Theor Appl Genet (2006) 112: 315–326
DOI 10.1007/s00122-005-0130-0

ORIGINAL PAPER

R.I. Bošković · B. Wolfram · K.R. Tobutt
R. Cerović · T. Sonneveld

Inheritance and interactions of incompatibility alleles in the tetraploid sour cherry

Received: 17 May 2005 / Accepted: 12 July 2005 / Published online: 24 November 2005

Abstract Three progenies of sour cherry (*Prunus cerasus*) were analysed to correlate self-(in)compatibility status with *S*-RNase phenotype in this allotetraploid hybrid of sweet and ground cherry. Self-(in)compatibility was assessed in the field and by monitoring pollen tube growth after selfing. The *S*-RNase phenotypes were determined by isoelectric focusing of stylar proteins and staining for RNase activity and, for the parents, confirmed by PCR. Seedling phenotypes were generally consistent with disomic segregation of *S*-RNase alleles. The genetic arrangements of the parents were deduced to be 'Köröser' (self-incompatible) $S_1S_4.S_BS_D$, 'Schattenmorelle' (self-compatible) $S_6S_{13}.S_BS_B$, and clone 43.87 (self-compatible) $S_4S_{13}.S_BS_B$, where "." separates the two homoeologous genomes. The presence of S_4 and S_6 alleles at the same locus led to self-incompatibility, whereas S_{13} and S_B at homoeologous loci led to self-compatibility. The failure of certain heteroallelic genotypes in the three crosses or in the self-incompatible seedlings indicates that S_4 and S_6 are dominant to S_B. However, the success of $S_{13}S_B$ pollen on styles expressing corresponding *S*-RNases indicates competitive interaction or lack of pollen-*S* components. In general, the universal compatibility of $S_{13}S_B$ pollen may explain the frequent occurrence of S_{13} and S_B together in sour cherry cultivars. Alleles S_B and S_D, that are presumed to derive from ground cherry, and S_{13}, presumably from sweet cherry, were sequenced. Our findings contribute to an understanding of inheritance of self-(in)compatibility, facilitate screening of progenies for self-compatibility and provide a basis for studying molecular interactions in heteroallelic pollen.

Keywords Incompatibility · Genetics · *Prunus cerasus* · Sour cherry · *S*-RNases

Communicated by H. Nybom

R.I. Bošković (✉) · K.R. Tobutt · T. Sonneveld
East Malling Research, New Road, East Malling,
Kent ME19 6BJ, UK
E-mail: radovan.boskovic@emr.ac.uk
Tel.: +44-1732-843833
Fax: +44-1732-849067

R.I. Bošković
Imperial College at Wye, Ashford, Kent, TN25 5AH, UK
E-mail: radovan.boskovic@emr.ac.uk
Tel.: +44-1732-843833
Fax: +44-1732-849067

B. Wolfram
Institut für Obstzüchtung, Pillnitzer Platz 2,
01326 Dresden, Germany

R. Cerović
Fruit and Grape Research Centre, Kralja Petra I/9,
32000 Čačak, Serbia and Montenegro

Introduction

The sour cherry (*Prunus cerasus*) is a tetraploid species, thought to be an allotetraploid derived from hybridisation of the diploid sweet cherry (*Prunus avium*) and the tetraploid ground cherry (*Prunus fruticosa*) (Oldén and Nybom 1968). Some cultivars are self-compatible, at least partially, whereas others are self-incompatible (Crane and Lawrence 1929; Hruby 1963; Montalti and Selli 1984; Redalen 1984b). Certain pairs of cultivars are cross-incompatible, reciprocally or unilaterally (Hruby 1963; Redalen 1984a; Apostol 1996).

In diploid sweet cherry, most individuals of which are self-incompatible, incompatibility was attributed to a multi-allelic *S* locus (Crane and Lawrence 1929), which comprises at least two parts, one operating in the style and the other in the pollen (Lewis 1949). So far, 19 distinct *S* alleles have been reported, S_1 to S_{16} (Sonneveld et al. 2003), and S_{17} to S_{22} (De Cuyper et al. 2005). The *S* alleles code for stylar ribonucleases (*S*-RNases) (Bošković and Tobutt 1996), as has been reported for the Solanaceae. All have now been cloned and sequenced, though for some, including S_{13}, the sequences are only partial (Sonneveld et al. 2001, 2003; De Cuyper

et al. 2005). Primers amplifying across the variable first or second introns of the *S*-RNase genes have been designed that allow the alleles to be distinguished on the basis of length polymorphism (Sonneveld et al. 2003; Sutherland et al. 2004). The corresponding pollen product in sweet cherry is likely to be an F-box protein encoded by the *S* locus (Yamane et al. 2003a; Ikeda et al. 2004; Sonneveld et al. 2005).

Some of the complexities of genetic control of self-(in)compatibility in tetraploids were recognised by Crane and Lawrence (1929) and Lawrence (1930). Subsequently, Crane and Lawrence (1938) built on these ideas to formulate an explanation for unilateral incompatibility between certain tetraploids. Lewis and Modlibowska (1942) concluded that the self-compatibility of a tetraploid mutant of *Pyrus communis* was a consequence of the compatibility of heteroallelic diploid pollen in the selfed style. Lewis, discussing evidence from other species, proposed that in heteroallelic diploid pollen of some tetraploids the two *S* alleles may interact competitively so that such pollen is not rejected in styles having one or both of the same *S* alleles (Lewis 1943, 1947, 1954), or else one allele can dominate the other so that the pollen is rejected in styles having the dominant allele (Lewis 1947, 1954). Recently, Chawla et al. (1997), investigating the breakdown of self-incompatibility in tetraploid *Lycopersicon peruvianum*, confirmed that only heteroallelic pollen achieved self-fertilisation. Luu et al. (2001), developing a model to explain the compatibility of heteroallelic pollen in tetraploid *Solanum chacoense* in terms of the competition effect, proposed a tetrameric structure for pollen-*S*.

In tetraploid sour cherry, studies on the inheritance of self-(in)compatibility include those of Yenikeyev (1973), Lansari and Iezzoni (1990) and Wolfram (1999); however few conclusions about the genetics were reached in these investigations. Hauck et al. (2002) studied the segregation of self-incompatible versus self-compatible phenotypes and of *S*-RNase alleles in a progeny from the cross of 'Rheinische Schattenmorelle' × 'Erdi Botermo' but they could not associate the two and presented no cosegregation data. Rather than competitive interaction, they advanced the hypothesis that self-compatibility in sour cherry results from non-functional *S*-RNase and pollen-*S* genes. Seedlings with certain unspecified *S* allele genotypes were said to segregate for self-incompatibility versus self-compatibility and the involvement of modifier genes was proposed.

Insights into the relationships between stylar ribonucleases and (in)compatibility in sour cherry have been provided by Tobutt et al. (2004) who, using isoelectric focusing of stylar proteins followed by activity staining, surveyed cultivars of known incompatibility status for *S*-RNase phenotype and undertook various test crosses and progeny analyses. They distinguished between RNase bands, e.g. 1, 4, 6 and 13, attributable to *S* alleles of sweet cherry origin and others, e.g. B and D, probably derived from ground cherry, and pointed out that their approach detected functional alleles in contrast to DNA methods that may detect non-functional ones. They stressed the significance of genotype and allelic arrangement in a largely allotetraploid species, using "." to separate the two homoeologous loci, one from sweet cherry and one from ground cherry, in somatic genetic formulae; and they stressed the importance of the nature of the interactions in the heteroallelic pollen and gave evidence for dominance and for competitive interaction in certain cultivars. In addition, they stressed how little was known of the incompatibility function of the presumed ground cherry alleles and of the functional implications of possible null alleles.

At Dresden, three sour cherry progenies were available that had been scored to study inheritance of (in)compatibility (Wolfram 1999). To establish correlations between self-(in)compatibility and *S*-RNase phenotype we have now re-scored these, by bagging and monitoring fruit set or by selfing and monitoring the pollen tube growth, and have also analysed them for *S*-RNases by protein analysis and, in the case of the parents, by PCR. We also considered the possible parental arrangements of the alleles, and thus the possible gamete genotypes, and compared the progeny phenotypes with the combinations possible. In cases where parents showed one or more *S* alleles in common, we considered the progeny phenotypes to see if any of the pollen genotypes had failed. In addition, we cloned the alleles S_B and S_D, to show that they have the sequence structure characteristic of *Prunus* *S*-RNases, and S_{13}, which had been only partially sequenced previously.

