Theor Appl Genet (2000) 101:234–240 © Springer-Verlag 2000

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Re-examination of (in)compatibility genotypes of two John Innes self-compatible sweet cherry selections

Received: 28 October 1999 / Accepted: 24 November 1999

Abstract The (in)compatibility genotypes of two self-compatible sweet cherry selections, JI 2420 and JI 2434, originating from the John Innes Institute were re-examined. The selections and seedlings derived from them were analysed for stylar ribonucleases, which are known to correlate with *S* alleles, and the outcome of test crosses was recorded. JI 2420, which had been reported previously as S_3S_4', where $'$ indicates loss of pollen activity, was deduced to have the genotype S_4S_4'. For JI 2434, which had been reported previously as $S_3S_4^0$, $S_3S_3^0$ or S_3S_3', where ^{0} indicates loss of pollen and stylar activity, two different clones were identified. One, at East Malling, was deduced to be $S_3'S_4$; the other, at Ahrensburg, appeared to be S_3S_3' or $S_3S_3^0$.

Key words Cherry · Genetics · Compatibility · Incompatibility · Isoelectric focusing · *Prunus avium* · Ribonuclease

Introduction

Most cultivars of sweet cherry (*Prunus avium)* are self-incompatible and many pairs of cultivars are cross-incompatible. This incompatibility was attributed by Crane and Lawrence (1929) to the gametophytic multiallelic locus *S*. A diploid style rejects a haploid pollen grain having one of the same *S* alleles. Six alleles, S_1 to S_6, have been assigned in various pairs to ten intra-incompatible inter-compatible groups of cultivars (Matthews and Dow 1969). The constitutions of two of these groups, V and VII, were revised recently and five more alleles were reported (Bošković et al. 1997).

With the aim of overcoming self-incompatibility, Lewis (1949) of the John Innes Institute, UK, made nominally incompatible crosses with pollen that had been X-irradiated during its development. From the cross of two cultivars belonging to Group III (S_3S_4), namely 'Emperor Francis' with irradiated pollen of 'Napoleon', several self-compatible seedlings were obtained. These included John Innes (JI) 2420 (formerly 3/45) and JI 2434 (formerly 11/45), both of which were reported to have an unmutated S_3 allele (Matthews and Lapins 1967). The genotypes of the two selections were deduced by making backcrosses to the parents and various other test crosses and noting the fruit set and, in some cases, segregation for self-compatibility in the resulting progenies (Matthews 1970). JI 2420 was reported to have the genotype S_3S_4' and JI 2434 the genotype $S_3S_4^0$, $S_3S_3^0$, or S_3S_3', where $'$ and ^{0} represent the mutation confirming self-incompatibility and indicate the loss of pollen activity and of pollen and style activity respectively. Both selections were used for further breeding at the John Innes Institute and at Summerland Research Station, British Columbia (Matthews and Lapins 1967). As both were thought to have an unmutated S_3 allele, they were crossed to cultivars of groups II, III, IV, V and VI, which were reported to have S_3, with the intention of yielding completely self-compatible progenies. The successful self-compatible cultivar 'Stella' came from a cross of 'Lambert', S_3S_4, ×JI 2420 (Lapins 1970), and the self-compatible cultivars 'Lapins' and 'Sunburst' are sister seedlings derived from 'Stella' (Lane and Schmid 1984).

JI 2420 and JI 2434 have also been used in the breeding programme at Ahrensburg, Germany. In the case of JI 2420, the reported genotype of S_3S_4' did not agree with some of the results obtained (Schmidt 1999). Unexpectedly, it set fruit with pollen of 'Büttners' and 'Ulster', both S_3S_4. Moreover, the progeny from the cross 'Sam', S_2S_4, ×JI 2420 did not segregate 1:1 for self-compatibility versus self-incompatibility but was completely self-compatible.

Communicated by G. Wenzel

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Recently, we have used the analysis of stylar ribonucleases to determine incompatibility genotypes (Bošković and Tobutt 1996; Bošković et al. 1997, 1999). We showed that unique ribonuclease bands correspond to the six original *S* alleles, S_1 to S_6, and to five new ones, and we re-assigned genotypes S_4S_5 and S_3S_5 respectively to groups V and VII, which had been critical in the original genotyping of JI 2420 and JI 2434. In addition, we showed that the band corresponding to the S_4' allele of 'Stella' appears identical to the S_4 band of, e.g., 'Napoleon'.

