Theor Appl Genet (1999) 98: 1146–1155 

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# Analysis of complex leaf and flower characters in *Rhododendron* using a molecular linkage map

Received: 28 September 1998 / Accepted: 5 November 1998

**Abstract** A molecular linkage map of *Rhododendron* has been constructed by using a segregating population from an interspecific cross. Parent-specific maps based on 239 RAPD, 38 RFLP, and two microsatellite markers were aligned using markers heterozygous in both parents. The map of the male parent 'Cunningham's White' comprised 182 DNA markers in 13 linkage groups corresponding to the basic chromosome number. In the female parent 'Rh 16' 168 markers were located on 18 linkage groups. An assignment of putative homologous linkage groups was possible for 11 groups of each parent. QTL analyses based on the non-parametric Kruskal-Wallis rank-sum test were performed for the characters "leaf chlorosis" and "flower colour" scored as quantitative traits. For leaf chlorosis, two genomic regions bearing QTLs with significant effects on the trait were identified on two linkage groups of the chlorosis-tolerant parent. RAPD marker analysis of additional lime-stressed genotypes tested under altered environmental conditions verified the relationship between marker allele frequencies and the expression of chlorosis. Highly significant QTL effects for flower colour were found on two chromosomes indicating major genes located in these genome areas. The prospects for utilization of a linkage map in *Rhododendron* are discussed.

**Key words** *Rhododendron* sp. · Flower colour · Leaf chlorosis · Molecular linkage map · Quantitative trait loci

Communicated by P. M. A. Tigerstedt

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## Introduction

The genus *Rhododendron* comprises about 1000 species representing the largest genus within the family *Ericaceae*. Bred since the 18th century, rhododendrons and azaleas are among the most important ornamental woody plants. In spite of its large botanical and economic importance, the genetics of *Rhododendron* is not well understood. Only in the evergreen azalea *Rhododendron simsii*, which is one of the most important pot plants in Europe and the USA, have a few systematic genetic studies focussing on flower characters been undertaken (Heursel and Horn 1977; Heursel 1981). Evergreen *Rhododendron* cultivars with large flowers have been mostly selected directly from $F_1$ populations often generated by the contribution of one or more wild species.

The heterozygous genome structure conserved by outcrossing, the long juvenile period and the limited knowledge about the inheritance of important traits, causes difficulties in specific cultivar-improvement efforts. Nevertheless, successful breeding projects which were aimed at abiotic stress factors such as freezing (Uosukainen and Tigerstedt 1988) and lime stress (Preil and Ebbinghaus 1994) were performed in the past. *Rhododendron* species growing in nature in humus soils with low pH values often show strong iron-deficiency chlorosis symptoms if they are grown on calcareous soil. This stress is not only one of the most serious difficulties in the cultivation of Ericaceous species but is generally recognized as a problem in many other crops, e.g. citrus, grape, or soybean (de Cianzio 1991). Genetic variability, which permits improvement by plant breeding, exists even within species. Among *Rhododendron* species *Rhododendron micranthum* could be identified as highly lime tolerant (Chaanin 1996).

The availability of a genetic linkage map for *Rhododendron* would be useful for both practical breeding and basic research. Molecular markers linked to genes

controlling flower characteristics, iron-chlorosis tolerance, and resistance to fungal diseases (e.g. *Phytophtora* wilting or powdery mildew) would allow an early simultaneous selection of desired gene combinations. The creation of superior *Rhododendron* cultivars growing on their own roots would be facilitated and could be a supplement to the common use of a restricted number of specific rootstocks that are often not well-compatible with the genotype used as a scion.

Over recent years, DNA markers have been successfully used to identify and map quantitative trait loci (QTLs) affecting many agriculturally important traits (Tanksley 1993). QTLs associated with parameters of growth and development have been identified in several woody plant species such as *Populus* (Bradshaw et al. 1995), *Eucalyptus* (Grattapaglia et al. 1995), *Pinus* (Plomion et al. 1996) and *Malus* (Conner et al. 1998). Ultimately, with the availability of mapped QTLs, candidate gene-mapping strategies can be employed to study the physiology of complex processes such as nutrient uptake, assimilate transport, and partitioning, that allow plants to adapt to unfavourable soil conditions (Prioul et al. 1997).

Within the *Ericaceae* molecular linkage maps have been developed only for blueberry (*Vaccinium* sp.) (Rowland and Levi 1994; Qu and Hancock 1997). Our main objectives were to construct a first molecular map of the *Rhododendron* genome and to evaluate the possibilities to map genes for flower colour and leaf-chlorosis expression in a genetically highly heterogeneous $F_1$ population with an interspecific pedigree.

## Materials and methods

### Plant materials

A cross between the cultivar 'Cunningham's White' ('CW') and the genotype Rh16 ('16') was used for raising the mapping population RD2. The male parent 'CW' is an old cultivar originating from an interspecific hybridization between *Rhododendron caucasicum* and *Rhododendron ponticum* more than 150 years ago. It is known as a valuable white-flowering cultivar and a rootstock with an excellent adaptibility and grows even in neutral or slightly alkaline soils without chlorotic leaves. The female parent '16' is a hybrid between *Rhododendron fortunei* and 'CW' (Preil 1990). This accession has a red-purple flower colour and is highly susceptible to lime-enriched soils as demonstrated by strong iron-deficiency chlorosis symptoms. For map construction, 68 $F_1$ individuals were choosen from the total population consisting of 164 genotypes. To evaluate the extent of environmental variation, 20 genotypes of RD2 have been cloned by an in vitro micropropagation method. The cross between 'CW' and '16' was repeated in 1994 and 80 plants of the resulting population RD3 were used for further phenotypic and marker analysis.

