Theor Appl Genet (2001) 103:475–485

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Genotyping cherry cultivars assigned to incompatibility groups, by analysing stylar ribonucleases

Received: 25 March 2000 / Accepted: 12 January 2001

Abstract Seventy cultivars of *Prunus avium* that had been assigned to incompatibility groups or to the O group of universal donors, primarily by the John Innes Institute, were analysed for stylar ribonucleases to check or determine their incompatibility, *S*, alleles. Three 'new' bands were detected and ascribed to new alleles S_{12} to S_{14}. For most of the groups that had previously been genotyped most of the cultivars had the genotypes expected, although various exceptions were found. In group VIII none of the cultivars tested had the correct genotype of S_2S_5 but this genotype occurred in 'Malling Black Eagle'. For the three groups not previously genotyped we assigned genotypes: group X, S_6S_9; group XI, S_2S_7; and group XII S_6S_{13}. We confirmed group XIV, which had been rejected by Canadian work. Group O comprised a range of genotypes. In collating these results and those of our previous ribonuclease studies we propose five new groups: group XV, S_5S_6; group XVI, S_3S_9; group XVII, S_4S_6; group XVIII, S_1S_9; and group XIX, S_3S_{13}. Several predictions were confirmed by test crossing; thus the three members of the proposed group XV, 'Colney', 'Erianne' and 'Zweitfruhe', were cross-incompatible, as were the two members of proposed group XVIII, 'Norbury's Early Black' and 'Smoky Dun.'

Keywords Cherry · Compatibility · Incompatibility · Ribonuclease · *Prunus avium*

Introduction

Most cultivars of sweet cherry (*Prunus avium*) are self-incompatible and various pairs of cultivars are cross-incompatible. Incompatibility in cherry was attributed to a multi-allelic gametophytic locus, *S*, by Crane and Lawrence (1929).

Communicated by H.C. Becker

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Work at the John Innes Institute, UK, and overseas, led to the publication of a table assigning some 190 cultivars and a few selections to 13 incompatibility groups (Matthews and Dow 1969). About 145 cultivars were assigned to one or other of the groups I to IX and XIII which were ascribed various pairs of the alleles S_1 to S_6. About 18 were placed in groups X, XI and XII, of unknown genotype. Thirty were placed in group O, the 'universal donors', which are compatible with members of groups I to XII, and usually with each other; it is likely that most members of group O have a unique genotype.

Way (1968) at Geneva, New York, USA, added "group" XIV, comprising one cultivar of known genotype, but Tehrani and Lay (1991) at Vineland, Ontario, Canada, subsequently rejected this group.

Recently, Bošković and Tobutt (1996) showed that for groups I to IX and XIII the bands revealed by separating stylar proteins using isoelectric focusing and staining for ribonuclease activity generally, though not always, correlated with the six reported *S* alleles. More recently, Bošković et al. (1997) reinterpreted the patterns revealed for groups V and VII, interchanging the genotypes assigned to these two groups; this reinterpretation cast doubt on the genotype that had been reported for group VIII. They also proposed two more *S* alleles in sweet cherries, S_8 and S_9, and three *S* alleles that occurred only in 'wild' cherries used as rootstocks or for timber production, S_7, S_{10} and S_{11}.

The ribonuclease analyses we now report extend these earlier studies and comprise five unequal parts. We have analysed: more cultivars of groups I to IV, VI, VII, IX and XIII to see if further cultivars of these groups had the same ribonuclease zymograms as revealed for the examples analysed originally; another two cultivars of group VIII to try and resolve the doubts about the genotype of that group; the representative of the new group, XIV, the status of which has been queried; cultivars of groups X, XI and XII to ascribe genotypes to these groups; and members of group O in the expectation of finding new combinations and new alleles.

Table 1 Incompatibility genotypes of cherry cultivars determined by stylar ribonuclease analysis

Group described by Matthews and Dow (1969) and reported genotype		Source[d]	Genotype determined	(New or previous group, if appropriate)
Group I S_1S_2				
Confirmed	Baumanns May A	B	S_1S_2	
	Bedford Prolific A	B	S_1S_2	
	Black Downton	B	S_1S_2	
	Carnation C	B	S_1S_2	
	Early Rivers[a]	EM	S_1S_2	
	Knight's Early Black A	B	S_1S_2	
	Ronald's Heart	B	S_1S_2	
	Roundel[a]	EM	S_1S_2	
Reassigned	Black Eagle A	B	S_3S_5	(To group VII)
	Flamentiner	B	S_6S_{12}	(To group O)
Added	Emperor Francis B	EM	S_1S_2	(Ex group II)
	F1/3[b]	EM	S_1S_2	
	Summit[b]	EM	S_1S_2	
	Ursula Rivers	B	S_1S_2	(Ex group IX)
Group II S_1S_3				
Confirmed	Belle Agathe	B	S_1S_3	
	Bigarreau de Schrecken	B	S_1S_3	
	Black Elton	B	S_1S_3	
	Caroon B	B	S_1S_3	
	Frogmore Early	B	S_1S_3	
	Merton Crane[a]	EM	S_1S_3	
	Van[a]	EM	S_1S_3	
	Venus	EM	S_1S_3	
	Victoria Black A	B	S_1S_3	
	Waterloo	B	S_1S_3	
	Windsor A	B	S_1S_3	
Reassigned	Emperor Francis B	EM	S_1S_2	(To group I)
Group III S_3S_4				
Confirmed	Bing[a]	EM	S_3S_4	
	Emperor Francis	EM	S_3S_4	
	Lambert	B	S_3S_4	
	Napoleon[a]	EM	S_3S_4	
	Querfurter Königskirsche	Ahr	S_3S_4	
	Vernon	B	S_3S_4	
	Star	Wad	S_3S_4	
Reassigned	Merton Marvel	EM	S_3S_6	(To group VI)
	Reverchon	B	S_3S_{13}	(To group XIX)
	Schneiders Späte Knorpelkirsche	EM	S_3S_{12}	(To group O)
Added	Bigarreau Esperen	Has	S_3S_4	(Ex group X)
	Büttners Späte Rote Knorpelkirsche	Hoh	S_3S_4	(Ex group VIII)
	Heinrichs Riesen	Aln	S_3S_4	(Ex group XIII)
	Ulster[a]	EM	S_3S_4	(Ex group XIII)
	Yellow Spanish	B	S_3S_4	(Ex group IV)
Group IV S_2S_3				
Confirmed	Allman Gulrod	B	S_2S_3	
	Kentish Bigarreau	B	S_2S_3	
	Late Amber	B	S_2S_3	
	Ludwig's Bigarreau	B	S_2S_3	
	Merton Premier	EM	S_2S_3	
	Velvet	B	S_2S_3	
	Victor[a]	EM	S_2S_3	
Reassigned	Knauffs Riesen (Knauffskirsche)	B	S_2S_6	(To group O)
	Yellow Spanish	B	S_3S_4	(To group III)
Added	Knight's Bigarreau	B	S_2S_3	(Ex group XI)
Group V S_3S_5, changed to S_4S_5 (Bošković et al. 1997)				
Confirmed	Turkey Heart[a]	B	S_4S_5	
	Late Black Bigarreau[a]	EM	S_4S_5	

