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Assignment of allelic configuration in polyploids using the MAC-PR (microsatellite DNA allele counting—peak ratios) method

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Abstract Polysomic inheritance frequently results in the simultaneous occurrence of several microsatellite DNA alleles on a single locus. The MAC-PR (microsatellite DNA allele counting—peak ratios) method was recently developed for the analysis of polyploid plants and makes use of the quantitative values for microsatellite allele peak areas. To date, this approach has only been used in plants with known genetic relationships. We report here the application of MAC-PR for the first time to random samples of unknown pedigrees. We analysed six microsatellite loci using a set of tetraploid ornamental rose (*Rosa* × *hybrida* L.) varieties. For each locus, all alleles were analysed in pairwise combinations in order to determine their copy number in the individual samples. This was accomplished by calculating the ratios between the peak areas for two alleles in all of the samples where these two alleles occurred together. The allele peak ratios observed were plotted in a histogram, and those histograms that produced at least two well-separated groups were selected for further analysis. Mean allelic peak ratio values for these groups were compared to the relationships expected between alleles in hypothetical configurations of the locus investigated. Using this approach, we were able to assign precise allelic configurations (the actual genotype) to almost all of the varieties analysed for five of the six loci investigated. MAC-PR also appears to be a very effective tool for detecting 'null' alleles in polyploid species.

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Introduction

The evolution of plant species is closely tied in with polyploidy. Tentative estimations suggest that approximately 70% of all plant taxa may have undergone polyploidization at least once (Masterson 1994). Polyploid speciation appears to take place mainly by interspecific hybridization between related taxa, which results in alloploids or autoalloploids depending on the degree of relatedness between the hybridizing genotypes. The same taxon may be formed by multiple unrelated hybridization events, which results in a considerable gain in genetic diversity (Soltis and Soltis 1999).

Polyploid plant taxa are often very successful and tend to be more widely distributed and to occur in more extreme habitats than their diploid ancestors (Soltis and Soltis 2000). Many of our most well-known and important crop plants are polyploid—for example, bread wheat (*Triticum aestivum*), potato (*Solanum tuberosum*), alfalfa (*Medicago sativa*) and strawberry (*Fragaria* × *ananassa*). In addition, the high basic chromosome number encountered in some genera like *Malus* (x=17) is in all likelihood the result of ancient polyploidization.

In spite of their undisputable importance, polyploid plant species are considerably less well studied than their diploid counterparts. This is especially evident in research fields where single-locus tools like allozyme and microsatellite DNA analysis have played an important role. In single-locus technology different alleles of, for instance, a microsatellite marker can be scored as differences in the mobility of bands and peaks on a sequencing gel. In diploid species, a maximum of two alleles per locus is expected (and found). The occurrence of one allele is interpreted as the plant being homozygous for that particular allele. Unfortunately, in polyploid species, the use of microsatellite markers is generally not so straightforward as in diploids. Although some (older) taxa have become diploidized and now show disomic inheritance, others have retained considerable genome integrity as evidenced by polysomic inheritance at the targeted loci. Some taxa represent intermediate stages and

display a combination of disomic and polysomic inheritance (Wu et al. 1992)—although regularly forming bivalents in meiosis, they may still exhibit polysomic inheritance for certain genome segments (Lerceteau-Köhler et al. 2003).

There is a major problem with defining which allele(s) occur in more than one copy when the number of displayed microsatellite DNA alleles in a sample is less than the possible maximum number for that ploidy level in species with polysomic inheritance. For a triploid plant with two detected alleles, the question is only which one of these occurs in two copies. For a tetraploid plant exhibiting three alleles for a locus, three different allelic configurations are possible: 2, 1, 1 or 1, 2, 1 or 1, 1, 2. With only two detected alleles, either could occur in three copies (and the other in a single copy) or both could occur in two copies. With higher ploidy levels, the number of possible allelic configurations becomes even larger.

Application of our recently developed MAC-PR (microsatellite DNA allele counting—peak ratios) method has enabled us to determine the number of allele copies in microsatellite loci in a series of tetra- and pentaploid dogrose species and their offspring from controlled crosses (Nybom et al. 2004). We report here the results of our investigation into the possibility of generalizing this approach by estimating genomic configurations in plant material that does not include known parent-offspring relations.