### Phenotypic evaluations

Expression of iron-deficiency chlorosis was evaluated in greenhouse experiments. Eight-month-old seedlings of the progenies RD2 and RD3 were grown for a period of between 3 months (RD2) and 1 year (RD3) in a peat/conifer-needle compost (1 : 1) enriched with 10 g/l (RD2) or 20 g/l (RD3) of $CaCO_3$. Leaf chlorosis was scored visually using a rating scale of nine stages ranging from 1 ( no chlorosis) to 5 (severe chlorosis with some necrosis), with 0.5 intervals. Twenty genotypes of RD2 were propagated vegetatively and 10–20 clones were stress-tested as larger plants, with a height of about 20–30 cm, using a watering procedure. Plants were watered 20 times over a period of 4 months with a solution of $K_2CO_3$ (ranging from 5 mM at the beginning up to 20 mM at the end) and scored as described. Flower colour in population RD2 was scored in the years 1995 to 1998 in the field as a quantitative trait using a rating scale of seven stages ranging from 1 (pure white) to 7 (dark red-purple).

### DNA marker analysis

#### *DNA isolations*

For map construction, *Rhododendron* DNA was extracted from young leaves using a protocol adapted from Doyle and Doyle (1987). Three grams of fresh or frozen leaf material were homogenized in a CTAB extraction buffer (2% CTAB, 0.1 M Tris-HCl pH 8.0, 20 mM EDTA, 1.4 M NaCl, 2% PVP-40, 1% 2-mercaptoethanol). After a 30-min incubation step (65°C) DNA was extracted twice with chloroform and precipitated with an 0.7 vol of isopropanol. DNA pellets were washed in 70% EtOH, air dried, re-suspended in TE buffer and then treated with RNAse A. DNA was precipitated again and prepared as specific concentrated solutions for RFLP and PCR analysis. DNA isolations using a scaled-down miniprep protocol were performed for additional RAPD analysis of RD2 and RD3 plants.

#### *RFLP analysis*

RFLP analysis was performed according to standard DNA hybridization procedures using [$\alpha$-$^{32}$P]dCTP-labelled probes (Sambrook et al. 1989). A genomic library was generated by cleaving *Rhododendron* DNA with the enzyme *Pst*I and cloning fragments with a size between 500 and 2000 bp in *Escherichia coli* DH10b. Selected single- and low-copy clones were first tested on a subset of the parents 'CW' and '16', and five progeny plants, using the restriction enzymes *Eco*RI and *Hin*dIII. Probes with hybridization patterns indicating segregating RFLP markers were applied to larger Southern blots containing 5 µg of digested DNA from each individual of the mapping population. Hybridizations were performed overnight at 65°C in a buffer containing 1% SDS, 1 M NaCl, 10% dextranesulphate, 50 mM of Tris-HCl pH 8.0 and 30 µg/ml of denaturated salmon-sperm DNA. Post-hybridization washes were at 65°C with $0.5 \times$ SSC, 1% SDS for $2–3 \times 30$ min, followed by autoradiography for 2–8 days at −80°C on Kodak XAR films.

#### *RAPD analysis*

In order to select RAPD primers of different length for efficient RAPD-based map construction, over 650 primers and primer combinations were screened for polymorphic markers between the parents 'CW' and '16'. In detail, the following primers were used: 220 10-mer primers from Operon, Alameda, USA (sets A, D, E, F, J, T, N, O, P, U, AR) and 50 10-mer primers from the University of British Columbia, Vancouver, Canada (Nos. 201–250); 25 15-mer primers (Nos. 469–493) and 25 20-mer primers (Nos. 494–518) designed on the basis of known 10-mer primer sequences (Debener and Mattiesch 1998) and synthesized by MWG-Biotech, Ebersberg, Germany; 300 primer combinations using the 20-mer primers and two 16-mer primers consisting of four repeats each of the tetranucleotid motives GACA and GATA, synthesized by

MWG-Biotech. PCR was performed in Hybaid Omnigene thermocyclers using 96-well microtiter plates and the simulated tube-control modus. The reaction mix of a 25-µl reaction comprised 25 ng of *Rhododendron* DNA, 400 nM of primer (or $2 \times 200$ nM for primer combinations), 100 µM each of dNTPs and 1 unit of *Taq* polymerase (Life Technologies, Eggenstein, Germany) in $1 \times$ reaction buffer (10 mM Tris-HCl, pH 8.3; 50 mM KCl; 2.0 mM $MgCl_2$; 0.01% gelatin). Annealing temperatures were 35°C for the 10-mer primers, 48°C for 15- and 16-mer primers and 55°C for the 20-mer primers.

*SSR analysis*

A size-selected genomic library was constructed from *Rhododendron* 'Cunningham's White' using *Sau*3A-digested DNA fragments of 0.2–0.6-kb length and screened for $(GA)_n$ and $(CA)_n$ dinucleotide repeats. Four primer pairs flanking GA repeats (GA102, GA211, GA512 and GA758) were choosen for mapping. PCR conditions were the same as described for RAPD analysis, with the exception that the annealing temperatures were adapted to the Tm of the specific primer pairs. The DNA fragments were separated by electrophoresis in 3% MetaPhor™ agarose gels (FMC, Rockland, USA) and stained with ethidium bromide.