Table 1 (continued)

Group described by Matthews and Dow (1969) and reported genotype		Source[d]	Genotype determined	(New or previous group, if appropriate)
Group VI S_3S_6				
Confirmed	Elton Heart	B	S_3S_6	
	Governor Wood[a]	EM	S_3S_6	
	Merton Heart[a]	EM	S_3S_6	
	Turkish Black	B	S_3S_6	
Added	Merton Marvel	EM	S_3S_6	(Ex group III)
Group VII S_4S_5, changed to S_3S_5 (Bošković et al. 1997)				
Confirmed	Bradbourne Black[a]	EM	S_3S_5	
	Fruhe Luxburger	Wad	S_3S_5	
	Hedelfingen[a]	EM	S_3S_5	
	Hooker's Black[a]	B	S_3S_5	
Reassigned	Bigarreau Burlat	B	S_3S_9	(To group XVI)
	Bigarreau Moreau	EM	S_3S_9	(To group XVI)
	Erianne	B	S_5S_6	(To group XV)
Added	Bigarreau Gaucher[b, c]	B	S_3S_5	(Ex group O)
	Black Eagle A	B	S_3S_5	(Ex group I)
Group VIII S_2S_5				
Reassigned	Büttners Späte Rote Knorpelkirsche	Hoh	S_3S_4	(To group III)
	Peggy Rivers[a]	EM	S_2S_4	(To group XIII)
	Schmidt	Lof	S_2S_4	(To group XIII)
Added	Malling Black Eagle	B	S_2S_5	(Ex group O)
Group IX S_1S_4				
Confirmed	Merton Late[a]	EM	S_1S_4	
	Merton Reward	B	S_1S_4	
	Rainier	EM	S_1S_4	
Reassigned	Ursula Rivers	B	S_1S_2	(To group I)
Group X we propose S_6S_9, S alleles previously unknown				
Confirmed	Bigarreau de Jaboulay	EM	S_6S_9	
	Bigarreau de Mezel	Bor	S_6S_9	
	Ramon Oliva	B	S_6S_9	
Reassigned	Bigarreau Esperen	Has	S_3S_4	(To group III)
	Rodmersham Seedling	B	S_3S_x	(To group O)
Added	Black Tartarian E	B	S_6S_9	(Ex group O)
Group XI we propose S_2S_7, S alleles previously unknown				
Confirmed	Cryall's Seedling	B	S_2S_7	
	Guigne d'Annonay	B	S_2S_7	
Reassigned	Knight's Bigarreau	B	S_2S_3	(To group IV)
Group XII we propose S_6S_{13}, S alleles previously unknown				
Confirmed	Noble	B	S_6S_{13}	
Group XIII S_2S_4				
Confirmed	Vic[a]	EM	S_2S_4	
Reassigned	Heinrichs Riesen	Aln	S_3S_4	(To group III)
	Ulster[a]	EM	S_3S_4	(To group III)
Added	Peggy Rivers[a]	EM	S_2S_4	(Ex group VIII)
	Ord	B	S_2S_4	(Ex group O)
	Schmidt	Lof	S_2S_4	(Ex group VIII)
Group XIV S_1S_5 (Matthews, personal communication in Way (1968))				
Confirmed	Valera	Dre	S_1S_5	
Added	Noir de Guben	B	S_1S_5	(Ex group O)
Group XV (new group, S_5S_6)				
	Erianne	B	S_5S_6	(Ex group VII)
	Zweitfruhe	B	S_5S_6	(Ex group O)
	Colney[b]	EM	S_5S_6	

Table 1 (continued)