Materials and methods

Material

A set of 83 commercially available rose varieties (*Rosa* × *hybrida*) together with a variable number of reference varieties that contained all of the alleles with respect to the analysed sequence-tagged microsatellite sites (STMS) were genotyped according to Esselink et al. (2003) with a few modifications. Young leaves of a single individual were harvested, immediately frozen in liquid nitrogen and stored at −80°C until use. DNA was extracted from freeze-dried leaves using the Qiagen DNeasy Plant Mini kit (Westburg, The Netherlands).

Method

Successful application of MAC-PR is completely dependent on the quality of the experimental data. This quality must be consistently high, with unambiguously scorable markers showing no or very few stutterbands. Six primer pairs with these characteristics were selected from a larger set of STMS markers derived from genomic DNA of the *Rosa* × *hybrida* variety *Sonia* (Esselink et al. 2003). Microsatellites were amplified by multiplex-PCR of three STMS markers, labelled with HEX, NED or 6-FAM, in a 20-µl reaction volume containing 10 ng genomic DNA, 2–4 pmol of each primer, 100 µ*M* of each dNTP, 10 m*M* Tris-HCL pH 9.0, 20 m*M* $(NH_4)_2SO_4$, 0.01% Tween 20, 1.5 m*M* $MgCl_2$ and 0.4 U Goldstar *Taq* DNA polymerase (Eurogentec, The Netherlands). The optimized PCR conditions were one cycle of 94°C for 3 min followed by 30 cycles of 94°C for 30 s, 50°C for 30 s, 72°C for 120 s and a final extension at 72°C for 3 min.

Following PCR amplification, the fluorescent-labelled products were detected using an ABI Prism 3700 DNA analyzer (Perkin Elmer Biosystems, Foster City, Calif.). Fragment sizes and peak areas were determined automatically using GENESCAN analysis software (release 1.1 3700 software; Perkin Elmer Biosystems). A database containing about 450 rose varieties enabled the selection of a set of varieties representing all of the alleles for each STMS marker detected within the group of *Rosa* × *hybrida*. These varieties were included in each run and used as a reference for allele determination. In this way alleles were assigned a name based on an exact match to the length of the corresponding allele present in the reference variety and not as a specific length in base pairs. Allele codes (A, B, etc) were transferred to a numeric database in an Excel spreadsheet for further analyses.

The MAC-PR approach makes use of the quantitative values for peak areas provided by the software. For each locus, all alleles were analysed in pairwise combinations in order to determine their copy number in the individual samples. This was accomplished by calculating ratios between the peak areas for two alleles in all of the samples in which these two alleles occurred together. The allele peak ratios that were obtained were plotted in a histogram, and those histograms that produced at least two well-separated groups were selected for further analysis. Mean allelic peak ratio values for these groups were compared to the relationships expected between alleles in hypothetical configurations of the investigated locus (Table 1). In a tetraploid individual having two alleles, we would expect the ratios 0.33 (x/yyy), 1 (xx/yy) and 3 (xxx/y) provided that the x and y alleles produce similar-sized peak areas. However, if the x-allele peak area is, for example, somewhat larger and the x/y ratio is, for example, 1.2, we need to multiply all of the expected ratios by 1.2. In individuals having three different alleles, relationships between each pair of alleles must be calculated separately (yielding a total of three ratios) and then compared to hypothetical ratios. On the basis of comparisons between the estimated group means and the expected ratios, a tentative model for allelic configuration can be worked out. Access to individuals with four different alleles can provide a baseline for the calculations since all of the six ratios obtained for such an individual must necessarily involve single-copy alleles.

Results

Amplification with primers for locus RhB303 yielded a total of six alleles—B, C, D, E, H and I—in 84 *Rosa* × *hybrida* samples. Nineteen of the plants analysed exhibited the maximum number of four alleles (configurations BCDH, BCDI, BCEI, BDEI or CDEI). These plants obviously had only single-copy alleles and thus provided a valuable baseline to which other peak ratios could be compared. Among the remaining samples, 43 had three different alleles, 21 had two different alleles and one had only one allele. Histograms of all allele peak ratios were constructed (for example, alleles E and I, see Fig. 1) and

Table 1 Expected ratios of all possible allele configurations for tetraploid samples containing two or three peaks after STMS genotyping if all alleles produce similar-sized peak areas. If, for example, the x-allele peak is larger and results in an x/y ratio of 1.2, all ratios given below should be multiplied by 1.2

