, and numerals on the left of the linkage groups indicate marker intervals (cM). * and ** indicate markers showing skewed segregation at 5% and 1% levels, respectively. # shows the markers linked in repulsion phase. Approximate locations of the markers inherited from both parents (designated by $) are shown by *open boxes*. *Arrows* indicate a mapped locus for anthocyanin pigmentation in flower tepals (*Lap*) and two putative QTLs for the number of spots per tepal area (*qSTck1* and *qSTck19*). Markers showing significance (*F*-value > 15) in the QTL analysis are *underlined*. *mon* = 'Montreux'; *ck* = 'Connecticut King'. *Lap* = *Lilium* anthocyanin pigmentation

proportion of skewed markers was almost the same among markers inherited from 'Montreux', 'Connecticut King' or both.

All PCR-based markers segregating in the F_1 plants, and one trait marker for anthocyanin pigmentation in the tepals, were used to construct linkage maps (Fig. 4). Of 133 markers inherited from 'Montreux' and 143 markers from 'Connecticut King', 88 and 103, respectively, were grouped with a LOD score of 4.0. Seven and 16 markers segregating at a 3:1 ratio in F_1 plants were placed on the linkage maps of 'Montreux' and 'Connecticut King', respectively. The maternal 'Montreux' map had a total of 95 markers in 26 linkage groups, and the paternal 'Connecticut King' had 119 markers in 24 linkage groups. Linkage groups were numbered sequentially from the longest to the shortest. The total map length was 867.5 cM and 1,114.8 cM for 'Montreux' and 'Connecticut King', respectively. The average and the standard deviations of 1:1 marker density were 14.1 ± 7.4 cM for 'Montreux' and 14.0 ± 8.6 cM for 'Connecticut King'.

Two 3:1 markers, P657-70 and P507-210, were mapped on the both maternal and paternal maps, i.e. P657-70 was placed on mon1 and ck1, and P507-210 was on mon4 and ck23 (Fig. 4). These results suggest that mon1 and ck1, and mon4 and ck23, were homologous linkage groups.

The trait locus for anthocyanin pigmentation in tepals (presence or absence of cyanidin 3-rutinoside) was placed between markers ASR35-180 and P506-40 in linkage group 1 of the 'Montreux' map, with map distances between the trait locus and the markers of 1.2 ± 1.2 cM (LOD = 22.0) and 2.6 ± 1.8 cM (LOD = 19.4), respectively (Fig. 4a). This locus was tentatively named as *Lap* (*Lilium* anthocyanin pigmentation). The dominant allele at *Lap* might control the presence of anthocyanin pigmentation because *Lap* was on the map of 'Montreux' which shows anthocyanin pigmentation.

A single-point analysis using 96 F_1 plants identified two putative QTLs for spot formation in the tepals, tentatively named as *qSTck1* and *qSTck19* according to the