Group described by Matthews and Dow (1969) and reported genotype		Source[d]	Genotype determined	(New or previous group, if appropriate)
Group XVI (new group, S_3S_9)				
	Bigarreau Burlat	B	S_3S_9	(Ex group VII)
	Bigarreau Moreau	EM	S_3S_9	(Ex group VII)
Group XVII (new group, S_4S_6)				
	Merton Glory	EM	S_4S_6	(Ex group O)
	Nutberry Black	B	S_4S_6	(Ex group O)
Group XVIII (new group, S_1S_9)				
	Norbury's Early Black	B	S_1S_9	(Ex group O)
	Smoky Dun	B	S_1S_9	(Ex group O)
Group XIX (new group, S_3S_{13})				
	Wellington A	B	S_3S_{13}	(Ex group O)
	Reverchon	B	S_3S_{13}	(Ex group III or O)
Group O *Universal Donors* (Compatible with cultivars in Groups I–XIX)				
	Bowyer Heart	B	S_1S_6	
	Dikkeloen	B	S_5S_{14}	
	Goodnestone Black	B	S_5S_{13}	
	Strawberry Heart	B	S_3S_x	
Reassigned	Bigarreau Gaucher[b, c]	EM	S_3S_5	(To group VII)
	Black Tartarian E	B	S_6S_9	(To group X)
	Malling Black Eagle	B	S_2S_5	(To group VIII)
	Merton Glory	B	S_4S_6	(To group XVII)
	Noir de Guben	B	S_1S_5	(To group XIV)
	Norbury's Early Black	B	S_1S_9	(To group XVIII)
	Nutberry Black	B	S_4S_6	(To group XVII
	Ord	B	S_2S_4	(To group XIII)
	Reverchon	B	S_3S_{13}	(To group XIX)
	Smoky Dun	B	S_1S_9	(To group XVIII)
	Wellington A	B	S_3S_{13}	(To group XIX)
	Zweitfrühe	B	S_5S_6	(To group XV)
Added	Charger[b]	EM	S_1S_7	
	Flamentiner	B	S_6S_{12}	(Ex group I)
	Inge[b]	EM	S_4S_9	
	Knauffs Riesen	B	S_2S_6	(Ex group IV)
	Orleans 171[b]	EM	$S_{10}S_{11}$	
	Rodmersham Seedling	B	S_3S_x	(Ex group X)
	Schneiders Späte Knorpelkirsche	EM	S_3S_{12}	(Ex group III)

[a] Cultivar genotyped by Matthews and Dow (1969) and analysed for stylar ribonucleases by Bošković and Tobutt (1996) or Bošković et al. (1997)
[b] Cultivar analysed for stylar ribonucleases by Bošković and Tobutt (1996) or Bošković et al. (1997) but not genotyped by Matthews and Dow (1969)
[c] 'Bigarreau Gaucher' regenotyped in accord with Sonneveld et al. (2001)
[d] Ahr=BAZ Ahrensburg, Germany; Aln=SUAS Alnarp, Sweden; Bor=INRA Bordeaux, France; B=NFC Brogdale, UK; Dre=IPK Dresden, Germany; EM=HRI East Malling, UK; Has=NBS Hasselt, Belgium; Hoh=IOGW Hohenheim, Germany; Lof=URC Lofthus, Norway; Wad=EFOWG Wadenswil, Switzerland

Materials and methods

Plant materials

The 70 accessions analysed are listed in Table 1. They include nearly all members nominally of groups I to XIV and O that grow in the collections at the National Fruit Collections, Brogdale, and at HRI, East Malling, but that had not been studied previously by Bošković and Tobutt (1996) or Bošković et al. (1997), and nine from overseas. In fact, self-incompatible cultivars analysed in these two earlier papers are included in the table for completeness. Several of the names have suffix letters, indicating that different forms with the same name had been received by the National Fruit Collections (Anonymous 1998), following the usage of Grubb (1949). 'A' denotes the one considered true to type and the one sampled where Matthews and Dow (1969) had not specified a particular clone, or had specified 'A.' Clones with other suffixes were used where it appeared Matthews and Dow (1969) had specified them.

Collection of styles was simpler than described by Bošković and Tobutt (1996). In some cases branches bearing buds were cut, stood in water-soaked florists' foam (Oasis) and 'forced' into flower in the laboratory. In others, flowers were collected direct from the orchard. We found it was not essential for the flowers to be newly opened and unpollinated. Flowers at the balloon stage, and even open flowers several days post-anthesis, gave satisfactory results and we took no precautions to avoid pollination.

Ribonuclease analyses

Extraction of stylar proteins essentially followed Bošković and Tobutt (1996) with the exception that only ten styles were used, with 1 ml of extraction solution. Likewise, electrophoretic separation generally followed Bošković and Tobutt (1996), with some modifications to running times to improve the separation of the increased number of alleles. Isoelectric focusing (IEF) conditions comprised 1 h at 150 V, 2 h at 300 V and 3 h at 450 V. To separate the bands representing alleles S_3 and S_9, non-equilibrium pH gradient electrofocusing (NEPHGE) was used: 1 h at 150 V, 2 h at 300 V and $1^3/_4$ h at 450 V. Staining followed Bošković and Tobutt (1996) as did the estimation, with IEF, of pI values of bands considered to represent new S alleles.

Pollination tests

Several crosses were made under glass using pollen tested for viability on 10% sucrose in agar gel to test some of the genotypes we predicted from ribonuclease analysis. 'Early Rivers', S_1S_2 was pollinated with 'Flamentiner' (116 flowers) which we determined as S_6S_{12} rather than S_1S_2, and 'Merton Late', S_1S_4, with 'Ursula Rivers' which we determined as S_1S_2 rather than S_1S_4. 'Bradbourne Black', S_3S_5, was pollinated with three cultivars which had previously been assigned to the same group, VII, 'Erianne' (130 flowers), 'Bigarreau Burlat' (165 flowers) and 'Bigarreau Moreau' (105 flowers), but which appeared to be S_5S_6, S_3S_9 and S_3S_9 respectively. 'Colney' was pollinated with 'Erianne' (208 flowers) and with 'Zweitfruhe' (167 flowers); all three appeared to be S_5S_6. To check a prediction made earlier (Bošković and Tobutt 1996, Bošković et al. 1997) 'Peggy Rivers', which we had determined to be S_2S_4 rather than S_2S_5, was pollinated with 'Vic', S_2S_4 (120 flowers), and the reciprocal cross was made (540 flowers). 'Smoky Dun' was pollinated with 'Norbury's Early Black' (116 flowers); these two had previously been assigned to group O, but appeared to be S_1S_9. Appropriate control crosses were made.