Materials and methods

Plant material

Three progenies from crosses of 'Köröser', self-incompatible, 'Schattenmorelle' (not 'Rheinische Schattenmorelle'), self-compatible, and clone 43.87 ('Köröser' × 'Schattenmorelle'), self-compatible, that were raised at Dresden and grown in the field for more than 10 years (Wolfram 1999), were chosen for determination of self-(in)compatibility and analysis of *S*-RNase constitution. They are: progeny 13, 'Schattenmorelle' × selection 43.87 (24 seedlings); progeny 11, 'Köröser' × 'Schattenmorelle' (21 seedlings); and progeny 12, 'Köröser' × selection 43.87 (20 seedlings). The *S*-RNase phenotypes of 'Köröser' and 'Schattenmorelle' are 1,4,B,D and 6,13,B, respectively (Tobutt et al. 2004).

The three parents were also used in PCR analysis to confirm their phenotype with four more sour cherry cultivars of known phenotype included for comparison, 'Bruine Waalse' (6,13,D), 'Čačanski Rubin' (1,4,13,B), 'Montmorency' (6,B,D) and 'Nabella' (13,B) (Tobutt et al. 2004).

Determination of parental *S* alleles by PCR

DNA extraction from the parents and reference cultivars, PCR amplification, separation and detection of PCR products were carried out in general accordance with Sonneveld et al. (2001, 2003). Two sets of primers were used to exploit the variation in length of the two introns associated with *Prunus S*-RNase genes: Pa ConsI-F + Pa ConsII-R (Sonneveld et al. 2003) based on signal peptide and C5 region of sweet cherry *S*-RNases, and EM-PC2consFD + EM-PC5consRD (Sutherland et al. 2004) based on C2 and C5 regions of *Prunus S*-RNases.

Cloning and sequencing of the *S*-RNase alleles S_B, S_D and S_{13}

Genomic DNA of 'Köröser' and 'Schattenmorelle', phenotypes 1,4,B, D and 6,13,B, was amplified by PCR with Pa ConsI-F + Pa ConsII-R primers. The amplified DNA was purified and concentrated and then cloned using the TA cloning kit (Invitrogen). Colony screening was carried out with M13-F + M13-R primers. The QIAprep Kit (Qiagen, Crawley, UK) was used for plasmid DNA extraction. The plasmids from three colonies corresponding to each of S_B and S_D from 'Köröser' and S_{13} and S_B from 'Schattenmorelle' were sequenced using M13 and internal primers.

The sequence contigs were assembled using SeqMan and EditSeq programs (DNAStar, Madison, WI, USA). The alignments of deduced amino acid sequences were carried out by the Clustal W method of the MegAlign computer program (DNAStar). Several published sequences were included for comparison.

Determination of self-(in)compatibility

Seedlings were assessed for fruit set after selfing in 1998. Between 100 and 200 flowers in bud were bagged; and at full bloom the flowers were self-pollinated, counted and rebagged and well developed fruits were counted after the June drop. To avoid confusion between self-incompatibility and low fertility, fruit set after open pollination was also recorded. In addition, a minimum of ten flowers per seedling were selfed and, 72 h after selfing, the styles were fixed and in due course squashed and the pollen tube growth in ten flowers was examined by fluorescence microscopy following the procedure described in Tobutt et al. (2004).

For some seedlings for which the initial studies indicated discrepancies between the bagging data and the pollen tube data, and for some randomly chosen seedlings, the bagging or the pollen tube studies were repeated in 2000. In the few cases where discrepancies remained, the pollen tube data were considered conclusive. Fruit set of 2% was taken as the threshold, even though this level does not represent commercially useful self-fertility.

Determination of parental and seedling *S* alleles by RNase analysis

The collection of styles, extraction of proteins, isoelectric focusing and staining for RNase activity was conducted as described by Tobutt et al. (2004). Where appropriate, the segregations were tested with χ^2.

Results

S-alleles of the parents of the segregating progenies

The banding patterns obtained with the two sets of primers designed to amplify *S*-RNase alleles (Fig. 1) could be correlated with the various combinations of *S*-RNases 1, 4, 6, 13, B and D revealed in the parents and reference cultivars, 'Bruine Waalse', 'Čačanski Rubin', 'Montmorency' and 'Nabella', by the IEF studies of Tobutt et al. (2004) (Fig. 1). Discrepancies concerning 'Bruine Waalse' and 'Montmorency' are dealt with later. In addition, the correspondence of the PCR products and RNase bands for the putative alleles S_B and S_D was confirmed by cosegregation analysis (data not shown). Thus 'Köröser' and 'Schattenmorelle' were, respectively, $S_1S_4S_BS_D$ and $S_6S_{13}S_B$. Selection 43.87 appeared to be $S_4S_{13}S_B$. Neither of the primer pairs amplified any product in the parents that could not be correlated with *S*-RNase phenotypes. The variations in size are consistent with the existence of the two introns.

Cloning and sequencing of the *S*-RNase alleles S_B, S_D and S_{13}

Figure 2 shows the alignment of deduced amino acid sequences of several putative *S*-RNases: SP-C5 genomic products for S_B 'Köröser' (S_B-K), and 'Schattenmorelle' (S_B-S), S_D from 'Köröser (S_D-K), and S_{13} from 'Schattenmorelle' (S_{13}-S). In addition amino acid sequences deduced from certain published cDNA or genomic sequences are included for comparison: S_a from 'Erdi Botermo' (Yamane et al. 2001) and from 'Rheinische Schattenmorelle' (Hauck et al. 2002), S_b and S_c from 'Rheinische Schattenmorelle' (Hauck et al. 2002), and S_{13} from sweet cherry 'Noble' (Sonneveld 2002). The RNase sequences for newly cloned S_B, S_D and S_{13} reveal the five conserved regions C1–C5 (Ushijima et al. 1998) that are characteristic for rosaceous *S*-RNases and have the two introns characteristic of *Prunus* in the right position.

It is apparent that the amino acid sequences corresponding to RNase S_B from 'Köröser' and 'Schattenmorelle' and to S_a from 'Erdi Botermo' and 'Rheinische Schattenmorelle' are identical. The sequence of S_{13} from 'Schattenmorelle' completely matches S_c from 'Rheinische Schattenmorelle' and the short sequence of S_{13} from 'Noble'. The alignment of deduced amino acid sequences of the presumed ground