Thus, the discrepancies in the results of crosses with JI 2420 at Ahrensburg, and the reassignment of *S* alleles to groups V and VII at East Malling, cast doubt on the accepted genotypes of JI 2420 and JI 2434, selections of crucial significance in breeding work and genetic studies concerning the important trait of self-compatibility in cherry. To resolve the doubts, we analysed the ribonucleases of the two JI selections and of some of the progenies available; we also made some test crosses. Initial work with JI 2434 revealed inconsistencies between the Ahrensburg and East Malling results; therefore we treated the Ahrensburg and East Malling accessions of JI 2434 as two different clones and compared them using AFLP analysis.

Materials and methods

Plant material

One clone of JI 2420 was available. The collection at East Malling had received material from Ahrensburg, which had come, via Giessen, from the John Innes Institute in the mid 1960s. JI 2434 was available at Ahrensburg and at East Malling, but the accessions had been received independently. The Ahrensburg clone, which we will refer to as JI 2434 AH, had come, via Giessen, from the John Innes Institute as before. The East Malling clone, JI 2434 EM, had come direct from the John Innes Institute in the early 1980s. In the ribonuclease analysis, the two parents, 'Emperor Francis' and 'Napoleon', were included for comparison.

Two progenies each of JI 2420 and JI 2434 AH which had been raised from controlled crosses at Ahrensburg (Schmidt 1999) were analysed for stylar ribonucleases. They were: 'Sam', S_2S_4 (Fischer 1995), ×JI 2420, 18 seedlings; 'Alma', S_1S_5 (Schmidt 1999), ×JI 2420, 15 seedlings; 'Ulster', S_3S_4 (Bošković and Tobutt 1996), ×JI 2434 AH, 18 seedlings; and 'Regina', S_1S_3 (Schmidt 1999), ×JI 2434 AH, 12 seedlings. In addition, one progeny which had been raised at East Malling, 'Van', S_1S_3 (Matthews and Dow 1969), ×JI 2434 EM, 35 seedlings, was analysed. The progenies are listed in Table 1.

Ribonuclease analysis

The collection of styles, protein extraction, isoelectric focusing and staining for ribonuclease activity followed the procedures described by Bošković et al. (1997).

AFLP analysis

The two accessions of JI 2434, AH and EM, were compared by AFLP analysis and the reported parents of JI 2434, 'Emperor Francis' and 'Napoleon', and the sibling, JI 2420, were included. DNA was extracted from buds using a method based on that described by Dellaporta et al. (1983) and the concentration was adjusted to 100 ng/µl. The restriction, ligation, pre-amplification and AFLP analysis were performed according to the System I protocol of 'Gibco/Life Technologies'. For selective amplification, two sets of primers were used, namely E-ACA/M-CTA and E-AGG/M-CTT, the two *Eco*RI primers being labelled with ^{33}P by 5' end-labelling. After selective amplification, an equal volume of formamide loading dye was added to the samples, which were then denatured for 3 min at 90°C. A 4-µl aliquot of the mixture was loaded on a 6% acrylamide gel which had been pre-run for 20 min at 55 W, and then run for $2^1/_2$ h at 55 W. Following electrophoresis, the gel was transferred to Whatman 3MM paper, covered with cellophane and dried under vacuum on a gel dryer for 40 min. A Kodak X-OMAT LS film was exposed to the gel for 72 h at room temperature.

Test crossing

Test crosses to check the predicted genotypes were made, on carefully emasculated trees in an insect-proof greenhouse at East Malling, of JI 2420 and its derivatives, 'Stella', 'Sunburst' and 'Lapins', and of JI 2434 EM, along with control crosses. In addition, JI 2434 EM was selfed and crossed with 'Napoleon'. The crosses are listed in Table 2. Generally, about 150–600 flowers were pollinated per cross.

Results

Stylar ribonuclease phenotypes of JI 2420 and JI 2434

Stylar ribonuclease zymograms of the JI selections and of the two parents, 'Emperor Francis' and 'Napoleon', both of which are known to have the genotype S_3S_4, are shown in Fig. 1. 'Napoleon' has the bands attributed to S_3 and to S_4 as described previously (Bošković and

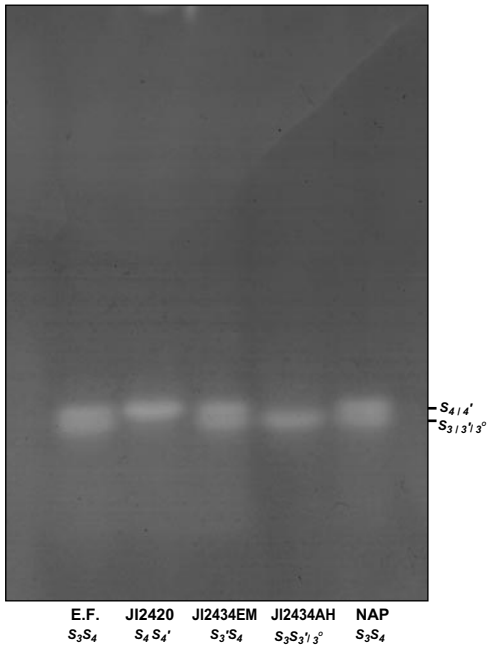

Fig. 1 Stylar ribonucleases of 'Emperor Francis' (E.F.), JI 2420, JI 2434 EM, JI 2434 AH and 'Napoleon' (NAP), showing bands for S_3 and/or S_4. The assignment of $'$ and ^{0} to alleles of the self-compatible JI selections is based on results of test crosses