Results

Group I

Figure 1 shows zymograms of the eight cultivars analysed that had been listed by Matthews and Dow (1969) as belonging to group I, of genotype S_1S_2, together with a ladder of the six original S alleles and two cultivars from group I that had already been analysed by Bošković and Tobutt (1996). 'Baumanns May A', 'Bedford Prolific A', 'Black Downton', 'Carnation C', 'Knight's Early Black A' and 'Ronald's Heart' revealed the same two bands as 'Early Rivers' and 'Roundel', which had been identified as the bands corresponding to S_1 and S_2 by Bošković and Tobutt (1996), in accord with the genotype given by Matthews and Dow (1969). However, 'Black Eagle A' and 'Flamentiner' showed different patterns. The former showed bands corresponding to S_3 and S_5 and thus to the genotype now assigned to group VII (Bošković et al. 1997). The latter showed the band corresponding to S_6 and a new band between those corresponding to S_1 and S_2 which we ascribe to S_{12}. The pI value of the S_{12} band was estimated as 8.80. The test cross of 'Early Rivers'×'Flamentiner' set 22.4% fruit, proving that 'Flamentiner' does not belong to group I.

Groups II, III, IV, V, VI, VII, IX and XIII

For several of these groups, some cultivars appeared to have patterns other than those corresponding to the reported genotype. In Fig. 2a we have included only the exceptions, run under IEF conditions, along with a 'ladder' showing the bands corresponding to the alleles S_1 to S_6. In Fig. 2b, run under NEPHGE conditions to clarify group VII, we have included a representative cultivar for S_9.

Group II

Ten of the cultivars analysed, 'Belle Agathe', 'Bigarreau de Schrecken', 'Black Elton', 'Caroon B', 'Frogmore

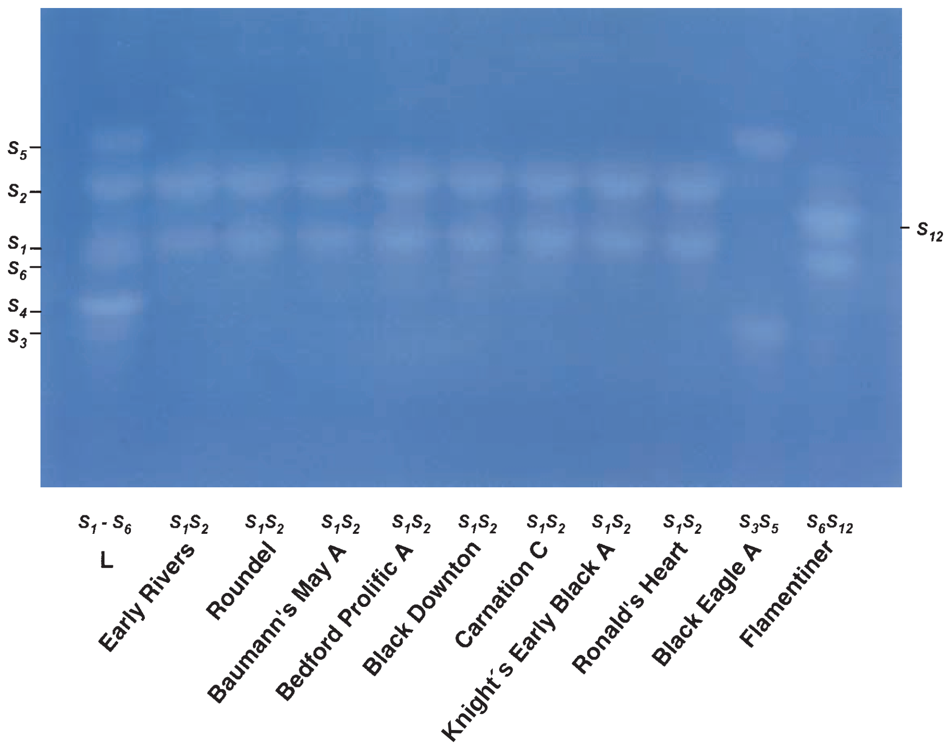
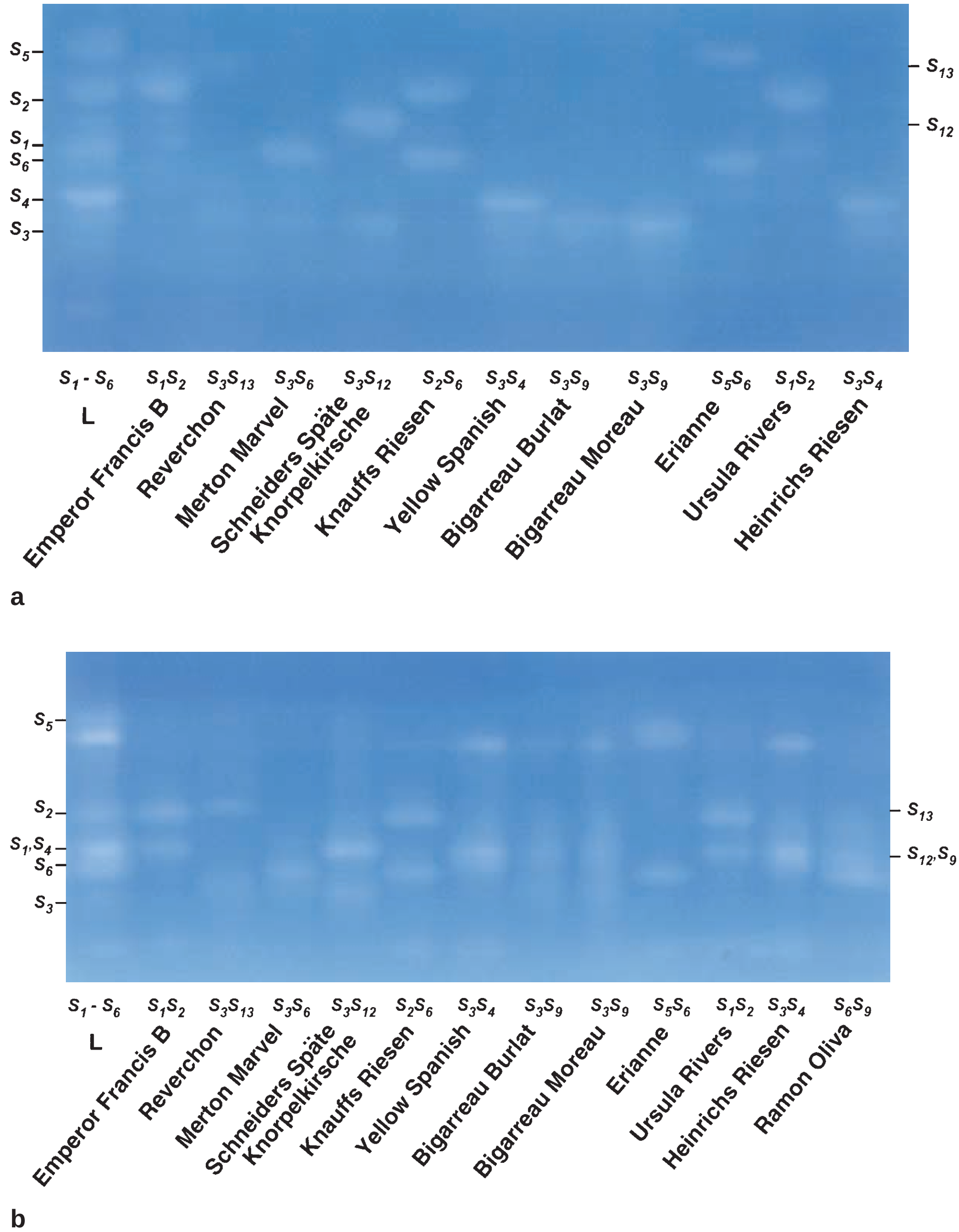