Map construction and QTL analysis

Two separate data sets were used for the construction of parent-specific linkage maps. Each data set contained the dominant markers segregating from the respective parent, all co-dominant markers, and the dominant markers present in both parents. Analysis of the expected segregation ratios of all markers and linkage mapping were performed with JoinMap version 2.0 (Stam and Van Ooijen 1995). A threshold LOD score of 4.0 was used for an assignment of the markers to the linkage groups. Map distances were computed using the Kosambi mapping function and a LOD threshold of 0.001 in the specific map-construction module. To map only well-fitting markers, causing a non-drastic increase in total chi-square value after being integrated in the map, a "jump" threshold of 5.0 was used as a criterion to discard problematic markers. The DrawMap software (Van Ooijen 1994) was used for a graphical construction of the map. The notations of RFLP markers (RDB) and SSR markers (GA) in the map are supplemented by additional letters (a, b, c, d) to indicate their segregation type. Markers with a one-letter appendix are dominant, and segregated for a single marker band from either one or both parents. The appendices 'ab' or 'abc' indicate co-dominant markers segregating for two or three alleles, respectively.

MapQTL version 3.0 (Van Ooijen and Maliepaard 1996) was used for QTL analysis of the data for the traits "leaf chlorosis expression" and "flower colour". As the data were not non-normally distributed, the Kruskal-Wallis rank-sum test was used for non-parametric mapping, which can be regarded as equivalent to the one-way analysis of variance (Van Ooijen and Maliepaard 1996). All molecular marker loci in which the calculated significance level of the K*-test statistic was $P \leq 0.05$, are marked on the map. Selected RAPD markers related to highly significant QTL effects were investigated in additional genotypes not included in the mapping population.

## Results

### Analysis of leaf and flower characters

Leaf-chlorosis symptoms of *Rhododendron* plants treated with lime were evaluated repeatedly during a period of 12 months. As demonstrated in Table 1, the $F_1$ plant material showed a broad spectrum of chlorosis expression under lime-stress conditions. In the mapping population, RD2 plants with no, or only slight, chlorosis were found beside individuals with intense chlorosis expression. A few RD3 genotypes showed no or slight leaf-yellowing symptoms even after 12 months of severe lime stress, whereas highly stressed plants of the same population would have died after a few months of culture if they were not re-potted into normal soil. Twenty additional RD2 genotypes not used for mapping were cloned from in vitro cultured seeds as starting material and tested for chlorosis tolerance using a watering procedure. Also in this material, the chlorosis values ranged from an average of 2.0 (slight chlorosis) in the best genotypes to 4.5 (severe chlorosis) in the most susceptible genotypes (Table 1). However, among the clones of a genotype the chlorosis values differed only to a relatively small extent and exceeded a value of more than one unit only in a few cases, demonstrating clearly the strong genotypic influence.

Although an oligogenic inheritance of the variability in flower colour studied in population RD2 is quite probable, a QTL approach seemed suitable due to the continuous variation of the flower colour. Scoring was also hampered because the colour of single flowers was not homogeneous and differed by one scoring unit between flowers of a single plant and between cloned material. The following frequency distributions of flower colours were obtained in the mapping population: 40% of the plants were white (as in parent 'CW'), 25% were white with a slightly reddish colouring, 15% were slightly red-purple (as in parent '16') and in the remaining 20% the flower colour was clearly more intense red-purple than in parent '16'.

**Table 1** Number of *Rhododendron* genotypes with a different expression of leaf chlorosis (1: no chlorosis, 5: severe chlorosis with necrosis)

| Plant material | Chlorosis expression | | | | | Number of genotypes tested |
|---|---|---|---|---|---|---|
| | 1–1.5 | 2–2.5 | 3–3.5 | 4–4.5 | 5 | |
| Population RD2 | 14 | 14 | 27 | 10 | 0 | 65 |
| RD2 clones[a] | 0 | 7 | 6 | 7 | 0 | 20 |
| Population RD3 | 2 | 23 | 23 | 12 | 10 | 70 |

[a] Chlorosis classification based on mean values

## DNA marker analysis

From a total of 280 RAPD markers scored in population RD2 239 markers were mapped into their respective linkage groups (Table 2). About 60% of the RAPDs were produced by using 20-mer primers in pairwise combinations, of which 195 RAPDs segregated as dominant markers from either the parent 'CW' or '16' (Table 2). Forty three dominant RAPDs segregating 3 : 1 from both parents and one co-dominant RAPD (F5-750ab) were included in the two data sets to enable an assignment of the "homologous" parent-specific linkage groups. However, as the information content of the ab × ab segregation-type of dominant markers is very limited, the RAPD framework map was supplemented by RFLP and SSR markers.

