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Mapping of qualitative and quantitative phenotypic traits in *Rosa* using AFLP markers

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Abstract A segregating population of 91 hybrids issued from a cross between a dihaploid rose, derived from the haploidisation of a modern cultivar, and a diploid species was used to construct linkage maps of the parental genomes. As in other recent genetic studies in *Rosa*, AFLPs were used as molecular markers. Two segregating qualitative traits, recurrent blooming and double corolla, already known to be inherited as single recessive and dominant genes, respectively, were recorded in the mapping population. A quantitative trait, thorn density of the shoots, was also evaluated in this population. Sixty eight and 108 AFLP markers located on 8 and 6 linkage groups could be analysed in the female and male parent, respectively. The two recorded qualitative phenotypic markers were mapped as well as the quantitative one, after having performed QTL analyses on the parental maps in the latter case. It appears that thorn quantity is controlled by a major and a minor QTL which are located on the same linkage group at 36.5 and 3.2 cM from the single seasonal-blooming gene, respectively.

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Introduction

Roses are the most important ornamental crop economically because of their popularity as garden, landscape and pot plants or cut flowers, and their use as a source of aromatic oils for the perfume industry (Gudin 2000). They represent a difficult material for genetic studies because of their high heterozygosity (Rowley 1966; Berninger 1992; Gudin and Mouchotte 1996), ploidy levels (Berninger 1992; Jacob et al. 1996), plus well-known difficulties in sexual reproduction, from pollination to seed germination, often resulting in small progenies (Buck 1960; Gudin 1995; Gudin and Mouchotte 1996). As a direct consequence, the inheritance of only a few morphological and physiological traits is known in roses. Thus, male sterility in *Rosa setigera* Michx., single seasonal blooming, moss character, dwarfness, resistance to blackspot disease, and double corolla are inherited as single dominant genes (Lewis and Basye 1961; Semeniuk 1971; De Vries and Dubois 1978, 1984; Dubois and De Vries 1987; Von Malek and Debener 1998; Debener 1999). Moreover, the pink colour of the flower (versus white) and the presence of thorns are inherited either as single dominant genes or pairs of complementary genes (Debener 1999).

Nowadays, molecular markers are commonly used in several crop species for genetic studies, including the construction of linkage maps and mapping of qualitative and quantitative traits. In the *Rosaceae*, maps have for instance been developed in *Malus, Prunus* and *Rosa* (Chaparro et al. 1994; Hemmat et al. 1994; Debener and Mattiesch 1999; Debener et al. 2001a; Rajapakse et al. 2001). These maps are a prerequisite to marker-assisted selection, which is of interest for breeders (Gudin 1999). Since it is difficult to produce segregating populations based on the inbred genotypes in *Rosa*, the pseudo-test-

cross strategy (Grattapaglia and Sederoff 1994) may be applied because of the high heterozygosity available in the genus. This strategy corresponds to the study of the segregation of a maximum of four alleles in the offspring of a cross between two heterozygous genotypes. It has been applied to *Rosa* by Debener and Mattiesch (1999) and Debener et al. (2001a) who used dominant markers, such as random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) markers, sequence-characterised amplified region (SCAR) markers, and codominant markers such as microsatellites and restriction fragment length polymorphism (RFLP) fragments, to establish a linkage map on material derived from crosses with *Rosa multiflora* Thunb. ex Murr., different garden roses, and open pollination. On these maps, three qualitative traits were located: a double corolla, pink flower colour and resistance to black spot disease. An additional qualitative trait, the presence of prickles on the petiole, was also located on another rose map (Rajapakse et al. 2001). Finally, Debener et al. (2001b) mapped quantitative trait loci (QTLs) controlling the number of petals.

The present work was made in an attempt (1) to confirm the location of the double corolla character on an original rose material, and (2) to map additional genes controlling other important morphological traits corresponding to both qualitative traits and QTLs on material resulting from a cross between a dihaploid *Rosa hybrida* L. and the diploid *Rosa wichuraiana* Crép. This original material, partly derived from the haploidisation of the modern tetraploid cv Zambra, has been developed in the frame of an introgression program of "wild" useful genes, and it has already been used in different genetic studies (El Mokadem et al. 2000; Crespel et al. 2001a, b; El Mokadem et al. 2001). As recently applied to roses (Debener and Mattiesch 1999; Debener et al. 2000; Crespel et al. 2001a, b; Rajapakse et al. 2001; Zhang et al. 2001), AFLPs were used in this study.

Materials and methods

Plant material

A segregating population (Hw) of 91 diploid (2n = 2*x* = 14) interspecific hybrids issued from a cross between H190 a dihaploid rose and the diploid species *R. wichuraiana*, was used as the mapping population. H190 resulted from the haploidisation of the 4*x R. hybrida* cv Zambra. It was obtained by in situ parthenogenesis induced by using irradiated pollen (γ-rays at 600 Gy) and embryo rescue, as described by Meynet et al. (1994). H190 is a recurrent blooming, double-flowered and thornless rose, whereas *R. wichuraiana* is single seasonal blooming, single flowered and thorny. The ploidy level of the hybrid progeny was determined by flow cytometry, as described by Jacob et al. (1996). The plants were maintained in a heated greenhouse at the National Agronomy Research Institute in Fréjus. The crosses were carried out and the plants cultivated as described by El Mokadem et al. (2000).