	Two alleles			Three alleles		
	xxyy	xyyy	xxxy	xyzz	xyyz	xxyz
x/y ratio	1	0.33	3.0	1	0.5	2
x/z ratio				0.5	1	2
y/z ratio				0.5	2	1

compared to the allele phenotype (i.e. which different combinations of alleles occurred in the different plants). Plants with three different alleles generate three peak ratios, thus providing an internal control since two of these ratios must refer to a 1:2 relationship and the third to a 1:1 relationship. Putative allelic configurations could be assigned for all but two of the samples without any overlapping between peak ratios for different suggested allelic proportions (Table 2). One of the samples for which the data were not conclusive had peak ratios suggesting that it may have a 'null' allele.

Analysis of locus RhEO506 yielded a total of five alleles—E, J, K, L and N—in the 88 samples investigated. Only five of these plants had the maximum number of four different alleles (three with EJKL, one with EKLN and one with JKLN), whereas 34 had three different alleles and 49 had two different ones. Putative allelic configurations could be assigned for all but one sample without any overlapping between peak ratios for different suggested allelic proportions (Table 3, Fig. 2, alleles E and L). The only sample for which the data were not conclusive had a peak ratio suggesting that it may have a null allele.

Analysis of locus RhP517 resulted in five alleles—B, C, D, E and F. Among the 86 plants investigated, 16 had four different alleles (BCDE, BCEF, BCDF and BDEF), 44 had three different alleles, 25 had two different alleles and one had only a single allele, namely B (thus having the configuration BBBB). Putative allelic configurations could be assigned for all but three of the investigated plants, however there were a couple of slightly overlapping ratios between alleles E and F (Table 4, Fig. 3, alleles B and E). All three plants for which data were not conclusive had allele ratios suggesting that they each had a null allele.

Analysis of locus RhD221 yielded a total of six alleles—A, D, E, F, H and I. Eight of these samples had four different alleles (AEFI, DEFI or EFHI), 19 had three different alleles, 44 had two different alleles and 18 had only one allele, namely F (and these were therefore FFFF, assuming no null alleles were present). Peak ratios were distributed in well-dispersed groups in the histograms.

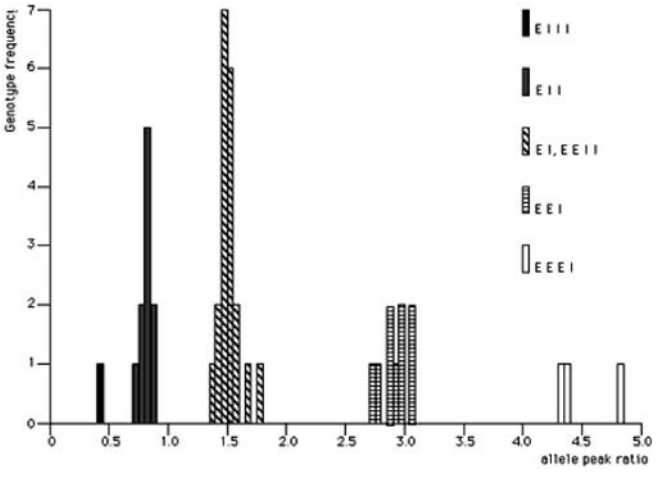

Fig. 1 Microsatellite allele peak ratios between alleles E and I in locus RhB303 for 43 tetraploid *Rosa* × *hybrida* cultivars

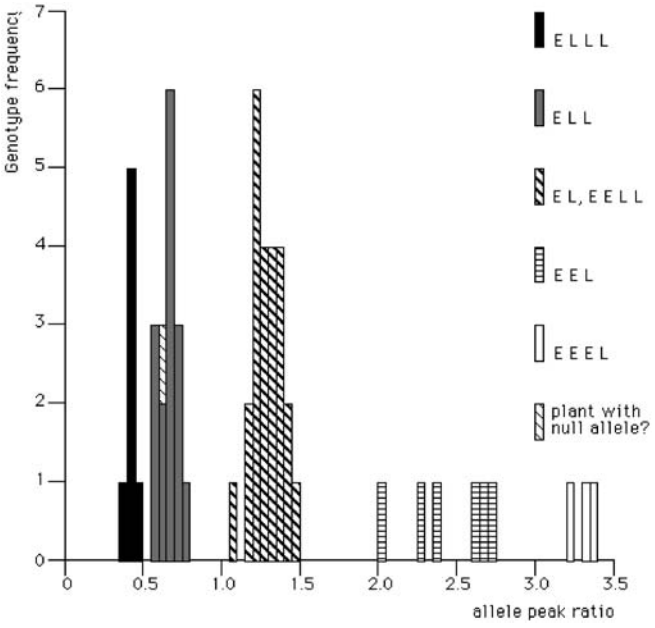

Fig. 2 Microsatellite allele peak ratios between alleles E and L in locus RhEO506 for 55 tetraploid *R.* × *hybrida* cultivars