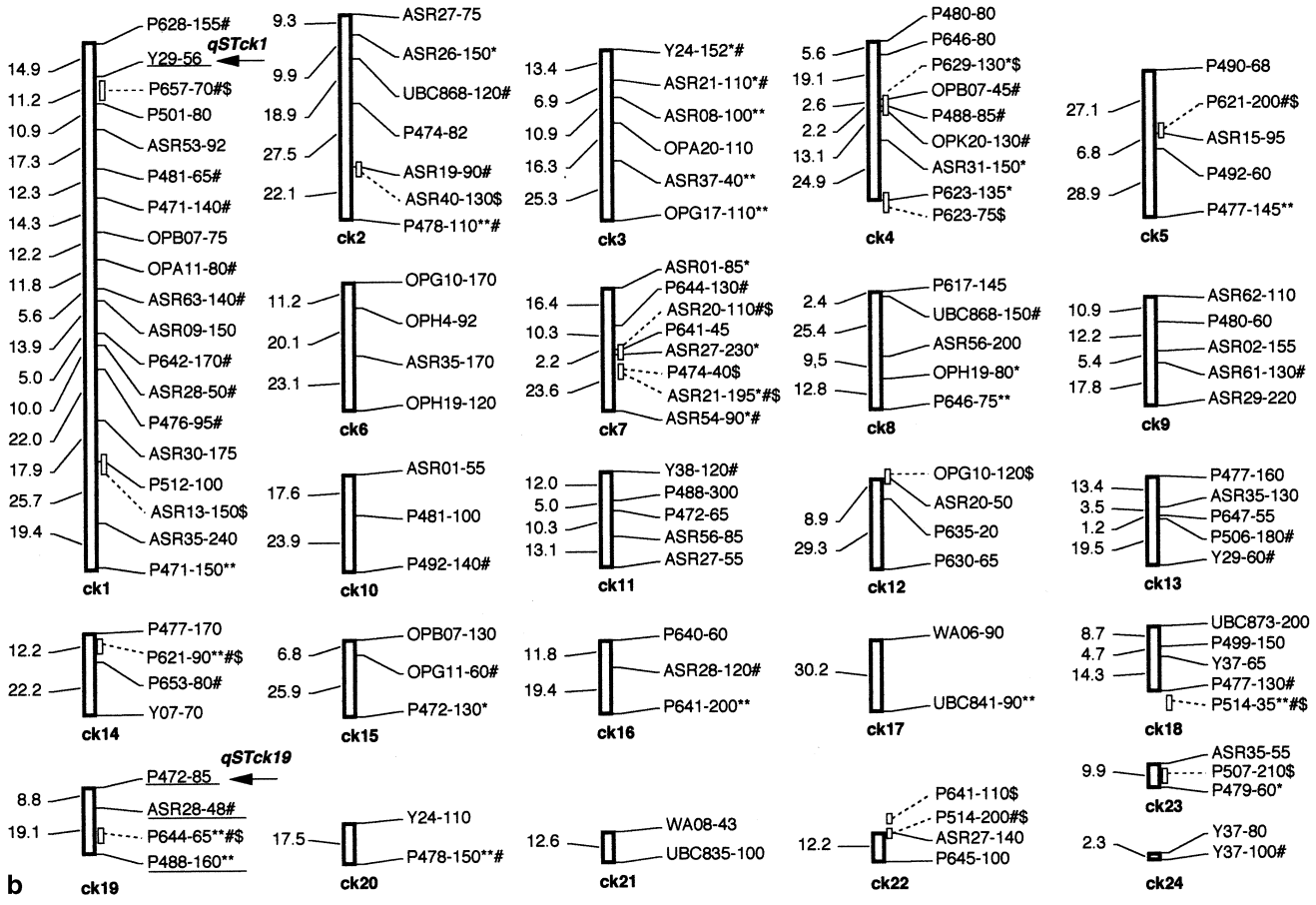

Fig. 4 (continued) Legend see page 1179

Table 3 Putative QTLs associated with the number of spots per square centimeter of tepals estimated by single-point analysis (F-value > 15)

QTL	Nearest marker	Linkage group	F-value	Variance explained (%)	
				Single	Multiple
qSTck1	Y29-56	ck1	15.9	16.9	68.6
qSTck19	P472-85	ck19	143.0	63.8	

linkage group number where each putative QTL was located (Fig. 4b, Table 3); *qSTck19* had a large effect explaining 63.8% of the total phenotypic variation. Multiple regression of the two putative QTLs explained 68.6% of the total phenotypic variation. Digenic interaction between the two putative QTLs was not recognized. Because both putative QTLs were on the linkage map of 'Connecticut King' having no spots on the tepals, their dominant alleles might suppress spot formation.

Discussion

Marker analysis and linkage map construction

In F_1 plants, 24–29% of markers showed segregation distortion (Table 2), almost the same as for other ornamental plant species, i.e. 23% and 11% of markers inherited from female and male parents, respectively, showed a skewed segregation in diploid rose (Debener and Mattiesch 1999), and segregation of 24% and 23% of markers from female and male parents, respectively, were distorted in *Rhododendron* (Dunemann et al. 1999). The causes of segregation distortion are not clearly understood. In rice, hybrid sterility genes and gametophytic selection genes have been identified as causes of segregation distortion in inter-specific and inter-subspecific

populations (Nakagahra 1972; Sano 1983, 1990; Harushima et al. 1996; Xu et al. 1997). Because the Asiatic hybrid lily is derived from inter-specific crosses among species in the section Sinomartagon, hybrid sterility genes and gametophytic selection genes may explain the observed segregation distortions.