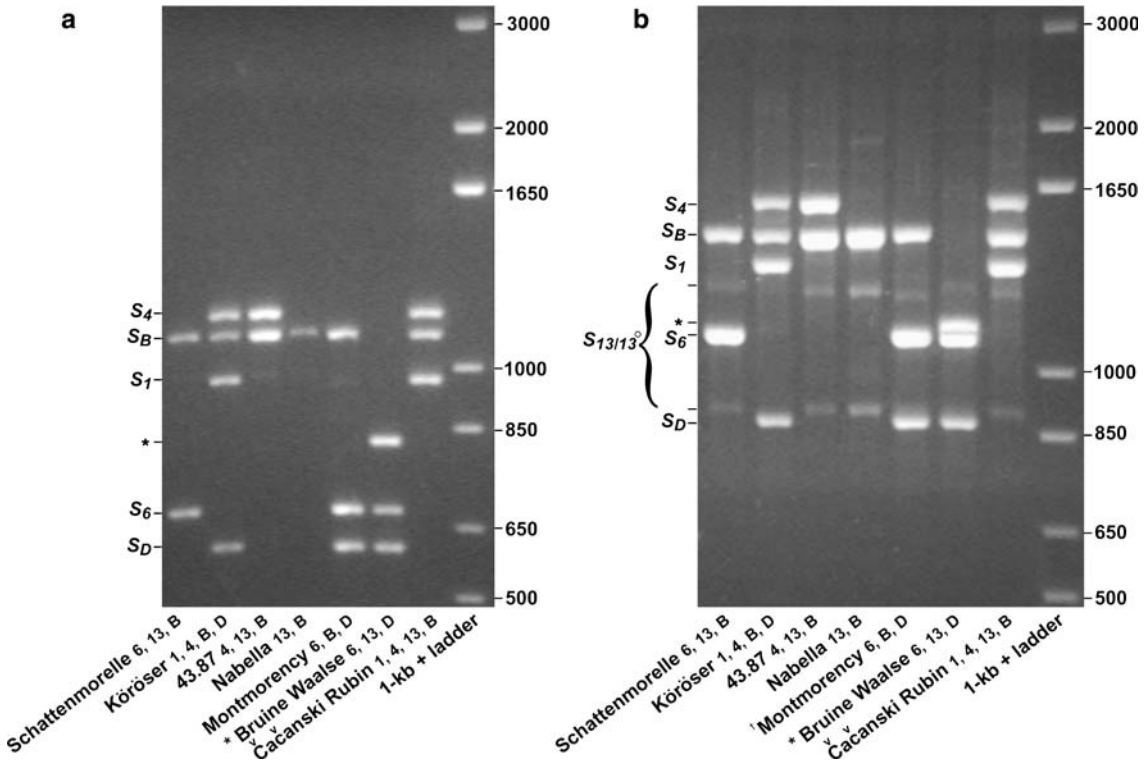

Fig. 1 Amplification patterns produced with primers based on *S*-RNase regions C2–C5 (**a**) and on SP-C5 (**b**) for the three parents and for the reference cultivars indicating the alleles S_1, S_4, S_6, S_{13}, S_B and S_D. In (**a**) conditions were adjusted, by increasing annealing temperature from 58 to 60°C, to avoid amplification of S_{13} the lower band of which comigrates with S_D. * 'Bruine Waalse' shows an additional band, not expected from its *S*-RNase phenotype, that was shown to be S_{14} by allele specific PCR (data not shown). † 'Montmorency' revealed the two weak bands characteristic of S_{13}, unexpected from its *S*-RNase phenotype, but confirmed by allele specific PCR (data not shown). *S*-RNase phenotypes (Tobutt et al. 2004) are given after each cultivar

cherry *S*-RNases showed that S_B and S_D cloned from 'Köröser' are different from each other and from S_b from 'Rheinische Schattenmorelle'.

Family 13 ('Schattenmorelle' × selection 43.87)

The results of the self-compatibility tests by bagging in the field, in comparison with fruit set after open pollination, and by monitoring pollen tube growth of the 24 seedlings from the cross 'Schattenmorelle' × selection 43.87 are given in Table 1. Twenty-one of the seedlings were determined as self-compatible by pollen tube growth studies. Of these only two set less than 2% fruit after selfing. Three seedlings were determined as self-incompatible by pollen tube studies and they set less than 2% fruit after selfing.

'Schattenmorelle' and selection 43.87 had the *S*-RNase phenotypes 6,13,B and 4,13,B, respectively; and four different seedling phenotypes were found, 4,6,B (three seedlings), 4,13,B (seven seedlings), 6,13,B (five seedlings) and 13,B (nine seedlings) (Fig. 3, Table 1). This seedling segregation, which approximates to 1:1:1:1 ($\chi^2 = 0.55$), is consistent with disomic inheritance and 'Schattenmorelle' having the genotype $S_6S_{13}.S_BS_B$ and selection 43.87 having the genotype $S_4S_{13}.S_BS_B$. 'Schattenmorelle' would produce ovules of genotype S_6S_B and $S_{13}S_B$, and selection 43.87 would produce pollen S_4S_B and $S_{13}S_B$. An alternative genotype for 'Schattenmorelle', $S_6S_{13}.S_BS_N$, where S_N is a null allele, is inconsistent with the segregation in family 11 described later. There was no evidence that the S_4S_B or the $S_{13}S_B$ pollen was rejected on the $S_6S_{13}.S_BS_B$ style. This indicates that S_B is not dominant to S_4 and that S_{13} and S_B interact competitively or lack the pollen-*S* products.

Comparison of the fruit set and pollen tube results with the *S*-RNase phenotypes shows that seedlings with the phenotype 4,6,B were self-incompatible, whereas those with the phenotypes 4,13,B, 6,13,B and 13,B were self-compatible.

By analogy with the proposed parental genotypes it is likely that the genotype of the self-incompatible seedlings was $S_4S_6.S_BS_B$. The pollen grains derived from this genotype would be S_4S_B and S_6S_B. As the pollen grains failed on selfing there was no competitive interaction in these heteroallelic combinations. It appears that S_4 is dominant to S_B and it is also likely that S_6 is dominant to S_B; the possibility that S_B is dominant to S_6 is inconsistent with the segregation in family 11 described later.

Again by analogy with the parental genotypes, the genotypes of the self-compatible seedlings are likely to be $S_4S_{13}.S_BS_B$, $S_6S_{13}.S_BS_B$ and $S_{13}S_{13}.S_BS_B$. Regarding the first of these, two classes of pollen are expected, S_4S_B and $S_{13}S_B$. We know (from the previous paragraph) that

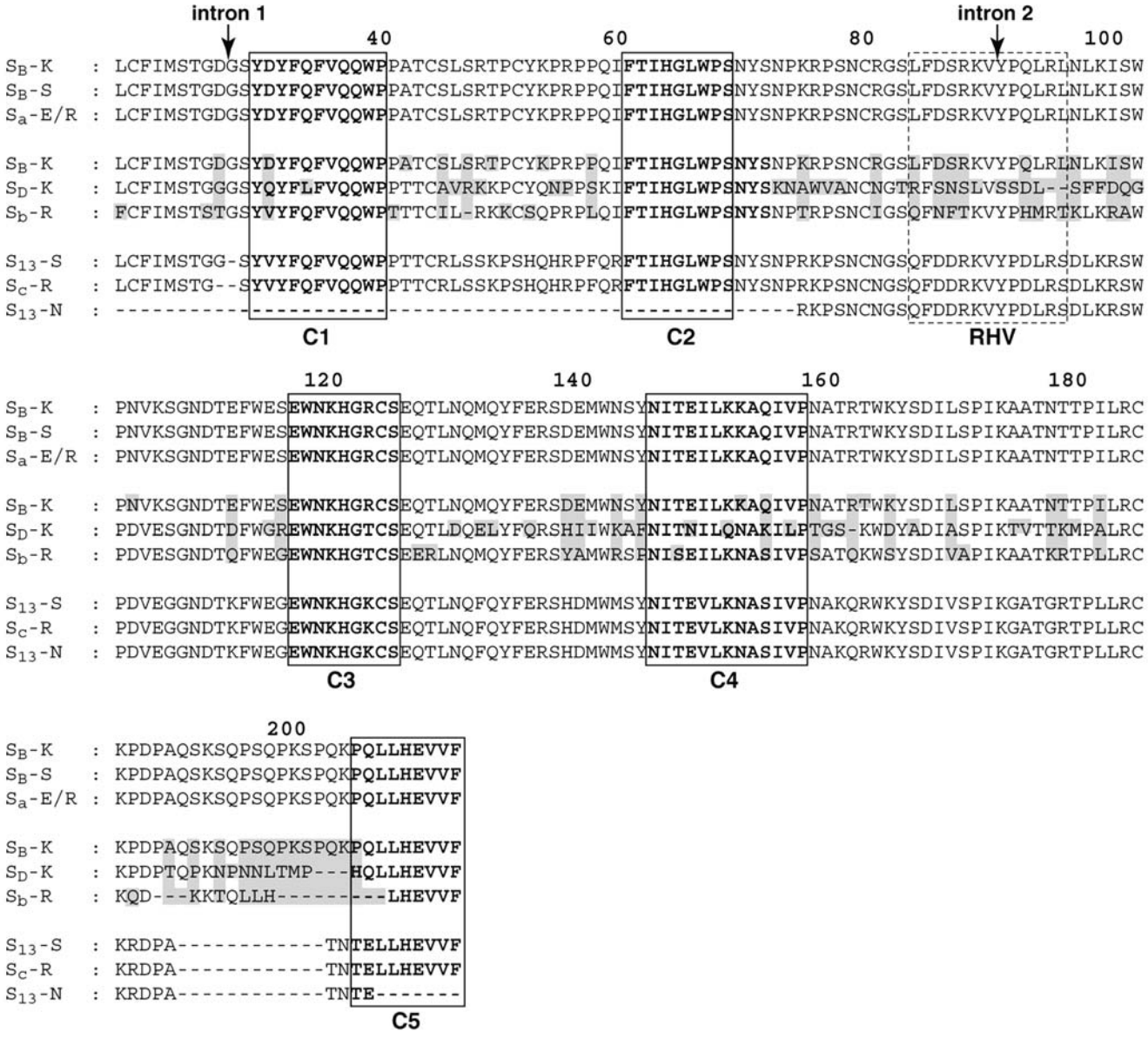

Fig. 2 The alignment of partial amino acid sequences of various *S*-RNases: S_B from 'Köröser' (S_B-K) and 'Schattenmorelle' (S_B-S), S_D from 'Köröser' (S_D-K), and S_{13} from 'Schattenmorelle' (S_{13}-S), S_a from 'Erdi Botermo' (Yamane et al. 2001) and from 'Rheinische Schattenmorelle' (Hauck et al. 2002), S_b and S_c from 'Rheinische Schattenmorelle' (Hauck et al. 2002), and S_{13} from sweet cherry 'Noble' (Sonneveld 2002). The five conserved regions (C1–C5) and hypervariable region (RHV), characteristic of rosaceous *S*-RNases (Ushijima et al. 1999) are *boxed*. The positions of two introns characteristic of *Prunus S*-RNases are indicated by *arrows*. The sequences are arranged to demonstrate: the identity of S_B with S_a; the differences between S_B, S_D and S_b; and the identity of S_{13} with S_c

S_4S_B pollen fails on selfing and so the self-compatibility can be attributed to the $S_{13}S_B$ pollen. Likewise regarding the second genotype, $S_6S_{13}.S_BS_B$. In the third genotype, $S_{13}S_{13}.S_BS_B$, all the pollen should be of the self-compatible $S_{13}S_B$ type.