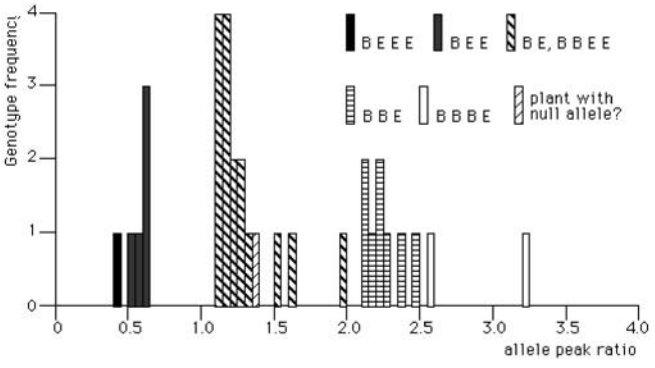

Fig. 3 Microsatellite allele peak ratios between alleles B and E in locus RhP517 for 32 tetraploid *R.* × *hybrida* cultivars

However, proper assignment of the alleles into tetraploid configurations could not be accomplished for 34 of the samples for which allele peak ratios were instead consistent with the assumption that a null allele was present. When we took this assumption into consideration, allelic configurations could be worked out for all of the plants analysed.

The most likely cause of non-amplification is an alteration to or the deletion of a primer site (Callen et al. 1993). Using the sequence information of the cloned microsatellite of marker RhD221 (Esselink et al. 2003), we designed a third primer 246 bp downstream of the reverse primer. This primer, when used along with the fluorescent-labelled forward primer, resulted in the amplification of additional alleles for 44 samples. All of the 34 samples—with one exception—which had been assumed to have a null allele showed an additional allele A and restored the expected peak ratios. In addition, of the previously 18 mono-allelic samples, ten showed allele A, resulting in a different genotype than the one shown with the original

Table 2 Locus RhB303. Allelic peak ratios calculated for all pairwise allele combinations in the 82 samples (out of 84 investigated *Rosa* × *hybrida* genotypes) for which allelic configurations could be determined using the MAC-PR method. The E/I ratios are shown as a histogram in Fig. 1

Alleles	Putative configurations, (number of samples) and range of peak values
B and C	BCC (2) 0.58; BC/BBCC (7) 1.06–1.13; BBC (3) 1.98–2.09
B and D	BD/BBDD (13) 0.95–1.19; BBD (1) 2.19
B and E	BEEE (4) 0.38–0.41; BEE (10) 0.54–0.64; BE/BBEE (15) 1.00–1.30; BBE (3) 1.94–2.35
B and H	BH (1) 1.88
B and I	BIII (1) 0.58; BII (7) 0.84–1.04; BI/BBII (11) 1.59–2.04; BBI (3) 3.09–3.72
C and D	CDD (1) 0.57; CD/CCDD (14) 0.97–1.12; CCD (1) 2.04
C and E	CEEE (2) 0.38–0.39; CEE (6) 0.54–0.58; CE/CCEE (20) 0.92–1.23; CCE (4) 2.16–2.28
C and H	CH (1) 1.72
C and I	CII (5) 0.79–0.95; CI/CCII (21) 1.50–1.86; CCI (3) 3.09–3.46
D and E	DEEE (4) 0.41–0.43; DEE (12) 0.56–0.63; DE/DDEE (18) 0.91–1.26
D and H	DH (2) 1.65–1.71
D and I	DII (2) 0.93–0.94; DI/DDII (18) 1.33–1.88; DDI (1) 3.12
E and H	EEH (1) 2.97
E and I	EIII (1) 0.41; EII (10) 0.75–0.90; EI/EEII (20) 1.37–1.76; EEI (9) 2.73–3.07; EEEI (3) 4.32–4.84

primer. In one sample, allele D showed up although it had not been found before, and in another sample (excluded from further analyses) a completely new allele was found. In summary, analysis using the new reverse primer resulted in the detection of 14 plants that had four different alleles (ADEI, ADFI, AEFI, AEFH, AEHI, DEFI or EFHI), 36 plants that had three different alleles, 29 plants that had two different alleles (plus one that had F and the new allele), and seven plants that had only the F allele. Peak ratios were for the most part distributed in well-dispersed groups (Table 5). Proper assignment of alleles into tetraploid configurations could be accomplished for 82 samples but not for the remaining four (three with three alleles and one with two alleles).