We made PCR-based linkage maps of the Asiatic hybrid lily. The maps of 'Montreux' and 'Connecticut King' had 26 and 24 linkage groups, respectively. Because the number of linkage groups is larger than the haploid number of chromosomes in *Lilium* (n = 12), the linkage maps obtained in this study were not saturated and further marker analyses will be necessary to complete them.

Genetic analysis of traits associated with anthocyanin pigmentation in tepals

The locus *Lap* controlling anthocyanin pigmentation in flowers was mapped in linkage group 1 of the 'Montreux' map. Markers ASR35-180, P506-40 and ASR53-70 were closely linked to *Lap* in coupling phase (Fig. 4). Identification of molecular markers closely linked to agronomically useful loci is a first step for MAS. QTL analysis clarified that putative QTLs *qSTck1* and *qSTck19* in linkage groups of 'Connecticut King' are associated with spot formation on the tepals. Especially, *qSTck19* had a large effect explaining 63.8% of the total phenotypic variation, indicating that a major gene is in this chromosomal region. These QTLs are a target for MAS.

Because a 3:1 marker P657-70 was closely linked to both the *LAP* (Fig. 4a) and the *qSTck1* locus (Fig. 4b), these two loci have a potential to be the same. On the other hand, the chromosomal position of *qSTck19*, which had a large effect on the spot formation, was different from the *LAP*. These results indicate that the traits of anthocyanin pigmentation in tepals and of spot formation are under distinct regulation, although both tepals and tepal spots contain the same anthocyanin pigment.

Many genes are necessary for anthocyanin biosynthesis. A number of structural genes, such as genes for chalcone synthase and dihydroflavonol reductase (DFR), and of regulatory genes, such as genes including the Myb and Myc motif, have been identified and precisely characterized (Mol et al. 1998; Winkel-Shirley 2001). These genes were mapped onto the linkage maps in petunia (Strommer et al. 2000) and in *Arabidopsis* (Winkel-Shirley 2001). However, when genetic analysis of trait loci for flower and fruit color was examined, a single locus that determines the presence or absence of anthocyanin pigments was detected as follows. A single-trait locus determining pink and white flowers has been mapped on the linkage map of diploid rose (Debener and Mattiesch 1999). The red and yellow skin color of apple fruits, which is correlated with anthocyanin accumulation, is controlled by a single dominant locus (Cheng et al. 1996). The single *LAP* locus was identified in Asiatic hybrid lily in this study. These results indicate that only a few genes determine the presence or absence of anthocyanin pigments in flowers and fruits, and a number of other genes for anthocyanin synthesis are functional even in cultivars without anthocyanins.

The dominant allele of two QTLs, *qSTck1* and *qSTck19*, might suppress the spot formation. To determine the functions of genes in these loci will be intriguing because only a few genes and trait loci have been shown to suppress anthocyanin synthesis. Although many regulator proteins positively control the spatially regulated expression of sets of genes needed for the anthocyanin biosynthesis pathway (Mol et al. 1998), the negative regulator *intensifier1* was identified in maize (Burr et al. 1996). Trait loci, of which the dominant allele suppressed the expression of sets of structural genes, were also characterized in *Arabidopsis* (*icx1*, Jackson et al. 1995) and snapdragon (*Eluta*, Martin et al. 1991), although their genes have not been cloned. The *BANYULS* gene that encodes a DFR-like structural protein (LCR) reduces the anthocyanin accumulation in *Arabidopsis* seed coats (Devic et al. 1999).

A candidate gene strategy that maps candidate genes in a linkage map, and compares gene loci with trait loci, may be useful to identify genes of traits if plants have a large genome and their genes are hardly isolated using a map-based cloning strategy (Faris et al. 1999). Because of the large genome size in *Lilium* species (approximately 3.6×10^{10} bp, Angiosperms C-values Database Query, Royal Botanic Gardens, Kew, http://www.rbgkew.org.uk/), the identification of candidate genes for anthocyanin pigmentation from *Lilium* species, and the determination of their expression pattern and of their locations in the linkage maps of this study, may provide additional information to understand the function of the *Lap* locus and the gene or genes placed at the *qSTck1* and *qSTck19* loci.