Phenotyping the double corolla and recurrent blooming characters

The segregating traits, recurrent blooming and double corolla, inherited as single recessive and dominant genes, respectively, were recorded in the mapping population. The scorings were realised during 1 year, following germination.

Recurrent blooming

It was recorded as the ability to form flowers continuously. As previously observed by De Vries and Dubois (1984), recurrent blooming plants started to flower a few weeks after germination whereas single seasonal blooming plants did not flower in the greenhouse during the first year of growth.

Double corolla

Only the recurrent blooming plants could be recorded for this trait. The flowers were inspected visually in the greenhouse. As practiced by Debener (1999), all the plants with an average petal number of more than seven petals were considered to be double, while plants with an average number of less than seven petals were considered to be single.

DNA isolation and AFLP analyses

Total DNA was isolated from frozen young leaf tissue as indicated in Zhang et al. (2001). The AFLP core reagent and starter primer kits were purchased from Life Technologies (Gibco BRL) and AFLP analyses were performed as described by Vos et al. (1995). Total genomic DNA (125 ng) was digested with 1.25 U of *Eco*RI and 1.25 U of *Mse*I in 12.5-µl reaction mixtures (Gibco BRL Life Technologies) at 37 °C for 2 h. After complete digestion, *Eco*RI and *Mse*I adapters, 0.5 U of T4 DNA ligase, and ligation buffer (Gibco BRL Life Technologies) were added and the mixture was incubated for 2 h at 20 °C.

The preamplification reaction was performed with 2.5 µl of template DNA (1:10 solution diluted from the restriction-ligation mixture), using a pair of primers based on the sequences of the *Eco*RI and the *Mse*I adaptaters, including one additional selective nucleotide at the 3′ end (E + 1, M + 1), as described by Vos et al. (1995). The selective amplification reaction was also performed with two primers based on the same sequences as the E + 1 and M + 1 primers, but with two additional selective nucleotides at the 3′ end of each primer. The *Eco*RI primers were labeled by phosphorylating the 5′ end with [γ-33P]ATP for fragment detection, as described by Vos et al. (1995).

The polymerase chain reaction (PCR) products were mixed with an equal volume of tracking dye (95% formamide, 10 mM of NaOH, 0.05% bromophenol blue, 12.5% saccharose, and 0.05% xylene cyanol), denatured at 92 °C for 3 min. Aliquots (4 µl) of each reaction were loaded onto a denaturing 5% polyacrylamide gel (acrymamide-bisacrylamide 29:1) in 0.5 × TBE (50 mM of Tris, 50 mM of boric acid, 1 mM of EDTA, pH 8) and 7.5 mM of urea; 0.5 × TBE was used as the electrophoresis buffer. Gels were run at constant power (55 W), fixed, dried and exposed to a Kodak BioMax X-ray film for 2–4 days before developing. Twenty AFLP primer combinations (Table 1) were used.

Scoring of data

PCR fragments generated in the AFLP analyses were scored as dominant markers. Only the polymorphic markers characterizing one parent (present in H190 and absent in *R. wichuraiana*, or vice versa) that segregate in the progeny were considered. Markers were recorded as present or absent, coded according to the JOINMAP program manual (Stam and Van Ooijen 1995).

Designation of AFLP markers

AFLP markers were designated after the names of the two primers (e.g. E1M1), followed by a number reflecting the fragment posi-

Table 1 Selective AFLP primers used to generate markers in the segregating population Hw

AFLP primer combination	*Eco*RI primers	*Mse*I primers
E1M1	E-AAC	M-CAA
E1M2	E-AAC	M-CAC
E1M3	E-AAC	M-CAG
E2M1	E-ACA	M-CAA
E2M2	E-ACA	M-CAC
E2M3	E-ACA	M-CAG
E2M4	E-ACA	M-CAT
E2M5	E-ACA	M-CTG
E3M2	E-ACT	M-CAC
E3M4	E-ACT	M-CAT
E3M6	E-ACT	M-CTT
E4M1	E-AGG	M-CAA
E4M2	E-AGG	M-CAC
E4M3	E-AGG	M-CAG
E4M5	E-AGG	M-CTG
E4M6	E-AGG	M-CTT
E5M3	E-ACC	M-CAG
E5M4	E-ACC	M-CAT
E5M7	E-ACC	M-CTA
E5M8	E-ACC	M-CTC

tion on the gel. The numbers given to the markers are classified according to ascending molecular weight.

Map construction

Map construction was performed with the software program JOINMAP version 2.0 (Stam and Van Ooijen 1995). The marker data were collected separately for markers present only in H190, and present only in *R. wichuraiana*. Markers that did not fit a 1:1 ratio were not used in mapping. Ratio conformity for each marker was tested by a χ^2 test ($P \leq 0.005$). Separate maternal (i.e. H190) and paternal maps (i.e. *R. wichuraiana*) were calculated. The determination of linkage groups of markers originating from H190 and *R. wichuraiana* were done with the logarithm of the odds ratio (LOD) thresholds of 6.0 and 7.0, respectively. The calculations of the linkage maps were done using all pairwise recombination estimates <0.499 and a LOD score >0.001 (ripple value = 1, jump threshold = 5, triplet threshold = 7, Kosambi mapping function).