Fig. 1 Stylar ribonuclease zymograms of ten cherry cultivars traditionally assigned to group I, S_1S_2, alongside a ladder representing alleles S_1 to S_6

Fig. 2a, b Stylar ribonuclease zymograms of 12 cherry cultivars previously assigned incorrectly to groups II, III, IV, VI, VII, IX and XIII alongside the ladder representing alleles S_1 to S_6, (**a**) run under IEF and (**b**) run under NEPHGE with cultivar indicating S_9. Note that under these latter conditions, used to separate S_3 from S_9, the migration of S_3 is anomalous in cultivars of genotype S_3S_4



Early', 'Merton Crane', 'Venus', 'Victoria Black A', 'Waterloo' and 'Windsor A', revealed the two bands corresponding to S_1 and to S_3 as previously reported by Bošković and Tobutt (1996) for 'Van' and in accord with the genotypes given by Matthews and Dow (1969). 'Emperor Francis B', however, showed the bands corresponding to S_1 and S_2, as just described for group I.

The inclusion of 'Emperor Francis B' in group II by Matthews and Dow (1969) was based on the report of Hruby (1962) (Matthews, personal communication). In that report, 'Emperor Francis B' is a red cherry (i.e. white fleshed), whereas in Grubb (1949) it has coloured juice (i.e. black flesh). So there are clearly two different clones of the same name.

Group III

Five cultivars, 'Emperor Francis', 'Lambert', 'Querfurter Königskirsche', 'Vernon' and 'Star', revealed the two bands previously reported in 'Napoleon' and 'Bing' and attributed to S_3 and S_4 (Bošković and Tobutt 1996). This accorded with the genotypes given by Matthews and Dow (1969). However, three cultivars showed other patterns. 'Merton Marvel' showed bands corresponding to S_3 and S_6 as previously assigned to group VI. 'Schneiders Späte Knorpelkirsche' showed the S_3 band and the band just seen in 'Flamentiner' and attributed to S_{12}. 'Reverchon' showed the S_3 band and another new band between those corresponding to S_5 and S_2 and ascribed to S_{13}. The estimated pI value of this band was 8.40.

It should be noted that Matthews and Dow (1969) listed ‘Reverchon’ or ‘Bigarreau Reverchon’ twice, once in group III and once in group O. ‘Merton Marvel’, which we found to be S_3S_6 and not S_3S_4, comes from a cross of ‘Noble’, which we find to be S_6S_{13}, and ‘White Bigarreau’, S_2S_3 (Anonymous 1997). So it is our genotype for ‘Merton Marvel’ and not that of Matthews and Dow (1969) that is consistent with its parentage.

Group IV

Six cultivars revealed the two bands previously seen in ‘Victor’ and attributed to S_2 and S_3 (Bošković and Tobutt 1996), and thus accorded with the genotypes of Matthews and Dow (1969). They were ‘Allman Gulrod’, ‘Kentish Bigarreau’, ‘Late Amber’, ‘Ludwig’s Bigarreau’, ‘Merton Premier’ and ‘Velvet.’ The cultivar ‘Yellow Spanish’ shows the bands corresponding to S_3 and S_4, as in Group III. ‘Knauffs Riesen’ shows bands for S_2 and S_6, a new combination of ‘old’ alleles.

Group V

No further cultivars were analysed.

Group VI

Two cultivars, ‘Elton Heart’ and ‘Turkish Black’, revealed the same two bands as previously reported for ‘Governor Wood’ and ‘Merton Heart’ and ascribed to S_3 and S_6 (Bošković and Tobutt 1996) in accord with Matthews and Dow (1969).