Family 11 ('Köröser' × 'Schattenmorelle')

The results of the self-compatibility tests in the field and by pollen tube growth studies of the 21 seedlings nominally from the cross 'Köröser' × 'Schattenmorelle' are given in Table 2. Nine of the seedlings were shown to be self-compatible by the pollen tube studies, of which two set less than 2% fruits after selfing. Ten seedlings were shown to be self-incompatible by pollen tube studies and they all set less than 2% fruit after selfing; two more seedlings, which were assessed only by recording fruit set after selfing, set 0% fruit and were deemed self-incompatible.

'Köröser' and 'Schattenmorelle' had the *S*-RNase phenotypes 1,4,B,D and 6,13,B, respectively, and, concerning 19 of the seedlings, six different phenotypes were observed, 1,6,B (one seedling), 1,6,B,D (one seedling),

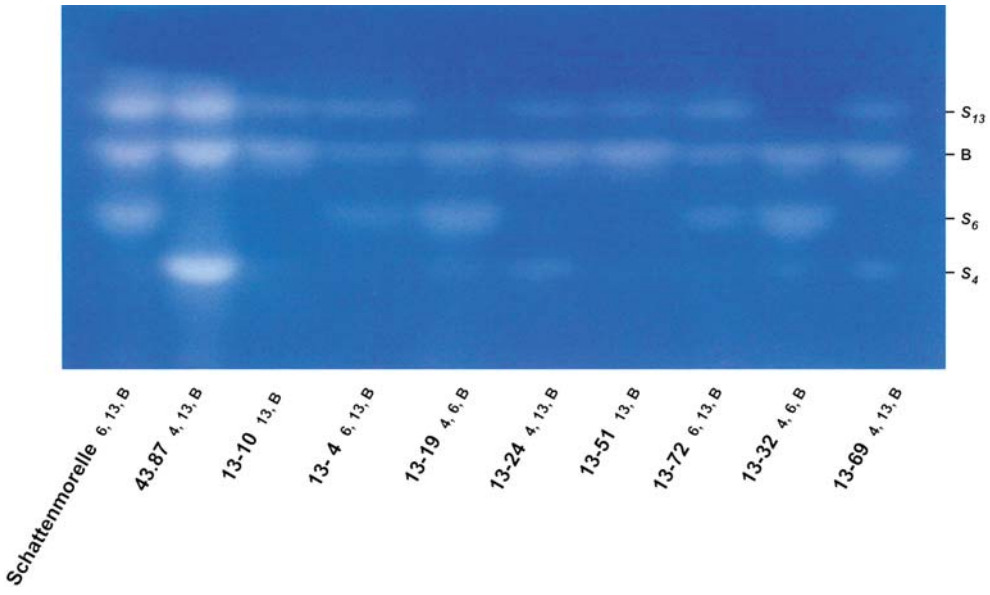

Fig. 3 *S*-RNase zymograms of sour cherry family 13 ('Schattenmorelle' × selection 43.87). *Tracks 1 and 2* show the parents and remaining tracks the range of seedling phenotypes observed

1,13,B (four seedlings), 4,6,B (nine seedlings), 4,13,B (three seedlings) and 4,13,B,D (one seedling) (Fig. 4, Table 2). In addition, one seedling had the phenotype 6,B, which may have resulted from tetrasomic segregation with double reduction in 'Köröser' giving an occasional ovule with the genotype S_BS_B; and another had the unexpected phenotype 6,13,B that may have resulted from outcrossing. As S_1 and S_4 from 'Köröser' are not inherited together, they are allelic; so the genotype of 'Köröser' can be written as $S_1S_4.S_BS_D$. Likewise, S_6 and S_{13} from 'Schattenmorelle' are not inherited together, supporting the genotype $S_6S_{13}.S_BS_B$ deduced earlier. Of the eight progeny phenotypes expected from these parental genotypes only 1,13,B,D and 4,6,B,D were not observed. The alternative genotype for 'Schattenmorelle' of $S_6S_{13}.S_BS_N$, where S_N is a null allele, can be rejected as that would predict 50% of the pollen and 25% of the seedlings would lack S_B. There was no evidence of S_6S_B or $S_{13}S_B$ pollen failing on the $S_1S_4.S_BS_D$ style, indicating that S_B is not dominant to S_6. Furthermore, as S_6S_B pollen failed on $S_4S_6.S_BS_B$ styles in family 13, we conclude S_6 is dominant to S_B.

By comparing the fruit set and pollen tube data to the *S*-RNase phenotypes, it is apparent that seedlings with

Table 1 Analysis of progeny 13 ['Schattenmorelle' (6,13,B) × selection 43.87 (4,13,B)] for self-compatibility, as determined by fruit set after bagging (with set after open pollination for comparison) and pollen tube growth after selfing, and for stylar ribonucleases

Seedling No.	% Fruit set after bagging 1998, 2000; SC = ≥2%		% Maximum fruit set after open pollination in 1998, 2000	Pollen tube growth assessment	Ribonuclease phenotypes
3	9.7	SC	23.2	SC	6,13,B
4	6.0	SC	19.2	SC	6,13,B
10	21.7	SC	21.7	SC	13,B
13	0.0, 0.5	SI	2.8	SC[a]	13,B
17	9.7	SC	7.2	SC	13,B
18	4.4	SC	5.6	SC[a]	6,13,B
19	0.0, 0.5	SI	4.9	SI	4,6,B
24	0.0, 1.9	SI	11.4	SC[a]	4,13,B
31	5.1	SC	12.3	SC	13,B
32	1.4	SI	13.6	SI[a]	4,6,B
33	16.3	SC	13.2	SC	4,13,B
39	11.4	SC	13.6	SC	4,13,B
45	4.7	SC	7.2	SC	4,13,B
47	3.0	SC	4.3	SC	4,13,B
49	3.3	SC	15.9	SC[a]	13,B
51	3.1	SC	3.1	SC	13,B
53	2.5	SC	12.8	SC	4,13,B
56	16.1	SC	29.1	SC	13,B
59	2.0	SC	10.3	SC	13,B
62	31.9	SC	28.2	SC	13,B
69	27.2	SC	23.9	SC	4,13,B
72	19.6	SC	8.3	SC	6,13,B
75	2.4	SC	14.0	SC	6,13,B
79	0.0	SI	1.2	SI[a]	4,6,B

[a]repeated and confirmed

Table 2 Analysis of progeny 11 ['Köröser'(1,4,B,D) × 'Schattenmorelle' (6,13,B)] for self-compatibility, as determined by fruit set after bagging (with set after open pollination for comparison) and pollen tube growth after selfing, and for stylar ribonucleases

Seedling No	% Fruit set after bagging 1998, 2000; SC = ≥2%		% Maximum fruit set after open pollination in 1998, 2000	Pollen tube growth assessment	Ribonuclease phenotypes
42	20.6	SC	23.8	SC	1,13,B
45	0.9	SI	18.3	SC	1,13,B
50	0.0	SI	6.3	SI	4,6,B
61	0.0	SI	5.4	–	1,6,B
62	0.0	SI	19.8	SI[a]	4,6,B
69	3.0	SC	1.4	SC	4,13,B
73	0.0	SI	0.0	SI[a]	4,6,B
114	0.0	SI	8.8	–	4,6,B
126	8.5	SC	3.8	SC	6,13,B[c]
127	0.0	SI	0.0	SI[a]	4,6,B
130	6.0	SC	7.4	SC	1,13,B
132	0.5	SI	2.8	SC	4,13,B
146	0.0	SI	18.8	SI[a]	4,6,B
148	1.5	SI?	6.7	SI[a]	1,6,B,D
149	0.0	SI	5.6	SI[a]	4,6,B
152	10.9	SC	15.3	SC	1,13,B
155	1.9, 0.0	SI	18.2	SI[a]	6,B[b]
158	4.7	SC	15.7	SC	4,13,B,D
162	0.9, 0.0	SI	23.0	SI[a]	4,6,B
166	0.0	SI	10.8	SI[a]	4,6,B
173	14.3	SC	16.7	SC	4,13,B

[a]repeated and confirmed
[b]unexpected phenotype, presumably resulting from tetrasomic segregation in 'Koroser'
[c]unexpected phenotype, presumably from outcrossing

the phenotypes 1,6,B, 4,6,B and 1,6,B,D are self-incompatible, whereas those with the phenotypes 1,13,B, 4,13,B and 4,13,B,D are self-compatible.