QTL mapping for the number of thorns

Thorns were numbered between the fifth and seventh nodes on at least three different shoots (all counted shoots had similar vigour, as appreciated by their length and diameter) for each parent and offspring individuals. Three scorings were made during the summer of 2000. High correlation coefficients characterized the three scorings. Hence, the average of the three scorings was used for QTL analysis.

The normality of the average number of thorns distribution was evaluated by using histograms and normal probability plots. The statistical analyses were performed by using the MINITAB statistical software.

QTL analysis was performed on each parental map with the MultiCrossQTL software (Rebaï et al. 1997), using interval mapping by the regression method. A LOD score threshold of 2.0 was set to identify the putative presence of a linked QTL in the interval, as used by Lander and Botstein (1989). The proportion of phenotypic variation relative to each QTL was estimated via linear regression between the identified region and the trait. When more than one identified region from a single linkage group was linked to a QTL, the R^2 values generally reached a peak at one position and decreased when moving away from this peak. The presence of a single QTL at this position was then hypothesized, and the peak was used to estimate its effect. If two peaks were present on the same linkage group and if they were more than 50 cM apart, two QTLs were hypothesized to be present at the corresponding positions.

When one or more QTLs with major effects were observed, their effects were eliminated to seek QTLs with more narrow effects, which are likely to be hidden otherwise (Lin et al. 1995). The residual variance used in the detection procedure was adjusted by subtracting the variance explained by the fixed QTL(s). However, in this case, the percentage of variation individually explained by the additionally detected QTLs could not be estimated.

Results

AFLP analysis

Among the 64 AFLP primer combinations (8 *Eco*RI and 8 *Mse*I), 20 (Table 1) were chosen for their ability to reveal a high polymorphism level in *R. hybrida*, as described by Zhang et al. (2001). A total of 288 segregating markers with an average of 14.6 markers per primer combination resulted from the AFLP analyses.

One hundred and thirty six and 152 markers were inherited from H190 and *R. wichuraiana*, respectively. The segregation ratios were tested with a standard χ^2 test. The markers with significant deviations from the expected segregation ratios at $P = 0.005$ varied from 28.9% to 50% (Table 2). The proportion of markers with skewed segregation ratios was higher for the markers inherited from H190 than for those inherited from *R. wichuraiana*.

Map construction

In H190, the two-point analysis grouped 68 AFLP markers in eight linkage groups (LOD score = 6.0). Among these linkage groups, six are major ones that include 4 to 29 markers and two are minor ones, with two markers each. During the mapping steps, five markers causing high χ^2-jumps during the ordering process or representing double recombinants were dropped. The average size of the eight linkage groups was 29.8 cM, ranging from 61.1 to 5.5 cM. The map covers a total length of 238.4 cM with an average distance between markers of 3.7 cM.

In *R. wichuraiana*, 108 AFLP markers were assembled in 6 linkage groups (LOD score = 7.0). The six linkage groups were formed by 4 to 30 markers. Two markers remained unlinked. Sixteen markers causing high χ^2-jumps during the ordering process or representing double recombinants were dropped. The average size of the six linkage groups was 47.8 cM, ranging from 65.3 to 32.7 cM. The whole map covers a total of 287.3 cM with an average marker density of 3.1 cM.

Several markers on linkage groups in H190 (A1, A4) and *R. wichuraiana* (B1, B2, B3, B4, B5) are strongly clustered (Fig. 1). The largest gap in the map is located on linkage group B4 between the markers E5M8.02 and E2M3.05. It corresponds to 20.7 cM.

Table 2 Analysis of marker segregation for two different marker types in Hw

Item	From H190	From *R. wichuraiana*	Total
Number of markers analysed in the segregating population	136	152	288
Expected segregation ratios	1:1	1:1	
Number of markers with deviation from the expected ratio at $P = 0.005$	68 (50%)	44 (28.9%)	112 (38.8%)