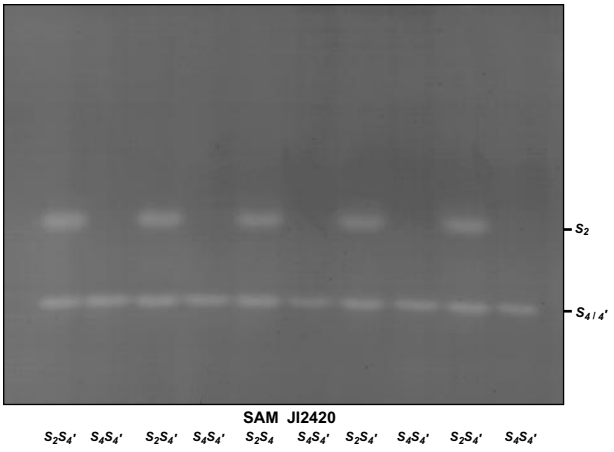

Fig. 2 Family 'Sam' (S_2S_4)×JI 2420 (S_4S_4') – stylar ribonucleases of parents, and eight seedlings segregating for two phenotypes

Tobutt 1996), as does 'Emperor Francis'. JI 2420 has only one band, in the S_4 position. This pattern is not consistent with the S_3S_4' genotype proposed by Matthews (1970). In view of JI 2420's parentage, and disregarding any other evidence at this stage apart from its self-compatibility, the single-banded phenotype could represent S_4S_4' or $S_4S_4^0$, or $S_3^0S_4$ if S_3^0 corresponds to a null band. JI 2434 AH showed a single band in the S_3 position. This pattern is consistent with two of the genotypes proposed by Matthews (1970), namely $S_3S_3^0$ and S_3S_3', but not with S_3S_4'; it would also be consistent with $S_3S_4^0$ if S_4^0 corresponds to a null band. Unlike JI 2434 AH, JI 2434 EM has two bands, in the S_3 and S_4 positions. This pattern is inconsistent with two of the three genotypes proposed by Matthews (1970), namely $S_3S_3^0$ and S_3S_3'. Instead, it could represent $S_3S_4^0$, the third genotype proposed by Matthews, or S_3S_4', $S_3^0S_4$ or $S_3'S_4$, as long as S_3^0 and S_4^0 do not correspond to null bands.

Analysis of stylar ribonucleases in progenies

When seedlings from the cross of 'Sam', S_2S_4, ×JI 2420 were analysed, two phenotypes were seen (Fig. 2, Table 1). Some seedlings showed two bands, in the S_2 and S_4 positions, and some showed a single band in the S_4 position. This is not consistent with JI 2420 having the genotype $S_3^0S_4$, where S_3^0 corresponds to a null band. In that case, both phenotypes should have had a single band, in the S_2 position in half the seedlings and in the S_4 position in the other half. The observed patterns are consistent with JI 2420 having a mutated S_4 allele and being either S_4S_4' or $S_4S_4^0$.

Analysis of the stylar ribonucleases of 'Alma', S_1S_5, ×JI 2420 showed that the progeny segregated for two phenotypes (Fig. 3, Table 1). All seedlings had two bands, in either the S_1 and S_4 positions or the S_4 and S_5 positions. Again, this is consistent with JI 2420 having the genotype S_4S_4' or $S_4S_4^0$, but not $S_3^0S_4$, as, in that case, two additional phenotypes would be expected with a single band in the S_1 position or in the S_5 position.