If, as seems likely by analogy with parental genotypes, the self-incompatible seedlings have the genotypes $S_1S_6.S_BS_B$, $S_1S_6.S_BS_D$ and $S_4S_6.S_BS_B$ then the pollen grains would variously have the genotypes S_1S_B, S_6S_B, S_4S_B, S_1S_D and S_6S_D. As the pollen grains failed on selfing there was no competitive interaction in these heteroallelic combinations, presumably because of the dominance of S_4 and S_6 over S_B proposed earlier and, maybe, the dominance of S_1 over S_B and of S_1 and S_6 over S_D or vice versa.

The genotypes of the self-compatible seedlings, again by analogy, are likely to be $S_1S_{13}.S_BS_B$, $S_4S_{13}.S_BS_B$ and $S_4S_{13}.S_BS_D$. In the first two genotypes, the self-compatibility can be explained by the $S_{13}S_B$ pollen, as S_1S_B pollen is expected to fail in the first and S_4S_B to fail in the second. In the third genotype, the S_4S_B pollen is expected to fail and the $S_{13}S_B$ pollen to succeed; the action of the S_4S_D and $S_{13}S_D$ pollen cannot be inferred from these data.

Family 12 ('Köröser' × selection 43.87)

The results of the self-incompatibility tests of the 20 seedlings from the cross 'Köröser' × selection 43.87 are given in Table 3. Apart from one seedling (12.73), all were found to be self-compatible by pollen tube tests. Six of them set less than 2% fruit after selfing, but it is worth noting that five of these seedlings set less than 4% fruit even when open pollinated, indicating poor fertility rather than genuine self-incompatibility.

'Köröser' and selection 43.87 had the *S*-RNase phenotypes 1,4,B,D and 4,13,B, respectively; and five different seedling phenotypes occurred, 1,4,13,B (three seedlings), 1,13,B (eight seedlings), 4,13,B (six seedlings), 4,13,B,D (one seedling) and 13,B (two seedlings) (Fig. 5, Table 3). As deduced previously, the parental genotypes appear to be $S_1S_4.S_BS_D$ and $S_4S_{13}.S_BS_B$. Presumably the ovules from 'Köröser' are S_1S_B, S_1S_D, S_4S_B and S_4S_D and pollens from selection 43.87 are either S_4S_B, which as explained earlier are expected to express S_4 and to fail on styles having S_4, or $S_{13}S_B$, which again as explained earlier do not fail on styles having S_B. Thus the expected seedling classes are 1,13,B, 1,13,B,D, 4,13,B and 4,13,B,D. Of these only 1,13,B,D was not found. The unexpected phenotypes 1,4,13,B and 13,B may be the result of tetrasomic segregation in the female parent 'Köröser' giving ovules of genotype S_1S_4 and, with double reduction, S_BS_B, respectively.

When the pollen tube data, supported in most cases by fruit set data, were compared with *S*-RNase phenotypes, it appeared that the seedlings with the phenotypes 1,4,13,B, 1,13,B, 4,13,B,D and 13,B were all self-compatible. Of the six seedlings sharing the 4,13,B phenotype, five appeared to be self-compatible, while one (12.73) was confirmed as self-incompatible.

The genotypes of the 'expected' self-compatible seedlings, again by analogy, are likely to be $S_1S_{13}.S_BS_B$, $S_4S_{13}.S_BS_D$, and $S_4S_{13}.S_BS_B$. Self-compatibility may be attributed to the $S_{13}S_B$ pollen, at least in part; the pollen with genotypes S_1S_B and S_4S_B is, from earlier evidence, unlikely to be self-compatible and the behaviour of pollen with genotypes S_4S_D and $S_{13}S_D$ remains unknown. The seedlings with phenotype 13,B presumably have the genotype $S_{13}S_{13}.S_BS_B$—giving self-compatible

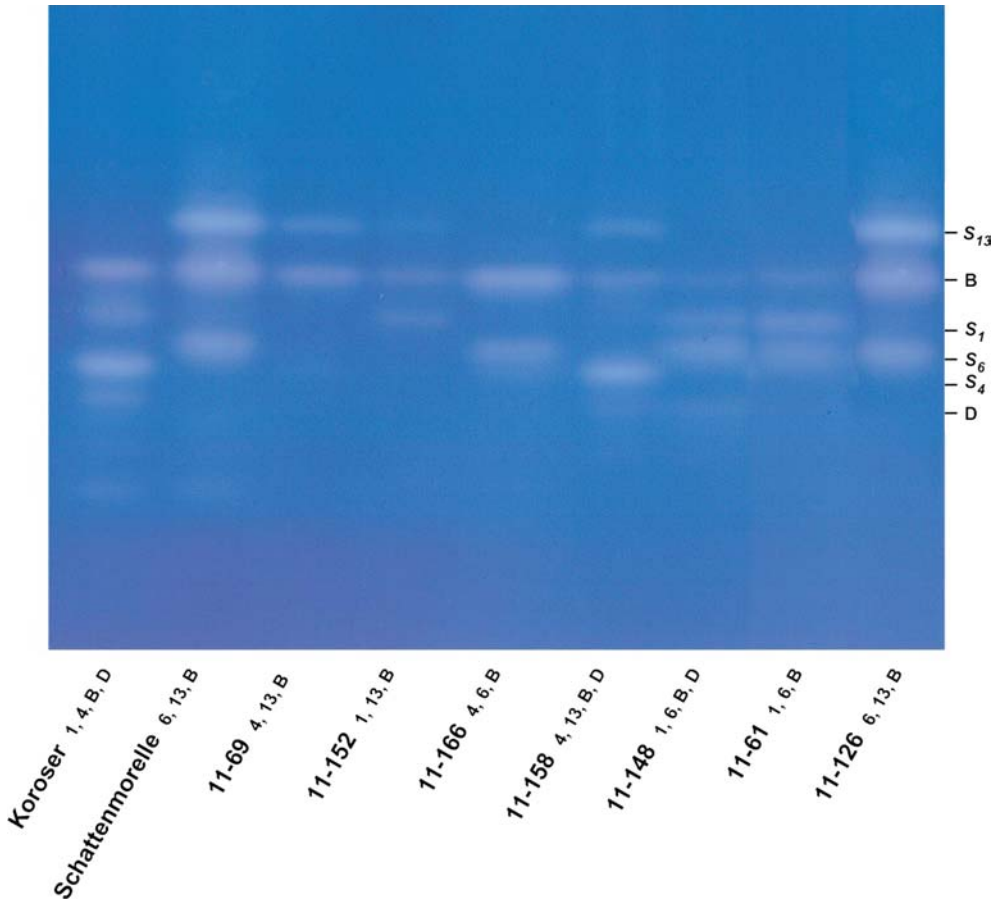

Fig. 4 *S*-RNase zymograms of sour cherry family 11

Table 3 Analysis of progeny 12 ['Köröser' (1,4,B,D) × selection 43.87 (4,13,B)] for self-compatibility, as determined by fruit set after bagging (with set after open pollination for comparison) and pollen tube growth after selfing, and for stylar ribonucleases