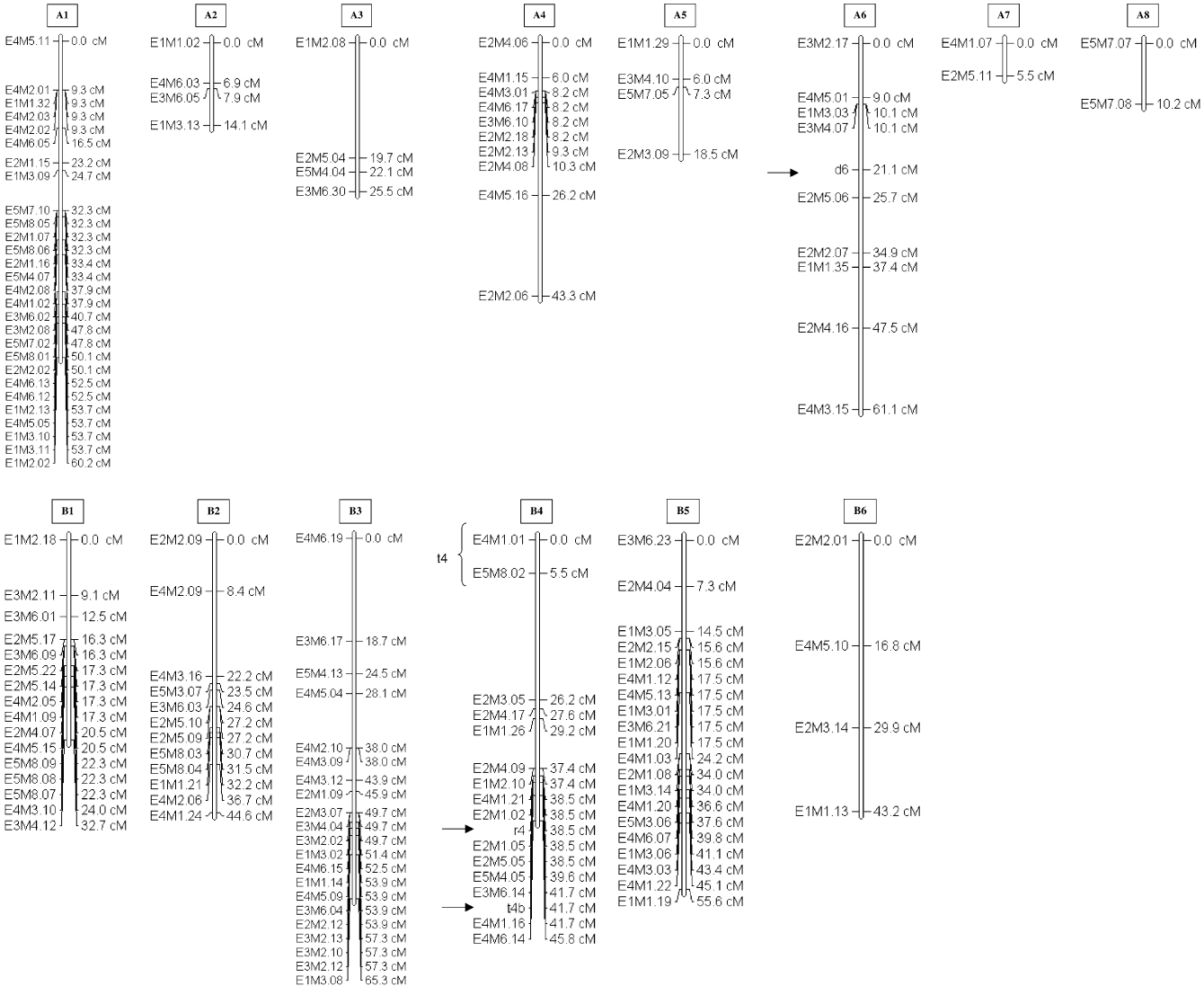

Fig. 1 Linkage maps of the dihaploid H190, parthenogenetically derived from *R. hybrida* cv Zambra (A1-A8), and of the diploid species *R. wichuraiana* (B1-B6). Map distances in cM are listed on the left and loci on the right of each linkage group. The localisations of the two genes controlling double corolla, *d6*, recurrent blooming, *r4*, and the minor QTL, *t4b*, are indicated by *arrows*. The major QTL region, *t4*, is indicated by a *brace*

Mapping of phenotypic markers

Recurrent versus single seasonal blooming, segregating in a 1:1 ratio, indicates monogenic inheritance ($\chi^2_{1\ df} = 2.47$, $0.05 < P < 0.1$). According to Debener (1999) and assuming that recurrent blooming H190 is homozygous recessive rr, this segregation ratio suggests that *R. wichuraiana* is heterozygous Rr. Tight linkage of the corresponding gene, *r4*, could be detected on linkage group B4 to the markers E4M1.21 (0 cM, LOD = 26.1), E2M1.02 (0 cM, LOD = 26.9), E2M1.05 (0 cM, LOD = 26.9), E2M5.05 (0 cM, LOD = 26.5), E2M4.09 (1.1 cM,

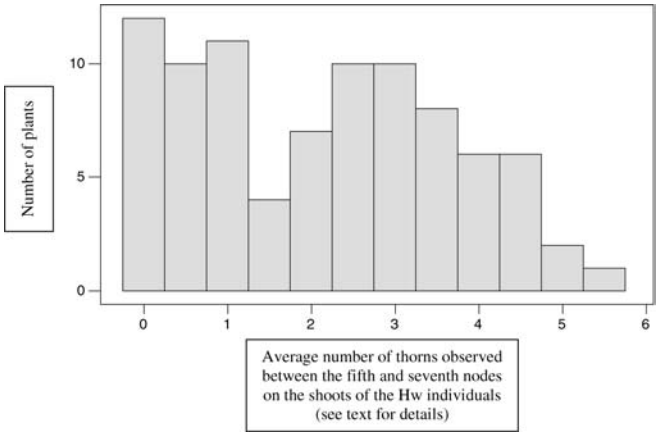

Fig. 2 Distribution of the average number of thorns in Hw

LOD = 24.1), E1M2.10 (1.1 cM, LOD = 23.5), E5M4.05 (1.1 cM, LOD = 24.5), E3M6.14 (3.1 cM, LOD = 21.3), E5M7.11 (3.1 cM, LOD = 20.2) and E4M1.16 (3.1 cM, LOD = 20.8), in coupling (Fig. 1).