Analysis of locus RhP519 produced a total of six alleles, two of which occurred at a low frequency; four samples had the B allele and a single sample had the C allele. Among the 87 samples analysed, allele E was moderately common (occurred in 16 samples) and alleles F, G and H were very common. Four of the samples had four different alleles (CEGH, EFGH), 38 had three different alleles, 42 had two different alleles and three had only one allele (the G allele, thus being GGGG, assuming no null alleles present). Unfortunately ratios involving the F allele varied widely, even among samples with the EFGH configuration, and in subsequent analyses values for the F allele were therefore omitted. In spite of this, we could still assign allelic configuration to all 38 plants that had three alleles and to 22 of the samples that had two alleles (Table 6).

Analysis of locus RhM405 yielded four alleles—B, C, D and E—all of which occurred simultaneously in 36 samples. Two of these alleles—B and E—showed comparatively stable peak ratios, varying from 0.94 to 1.51, with a mean of 1.20. In another four samples having only the B and E alleles, ratios differed between 1.13 and 1.36, suggesting that they were BBEE. In contrast, all peak ratios involving the C and D alleles showed widely ranging ratios which did not produce any clear-cut groupings in the histograms. Thus, the B/C ratio varied

Table 3 Locus RhEO506. Allelic peak ratios calculated for all pairwise allele combinations in the 87 samples (out of 88 investigated *R.* × *hybrida* genotypes) for which allelic configurations could be determined using the MAC-PR method. The E/L ratios are shown as a histogram in Fig. 2

Alleles	Putative configurations, (number of samples) and range of peak values
E and J	EJJJ (1) 0.39; EJJ (2) 0.59–0.61; EJ/EEJJ (6) 0.93–1.31; EEJ (2) 2.31–2.40
E and K	EKK (7) 0.52–0.62; EK/EEKK (20) 0.97–1.23; EEK (4) 1.53–2.19; EEEK (1) 2.74
E and L	ELLL (7) 0.38–0.46; ELL (15) 0.57–0.79; EL/EELL (24) 1.09–1.46; EEL (6) 2.00–2.71; EEEL (3) 3.21–3.40
E and N	ENN (1) 0.71; EN (1) 1.54
J and K	JKK (2) 0.59–0.67; JK/JJKK (5) 0.95–1.06
J and L	JLLL (4) 0.34–0.41; JLL (2) 0.52–0.68; JL/JJLL (8) 1.12–1.32; JJL (2) 2.11–2.38
J and N	JN (1) 1.38
K and L	KLLL (9) 0.44–0.55; KLL (16) 0.57–0.74; KL/KKLL (15) 1.08–1.34; KKL (7) 2.10–2.24; KKKL (2) 2.74–3.25
K and N	KN (3) 1.32–1.36
L and N	LNN (1) 0.56; LN (2) 1.12–1.13; LLN (1) 1.78

Table 4 Locus RhP517. Allelic peak ratios calculated for all pairwise allele combinations in the 82 samples (out of 86 investigated *R.* × *hybrida* genotypes, one of which had BBBB) for which allelic configurations could be determined using the MAC-PR method. The B/E ratios are shown as a histogram in Fig. 3