Seedling No.	% Fruit set after bagging 1998, 2000; SC = ≥2%		% Maximum fruit set after open pollination in 1998, 2000	Pollen tube growth assessment	Ribonuclease phenotypes
29	0.5	SI	0.5	SC	1,13,B
33	0.0, 0.0	SI	3.3	SC[a]	1,4,13,B[b]
37	4.2	SC	6.2	SC	1,13,B
41	13.0	SC	5.4	SC	4,13,B
45	0.0, 1.1	SI	5.7	SC[a]	1,13,B
50	2.4	SC	4.1	SC	4,13,B
52	0.0, 0.5	SI	3.6	SC	4,13,B
55	0.8, 1.0	SI	3.3	SC[a]	4,13,B,D
62	8.4	SC	19.7	SC	1,4,13,B[b]
66	1.8, 8.7	SC	11.6	SC	1,13,B
67	2.0, 2.0	SC	8.0	SC	1,13,B
73	0.0, 0.0	SI	10.4	SI	4,13,B
82	23.3	SC	15.6	SC	1,13,B
87	3.0	SC	10.4	SC	4,13,B
88	2.2	SC	4.9	SC	13,B[b]
92	17.4	SC	22.3	SC	1,4,13,B[b]
103	1.4, 45.0	SC	44.0	SC	13,B[b]
110	2.1	SC	1.7	SC	1,13,B
114	14.1	SC	22.3	SC	4,13,B
128	0.0, 0.5	SI	3.8	SC	1,13,B

[a]repeated and confirmed
[b]unexpected phenotype, presumably resulting from tetrasomic segregation in 'Koroser'

$S_{13}S_B$ pollen. In the case of the seedlings with the phenotype 1,4,13,B, two genotypes seem likely, $S_1S_{13}.S_4S_B$ and $S_4S_{13}.S_1S_B$, their self-compatibility again due to the $S_{13}S_B$ pollen. The genotype of the self-incompatible seedling of phenotype 4,13,B may be $S_4S_4.S_{13}S_B$ but it is difficult to reconcile with parental arrangements.

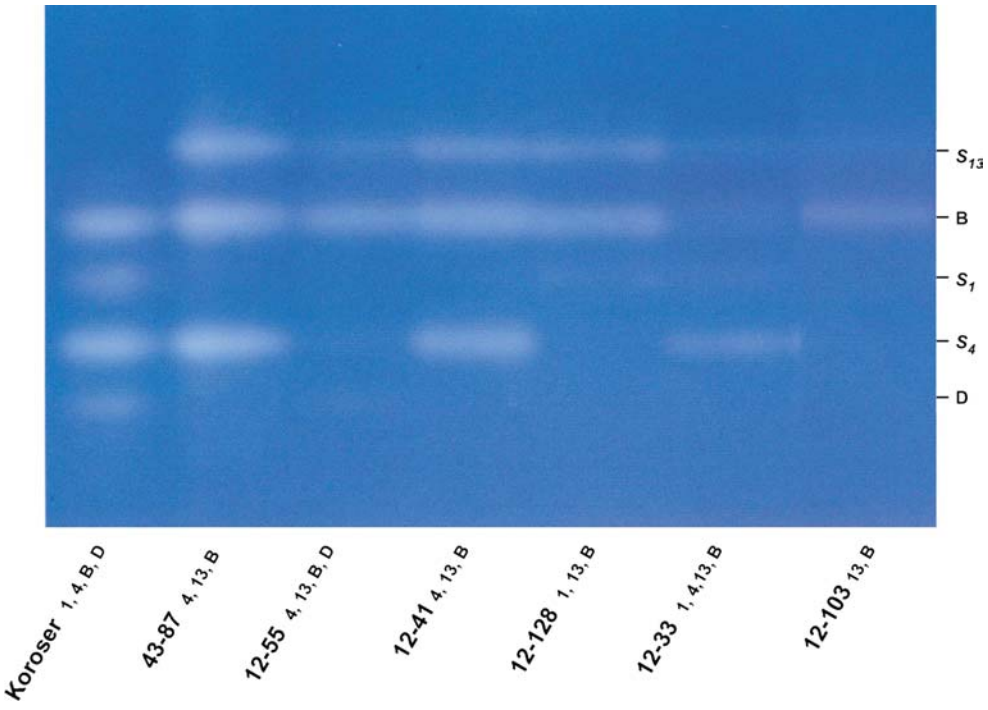

Fig. 5 *S*-RNase zymograms of sour cherry family 12 ('Köröser' × selection 43.87). *Tracks 1 and 2* show the parents and remaining tracks the range of seedling phenotypes observed

Discussion

The PCR products amplified in 'Köröser', 'Schattenmorelle' and selection 43.87 with *S*-RNase consensus primers corresponded to the bands, seen on RNase zymograms, attributable to the alleles S_1, S_4, S_6, S_{13}, S_B and S_D. Cloning and sequencing showed that S_B and S_D, the presumed ground cherry stylar RNase alleles, have the structure typical of rosaceous *S*-RNase genes. Interestingly, two of the reference cultivars, 'Bruine Waalse' and 'Montmorency', showed some discrepancy between PCR results and zymograms, as discussed later.

In addition, by determining the *S*-RNase phenotypes of 65 seedlings in three progenies from controlled crosses, we have been able to deduce the genotypes and arrangements of the *S*-RNase alleles in the parents and seedlings. Furthermore, by comparing *S*-RNase phenotypes/genotypes with incompatibility phenotypes we have arrived at a better understanding of the mediation of (in)compatibility in sour cherry by *S*-RNase alleles.

Parental *S* alleles

The PCR confirmed IEF scores and did not indicate the existence of null *S*-RNase alleles in 'Köröser', 'Schattenmorelle' and selection 43.87. Thus, it is unlikely that our interpretation of parental and seedling genotypes needs to consider the presence of null *S*-RNase alleles.

The deduced amino acid sequence for S_{13} from 'Schattenmorelle' matched that for S_{13} from 'Noble' (Sonneveld 2002) and for S_c (Hauck et al. 2002). Thus the complete sequence of S_{13} is now available. In addition we reconciled our sequence for S_B with S_a (Yamane et al. 2001; Hauck et al. 2002). The sequences of S_B and S_D RNase alleles, that so far have been found only in sour cherry and in ground cherry (Tobutt et al. 2004) and are thought to originate from the latter, had the sequence structure (conserved regions, RHV region, position and number of introns) typical of *Prunus* *S*-RNases (Ushijima et al. 1988) but different from *Prunus* non-*S*-RNases (Ma and Oliveira 2000; Yamane et al. 2003b). The cloning of S_B and S_D brings to three the number of *S*-RNase sequences that have been found in sour cherry but are absent from sweet cherry.

Genomic arrangement of *S* alleles

From the genotypes of the seedlings analysed, the genomic arrangements of the *S*-RNase alleles in the three parents 'Schattenmorelle', selection 43.87 and 'Köröser' have been deduced. In each case, the *S*-RNases thought to be derived from sweet cherry (S_1, S_4, S_6 or S_{13}) were allelic, representing one of the two loci in the allotetraploid, and the *S*-RNases thought to be derived from ground cherry (S_B or S_D) represented the other locus. Although most seedling patterns were consistent with disomic inheritance there was some evidence of tetrasomic inheritance. Previously, Tobutt et al. (2004) gave the genomic arrangement of 'Erdi Botermo B'and 'Marasca Luxardo' and inferred the genomic arrangement of 'Amarena di Verona P.C.' and 'Montmorency'.

Of the 36 sour cherry cultivars analysed by Tobutt et al. (2004), 30 are of known self-(in)compatibility status, and 14 of these have *S*-RNase phenotypes similar to

those observed in the seedlings analysed in this work. The most likely genomic arrangement of their *S* alleles can now be predicted. Seven cultivars have the phenotype 6,13,B, four the phenotype 13,B and one the phenotype 1,13,B. Consistent with their known self-compatibility would be the genotypes and arrangements $S_6S_{13}.S_BS_B$, $S_{13}S_{13}.S_BS_B$ and $S_1S_{13}.S_BS_B$, respectively. 'Čačanski Rubin', with phenotype 1,4,13,B and also self-compatible, could have the genotype and arrangement $S_1S_{13}.S_4S_B$ or $S_4S_{13}.S_1S_B$. 'Chase Morello', 4,13,B, which is self-incompatible, could have the genotype and arrangement $S_4S_4.S_{13}S_B$, as later proposed for the self-incompatible selection with the phenotype 4,13,B.

S allele interactions in heteroallelic diploid pollen and self-(in)compatibility

By correlating (in)compatibility status with the *S*-RNase phenotypes/genotypes, we were able to make certain deductions about the interactions in heteroallelic pollen. When the sweet cherry alleles S_1, S_4 or S_6 are present with S_B, the pollen behaves as S_1, S_4 or S_6, i.e. there is no competitive interaction, possibly because S_B has no activity in pollen. In contrast, when S_{13} is present with S_B in heteroallelic pollen, there is no evidence of a dominant/recessive relationship as $S_{13}S_B$ pollen is not rejected by the style expressing the corresponding RNases. S_1 and S_6 show no competitive interaction with S_D; there were no data on how S_4 or S_{13} interact with S_D.