For RFLP marker development about 800 clones of a genomic *Pst*I library were screened for their single- to low-copy character by colony hybridization with total genomic DNA as a probe. Eighty pre-selected clones were screened to detect usable polymorphisms for mapping. Of the 48 RFLP loci identified, 38 markers, which were generated by a total of 27 genomic clones, could be located on the map. Seventeen RFLPs segregated as a single band from either one or both parents. Twenty-one RFLPs could be unambiguously scored as co-dominant markers segregating for two or three alleles (Table 2). All of the four SSR primer pairs generated segregating markers, but only two SSRs could be mapped. The marker GA211 was detected as a highly informative SSR locus segregating for four alleles. The other mapped SSRs segregated for a single amplicon. The three-allelic SSR GA758abc could not be integrated into the map using the "stringent" mapping conditions described above.

Genotypic segregation data of 279 mapped DNA markers were analysed for a significant deviation from the expected segregation ratio using the appropriate JoinMap 2.0 software module. If the two parent-specific data sets were checked separately, 41 markers showed skewed segregation at the 5% level of probability. This corresponds to percentages of 23% ('CW') and 24% ('16'), respectively.

## Map construction

A total of 332 DNA markers that included 280 RAPDs, 48 RFLPs and 4 SSRs were analysed to construct linkage maps. Two hundred and seventynine markers were linked to at least three other markers and two parent-specific maps were generated using a LOD score of 4.0 (Fig. 1). Fifty one markers (15% of the total) remained unlinked or could not be placed unambiguously onto the map because they exceeded the "jump threshold" value. For parent 'CW', the map comprised 148 RAPD-, 32 RFLP- and two SSR-markers, which were arranged in 13 linkage groups corresponding to the basic chromosome number of *Rhododendron*. In parent '16', 135 RAPDs, 32 RFLPs and one SSR marker were located on 18 linkage groups covering a total map length of 720 cM with the linkage groups ranging from 7 to 86 cM. The calculated total map length for 'CW' is 556 cM with about the same range regarding the linkage-group lengths. Considering the number of markers in 'CW' (182) and '16' (168), the average marker distances are 3.1 cM ('CW') and 4.3 cM ('16'), respectively.

An assignment of putative homologous linkage groups was possible for 11 groups of each parent. In nine groups, 4–6 co-dominant RFLPs and 3 : 1-segregating RAPDs allowed an alignment of the corresponding groups. In four groups (6, 8, 9 and 10) the marker order was identical in both parents. In the groups 1, 2, 3, 4, 7 and 11 some rearrangements in marker order and minor differences in the calculated

**Table 2** Number and segregation types of DNA markers used for parent-specific linkage group construction in *Rhododendron*

| Marker type | Segregation/segregation type[a] | Number of markers mapped | | |
|---|---|---|---|---|
| | | Parent 'CW' | Parent '16' | Total |
| RAPD | 1 : 1 (ab × aa) | 104 | 91 | 195 |
| RAPD | 3 : 1 (ab × ab) | 43 | 43 | 43 |
| RAPD | 1 : 2 : 1 (ab × ab) | 1 | 1 | 1 |
| | | | | 239 |
| RFLP | 1 : 1 (ab × aa) | 6 | 6 | 12 |
| RFLP | 3 : 1 (ab × ab) | 5 | 5 | 5 |
| RFLP | 1 : 2 : 1 (ab × ab) | 10 | 10 | 10 |
| RFLP | 1 : 1 : 1 : 1 (ab × ac) | 11 | 11 | 11 |
| | | | | 38 |
| SSR | 1 : 1 (ab × aa) | 1 | – | 1 |
| SSR | 1 : 1 : 1 : 1 (ab × cd) | 1 | 1 | 1 |
| Totals | | 182 | 168 | 279 |

[a] According to the JoinMap version 2.0 format

map distances were observed. In group 5, a tri-allelic RFLP (RDB419abc) was found as an allelic bridge. A comparison of the map lengths of joined linkage groups suggests that some of the groups are not yet complete. Examples are groups 16-1, 16-2, 16-3 and 16-4, which are significantly shorter than their 'CW' counterparts. However, among the seven linkage groups of '16' not yet joined with 'CW', at least the five smaller groups (16-14 to 16-18) are candidates for filling up the missing areas. A clustering of RAPD markers was observed on a few linkage groups (CW-9, CW-12, 16-11). In groups 2, 6 and 9 it appears that clustering occurred in the same regions of the corresponding linkage groups, possibly indicating the centromere regions of the chromosomes. Markers with distorted segregation ratios were not equally distributed over the