H190 is characterized by double corollas (>15 petals) and recurrent blooming, whereas *R. wichuraiana* is single flowered (five petals in the corolla) and with single seasonal blooming. Double versus simple corollas, segregating in a 1:1 ratio, indicates monogenic inheritance ($\chi^2_{1\ df}$ = 0.23, 0.5 < *P* < 0.7). According to Debener (1999) and assuming that single-flowered *R. wichuraiana* is homozygous recessive dd, this segregation ratio suggests that H190 is heterozygous Dd. Tight linkage of the corresponding gene, *d6*, could be detected on linkage group A6 between the markers E3M4.07 (10.9 cM, LOD = 4.8) and E2M5.06 (4.7 cM, LOD = 6.1), in repulsion (Fig. 1).

QTL mapping for the number of thorns

The frequency distributions of the number of thorns for the mapping population are shown in Fig. 2. Normal probability plot tests rejected the normal distribution hypothesis for thorn quantity (*P* < 0.05). The distribution of the number of thorns in the progeny was bimodal, suggesting the effect of a major genetic factor. The average number of thorn values in the mapping population display a continuous range from totally thornless to very thorny plants, suggesting the action of QTLs (Fig. 2). Interestingly, 11 individuals had significantly more thorns than the "thorny" parent, *R. wichuraiana*, characterised by an average of 4.25 thorns between the fifth and seventh nodes.

A significant (*P* < 0.05) correlation was observed between the average number of thorns and single seasonal blooming (*r* = 0.45).

As a result of the MultiCrossQTL analysis, the number of thorns is controlled by a major QTL, *t4*, which is responsible for 66.4% of the phenotypic variation. The peak of the LOD score (LOD = 36.4) for *t4* is located between E4M1.01 and E5M8.02 on the group B4 of the *R. wichuraiana* map. Once *t4* is fixed, a minor QTL *t4b*, appears, which explains 13.8% of the phenotypic variance. The peak of the LOD score (LOD = 2.9) for *t4b* is located on marker E5M7.11, at a distance of 39.7 and 3.2 cM from *t4* and *r4*, respectively.

Discussion

Map construction

As previously done in roses by Debener and Mattiesch (1999) and Rajapaske et al. (2001), the double pseudotestcross strategy was used in combination with the AFLP technique to construct the first female and male linkage map based on an interspecific cross between a dihaploid rose derived from *R. hybrida* cv Zambra and diploid *R. wichuraiana*. Because AFLPs are dominant markers, two types of segregation are generated using this format. The markers which are heterozygous in only one parent segregate in a 1:1 present:absent ratio in the progeny, and markers heterozygous in both parents segregate in a 3:1 present:absent ratio. One interest of the double heterozygous markers is that they allow homologous linkage groups from the parental maps to be aligned, through the use of "locus bridges" (Echt et al. 1993). However, these markers were not used to construct the present maps because double heterozygous markers are less informative than single heterozygous ones, as the dominant-allele progenies comprise three indistinguishable genotypes (+ +, + –, and – +).

Because of the wide genetic diversity of the rose species and varieties at the origin of the material used by previous authors (Debener and Mattiesch 1999; Rajapaske et al. 2001) and in the present study, the different maps obtained are difficult to compare. As previously shown by Debener et al. (2001a), each parental map should contain seven linkage groups, consistent with the seven chromosomes corresponding to the haploid number in rose. The eight and six groups found in H190 and *R. wichuraiana*, respectively, indicate that gaps or breakpoints still remain in these maps. It will thus be necessary to increase the number of molecular markers and the size of the segregating population in order to saturate these linkage maps, and eventually bridge the gaps. The first rose maps based on RAPD and AFLP markers, using a population of 60 F1 hybrids resulting from a cross between two diploid half-sib rose genotypes, covered a total of 326 and 370 cM for the female and male parent, respectively (Debener and Mattiesch 1999). More recently, 52 F2 seedlings resulting from the open pollination of a F1 tetraploid hybrid issued from a cross between a blackspot resistant amphidiploid and the tetraploid sensitive cv "Basye's Blueberry" were used to construct new maps (Rajapaske et al. 2001). The amphidiploid had been obtained by an in vitro colchicine treatment of the meristems of a hybrid issued from a cross between *R. wichuraiana* "Basye's Thornless" and *Rosa*

rugosa var. rubra. The lengths of the resulting female and male maps corresponded to 682 and 902 cM, respectively.They have to be divided by two, to be compared with Debener and Mattiesch's or the present results, as they corresponded to a tetraploid material. These values from earlier studies are comparable with the ones of the present maps. Although they are much smaller than map lengths from other plant species, such as apple with 950 cM (Hemmat et al. 1994), tomato with 1,276 cM (Tanksley et al. 1992) and rice with 1,670 cM (Nagamura et al. 1993), they are comparable to what was obtained in *Arabidopsis* with 520 cM (Hauge et al. 1993), sugar beet with 508 cM (Nilsson et al. 1997), and diploid strawberry with 445 cM (Davis and Yu 1997). The average distances between the markers in the present study provide a density marker coverage comparable to that of the Debener and Mattiesch (1999) and Rajapaske et al. (2001) studies. This coverage can be considered as "medium", compared to the high-density maps obtained in tomato (Tanksley et al. 1992) and *Arabidopsis* (Hauge et al. 1992), or the low-density maps obtained in strawberry (Davis and Yu 1997) and *Citrus* (Cai et al. 1994).