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References

Asano Y (1989) *Lilium* L. In: Tsukamoto Y (ed) The grand dictionary of horticulture, vol. 5 (in Japanese). Syogakukan, Tokyo, pp 198–209

Banba H (1967) Pigments of lily flowers I. Survey of anthocyanin (in Japanese). J Jpn Sci Hort Sci 36:61–65

Burr FA, Burr B, Scheffler BE, Blewitt M, Wienand U, Mats EC (1996) The maize repressor-like gene *intensifier1* shares homology with the *r1/b1* multigene family of transcription factors and exhibits missplicing. Plant Cell 8:1249–1259

Cheng F, Weeden N, Brown S (1996) Identification of co-dominant RAPD markers tightly linked to fruit skin color in apple. Theor Appl Genet 93:222–227

Conner PJ, Brown SK, Weeden NF (1998) Molecular-marker analysis of quantitative traits for growth and development in juvenile apple trees. Theor Appl Genet 96:1027–1035

Debener T, Mattiesch L (1999) Construction of a genetic linkage map for roses using RAPD and AFLP markers. Theor Appl Genet 99:891–899

Devic M, Guilleminot J, Debeaujon I, Bechtold N, Bensaude E, Koornneef M, Pelletier G, Delseny M (1999) The *BANYULS* gene encodes a DFR-like protein and is a marker of early seed coat development. Plant J 19:387–398

Dunemann F, Kahnau R, Stange I (1999) Analysis of complex leaf and flower characters in *Rhododendron* using a molecular linkage map. Theor Appl Genet 98:1146–1155

Faris JD, Li WL, Liu DJ, Chen PD, Gill BS (1999) Candidate gene analysis of quantitative disease resistance in wheat. Theor Appl Genet 98:219–225

Garcia MR, Asins MJ, Forner J, Carbonell EA (1999) Genetic analysis of apomixis in *Citrus* and *Poncirus* by molecular markers. Theor Appl Genet 99:511–518

Garcia MR, Asins MJ, Carbonell EA (2000) QTL analysis of yield and seed number in *Citrus*. Theor Appl Genet 101:487–493

Grattapaglia D, Sederoff R (1994) Genetic linkage maps of *Eucalyptus grandis* and *Eucalyptus urophylla* using a pseudo-testcross: mapping strategy and RAPD markers. Genetics 137: 1121–1137

Harushima Y, Kurata N, Yano M, Nagamura Y, Sasaki T, Minobe Y, Nakagahra M (1996) Detection of segregation distortion in an indica-japonica rice cross using a high-resolution molecular map. Theor Appl Genet 92:145–150

Herran A, Estioko L, Becker D, Rodriguez MJB, Rohde W, Ritter E (2000) Linkage mapping and QTL analysis in coconut (*Cocos nucifera* L). Theor Appl Genet 101:292–300

Hittalmani S, Parco A, Mew TV, Zeigler RS, Huang N (2000) Fine mapping and DNA marker-assisted pyramiding of the three major genes for blast resistance in rice. Theor Appl Genet 100:1121–1128

Jackson JA, Fuglevand G, Brown BA, Shaw MJ, Jenkins GI (1995) Isolation of *Arabidopsis* mutants altered in the light-regulation of chalcone synthase gene expression using a transgenic screening approach. Plant J 8:369–380

King GJ, Maliepaard C, Lynn JR, Alston FH, Durel CE, Evans KM, Griffon B, Laurens F, Manganaris AG, Schrevens E, Tartarini S, Verhaegh J (2000) Quantitative genetic analysis and comparison of physical and sensory descriptors relating to fruit flesh firmness in apple (*Malus pumila* Mill.). Theor Appl Genet 100:1074–1084

Leslie AC (1982) The international lily register, 3rd edn. The Royal Horticultural Society, London

Lespinasse D, Rodier-Goud M, Grivet L, Leconte A, Legnate H, Seguin M (2000) A saturated genetic linkage map of rubber tree (*Hevea* spp.) based on RFLP, AFLP, microsatellite, and isozyme markers. Theor Appl Genet 100:127–138