The stylar ribonuclease zymograms of the seedlings of 'Ulster', S_3S_4, ×JI 2434 AH were of two phenotypes (Fig. 4, Table 1). Some seedlings showed two bands, in the S_3 and S_4 positions, and others showed a single band in the S_3 position. This pattern is inconsistent with the genotype of JI 2434 AH being $S_3S_4^0$, where S_4^0 corresponds to a null band as, in that case, about half the seedlings would have shown a single band in the S_3 posi-

Table 1 Progenies analysed for stylar ribonucleases to deduce *S* genotypes of JI 2420 and JI 2434

Parents				Predicted seedling genotypes	Observed seedling RNase phenotypes	Accordance of observed RNase phenotypes with predicted genotypes	χ^2
♀ (known genotype)	×	♂ (possible genotypes according to ribonuclease phenotype)					
'Sam' (S_2S_4)	×	JI 2420	($S_3^0S_4$)	$1S_2S_3^0$:$1S_3^0S_4$	$11S_2S_4$:$7S_4S_4$	×	–
			(S_4S_4')	$1S_2S_4'$:$1S_4S_4'$		✔	0.89
			($S_4S_4^0$)	$1S_2S_4^0$:$1S_4S_4^0$		✔	0.89
'Alma' (S_1S_5)	×	JI 2420	($S_3^0S_4$)	$1S_1S_3^0$:$1S_1S_4$:$1S_3^0S_5$:$1S_4S_5$	$7S_1S_4$:$8S_4S_5$	×	–
			(S_4S_4')	$1S_1S_4$:$1S_1S_4'$:$1S_4S_5$:$1S_4'S_5$		✔	0.07
			($S_4S_4^0$)	$1S_1S_4$:$1S_1S_4^0$:$1S_4S_5$:$1S_4^0S_5$		✔	0.07
'Ulster' (S_3S_4)	×	JI 2434 AH	(S_3S_3')	$1S_3S_3'$:$1S_3'S_4$	$14S_3S_3$:$4S_3S_4$	✔	5.56*
			($S_3S_3^0$)	$1S_3S_3^0$:$1S_3^0S_4$		✔	5.56*
			($S_3S_4^0$)	$1S_3S_4^0$:$1S_4S_4^0$		×	–
'Regina' (S_1S_3)	×	JI 2434 AH	(S_3S_3')	$1S_1S_3'$:$1S_3S_3'$	$3S_1S_3$:$9S_3S_3$	✔	3.00
			($S_3S_3^0$)	$1S_1S_3^0$:$1S_3S_3^0$		✔	3.00
			($S_3S_4^0$)	$1S_1S_4^0$:$1S_3S_4^0$		×	–
'Van' (S_1S_3)	×	JI 2434 EM	($S_3'S_4$)	$1S_1S_3'$:$1S_1S_4$:$1S_3S_3'$:$1S_3S_4$	$13S_1S_3$:$8S_1S_4$:$9S_3S_3$:$5S_3S_4$	✔	3.74
			($S_3^0S_4$)	$1S_1S_3^0$:$1S_1S_4$:$1S_3S_3^0$:$1S_3S_4$		✔	3.74
			(S_3S_4')	$1S_1S_4'$:$1S_3S_4'$		×	–
			($S_3S_4^0$)	$1S_1S_4^0$:$1S_3S_4^0$		×	–

* Observed ratio significantly different from expected at P=0.05

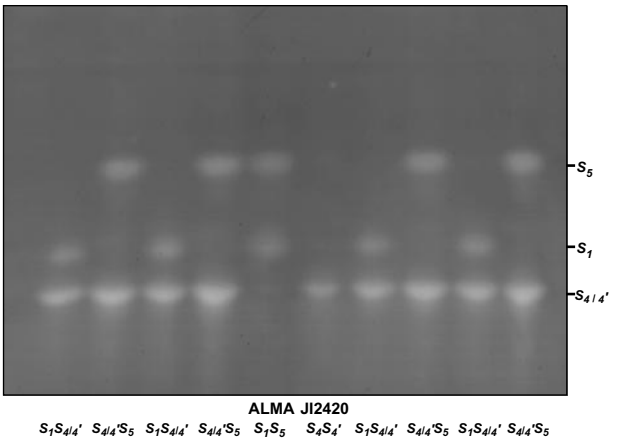

Fig. 3 Family 'Alma' (S_1S_5)×JI 2420 (S_4S_4') – stylar ribonucleases of parents, and eight seedlings segregating for two phenotypes

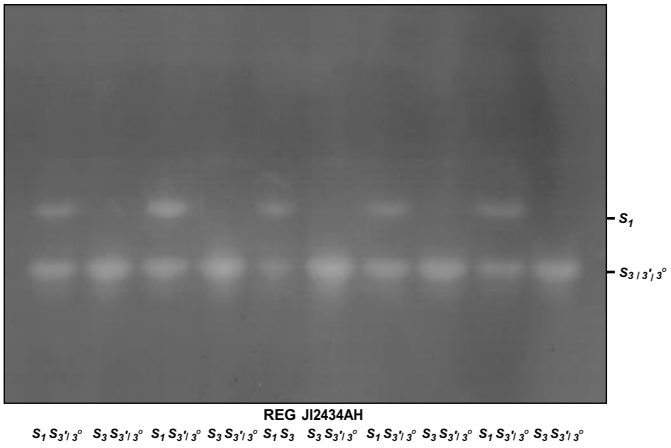

Fig. 5 Family 'Regina' (REG) (S_1S_3)×JI 2434 AH ($S_3S_3'/_3{}^0$) – stylar ribonucleases of parents, and eight seedlings segregating for two phenotypes