In the case of $S_{13}S_B$, it may be that there is competitive interaction or that neither allele in these sour cherries has an active pollen component, in which case this variant of S_{13} could be denoted S_{13}' in the same way that S_3' is used to denote the variant S_3 that lacks pollen activity (Matthews 1970). Sonneveld et al. (2005) have recently demonstrated that the self-compatibility of S_3' is attributable to the deletion of the pollen-S_3 F-box allele. However, with primers based on conserved regions of pollen-*S* F-box alleles (Yamane et al. 2003a; Ikeda et al. 2004), genomic sequences corresponding to S_{13} and S_B F-box alleles have been detected in 'Schattenmorelle' and selection 43.87 (data not shown). This indicates that lack of pollen expression rather than deletion of the pollen component could be an alternative to competitive interaction as an explanation of self-compatibility. It would be difficult to discriminate between these possibilities by pollination tests, but sequences for putative pollen-*S* F-box alleles could be used to check for expression in pollen.

So long as the sweet cherry alleles such as S_1, S_4, S_6 and S_{13} remain at one locus of the allotetraploid there is no opportunity to see how they interact with each other in heteroallelic pollen; likewise for the presumed ground cherry alleles such as S_B and S_D.

According to the model proposed by Luu et al. (2001), if the pollen components of S_{13} and S_B are expressed and show competitive interaction this is a result of heterotetramers forming between S_{13} and S_B pollen-specific proteins—the heterotetramers would not bind to the S_{13} and S_B RNases and thus not prevent the inhibition of the *S*-RNases. The model does not explicitly address the possible lack of competitive interaction in heteroallelic pollen such as observed in, e.g. S_4S_B, where S_4 is expressed specifically but S_B is not. In this case, if both alleles are active in pollen, it may be that many more copies of S_4 pollen-specific protein are produced than of S_B pollen-specific protein; this would allow the formation of S_4 homotetramers that could bind to the S_4-RNases and prevent their inhibition.

It is apparent that the lack of pollen function of S_{13} and S_B, or their competitive interaction and the effective dominance of S_4 over S_B, can explain without exception the self-(in)compatibility phenotypes of the cultivars discussed in the previous section. The high frequency of S_{13} and S_B together in sour cherry cultivars may be a reflection of the universal compatibility of $S_{13}S_B$ pollen.

Inheritance and expression of self-(in)compatibility

The mode of inheritance of self-(in)compatibility in sour cherry is addressed in part in Table 4. This shows the segregation for self-compatible (SC) versus self-incom-

Table 4 Predicted segregation for self-compatibility (SC) versus self-compatibility (SI) in progenies resulting from crosses between various sour cherry genotypes

♀ \ ♂	$S_1S_6.S_BS_B$ (SI)	$S_1S_{13}.S_BS_B$ (SC)	$S_4S_6.S_BS_B$ (SI)	$S_4S_{13}.S_BS_B$ (SC)	$S_6S_{13}.S_BS_B$ (SC)	$S_{13}S_{13}.S_BS_B$ (SC)
$S_1S_6.S_BS_B$ (SI)	–	all SC	all SI	50SC:50SI	all SC	all SC
$S_1S_{13}.S_BS_B$ (SC)	50SC:50SI	all SC	50SC:50SI	75SC:25SI	75SC:25SI	all SC
$S_4S_6.S_BS_B$ (SI)	all SI	50SC:50SI	–	all SC	all SC	all SC
$S_4S_{13}.S_BS_B$ (SC)	50SC:50SI	75SC:25SI	50SC:50SI	all SC	75SC:25SI	all SC
$S_6S_{13}.S_BS_B$ (SC)	50SC:50SI	75SC:25SI	50SC:50SI	75SC:25SI	all SC	all SC
$S_{13}S_{13}.S_BS_B$ (SC)	all SC	all SC	all SC	all SC	all SC	all SC

patible (SI) seedlings that we could predict in progenies resulting from crosses between all but two of the various expected seedling genotypes for which we have proposed arrangements in this paper. We have assumed that the interactions in heteroallelic pollen are as summarised in the previous section. We have excluded $S_1S_6.S_BS_D$ and $S_4S_{13}.S_BS_D$ as the interactions of S_4 and S_{13} with S_D are currently unknown.

In the progenies from these crosses of SI × SI, we would expect the seedlings to be all SI. In the progenies from crosses of SI × SC or SC × SI, the seedlings would all be SC or would segregate 50:50. In progenies from crosses of SC × SC, seedlings would be SC or would segregate 75SC:25SI.

Two further points are noteworthy. Cultivars with genotype $S_{13}S_{13}.S_BS_B$ appear to be universal donors of self-compatibility, whether used as male or female parent. And the progenies from cross $S_6S_{13}.S_BS_B \times S_4S_6.S_BS_B$ and the reciprocal give different segregations. (Incidentally, we would expect the cross $S_6S_6.S_BS_D \times S_4S_6.S_BS_B$ to succeed, but the reciprocal to fail.)

In general, seedlings with the S_4 and S_6 alleles at the same locus should be self-incompatible whereas those with S_{13} and S_B at homoeologous loci should be self-compatible. This information could be used as a basis for screening for self-compatibility seedling progenies from appropriate controlled crosses at an early stage. However, as apparent from family 12, the combination of S_{13} with S_B, while associated with self-compatibility, is not indicative of satisfactory cropping. The poor fertility of 'Köröser', a parent of family 12, was studied by Murawski and Endlich (1962) and attributed to irregular meiosis.

Possible tetrasomic segregation

To account for some unexpected genotypes we have suggested occasional tetrasomic segregation in 'Köröser' as we have already suggested in 'Erdi Botermo B' (Tobutt et al. 2004). Beaver and Iezzoni (1993) presented data consistent with occasional tetrasomic segregation at some non-*S* loci in sour cherry progenies.

In the disomic segregation of an allotetraploid, the genotype $S_1S_2.S_AS_B$ would give the gametes S_1S_A, S_1S_B, S_2S_A and S_2S_B in equal proportions. In tetrasomic segregation, without double reduction, the genotype $S_1S_2S_AS_B$ would additionally give, in the same proportions, gametes S_1S_2 and S_AS_B. However, for loci distant from the centromere, tetrasomic segregation can involve double reduction. In that case the expected gametes would also include the homoallelic genotypes S_1S_1, S_2S_2, S_AS_A and S_BS_B, the frequency of each of these genotypes being a quarter that of each of the heteroallelic genotypes. Ballester et al. (1998) reported that the *S* locus in *Prunus* lies near the end of the linkage group, but the location of the centromere of the corresponding chromosome is unknown. Bigger progenies would be needed to establish unambiguously the degree of tetrasomy and possible double reduction. Even though the role of tetrasomic segregation is speculative, the correlations established between *S*-RNase phenotype and self-(in)compatibility are not dependent on it.

If occasional tetrasomic segregation does occur in allotetraploid sour cherry it would make self-incompatibility less absolute than in diploid sweet cherry if the sour cherry genotype has two homeologous *S* alleles that can interact competitively.

Other work

Our findings can be used as a basis for interpreting aspects of the allelic segregations reported by Hauck et al. (2002), in the progeny from the cross of two self-compatible cultivars, 'Rheinische Schattenmorelle' ($S_aS_bS_cS_6$) × 'Erdi Botermo' ($S_aS_4S_{6m}$). As mentioned before, their S_a corresponds to our S_B and their S_c to our S_{13}; S_{6m} is a self-compatible mutant of S_6 allele in which the self-compatibility function is attributed to the inactivation of corresponding *S*-RNase but presence of an active pollen *S* component (Yamane et al. 2003c). Our detection by PCR but not by activity staining of S_{14} and S_{13} in 'Bruine Waalse' and 'Montmorency,' respectively, may be attributable to similar inactivation. The likely genotype and arrangement of 'Erdi Botermo' with our nomenclature is $S_4S_{6m}.S_BS_B$ or even $S_4S_{6m}.S_BS_N$. That none of the seedlings from the cross inherited S_{6m} from 'Erdi Botermo' implies that the pollen grains carrying the S_{6m} allele in combination with S_B behave as S_6. The failure of the $S_{6m}S_B$ pollen is, if occasional tetrasomic segregation occurs, sufficient to explain the transmission of S_4 to the great majority of seedlings; the S_4S_B pollen is not rejected. It is unnecessary to assume two copies of S_4 in 'Erdi Botermo'. In the absence of competitive interaction of S_4 or S_6 with S_B, we can predict that 'Erdi Botermo' ($S_4S_{6m}.S_BS_B$), though self-compatible, will fail to pollinate sweet cherry cultivars of genotype S_4S_6, e.g. 'Merton Glory'. If only disomic segregation operated, all the seedlings should have inherited S_4 as the allele S_{6m} could not succeed on the 'Rheinische Schattenmorelle' style expressing allele S_6. However eight out of 85 seedlings did not inherit S_4 from 'Erdi Botermo', which is consistent with some tetrasomic segregation.