Alleles	Putative configurations, (number of samples) and range of peak values
B and C	BCC (1) 0.54; BC/BBCC (14) 0.91–1.34; BBC (12) 1.78–2.44
B and D	BDD (4) 0.53–0.55; BD/BBDD (25) 0.91–1.84; BBD (12) 1.92–2.82
B and E	BEEE (1) 0.42; BEE (5) 0.55–0.64; BE/BBEE (16) 1.11–1.97; BBE (8) 2.11–2.46; BBBE (2) 2.56–3.22
B and F	BFF (5) 0.58–0.67; BF/BBFF (19) 1.09–1.82; BBF (6) 2.12–2.85; BBBF (3) 3.23–3.47
C and D	CDDD (1) 0.37; CDD (1) 0.57; CD/CCDD (20) 0.99–1.94; CCD (3) 1.99–2.12
C and E	CEE (2) 0.55–0.60; CE/CCEE (7) 1.14–1.79; CCE (2) 2.24–2.28
C and F	CFF (1) 0.58; CF/CCFF (14) 1.12–1.97; CCF (2) 2.33–2.34
D and E	DEE (5) 0.56–0.60; DE/DDEE (12) 1.11–1.57; DDE (5) 2.14–2.88
D and F	DFF (2) 0.58–0.59; DF/DDFF (17) 1.11–2.00; DDF (2) 2.24–2.26; DDDF (1) 4.00
E and F	EFF (4) 0.54–0.59; EF/EEFF (10) 1.12–1.95; EEF (4) 1.86–2.43

from 0.36 to 1.40 in the 36 plants that obviously had one copy of each allele. In this same plant material, the B/D ratio varied from 0.41 to 2.37, the C/D ratio from 1.10 to 3.35, the C/E ratio from 0.84 to 3.79 and the D/E ratio from 0.48 to 3.21. Clearly, the MAC-PR method cannot be used when peak ratios are this variable.

Discussion

An understanding of just how chromosomes are transmitted from one generation to the next is an essential prerequisite for plant genetic studies in polyploid species. Locus-specific microsatellite markers could potentially become an important tool for such studies but, unfortunately, analysis of polysomically inherited loci often produces complex band profiles that can be both very difficult to score and to interpret. Using microsatellite DNA analysis, Amsellem et al. (2001) found up to four alleles per locus in tetraploid *Rubus alceifolius*, whereas up to five alleles were found in a set of interspecific, pentaploid dogrose hybrids, *Rosa* Sect. *Caninae* (Nybom et al. 2004). Up to six alleles have been reported in hexaploid *Elymus athericus* (Bockelmann et al. 2003), hexaploid *Phleum pratense* (Cai et al. 2003) and also in some materials for which the ploidy levels are unknown—for example, in a set of *Rosa* genotypes (Esselink et al. 2003) and in *Salix reinii* (Lian et al. 2001). In contrast, tetraploid durum wheat *Triticum durum* showed disomic segregation for all of the SSR loci investigated (Macaferri et al. 2003), while octoploid wild strawberry *Fragaria virginiana* showed disomic segregation for all investigated loci but one (Ashley et al. 2003).

Complex microsatellite banding patterns can also be obtained for species that are normally not perceived as being polyploid. In a diploid species, more than two alleles can be expected for markers present in parts of the genome that are duplicated. However, this type of marker is usually

Table 5 Locus RhD221. Allelic peak ratios calculated for all pairwise allele combinations in 75*R.* × *hybrida* genotypes (out of 86 analysed samples, seven of which had only the F allele) for which allelic configurations could be determined using the MAC-PR method

Alleles	Putative configurations, (number of samples) and range of peak values
A and D	ADDD (1) 0.23; ADD (11) 0.24–0.36; AD/AADD (9) 0.40–0.70; AAD (3) 0.70–1.29
A and E	AE (5) 0.60–0.77
A and F	AFFF (5) 0.19–0.24; AFF (10) 0.22–0.36; AF/AAFF (21) 0.46–0.88; AAF (2) 0.72–1.25
A and H	AH (2) 0.65–0.78
A and I	AI (12) 0.88–1.36; AAI (1) 1.89
D and E	DEE (1) 0.44; DE (7) 0.97–1.19
D and F	DFFF (5) 0.32–0.39; DFF (7) 0.45–0.57; DF/DDFF (19) 0.89–1.10; DDF (12) 1.75–2.18; DDDF (1) 2.97
D and I	DII (2) 0.94; DI (13) *0.32*[a] 0.79–1.94; DDI (3) 2.91–3.62; DDDI (2) 4.48–4.86
E and F	EFF (1) 0.45; EF/EEFF (10) 0.89–1.08; EEF (1) 2.42
E and H	EH (3) 0.96–1.09
E and I	EI (12) *0.28*[a] 1.22–1.63
F and H	FH (2) 0.89
F and I	FIII (1) 0.61; FII (3) 0.78–0.91; FI/FFII (14) *0.31*[a] 1.12–1.67; FFI (8) 1.60–3.55
H and I	HI (2) 1.47–1.50