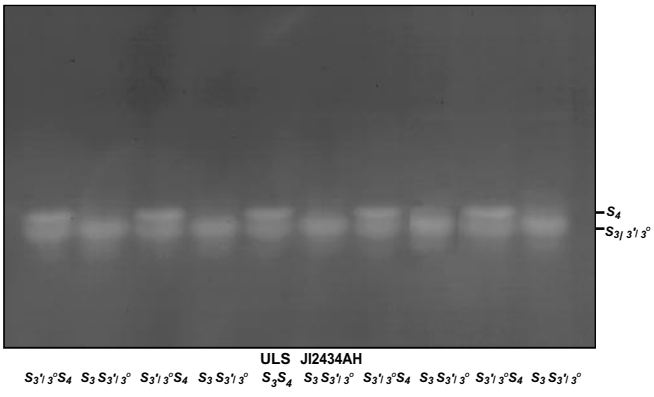

Fig. 4 Family 'Ulster' (ULS) (S_3S_4)×JI 2434 AH ($S_3S_3'/_3{}^0$) – stylar ribonucleases of parents, and eight seedlings segregating for two phenotypes

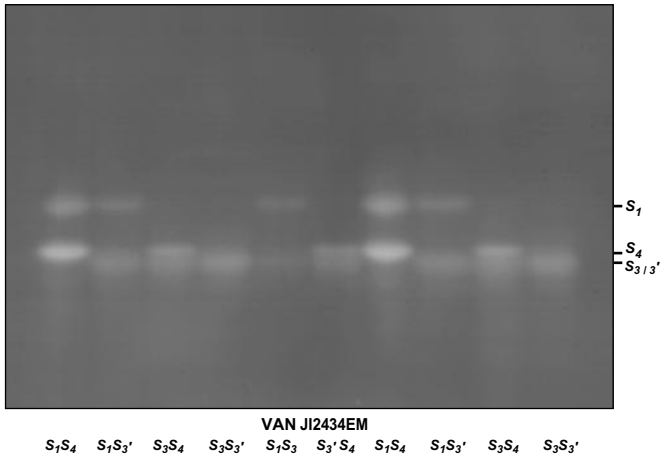

Fig. 6 Family 'Van' (S_1S_3), JI 2434 EM ($S_3'S_4$) – stylar ribonucleases of parents, and eight seedlings segregating for four phenotypes

tion and the other half a single band in the S_4 position. The observed patterns are reasonably consistent with JI 2434 AH having a mutated S_3 allele and being S_3S_3' or $S_3S_3{}^0$, as long as $S_3{}^0$ is not associated with a lack of ribonuclease activity, though the observed phenotypes showed a significant departure from the expected 1:1 segregation.

In the progeny of 'Regina', S_1S_3, ×JI 2434 AH, two phenotypes were seen (Fig. 5, Table 1), one with two bands corresponding to S_1 and S_3 and one with a single band in the S_3 position. Again, these patterns are consistent with the genotype of JI 2434 AH being S_3S_3' or $S_3S_3{}^0$, but not $S_3S_4{}^0$.

In the progeny of 'Van', S_1S_3, ×JI 2434 EM, four phenotypes were seen (Fig. 6, Table 1), indicating the cross was fully compatible. There were three two-banded phenotypes, with bands corresponding to S_1 and S_4, to S_3 and S_4, and to S_1 and S_3, and a fourth phenotype with a single band in the S_3 position. These last two phenotypes indicate that JI 2434 carries a mutated S_3 allele and has the genotype $S_3{}'S_4$ or $S_3{}^0S_4$; if the mutation had been in the S_4 allele, these two phenotypes would have been absent.

Comparison of JI 2434 AH and EM by AFLP

With both primer combinations, the AFLP analysis revealed many bands common to the JI selections and the reported parents, as well as some variable bands. A section of the autoradiograph resulting from the use of the combination E-ACA/M-CTA is shown in Fig. 7. With each primer combination, one band allowed JI 2434 AH and EM to be discriminated from each other and both could be distinguished from JI 2420. None of the bands seen in the JI clones were absent from 'Emperor Francis' and 'Napoleon'; thus the patterns were consistent with the reported parentage.