Ling P, Duncan LW, Deng Z, Dunn D, Hu X, Huang S, Gmitter Jr FG (2000) Inheritance of citrus nematoda resistance and its linkage with molecular markers. Theor Appl Genet 100: 1010–1017

Maliepaard C, Alston FH, van Arkel G, Brown LM, Chevreau E, Dunemann F, Evans KM, Gardiner S, Guilford P, van Heusden AW, Janse J, Laurens F, Lynn JR, Manganaris AG, den Nijs APM, Periam N, Rikkerink E, Roche P, Ryder C, Sansavini S, Schmidt H, Tartarini S, Verhaegh JJ, Vrielink-van Ginkel M, King GJ (1998) Aligning male and female linkage maps of apple (*Malus pumila* Mill.) using multi-allelic markers. Theor Appl Genet 97:60–73

Manly KF, Cudmore RH (1998) MAP MANAGER XP. Plant and Animal Genome VI, San Diego, California, January 18–22, C10

Martin C, Prescott A, Mackay S, Bartlett J, Vrijlandt E (1991) Control of anthocyanin biosynthesis in flowers of *Antirrhinum majus*. Plant J 1:37–49

Mol J, Grotewold E, Koes R (1998) How genes paint flowers and seeds. Trends Plant Sci 3:212–217

Nakagahra M (1972) Genetic mechanism on the distorted segregation of marker genes belonging to eleventh linkage group in cultivated rice. Japan J Breed 22:232–238

Nelson JS (1997) QGENE: software for marker-based genomic analysis and breeding. Mol Breed 3:239–245

Nikaido AM, Ujino T, Iwata H, Yoshimura K, Yoshimaru H, Suyama Y, Murai M, Nagasaka K, Tsumura Y (2000) AFLP and CAPS linkage maps of *Cryptomeria japonica*. Theor Appl Genet 100:825–831

Nørbæk R, Kondo T (1999) Anthocyanins from flowers of *Lilium* (Liliaceae). Phytochemistry 50:1181–1184

Risterucci AM, Grivet L, N'Goran JAK, Pieretti I, Flament MH, Lanaud C (2000) A high-density linkage map of *Theobroma cacao* L. Theor Appl Genet 101:948–955

Sano Y (1983) A new gene controlling sterility in F_1 hybrids of two cultivated rice species. J Hered 74:435–439

Sano Y (1990) The genetic nature of gamete eliminator in rice. Genetics 125:183–191

Scovel G, Ben-Meir H, Ovadis M, Itzhaki H, Vainstein A (1998) RAPD and RFLP markers tightly linked to the locus controlling carnation (*Dianthus caryophyllus*) flower type. Theor Appl Genet 96:117–122

Strommer J, Gerats AGM, Sanago M, Molnar SJ (2000) A gene-based RFLP map of petunia. Theor Appl Genet 100:899–905

Smyth DR, Kongsuwan K, Wisudharomn S (1989) A survey of C-band patterns in chromosomes of *Lilium* (*Liliaceae*). Plant Syst Evol 163:53–69

Tanksley SD (1993) Mapping polygenes. Annu Rev Genet 27:205–233

Winkel-Shirley B (2001) Flavonoid biosynthesis. A colorful model for genetics, biochemistry, cell biology, and biotechnology. Plant Physiol 126:485–493

Xu Y, Zhu L, Xiao J, Huang N, McCouch SR (1997) Chromosomal regions associated with segregation distortion of molecular markers in F_2, backcross, doubled-haploid, and recombinant inbred populations in rice (*Oryza sativa* L.). Mol Gen Genet 253:535–545

Yamagishi M (1995) Detection of section-specific random amplified polymorphic DNA (RAPD) markers in *Lilium*. Theor Appl Genet 91:830–835

Yamagishi M, Abe H, Nakano M, Nakatsuka A (2002) PCR-based molecular markers in Asiatic hybrid lily. Scientia Hortic (in press)

Yano M, Sasaki T (1997) Genetic and molecular dissection of quantitative traits in rice. Plant Mol Biol 35:145–153