[a]One plant with alleles DEFI has an unusually small peak area for the I allele, resulting in extremely low ratios for D/I, E/I and F/I, reported here in italics followed by the range for the remaining values

Table 6 Locus RhP519. Allelic peak ratios calculated for all pairwise allele combinations in 64 samples of *R.* × *hybrida* for which allelic configurations could be determined using the MAC-PR method. Another 20 samples were excluded from the analysis since they had only two alleles, one which was the F allele which showed inconsistent peak ratios

Alleles	Putative configurations, (number of samples) and range of peak values
B and E	BE (1) 0.76
B and G	BGG (3) 0.63–0.66; BG (1) 1.12
B and H	BHH (1) 0.66; BH (1) 1.38
E and G	EGGG (2) 0.50–0.55; EGG (4) 0.64–0.89; EG (8) 1.27–1.59)
E and H	EHH (4) 0.77–0.91: EH (6) 1.33–1.74
G and H	GHHH (2) 0.40–0.42; GHH (11) 0.49–0.64; GH/GGHH (23) 0.95–1.92; GGH (13) 1.97–2.78; GGGH (8) 2.74–3.39

discarded. Also, the use of a single primer pair has occasionally yielded more than the expected number of alleles in some species suspected of ancient polyploidization—such as apple ($2n$=34; Liebhard et al. 2002) and *Actinidia chinensis* ($2n$= 58; Huang et al. 1998).

To date, efforts to determine the copy number of microsatellite alleles in polyploid species has been described as mostly unsuccessful (Falque et al. 1998). Even unambiguously scored bands have therefore been interpreted as phenotypic banding patterns, and no attempts have been made to assign precise allelic configurations (Provan et al. 1996; Becher et al. 2000; McGregor et al. 2000; Mengoni et al. 2000; Andrew et al. 2003; Bockelmann et al. 2003). Although the evaluation of multilocus band patterns can be very useful—for example, for estimating genetic distances or identifying cultivars (Esselink et al. 2003)—there are many applications where considerably more information would be gained from a proper quantification of the alleles in the loci analysed, such as population genetics and paternity analysis.

Using the MAC-PR method, we were able to assign tetraploid allelic configurations in four loci (RhB303, RhEO506, RhO517 and RhD221) for almost all of the rose plants investigated. For another locus, RhM405, only two of the four alleles produced peaks that yielded sufficiently stable peak ratios. This locus is a hexanucleotide microsatellite with absolutely no stutters, and it appears to produce unambiguously scorable markers. A possible explanation for our failure to obtain good results with this locus is differential amplification of alleles or a plateau effect in the amplification reaction. In yet another locus, RhP519, one of the six alleles scored did not produce useful ratios. Despite this, we were able to assign putative configurations to all of the samples that contained a minimum of two of the other alleles.

For the assignment of putative allelic configurations, access to at least some plants with the maximum number of alleles for that ploidy level is very helpful. Peak ratios calculated in such plants represent the ratios one should expect for 1:1 relationships. In a histogram, samples with 1:1 peak ratios should be carefully gathered. Samples with other ratios should show clear-cut groupings in the histogram. The allelic configurations behind these other ratios are then easily evaluated by using the known 1:1 ratio as a base line. Known (or putative) parent-offspring relationships in the material are also very helpful but definitely not a prerequisite.

In conclusion, we believe that the MAC-PR approach offers a promising possibility to extract quantitative information from microsatellite DNA analyses in polyploid plants. We also believe that MAC-PR can be used to identify possible 'null alleles'. Obviously, not all markers are equally suited for a MAC-PR approach. This clearly depends on their scoring characteristics (quality). However, even with high-quality markers, results must be treated with caution since possible artefacts, which would interfere with the relationships between the number of allele copies and peak areas, have not yet been properly investigated. Differential amplification between alleles could possibly result from point mutations in the primer binding sites or in the sequence downstream of a primer, or as an effect of the repeat structure itself (Esselink et al. 2003). Real-time PCR using fluorescence monitoring (Wittwer et al. 1997) could be a suitable means to measure the amplification profile and to be certain that allele amplification is still in the exponential phase when MAC-PR is used on the quantitative data. This would make the MAC-PR even more accurate and precise, and possibly even more loci could then be used in this type of analysis.

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