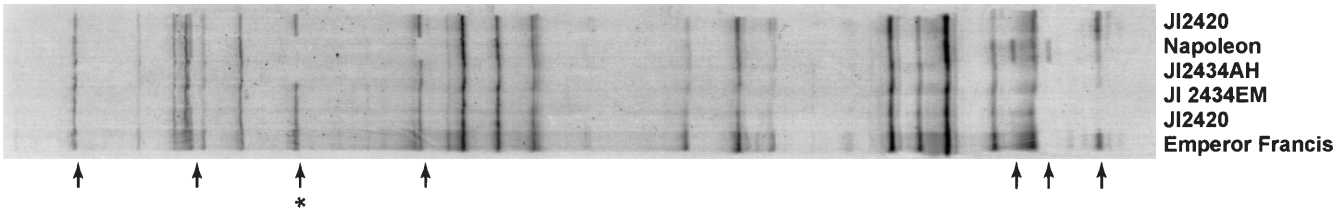

Fig. 7 AFLP fingerprints of 'Emperor Francis', JI 2420, JI 2434 EM, JI 2434 AH, 'Napoleon' and JI2420 again produced with primer combination E-ACA/M-CTA. The *asterisked band* distinguishes JI 2434 EM from AH. The *arrowed bands* are polymorphic

Table 2 Test crosses made at East Malling to clarify (in)compatibility genotypes of JI 2420 and JI 2434 EM

Parents					Number of pollinated flowers	Number of fruit set	Accordance of fruit set and proposed genotype[b]
♀		×	♂				
JI 2420	(S_4S_4')[a]	×	'Napoleon'	(S_3S_4)	146	30	✔
'Stella'	(S_3S_4')[a]	×	'Napoleon'	(S_3S_4)	672	2	✔
'Stella'	(S_3S_4')[a]	×	'Van'	(S_1S_3)	221	39	✔
'Sunburst'	(S_3S_4')[a]	×	'Napoleon'	(S_3S_4)	730	2	✔
'Sunburst'	(S_3S_4')[a]	×	'Van'	(S_1S_3)	360	22	✔
'Lapins'	(S_1S_4')	×	'Merton Late'	(S_1S_4)	403	0	✔
'Lapins'	(S_1S_4')[a]	×	'Van'	(S_1S_3)	420	219	✔
JI 2434 EM	($S_3'S_4$)[a]	×	'Napoleon'	(S_3S_4)	146	0	✔
'Napoleon'	(S_3S_4)	×	JI 2434 EM	($S_3'S_4$)[a]	350	35	✔
JI 2434 EM	($S_3'S_4$)[a]	×	JI 2434 EM	($S_3'S_4$)[a]	71	34	✔

[a] Proposed genotypes
[b] Observed fruit set is in accordance with proposed genotype

Outcome of test crosses

The cross of JI 2420×'Napoleon', S_3S_4, proved to be compatible, setting 30 fruit from 146 cross-pollinated flowers (Table 2). Previously, JI 2420 set fruit when pollinated with 'Büttners' and with 'Ulster', both S_3S_4 (Schmidt 1999). These results do not help to distinguish between the two possible genotypes for JI 2420 consistent with the stylar ribonuclease zymograms of JI 2420 and the progenies, namely S_4S_4' and $S_4S_4^0$, but they support the rejection of the genotype proposed by Matthews (1970), S_3S_4'.

The test cross of 'Stella', a self-compatible seedling of JI 2420 [known to have stylar ribonuclease bands in the S_3 and S_4 positions (Bošković and Tobutt 1996, Bošković et al. 1999) and confirmed by Schmidt (1999) to be S_3S_4'], by 'Napoleon', S_3S_4, was more informative. It failed, 672 flowers giving only two fruit. This indicates that the mutated S_4 allele that 'Stella' inherits from JI 2420 retains the ability to reject S_4 pollen and is thus S_4' rather than S_4^0. In addition, similar test crosses of two self-compatible seedlings of 'Stella', 'Sunburst' and 'Lapins', failed. In 'Sunburst' [having stylar ribonuclease bands in the S_3 and S_4 positions (Bošković and Tobutt 1996) and confirmed by Schmidt (1999) to be S_3S_4']×'Napoleon', S_3S_4, 730 flowers gave two fruit. In 'Lapins' [having bands in the S_1 and S_4 positions (Bošković and Tobutt 1996) and confirmed by Schmidt (1999) to be S_1S_4']×'Merton Late', S_1S_4, 403 flowers gave 0 fruit. In the control crosses, 'Stella', 'Sunburst' and 'Lapins' set well when pollinated with 'Van'. These results support the conclusion that the mutated S_4 allele derived from JI 2420 is S_4' rather than S_4^0. Thus the genotype of JI 2420 appears to be S_4S_4'. Data for these crosses and for the control crosses are given in Table 2.