Group VII

‘Fruhe Luxburger’ revealed the bands for S_3 and S_5 in accord with the revised genotype we recently proposed for group VII, S_3S_5, on the basis of our analysis of ‘Bradbourne Black’ and others (Bošković et al. 1997). However, ‘Erianne’ showed bands corresponding to S_5 and to S_6, a combination of ‘old’ alleles recently reported in ‘Colney’ (Bošković et al. 1997). We thus predict that these two are inter-incompatible and propose a new group, XV, with the genotype S_5S_6. In the test crosses, ‘Bradbourne Black’×‘Erianne’ set 23.8%, whereas ‘Colney’×‘Erianne’ set 0.4%. Under IEF conditions ‘Bigarreau Burlat’ and ‘Bigarreau Moreau’ both appeared to show a single band corresponding to S_3. However, under NEPHGE conditions a second occurred, corresponding to S_9. We propose ‘Bigarreau Burlat’ and ‘Bigarreau Moreau’ constitute a new group, XVI, with the genotype S_3S_9. The test crosses of ‘Bradbourne Black’×‘Bigarreau Burlat’ and ‘Bigarreau Moreau’ set 13.3% and 14.2% respectively, demonstrating that these two do not belong to group VII.

Our conclusion that ‘Erianne’ and ‘Bigarreau Burlat’ do not belong to group VII accords with reports that ‘Hedelfinger’ is compatible with ‘Erianne’ (Johansson and Callmar 1936) and with ‘Bigarreau Burlat’ (Saunier 1988). Our establishment of a new group for ‘Bigarreau Burlat’ and ‘Bigarreau Moreau’ accords with the finding of Saunier (1988) that these two are cross-incompatible.

Group IX

Two of the cultivars, ‘Merton Reward’ and ‘Rainier’, revealed the two bands corresponding to S_1 and S_4 as previously reported for ‘Merton Late’ (Bošković and Tobutt 1996) in accord with Matthews and Dow (1969). ‘Ursula Rivers’, however, showed the bands corresponding to S_1 and S_2, and hence the same phenotype as group I. The test cross of ‘Merton Late’×‘Ursula Rivers’ set 63.6% fruit, proving that the accession of ‘Ursula Rivers’ we analysed does not belong to group IX.

The genotype reported for ‘Ursula Rivers’ by Matthews and Dow (1969), S_1S_4, is consistent with the genotype, S_4S_6, that we have determined for ‘Merton Glory’, which comes from the cross ‘Ursula Rivers’× ‘Noble’ (Anonymous 1997). We conclude that the accession of ‘Ursula Rivers’ at the National Fruit Collection that we analysed is different from the ‘Ursula Rivers’ genotyped at the John Innes Institute and used as a parent of ‘Merton Glory.’

Group XIII

The only member of this group that we analysed, ‘Heinrichs Riesen’, had the bands not for S_2 and S_4, but for S_3 and S_4, and thus appeared to be a member of group III.

Groups VIII, X, XI, XII and XIV

These groups have either not been well characterised previously, X, XI and XII, or doubts have been raised about their genotypes, VIII and XIV. In Fig. 3 we show the cultivars we analysed that had been assigned by Matthews and Dow (1969) to these groups, along with a ladder of S_1 to S_6 and representative cultivars for S_7 and S_9.

Group VIII

None of the accessions analysed as representatives of group VIII showed the bands for S_2 and S_5 that would be expected from the genotype given by Matthews and Dow (1969). ‘Schmidt’ showed the bands for S_2 and S_4, i.e. the genotype of group XIII, and thus the same pattern as ‘Peggy Rivers’, the one representative of group VIII analysed previously (Bošković and Tobutt 1996;

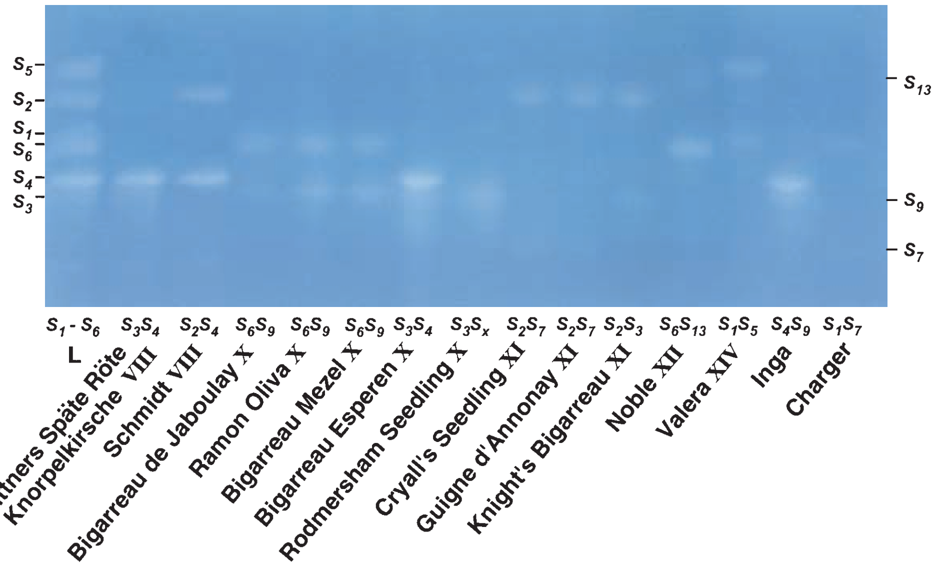


Fig. 3 Stylar ribonuclease zymograms of 13 cherry cultivars traditionally assigned to groups VIII, X, XI, XII and XIV, with a ladder representing alleles S_1 to S_6 and two cultivars indicating S_7 and S_9

Bošković et al. 1997). The test crosses of 'Peggy Rivers'×'Vic' S_2S_4 and the reciprocal set no fruit. 'Büttners Späte Rote Knorpelkirsche' showed the S_3 and S_4 bands, the pattern indicating group III.