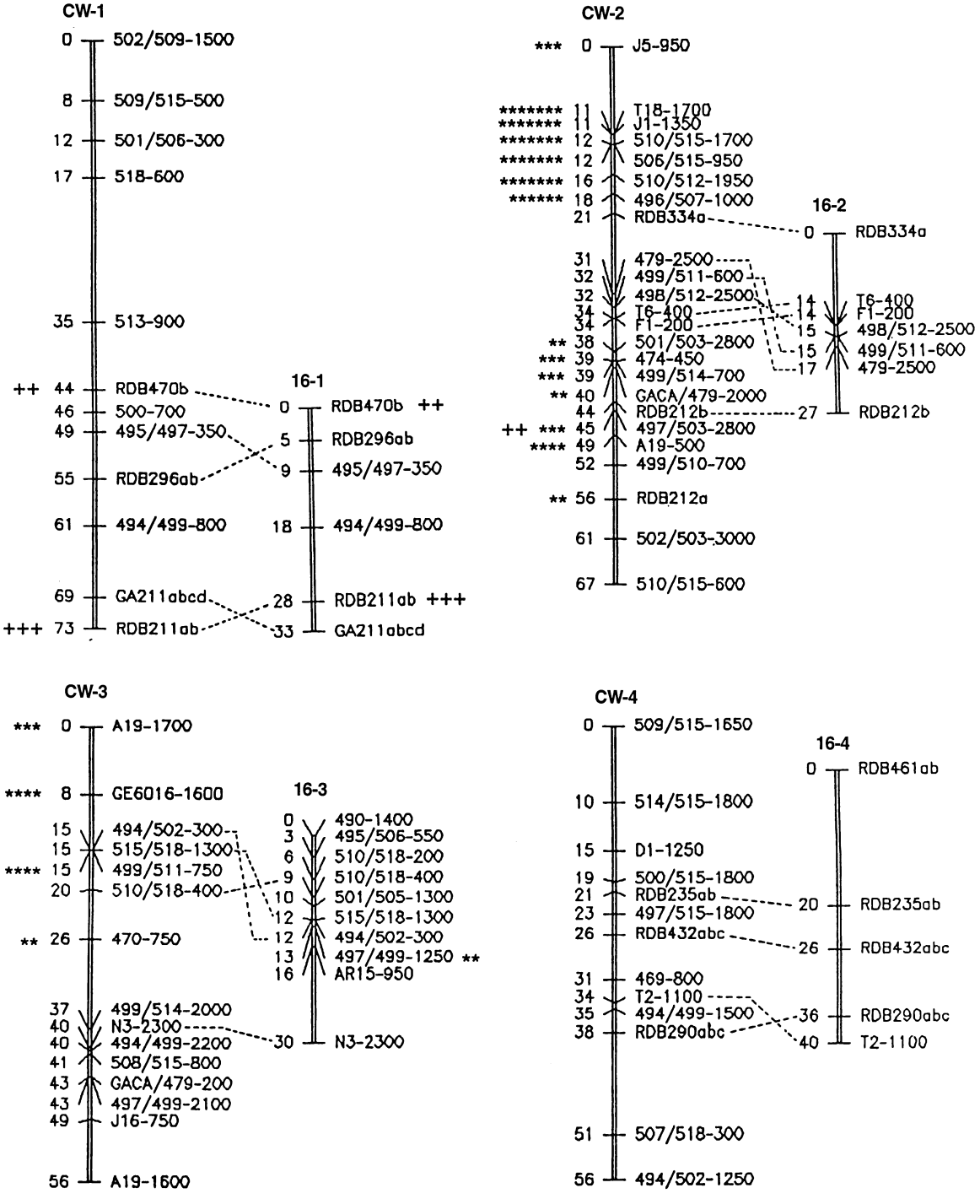


**Fig. 1** See page 1152 for legend

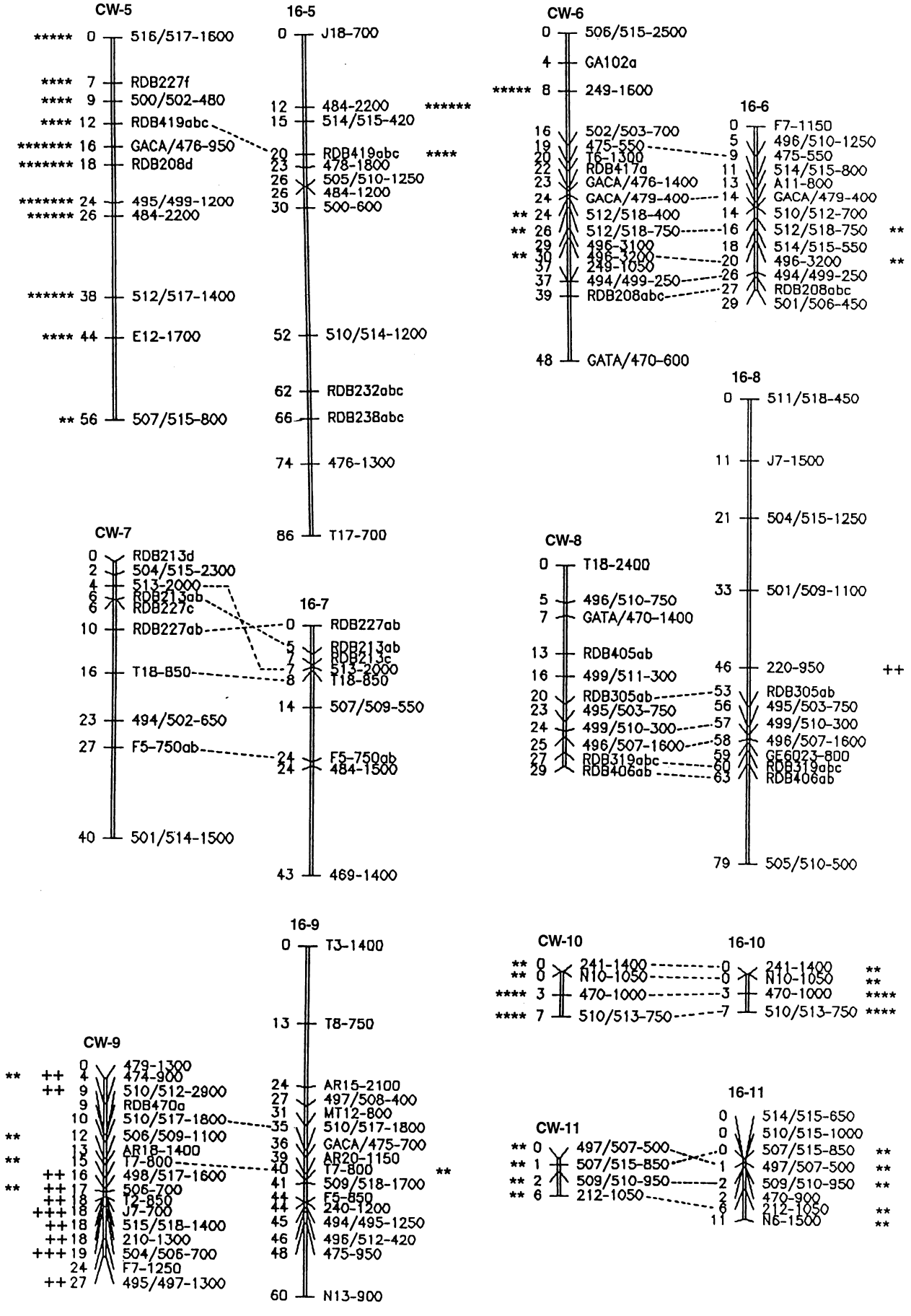


**Fig. 1** See page 1152 for legend

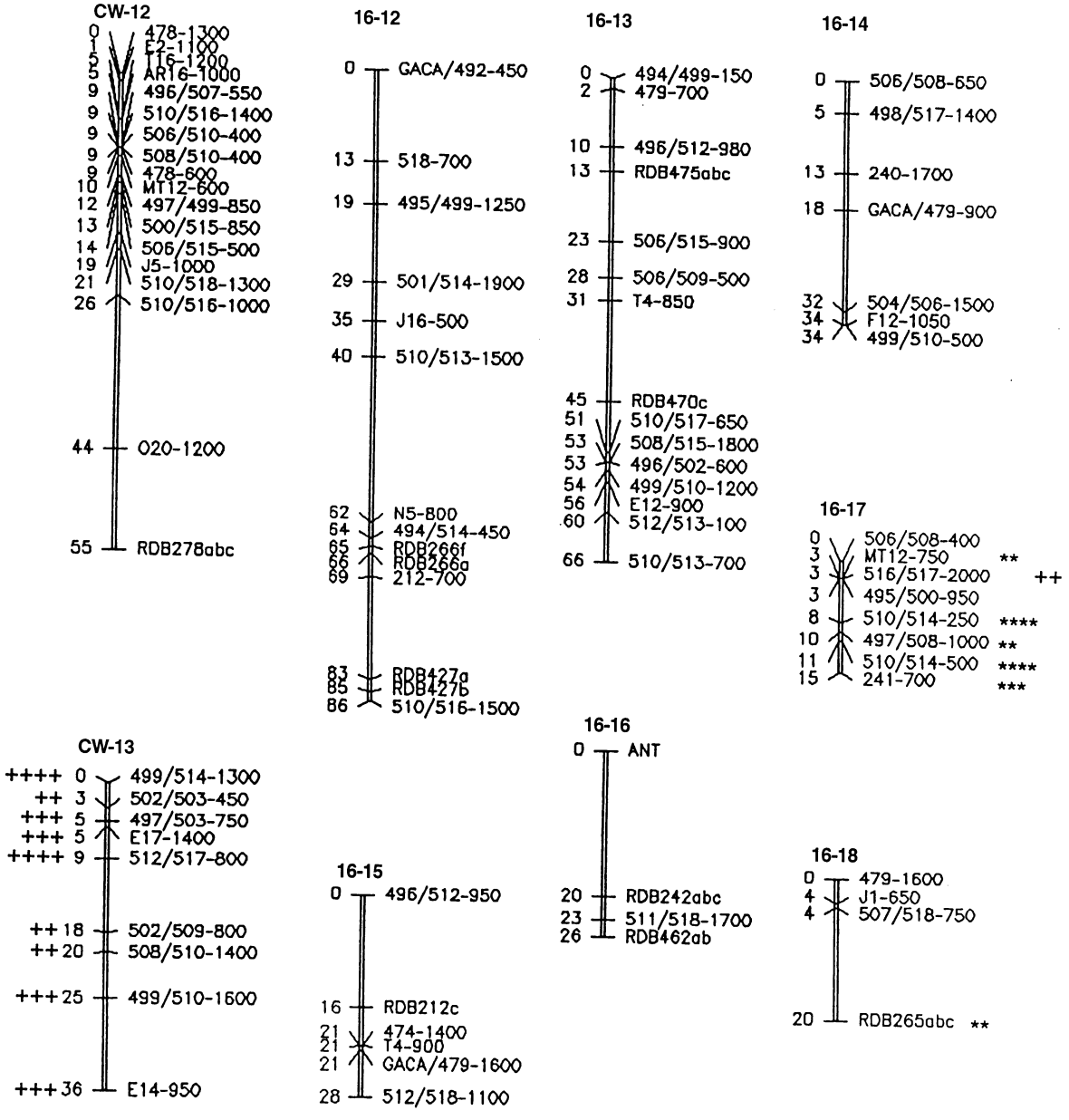


**Fig. 1** Molecular linkage maps of rhododendron 'Cunningham's White' and 'Rh 16'. Allelic bridges are indicated by *dotted lines* between parent-specific maps. Marker loci linked to a QTL are marked by *plus* signs (leaf chlorosis) and *asterisks* (flower colour) if the Kruskal-Wallis test statistic is significant at a level of $P = 0.05$ (++/**), $P = 0.01$ (+++/***), $P = 0.005$ (++++/****), $P = 0.001$ (*****), $P = 0.0005$ (******) and $P = 0.0001$ (*******)

map. Especially in groups 4 and 5, arrangements of 3–5 linked markers with skewed segregation were located in corresponding genome areas.