Previously, Schmidt (1999) reported that JI 2434 AH is compatible in both directions with cultivars of the genotype S_3S_4. That finding is consistent with both of the two possible genotypes for JI 2434 that were consistent with the stylar ribonuclease zymograms of JI 2434 AH and its progenies, i.e. S_3S_3' and $S_3S_3^0$. Had the genotype been $S_3'S_4$, the cross of JI 2434 AH×a cultivar with genotype S_3S_4 should have failed.

The test cross of JI 2434 EM×'Napoleon', S_3S_4, failed to set fruit, i.e. was incompatible, setting 0 fruit from 146 flowers; whereas 'Napoleon'×JI 2434 EM succeeded, setting 35 fruit from 350 flowers (Table 2). Of the two possible genotypes for JI 2434 EM that were consistent with the stylar ribonuclease zymograms of JI 2434 EM and its progenies, these test cross results are consistent only with $S_3'S_4$. Had the genotype been $S_3^0S_4$, both test crosses should have been compatible.

JI 2434 EM set 34 fruits from selfing 71 flowers, confirming that this selection is self-compatible.

Discussion

In the light of our observations, we propose a genotype for JI 2420 different from that proposed by Matthews

(1970). The genotype of JI 2420 appears to be S_4S_4', rather than S_3S_4'. In the case of JI 2434, we have detected two clones. JI 2434 EM appears to be $S_3'S_4$ rather than $S_3S_4^0$, $S_3S_3^0$ or S_3S_3', and JI 2434 AH appears to be $S_3S_3^0$ or S_3S_3'.

The revised genotype for JI 2420, S_4S_4', is inconsistent with data presented by Matthews (1970). He reported that the cross JI 2420×'Napoleon', S_3S_4, was incompatible, and Lewis and Crowe (1954) gave the data (for 3/45=JI 2420) as 0 fruit from 60 flowers pollinated in an insect-proof greenhouse. However, this cross succeeded at East Malling, as did the similar crosses of JI 2420×'Büttners' and ×'Ulster', both S_3S_4, at Ahrensburg (Schmidt 1999). An apparent discrepancy of the new genotype with the observation of Matthews (1970) that 'Late Black Bigarreau', S_3S_5, ×JI 2420 gives a wholly self-compatible progeny, disappears when the genotype of 'Late Black Bigarreau' is revised to S_4S_5 in accordance with Bošković et al. (1997). Indeed, when the genotype of 'Late Black Bigarreau' is revised in this way, the conclusion of Matthews (1970) that S_3S_4' is the genotype for JI 2420 is no longer consistent with the results of that cross. Schmidt (1999) found all seedlings from the cross 'Sam', S_2S_4, ×JI 2420 to be self-compatible; this accords with the genotype S_4S_4' that we propose, but not with the genotype S_3S_4' proposed by Matthews (1970).

Also, the revised genotype for JI 2434 EM, $S_3'S_4$, is not consistent with the data of Matthews (1970). He reported that the cross JI 2434×'Napoleon', S_3S_4, was compatible; whereas we found that this cross failed. Initially, it might appear that our assignment of $S_3'S_4$ is inconsistent with the report by Matthews (1970) that the crosses of an unspecified cultivar, S_4S_5, ×JI 2434 and of 'Hedelfinger', considered to be S_4S_5, ×JI 2434 segregated 1:1 for self-compatibility versus self-incompatibility. However, Bošković et al. (1997) revised the genotype of 'Hedelfinger' to S_3S_5, and it is likely that the unspecified cultivar was also S_3S_5. With these revisions, our genotype for JI 2434 EM, $S_3'S_4$, accords with the 1:1 segregations, whereas the three options of Matthews (1970) do not. Matthews (1970) also reported that all seedlings of 'Windsor', S_1S_3, ×JI 2434 were self-compatible rather than segregating 1:1 and this appears to be inconsistent with the genotype we propose for JI 2434 EM; however, only ten seedlings were involved.

Our proposed genotype for JI 2434 AH, $S_3S_3^0$ or S_3S_3', fits two of the three options proposed by Matthews (1970). He reported that the cross JI 2434×'Napoleon', S_3S_4, is compatible and Schmidt (1999) reported that 'Ulster' and 'Büttners', both S_3S_4, ×JI 2434 AH, set fruit. Matthews (1970) also reported that all the seedlings of 'Windsor', S_1S_3, ×JI 2434 were self-compatible. Our reassignment of 'Hedelfinger', and perhaps the unspecified cultivar, from S_4S_5 to S_3S_5 is not consistent with the 1:1 segregation for self-compatibility reported by Matthews (1970) in progenies resulting from pollinating these cultivars with JI 2434.

Our proposal of a different genotype for JI 2420 does not necessarily indicate that we have analysed a clone different from that analysed by Matthews (1970). As described above, the genotype he ascribed to this selection was not entirely consistent with what we now know to be the genotypes of one of the cultivars used in test crosses.