The genotype we determined for 'Schmidt', S_2S_4, is in accord with the genotype we determined for 'Ulster', S_3S_4, (Bošković and Tobutt 1996) which came from the cross 'Schmidt'×'Lambert', S_3S_4 (Anonymous 1997), whereas the genotype assigned to 'Schmidt' by Matthews and Dow (1969), S_2S_5, was not consistent with this pedigree. 'Büttners Späte Rote Knorpelkirsche' is supposedly of distinct origin from Büttners Rote Knorpelkirsche', but for many years these two have been considered to be the same (Krümmel 1956) and our score for the former is the same as the score of Matthews and Dow (1969) for the latter.

As S_2S_5 is the genotype reported by, e.g. Matthews and Dow (1969) for Group VIII we suggest retaining it even though none of the cultivars assigned originally to this group has this genotype. As mentioned later, we find that 'Malling Black Eagle' has the bands for S_2 and S_5 and we suggest that it be considered the type member of this group.

Group X

Three of the five cultivars of this group that has not previously been genotyped showed the same pattern. 'Bigarreau de Jaboulay', Bigarreau de Mezel' and 'Ramon Oliva' all showed the S_6 band and the S_9 band. However, 'Bigarreau Esperen' showed the bands corresponding to S_3 and S_4 as described for group III. And 'Rodmersham Seedling' appeared to have only a single band, wider than but apparently containing S_3; maybe the genotype is best given as $S_3 S_x$ where S_x is an unknown allele.

'Bigarreau Esperen' is often considered to be a synonym for 'Napoleon' (Hedrick 1915) which indeed belongs to group III.

With three of the five cultivars analysed being S_6S_9 we propose this as the genotype of Group X.

Group XI

Two of the representatives of this group, another which has not previously been genotyped, had the bands corresponding to S_2 and S_7; they were 'Cryall's Seedling' and 'Guigne d'Annonay.' The third representative, 'Knight's Bigarreau', revealed the bands corresponding to S_2 and S_3 and thus appears to belong to group IV.

We have recently been able to borrow the notebooks from which Matthews and Dow (1969) compiled their classic table. Against the assignment of 'Knight's Bigarreau', group XI, is written a reference to Crane and Brown (1955) and "John Innes variety is not that described by Grubb since ours is black." Thus the accession we analysed, which accords with that of Grubb (1949), is different from that genotyped at the John Innes Institute.

We propose S_2S_7 as the genotype of group XI.

Group XII

We could analyse only one representative of this group, another group that has not been genotyped previously. This was 'Noble'; it had the bands corresponding to S_6 and to S_{13}, the latter being the new allele we have just described in 'Reverchon'. As 'Noble' was a founder member of group XII we propose S_6S_{13} as the genotype of group XII.

Group XIV

'Valera' indeed had the bands corresponding to S_1 and to S_5, in accord with the originally published genotype

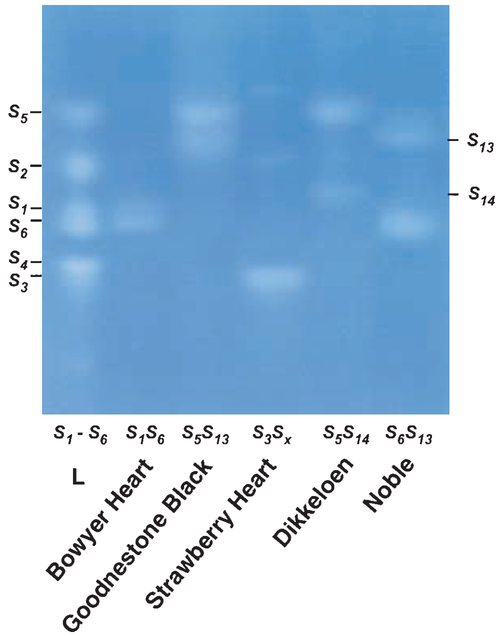


Fig. 4 Stylar ribonuclease zymograms of four cherry cultivars remaining in group O run alongside a ladder representing alleles S_1 to S_6 and 'Noble' indicating S_{13}

[Matthews, personal communication, in Way (1968)]. Tehrani and Lay (1991) stated that the combination S_1S_5 could not have resulted from the cross that gave rise to 'Valera', namely 'Hedelfinger'×'Windsor', S_1S_3. However, that would be true only if 'Hedelfinger' were known to lack S_5. 'Valera' was originally called V350427 (Anonymous 1997) and, under this name, Matthews and Dow (1969) placed it in Group O but said it was cross-incompatible with 'Noir de Guben.'

Group O

In addition to 'Reverchon', which Matthews and Dow (1969) assigned to both groups III and O and on which we reported earlier, 14 representatives were analysed and, as expected, revealed a wide range of genotypes.

As mentioned already, 'Malling Black Eagle' showed the bands corresponding to S_2 and S_5, and we thus regard it as a representative of group VIII. 'Black Tartarian E' showed the two bands, corresponding to S_6 and S_9, as just described for group X. 'Ord' showed the bands described for S_2 and S_4, characteristic of group XIII. 'Noir de Guben' showed the bands described for S_1 and S_5 as just confirmed for group XIV and in accord with its reported cross-incompatibility with V350427, now 'Valera' (Matthews and Dow 1969). 'Zweitfruhe' appeared to be S_5S_6, the same genotype as just reported for 'Erianne', and previously for 'Colney', and is thus an addition to the new group XV. The cross 'Colney'×'Zweitfruhe' set 0.5% fruit. 'Merton Glory' and 'Nutberry Black' both appeared to be S_4S_6 and we feel justified in creating a new group for these two, group XVII. 'Norbury's Early Black' and 'Smoky Dun' both appeared to be S_1S_9 and we propose creating group XVIII for them. The test cross 'Smoky Dun'×'Norbury's Early Black' set no fruit. 'Reverchon', described earlier, appeared to be S_3S_{13} as did 'Wellington A' and we propose another new group, XIX.