## QTL analysis

Approaches to map "leaf chlorosis" and "flower colour" as qualitative characteristics under the assumption of a monogenic or digenic control proved insufficient, especially for leaf chlorosis. Therefore, QTL analyses using the Kruskal-Wallis rank-sum test were performed separately for each parent and each character. There were highly significant QTL effects for the expression of chlorosis on chromosomes CW-9 and CW-13. QTL effects of the highly susceptible parent '16' were less pronounced for this characteristic (Fig. 1). All markers of group CW-13 showed significant effects with the Kruskal-Wallis statistic ($K^* = 9.9$) at $P = 0.005$ for marker position 512/517-800, and other significant $K^*$ values in the neighbouring area around this marker. As the marker 512/517-800 is linked with the assumed QTL in repulsion, the resulting data allowed the following interpretation. The genotypic class with the marker has an average chlorosis value of 3.0 whereas the mean chlorosis expression of the genotypes

without the marker was only 2.2. Assuming that the groups CW-9 and CW-13 have putative genes involved in iron-chlorosis tolerance, the relationship between marker presence or absence and chlorosis expression should also be found in plants stress-tested under altered environmental conditions. RAPD markers with high QTL effects were therefore investigated in the five best genotypes (mean chlorosis values approximately 2.0) and the five worst genotypes (mean values approximately 4.0) of the cloned RD2 plant material, which was stress-tested 2 years later using a completely distinct stress-application system. For the RAPD markers showing highly significant QTL effects, e.g. 499/514-1300, 497/503-750 and 512/517-800 at the top of group CW-13, a correspondence between marker presence or absence and high or low chlorosis exceeding 80% was observed. In the lower half of group CW-9 up to 70% correspondence was found for markers 504/506-700 ($K^* = 7.04$) and 498/517-1600 ($K^* = 5.83$). In population RD3 grown under extreme lime stress, the correspondence between markers and chlorosis expression in the 15 best and 15 worst genotypes was about 70% on both linkage groups. In addition, contingency chi-square tests for the relationship between markers and chlorosis tolerance were significant (data not shown).

For the trait "flower colour" on linkage groups CW-2 and CW-5 highly significant QTL effects were found, which indicated major genes located in these genome areas. The highest $K^*$ (24.74; $P = 0.0001$) was calculated at marker position 495/499-1200 on group CW-5.

## Discussion

### Mapping population and marker analysis

The genetic structure of the *Rhododendron* progeny RD2 allowed an efficient selection and mapping of polymorphic DNA markers. The genomes of RD2 individuals are complex mixtures due to a contribution of the three species *R. caucasicum*, *R. ponticum* and *R. fortunei*, which belong to the large subgenus *Hymenanthes*. Although RD2 is a backcross offspring of 'CW', the term "double pseudo-testcross" (Grattapaglia and Sederoff 1994) seems to appropriately characterize the genetic situation of the full-sib $F_1$ family RD2. Based on AFLP fingerprinting data the genetic similarity index, calculated as Jaccard's coefficient, was only 0.35 between the parents 'CW' and '16', which indicates a relatively high genetic distance (Dunemann and Kahnau 1998).

In parent 'CW' 104 and in parent '16', 91 RAPD markers segregating 1 : 1 were found to be linked in coupling phase and were used to construct two parent-specific framework maps. A relatively high number of 3 : 1-segregating RAPDs was found, which could be explained by the backcross offspring as indicated above. Despite its theoretically lower information content, this marker type was integrated in the data sets to enhance the number of markers suited for an alignment of parent-specific linkage groups. The detection of DNA polymorphism using the RAPD marker technique was more effective if 20-mer primers were used in pairwise combinations instead of single primers. If two different RAPD primers are used in combination, amplification patterns different from those obtained with each single primer can be obtained (Welsh and McClelland 1991; Micheli et al. 1993). Moreover, as shown by Debener and Mattiesch (1998), in *Rosa* the number of new fragments increased with increasing primer length. The application of 16-mer PCR primers, designed on the basis of four repeats each of the tetra-nucleotide motives GACA and GATA, resulted in DNA fingerprints comparable to the RAPD patterns. Especially in pairwise combinations with 15-mer RAPD primers, highly informative fingerprints yielding up to five unlinked markers were obtained. In plant nuclear genomes, on average a repeat longer than 20 bp in length occurs every 33 kb (Powell et al. 1996). New bands not present in the patterns of the 15-mer primers could indicate that stretches of DNA consisting of tandemly repeating tetranucleotide motives were responsible for these markers. Two of four SSR loci analyzed in RD2 population proved to have a high information content for linkage mapping. As a most-informative DNA marker, the SSR GA211 segregated for four different alleles. Multi-allelic SSRs have been shown to be an alternative to co-dominant RFLPs for the alignment of parent-specific maps of apple (Gianfranceschi et al. 1998; Maliepaard et al. 1998).