The clustering of markers on specific areas of the linkage groups is a commonly observed phenomenon, which could be explained by a reduced recombination rate around the centromeres (Tanksley et al. 1992; AlonsoBlanco et al. 1998) and by the tendency of some marker types such as AFLPs to map in clusters (Nilsson et al. 1997; AlonsoBlanco et al. 1998; Debener and Mattiesch 1999).

Segregation distortion

A notable proportion of markers (38.8%) displayed distorted segregation ($P = 0.005$). Such high levels of segregation distortion have already and frequently been reported: 16–45.3% in roses (Debener and Mattiesch 1999; Crespel et al. 2001b), 25.5% in potato (Gebhardt et al. 1989), 12–59% in rape (Kianian and Quiros 1992), and 30% in banana (Faure et al. 1993). Distorted ratios are most commonly explained by pre- and/or post-zygotic selection. Since there was no selection in Hw and seedling survival was >70%, post-zygotic selection is unlikely to have occurred. However, sources of unintentional seedling selection caused by putative low vigour cannot be excluded. A gametophytic incompatibility system has been described in *R. rugosa* (Ueda et al. 1996). The existence of interparental incompatibility alleles could explain the distorted segregations that were observed. Chromosomal rearrangements (Tanksley 1984; Kianian and Quiros 1992; Faure et al. 1993) or gametic selection due to certation as shown in rice (Nakagahra 1986), can also result in distorted segregations. In the present experiment, segregation distortion was much more frequent in the female than in the male parent, suggesting the occurrence of some form of selection at the female gamete level. Distorted segregations have indeed been previously described as a consequence of genetic divergence between the parent plants (Kianian and Quiros 1992).

QTLs for the number of thorns

QTL mapping studies using a pseudo-testcross format differ from those using inbred populations as in the former case up to four different QTL alleles may be present in the two parents. Because the two parents are not derived from the same F1, their QTL alleles may indeed differ. If the genotype of one parent is Q_1Q_2 at the QTL locus, and the other parent is Q_3Q_4, for single heterozygous markers, the QTL analysis compares the difference between the average trait value of ($Q_1Q_3 + Q_1Q_4$) versus ($Q_2Q_3 + Q_2Q_4$) in the first parent, and ($Q_3Q_1 + Q_3Q_2$) versus ($Q_4Q_1 + Q_4Q_2$) in the second one (Grattapaglia et al. 1995). Thus, the quantitative value of alternative marker genotypes is measured as the effect of one allelic substitution averaged over potentially two alternative alleles inherited from the other parent. In the event of a QTL linked to a double heterozygous marker, with band-absent marker alleles linked to Q_2 and Q_4, the QTL analysis compares the difference between the trait value of (Q_2Q_4) versus the average of ($Q_1Q_3 + Q_1Q_4 + Q_2Q_3$). Specific intralocus interactions cannot be tested, because the QTL genotype of each parent is unknown and the information provided by dominant markers is limited. As a consequence, more variation occurs in this system because the effect of the QTL allele-substitution is measured against a heterogeneous background. Hence, this study may have only detected loci with specific genetic configurations and/or strong effects. In addition, the additivity/dominance ratio of QTLs could not be estimated, either due to the lack of a homozygous dominant class or because the homozygous dominant class could not be differentiated from the heterozygous one.

Despite these limitations, the *t4* and *t4b* QTLs presently detected can explain a large part of the phenotypic variation for the number of thorns. Moreover, the presence of 11 transgressive individuals in Hw which do not fit in the parental variation range for the number of thorns is in favour of the existence of two independent controlling loci (De Vicente and Tanksley 1993). This is in accordance with the important distance observed between *t4* and *t4b*. However some additional QTLs may have remained undetected. QTLs putatively located on chromosomal segments that are not yet mapped cannot be identified. Furthermore, QTLs with minor effects are known to be difficult to detect when the population size is limited (Lander and Botstein 1989; Moreno-Gonzalez 1992; Darvasi et al. 1993), as it was in the present study. The QTL *t4* has a major effect on the number of thorns and is probably responsible for the bimodal shape of the distribution for the studied trait. Moreover, when the plants with the average numbers of thorns <1 and ≥1 were pooled in "thornless" and "thorny" groups, respec-

tively, the observed segregation did not fit a 1:1 (thornless:thorny) ratio ($P < 0.05$). These results confirm the oligogenic nature of the presence/absence of thorns, as previously proposed by Debener (1999).

The single seasonal blooming character appears to be poorly correlated to thorn quantity. The single seasonal blooming gene *r4* is located at 36.5 and 3.2 cM from *t4* and *t4b*, respectively. The genetic control of these traits has not been shown to be independent until now. They could thus be linked and this would explain the observed correlation.