For JI 2434, we have detected two different clones on the basis of their compatibility relationships and confirmed this by AFLP analysis. Both are self-compatible, as shown by Schmidt (1999) for JI 2434 AH and by the fruit set after our test selfing of JI 2434 EM. At least one of them must be different from that analysed by Matthews (1970), unless he analysed a 'mixed' clone. As JI 2434 EM and JI 2434 AH showed AFLP patterns consistent with the parentage 'Emperor Francis'×'Napoleon', an error may have occurred prior to the despatch of material from John Innes. Lewis and Crowe (1954) described the compatibility relationships of another seedling of the same parentage, 12/45, and indicated they may have analysed additional similar seedlings. Possibly a sister clone was mislabelled JI 2434. Matthews (personal communication) noted inconsistencies between early descriptions of JI 2434 and his later descriptions. It is unclear which, if either, of the two clones we analysed is the one analysed by Matthews (1970).

Although JI 2420 and JI 2434 were sent to Summerland, British Columbia (Matthews and Lapins 1967), they are no longer held there (Kappel, personal communication).

It was thought that JI 2420 and JI 2434 both had an unmutated S_3 allele and the strategy employed for generating wholly self-compatible progenies from these two selections had been to use them, as pollen parents, on cultivars having an S_3 allele (Matthews and Lapins 1967). In light of our findings, it now appears that, although this strategy should be effective for JI 2434 AH, the correct strategy for JI 2420 and JI 2434 EM would be to cross them on to cultivars having an S_4 allele.

Williams and Brown (1956) speculated that the S_3 allele has a selective advantage in cherry breeding, because either S_3 itself, or genes linked with it, have a beneficial effect on economic characters. Greater use of the two forms of JI 2434 for breeding for self-compatibility with the mutated S_3 allele may be worthwhile. In addition, although JI 2420 and JI 2434 have been described as small-fruited (Matthews and Lapins 1967), the fruit size and quality of JI 2434 EM is superior to that of JI 2420.

S_3' , which we conclude is present in JI 2434 EM, has not previously been identified unambiguously in cherry. Like S_4', its ribonuclease activity is not affected. Though we can deduce there is a mutant S_3 allele in JI 2434 AH, we cannot distinguish between S_3' and S_3^0 from the evidence currently available. To resolve this in future, a seedling with S_3 and S_4 bands from the cross of 'Ulster' 'S_3S_4'×JI 2434 AH 'S_3S_3'/S_3^0' could be crossed reciprocally with 'Napoleon' 'S_3S_4''.

The cause of the loss of pollen activity in S_3' and S_4' is unknown. Originally, such alleles were attributed to a mutation in the pollen part of the *S* gene (Lewis and Crowe 1954). There are two current models to explain

the inhibition of pollen-tube growth in the incompatibility reaction, the receptor model and the inhibitor model (Thompson and Kirch 1992). With the former model, the S_3' or S_4' mutations presumably result in the pollen avoiding uptake of the S_3 or S_4 ribonuclease; with the latter, they presumably result in the pollen inhibiting the S_3 or S_4 ribonuclease. Alternatively, Brewbaker and Natarajan (1960) speculated that some pollen-part mutants in *Prunus* could be attributed to a centric fragment or translocation leading to diallelic pollen able to grow down self styles. Lewis (1961) cast doubt on the centric fragment explanation for *Prunus*, but did not adduce any data. For S_4', the finding by Tehrani [personal communication in Schmidt (1999)] that at least 94% of seedlings from the cross 'Van' 'S_1S_3'×'Stella' 'S_3S_4' were self-compatible does not support the centric fragment explanation as that would require 50% of the seedlings to be self-compatible. For S_3', if the centric fragment contains the pollen component of S_4, we would expect a preponderance of seedlings from our cross of 'Van' 'S_1S_3' ×JI 2434 EM '$S_3'S_4$' to show the S_1S_4 or S_3S_4 phenotypes; however, this was not the case. The duplication of the pollen component of S_3 and the translocation of one copy to close linkage with the S_4 gene could account for S_4', and the reciprocal events could account for S_3'.

JI 2420 and JI 2434 EM and AH could be useful in the search for the pollen component. Polymorphisms in the regions flanking the S_3 and S_4 ribonucleases of the wild-type and mutant forms could indicate a mutation, deletion or translocation of the pollen component.

Acknowledgements This work was funded by the UK Ministry of Agriculture, Fisheries and Food and the Biological and Biotechnological Sciences Research Council, and by the European Commission (Contract AIR 3-CT93–1585). Radovan Bošković is grateful for an HRI scholarship. We thank Celia James for guidance with AFLP analysis.

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