### Map construction

A RAPD-based linkage map supplemented with RFLPs and SSRs was developed for *Rhododendron*. Two separate parental maps were constructed for an efficient mapping of dominant RAPD markers linked in coupling phase. This mapping strategy has been proved as useful, especially in highly heterozygous outcrossing forest-tree species (Grattapaglia and Sederoff 1994; Kubisiak et al. 1995) and in fruit crops (Lodhi et al. 1995; Conner et al. 1997; Maliepaard et al. 1998). Only in the male parent 'CW' is the number of linkage groups identical with the expected basic chromosome number. The map of female parent '16' has still not coalesced into the 13 chromosomal linkage groups. The difference in total map length, with a longer map in '16', could be due to several factors. One reason could be attributed to the complex interspecific genome composition of the parents. A reduction of male map length was observed after interspecific crosses in potato (Gebhardt et al. 1991) and tomato (Van Ooijen et al. 1994). It has been explained by a reduced recombination at

male meiosis, perhaps together with gametophytic selection against recombinant genotypes acting on at least two loci of a chromosome. However, we could not observe major differences in recombination frequencies if map regions carrying markers common to both maps were examined.

Segregation distortion was observed for approximately a quarter of the marker loci scored. This distortion could result from the interspecific nature of the cross between 'CW' and '16', as was assumed for interspecific mapping populations of *Brassica* (Kianian and Quiros 1992) and *Prunus* (Foolad et al. 1995). Also the influence of lethal genes on gametic selection cannot be excluded, because we detected a considerably high extent of inbreeding depression after selfing 'CW'.

Neither the DNA content nor the total recombinational length of the *Rhododendron* genome are known. In diploid *Vaccinium* species, also members of the family *Ericaceae*, the genome size has been estimated as approximately 1.2 pg of DNA/2C (Costich et al. 1993), which indicates a relatively small genome. The total lengths of two published *Vaccinium* maps were 954 cM in a diploid and 1288 cM in a tetraploid population (Rowland and Levi 1994; Qu and Hancock 1997). If we consider the longer group in each of the homologous linkage groups, an "integrated map length" of more than 900 cM is obtained. Using a map-length criterion of 50 cM as the minimal number of map units per chromosome (Meagher et al. 1988), 11 such linkage groups should exist, which indicates that the map is almost complete.

## QTL analyses

Separate QTL analyses were carried out using the parent-specific linkage maps. For the character "leaf chlorosis", two genomic regions were identified in parent 'CW' encompassing the whole linkage group. Whether each of these regions contains only a single QTL or a cluster of linked genes, could not be determined in this investigation. In addition, the precise location of the QTL remained unsettled. However, there are several indications that both linkage groups contain genes controlling iron-chlorosis tolerance in *Rhododendron*. Firstly, only in the chlorosis-tolerant parent 'CW' are large QTL effects present, which can be explained by a dominant QTL allele linked in coupling with the mapped markers. This result was expected considering the strong chlorosis-expression of the susceptible parent '16'. Nonetheless QTLs with allelic effects opposite to those predicted by the phenotype of the parent from which they originated may be detected if transgressive segregation is observed (Tanksley 1993). However, transgressive segregation was unlikely in the plant material studied. Secondly, marker analyses of 40 additional genotypes tested under altered environmental conditions verified the relationship between marker-allele frequencies and the expression of the trait.

The highly significant QTL effects for flower colour detected in both maps could be explained by the location of major genes in these genome areas. In the evergreen azalea *R. simsii* the inheritance of anthocyanins and flavonols is complex. At least six loci were reported to be involved (Heursel and Horn 1977). We assume that an analogue to the gene *W*, which is responsible for the production of anthocyanin, is located in the highly significant QTL area of linkage group 5. After a re-classification of the data on the basis of only two classes (white and coloured) the "non-white flower colour" mapped in the same region of group 5. More detailed mapping studies with flower colour genes in the sub-genus *Hymenanthes* would require quantitative anthocyanin and flavonol determinations.

A single-marker QTL analysis was performed using the non-parametric Kruskal-Wallis rank-sum test. This test has the advantage of being unaffected by distorted segregation ratios and uses scoring data, evaluated on an ordinal scale, that are non-normally distributed. Using this QTL mapping method, genes for bacterial canker resistance in tomato (Sandbrink et al. 1995) and for $Pl_2$ powdery mildew resistance in apple (Seglias–Hodel 1997) were localized. For the character "leaf chlorosis" the power of QTL detection was limited due to the small population, the heterogeneous nature of the QTL mapping design, and the QTL mapping method employed. Nevertheless, genome areas with a sufficiently large phenotypic effect on leaf chlorosis could be detected by this approach, thereby justifying further attempts to map genes involved in iron-deficiency chlorosis tolerance of *Rhododendron*. QTL mapping in soybean confirmed the hypothesis that lime-induced iron chlorosis is controlled by a single major gene with modifying genes (Lin et al. 1997). The physiological mechanisms of iron-chlorosis tolerance are not clearly understood, although several plant strategies for improving iron nutrition are known (reviewed in Guerinot et al. 1994). In the future it may be possible to identify some of the genetic factors associated with these processes by matching QTLs with candidate genes.

**Acknowledgements** The authors thank Dr. Amin Chaanin for providing the RD2 population and for valuable suggestions in managing the lime-stress tests and other advice in plant nutrition.

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