Application of the map

The current maps can be used to locate genes of important ornamental traits for both cut and garden roses, as predicted by De vries and Dubois (1996) and Krüssmann (1986). Tightly linked molecular markers were obtained for the three studied traits, double corolla, single seasonal blooming and thorn quantity. Rose breeders generate large hybrid populations in order to recombine traits from different varieties, and actual plant breeding is mostly based upon phenotypic selection. Markers that allow selection at the seedling stage of traits expressed lately during plant development could be especially useful in most of the woody species because of their long generation cycle.

The population used in this study is of special interest as it segregates for the qualitative and quantitative traits presently analysed, but it also seems to segregate for powdery mildew resistance (data not shown). This resistance could soon be characterized in the population using a recently described standardized in vitro test (Ferrero et al. 2001).

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References

AlonsoBlanco C, Peters AJM, Korneef M, Lister C, Dean C, van den Bosch N, Pot J, Kuiper MTR (1998) Development of an AFLP based linkage map of Ler, Col and Cvi *Arabidopsis thaliana* ecotypes and construction of a Ler/Cvi recombinant inbred line population. Plant J 14:259–271

Berninger E (1992) Le rosier de serre pour fleurs à couper. In: Gallais A, Bannerot H (eds) Amélioration des espèces végétales cultivées, objectifs et critères de sélection. INRA Editions, Paris, pp 490–504

Buck GJ (1960) Progress report on breeding hardy ever-blooming roses. Am Rose Annu 45:95–99

Cai Q, Guy CL, Moore GA (1994) Extension of the linkage map in *Citrus* using random amplified polymorphic DNA (RAPD) markers and RFLP mapping of cold-acclimation-responsive loci. Theor Appl Genet 89:215–221

Chaparro JX, Werner DJ, O'Malley D, Sederoff RR (1994) Targeted mapping and linkage analysis of morphological, isozyme, and RAPD markers in peach. Theor Appl Genet 87:805–815

Crespel L, Meynet J, Jacob Y, Zhang D, Gudin S (2001a) Identification of apomictic plants in *Rosa hybrida* L. by AFLPs. Acta Hort 547:51–55

Crespel L, Meynet J, Zhang D, Gudin S (2001b) Estimation of heterozygosity in two botanic rose species using AFLP markers. Acta Hort 546:187–191

Darvasi A, Weinreb A, Minke V, Weller JI, Soller M (1993) Detecting marker-QTL linkage and estimating QTL gene effect and map location using a saturated genetic map. Genetics 134:943–951

Davis TM, Yu H (1997) A linkage map of the diploid strawberry *Fragaria vesca*. J Hered 88:215–221

Debener T (1999) Genetic analysis of horticulturally important characters in diploid roses. Gartenbauwissenschaft 64:14–20

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

Debener T, Janakiram T, Mattiesch L (2000) Sports and seedlings of rose varieties analysed with molecular markers. Plant Breed 119:71–74

Debener T, Mattiesch L, Vosman B (2001a) A molecular marker map for roses. Acta Hort 547:283–287

Debener T, von Malek B, Mattiesch L, Kaufmann H (2001b) Genetic and molecular analysis of important characters in roses. Acta Hort 547:45–49

De Vicente MC, Tanksley SD (1993) QTL analysis of transgressive segregation in an interspecific tomato cross. Genetics 134:585–596

De Vries DP, Dubois LAM (1978) On the transmission of the yellow flower colour from *Rosa foetida* to recsurrent flowering hybrid tea-roses. Euphytica 27:205–210

De Vries DP, Dubois LAM (1984) Inheritance of the recurrent flowering and moss characters in F1 and F2 Hybrid Tea × *R. centifolia muscosa* (Aiton) Seringe populations. Gartenbauwissenschaft 49:97–100

De vries DP, Dubois LAM (1996) Rose breeding: past, present, prospects. Acta Hort 424:241–248

Dubois LAM, De Vries DP (1987) On the inheritance of the dwarf character in Polyantha × *Rosa chinensis minima* (Sims) Voss F1 populations. Euphytica 36:535–539

Echt CS, Kidwell KK, Knapp SJ, Osborn TC, McCoy TJ (1993) Linkage mapping in diploid alfalfa (*Medicago sativa*). Genome 37:61–71

El Mokadem H, Meynet J, Jacob Y, Gudin S (2000) Utilisation of parthenogenetic diploid plants of *Rosa hybrida* L. in interspecific hybridizations. Acta Hort 508:185–190

El Mokadem H, Crespel L, Meynet J, Gudin S, Jacob Y (2001) Gametic behaviour of parthenogenetic plants of *Rosa canina* L. and *R. hybrida* L. Acta Hort 547:289–296

Faure S, Noyer JL, Horry JP, Bakry F, Lanaud C, Gonzalez de Leon D (1993) A molecular marker-based linkage map of diploid bananas (*Musa acuminata*). Theor Appl Genet 87:517–526

Ferrero F, Cadour-Marvaldi P, Guilloteau E, Jacob Y, Coudray A, Sallanon H, Urban L (2001) Evaluation of the resistance to powdery mildew, *Sphaerotheca pannosa* var. *rosae*, of rose tree species and hybrids. Biological test on excised leaflets microscopic observations of the fungus, physical evaluation of the leaf cuticle protection. Acta Hort (in press)

Gebhardt C, Ritter E, Debener T, Schachtschabel U, Walkemeier B, Uhrig H, Salamini F (1989) RFLP analysis and linkage mapping in *Solanum tuberosum*. Theor Appl Genet 78:65–75