Concluding remarks

We have shown that the stylar RNases, presumed to derive from ground cherry, share the sequence structure of *S*-RNases from sweet cherry. In addition this study has given an insight into the genomic arrangement of the *S* alleles, the interaction of particular allelic combinations in diploid pollen and the inheritance of self-(in)compatibility in allotetraploid sour cherry. Our findings could be used for designing crosses to yield only

self-compatible seedlings and for screening certain segregating progenies for self-compatibility soon after germination by PCR. They also provide models to study the molecular basis of competitive interactions and dominant/recessive relationships of *S* alleles in heteroallelic pollen. Further research should investigate expression of the pollen component as well as the allelic arrangements and interactions not considered by this work.

Acknowledgements Cherry genetics at East Malling is supported by the Department for Environment, Food and Rural Affairs. Radovan Bošković acknowledges a grant from the Mount Trust and Tineke Sonneveld, a studentship from Horticulture Research International and University of Nottingham.

References

Apostol J (1996) Sour and sweet cherry breeding and production in Hungary. Acta Hort 410:101–104

Ballester J, Bošković R, Batlle I, Arus P, Vargas F, de Vicente MC (1998) Location of the self-incompatibility gene on the almond linkage map. Plant Breed 117:69–72

Beaver JA, Iezzoni AF (1993) Allozyme inheritance in tetraploid sour cherry (*Prunus cerasus* L.). J Am Soc Hort Sci 118:873–877

Bošković R, Tobutt KR (1996) Correlation of stylar ribonuclease zymograms with incompatibility alleles in sweet cherry. Euphytica 90:245–250

Chawla B, Bernatzky R, Liang W, Marcotrigiano M (1997) Breakdown of self-incompatibility in tetraploid *Lycopersicon peruvianum*: inheritance and expression of *S*-related proteins. Theor Appl Genet 95:992–996

Crane MB, Lawrence WJC (1929) Genetical and cytological aspects of incompatibility and sterility in cultivated fruits. J Pomol Hort Sci 7:276–301

Crane MB, Lawrence WJC (1938) The genetics of garden plants. Macmillan, London

De Cuyper B, Sonneveld T, Tobutt KR (2005) Determining self-incompatibility genotypes in Belgian wild cherries. Mol Ecol 14:945–955

Hauck NR, Yamane H, Tao R, Iezzoni AF (2002) Self-compatibility and incompatibility in tetraploid sour cherry (*Prunus cerasus* L.). Sex Plant Reprod 15:39–46

Hruby K (1963) Pollination interrelations in some sour cherry cultivars. Biol Plant 5:124–128

Ikeda K, Igic B, Ushijima K, Yamane H, Hauck RH, Nakano R, Sassa H, Iezzoni AF, Kohn JR, Tao R (2004) Primary structural features of the *S* haplotype-specific F-box protein, SFB, in *Prunus*. Sex Plant Reprod 16:235–243

Lansari A, Iezzoni A (1990) A preliminary analysis of self-incompatibility in sour cherry. HortScience 25:636–1638

Lawrence WJC (1930) Incompatibility in polyploids. Genetica 12:269–296

Lewis D (1943) Physiology of incompatibility in plants III. Autopolyploids. J Genet 45:171–185

Lewis D (1947) Competition and dominance of incompatibility alleles in diploid pollen. Heredity 1:85–108

Lewis D (1949) Structure of the incompatibility gene II. Induced mutation rate. Heredity 3:339–355

Lewis D (1954) Comparative incompatibility in angiosperms and fungi. Adv Genet 6:235–285

Lewis D, Modlibowska I (1942) Genetical studies in pear IV. Pollen tube growth and incompatibility. J Genet 43:211–222

Luu D-T, Qin X, Laublin G, Yang Q, Morse D, Cappadocia M (2001) Rejection of *S*-heteroallelic pollen by a dual-specific *S*-RNase in *Solanum chacoense* predicts a multimeric SI pollen component. Genetics 159:329–335

Ma R-C, Oliveira MM (2000) The RNase PD2 gene of almond (*Prunus dulcis*) represents an evolutionary distinct class of S-like RNase genes. Mol Gen Genet 263:925–933

Matthews P (1970) The genetics and exploitation of self-fertility in the sweet cherry. Proceedings of Eucarpia fruit breeding symposium, Angers, September 1970, pp307–316

Montalti P, Selli R (1984) Osservazioni sulla fertilita del ciliego acido. Rivista di Frutticol 46(9/10):57–62

Murawski H, Endlich J (1962) Beiträge zur Züchtungsforschung an Kirschen. II. Befruchtungsbiologische und embriologische Untersuchungen an der Sauerkirschen-sorte Köröser Weichsel. Arch Gartenbau 10:616–646

Oldén EJ, Nybom N (1968) On the origin of *Prunus cerasus* L. Hereditas 59:327–345

Redalen G (1984a) Cross pollination of five sour cherry cultivars. Acta Hort 149:71–76

Redalen G (1984b) Fertility in sour cherries. Gartenbauwissenschaft 49:212–217

Sonneveld T, Robbins TP, Bošković R, Tobutt KR (2001) Cloning of six cherry self-incompatibility alleles and development of allele-specific PCR detection. Theor Appl Genet 102:1046–1055

Sonneveld T (2002) The molecular genetics of self-incompatibility in sweet cherry (Prunus avium). PhD Thesis, University of Nottingham, Nottingham, UK

Sonneveld T, Tobutt KR, Robbins TP (2003) Allele-specific PCR detection of sweet cherry self-incompatibility (*S*) alleles S_1 to S_{16} using consensus and allele-specific primers. Theor Appl Genet 107:1059–1070

Sonneveld T, Tobutt KR, Vaughan S, Robbins TP (2005) Loss of pollen-*S* function in two self-compatible selections of *Prunus avium* is associated with deletion/mutation of an *S* haplotype-specific F-box gene. Plant Cell 17:37–51

Sutherland BG, Robbins TP, Tobutt KR (2004) Primers amplifying a range of *Prunus S*-alleles. Plant Breed 123:582–584

Tobutt KR, Bošković R, Cerović R, Sonneveld T, Ružić Đ (2004) Identification of incompatibility alleles in the tetraploid species sour cherry. Theor Appl Genet 108:775–785

Ushijima K, Sassa H, Tao R, Yamane H, Dandekar AM, Gradziel TM, Hirano H (1998) Cloning and characterization of cDNAs encoding *S*-RNases from almond (*Prunus dulcis*): primary structural features and sequence diversity of the *S*-RNases in Rosaceae. Mol Gen Genet 260:261–268

Wolfram B (1999) Self-fertility as a factor of yield potential in progenies of sweet and sour cherries. Acta Hort 484:291–296

Yamane H, Tao R, Sugiura A, Hauck NR, Iezzoni AF (2001) Identification and characterization of *S*-RNases in tetraploid sour cherry (*Prunus cerasus*). J Am Soc Hort Sci 126:661–667

Yamane H, Ikeda K, Ushijima K, Sassa H, Tao R (2003a) A pollen-expressed gene for a novel protein with an F-box motif that is very tightly linked to a gene for *S*-RNase in two species of cherry, *Prunus cerasus* and *Prunus avium*. Plant Cell Physiol 44:764–769

Yamane H, Tao H, Sugiura A (2003b) Identification of non-*S* RNase, a possible ancestral form of *S*-RNases, in *Prunus*. Mol Gen Genom 269:90–100

Yamane H, Ikeda K, Hauck NR, Iezzone AF, Tao R (2003c) Self-incompatibility (*S*) locus region of the mutated S^6-haplotype of sour cherry (*Prunus cerasus*) contains a functional pollen *S* allele and a non-functional pistil *S* allele. J Exp Bot 54:2431–2437

Yenikeyev KK (1973) Development of self-fertile sour cherry varieties (C. vulgaris Mill.) in the central areas of the USSR. Atti Convegno del Ciliego, Verona, June 1972, pp19–23