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

Grattapaglia D, Bertolucci FL, Sederoff RR (1995) Genetic mapping of QTLs controlling vegetative propagation in *Eucalyptus grandis* and*E. urophylla* using a pseudo-testcross mapping strategy and RAPD markers. Theor Appl Genet 90:933–947

Gudin S (1995) Rose improvement, a breeder's experience. Acta Hort 420:125–128

Gudin S (1999) Improvement of rose varietal creation in the world. Acta Hort 495:283–291

Gudin S (2000) Rose: genetics and breeding. Plant Breed Rev 17:159–189

Gudin S, Mouchotte J (1996) Integrated research in rose improvement. A breeder's experience. Acta Hort 424:285–292

Hauge BM, Hanley SM, Cartinhour S, Cherry JM, Goodmann HM, Korneef M, Stam P, Chang C, Kemplin S, Medrano L, Meyerowitz EM (1993) An integrated genetic/RFLP map of the *Arabidopsis thaliana* genome. Plant J 3:745–754

Hemmat M, Weeden NF, Manganaris AG, Lawson DM (1994) Molecular marker linkage map for apple. J Hered 85:4–11

Jacob Y, Teyssier C, Reynders-Aloisi S, Brown SC (1996) Use of flow cytometry for the rapid determination of ploidy level in the genus *Rosa*. Acta Hort 424:273–278

Kianian SF, Quiros CF (1992) Generation of a *Brassica oleracea* composite RFLP map: linkage arrangements among various populations and evolutionary implications. Theor Appl Genet 87:544–554

Krüssmann G (1986) Rosen, rosen, rosen. Verlag Paul Parey, Berlin Hamburg

Lander ES, Botstein D (1989) Mapping Mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185–199

Lewis WH, Basye RE (1961) Analysis of nine crosses between diploid *Rosa* species. Proc Am Soc Hort Sci 78:573–579

Lin Y-Y, Schertz KF, Paterson AH (1995) Comparative analysis of QTLs affecting plant height and maturity across the *Poaceae*, in reference to an interspecific sorghum population. Genetics 141:391–411

Meynet J, Barrade R, Duclos A, Siadous R (1994) Dihaploid plants of roses (*Rosa* × *hybrida* cv Sonia) obtained by parthenogenesis induced using irradiated pollen and in vitro culture of immature seeds. Agronomie 2:169–175

Moreno-Gonzalez J (1992) Genetic models to estimate additive and non-additive effects of marker-associated QTL using multiple regression techniques. Theor Appl Genet 85:435–444

Nagamura Y, Yamamoto K, Harushma Y, Wu J, Antonio BA, Sue N, Shomura A, Lin S-Y, Miyamoto Y, Toyama T, Kirihara T, Shimizu T, Wang Z-X, Tamura Y, Ashikawa I, Yano M, Kurata N (1993) A high density STS and EST linkage map of rice. Rice genome 2:11–13

Nakagahra (1986) Geographic distribution of gametophyte genes in wide crosses of rice cultivars. In: Proc Int Rice Genet Symp. IRRI, Manila, The Philippines, pp 73–82

Nilsson NO, Hallden C, Hansen M, Hjerdin A, Säll T (1997) Comparing the distribution of RAPD and RFLP markers in a high density linkage map of sugar beet. Genome 40:644–651

Rajapakse S, Zhang L, Ballard RE, Byrne DH (2001) AFLP marker development in rose for genetic mapping: comparison of three restriction enzyme pairs. Acta Hort 546:619–627

Rebaï A, Jourjon MF, Mangin B, Goffinet B (1997) MultiCrossQTL: software for QTL mapping in multiple crossing designs. Plant and Genome V Conference, San Diego, Poster 389

Rowley GD (1966) The experimental approach to rose breeding. Scientia Hort 18:131–135

Semeniuk P (1971) Inheritance of recurrent blooming in *Rosa wichuraiana*. J Hered 62:203–204

Stam P, Van Ooijen JW (1995) JOINMAP version 2.0: Software for the calculation of genetic linkage maps, CPRO-DLO, Wageningen, The Netherlands

Tanksley SD (1984) Linkage relationships and chromosomal locations of enzyme-coding genes pepper, *Capsicum annuum*. Chromosoma 89:352–360

Tanksley SD, Ganal MW, Prince JW, de Vicente MC, Bonierbale MW, Broun P, Fulton TM, Giovannoni JJ, Grandillo S, Martin O, Röder MS, Wing RA, Wu W, Young ND (1992) High density molecular linkage maps of the tomato and potato genomes. Genetics 132:1141–1160

Ueda Y, Takeshita D, Ando T (1996) Pollination in *Rosa rugosa* Thunb. ex Murray. Acta Hort 424:309–310

von Malek B, Debener T (1998) Genetic analysis of resistance to blackspot (*Diplocarpon rosae*) in tetraploid roses. Theor Appl Genet 96:228–231

Vos P, Hogers R, Bleeker M, Rijans M, Van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23:4407–4414

Zhang D, Besse C, Cao M, Gandelin MH (2001) Evaluation of AFLPs for variety identification in modern rose. Acta Hort 546:351–357