This leaves four of the group O cultivars, zymograms of which are shown in Fig. 4 along with a ladder and a reference cultivar. 'Bowyer Heart' has bands for S_1 and S_6 and 'Goodnestone Black' has bands for S_5 and S_{13}. 'Strawberry Heart' shows one wide band apparently including S_3, as in 'Rodmersham Seedling' mentioned earlier; the faint bands are non-*S* ribonucleases occasionally seen in various cultivars. 'Dikkeleon' shows the S_5 band and a new band between those corresponding to S_6 and S_{12} which we ascribe to S_{14}, the only example in this paper. This band has an estimated pI value of 8.85.

The determination of different genotypes for 'Smoky Dun' and 'Strawberry Heart' is unexpected as Matthews and Dow (1969) reported this pair of cultivars within group O as cross-incompatible.

Discussion

Thus by analysing the stylar ribonucleases of cherry cultivars we have clarified the membership of incompatibility groups I to IX and XIII which had previously been genotyped. In the case of group VIII we found that the reported representatives did not have the expected genotype but we have nominated another representative, 'Malling Black Eagle.' We have proposed genotypes for the three groups that had not previously been genotyped: groups X, S_6S_9; XI, S_2S_7; and XII, S_6S_{13}. We confirmed the genotype of Group XIV that had been disputed. Several cultivars from group O, and several nominally from other groups but of unexpected genotype, fell into five new groups: XV, S_5S_6; XVI, S_3S_9; XVII, S_4S_6; XVIII, S_1S_9; and XIX, S_3S_{13}. Groups XV and XVIII were confirmed by test crossing. Two representatives of each of these new groups are shown in Fig. 5, along with a ladder of six alleles and two reference cultivars. Of the 15 representatives of Group O tested, just four remain in this group, i.e. they have unique or unresolved genotypes, and four have been added variously from groups I, III, IV and X. 'Rodmersham Seedling' and 'Strawberry Heart' may belong to a new group but we have left them in group O until their genotype and cross-incompatibility is confirmed. Table 1 is in effect a revision of that presented by Matthews and Dow (1969), revised in the light of recent studies. As well as the 70 cultivars just analysed it includes 25 cultivars assigned genotypes by Bošković and Tobutt (1996) or Bošković et al. (1997). The genotype of one of these, 'Bigarreau Gaucher', has been changed in accord with Sonneveld et al. (2001), who showed S_8 is functionally identical with S_3.

Of the cultivars previously placed in groups I to XIII (Matthews and Dow 1969) we have moved 18 to different groups as a result of this work, and have moved three

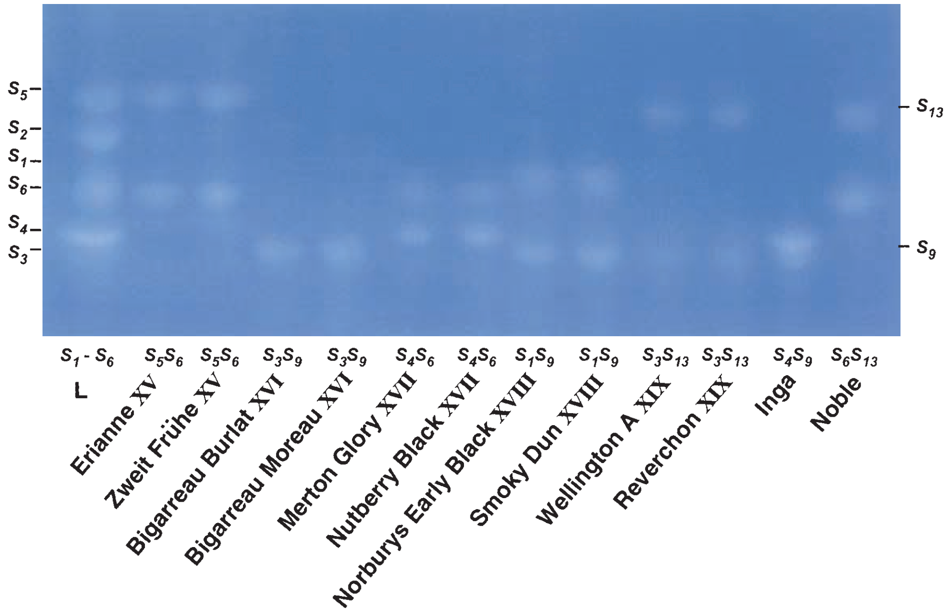


Fig. 5 Stylar ribonuclease zymograms of two representatives of five new groups, XV to XIX, alongside a ladder representing alleles S_1 to S_6 and two cultivars indicating S_9 and S_{13}

Table 2 Frequency of cherry incompatibility genotypes in Table 1, and allelic frequencies

	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_9	S_{10}	S_{11}	S_{12}	S_{13}	S_{14}	S_x	No. alleles	Frequency of alleles
S_1	–	12	11	3	2	1	1	2							32	0.17
S_2		–	8	4	1	1	2								28	0.15
S_3			–	12	6	5		2			1	2		2	49	0.26
S_4				–	2	2		1							24	0.13
S_5					–	3						1	1		16	0.08
S_6						–		4			1	1			18	0.09
S_7							–								3	0.02
S_9								–							11	0.06
S_{10}									–	1[a]					1	0.01
S_{11}										–					1	0.01
S_{12}											–				2	0.01
S_{13}												–			4	0.02
S_{14}													–		1	0.01
S_x														–	2	0.01
															190	

[a] Orleans 171

cultivars from group O to groups I to XIII. Leaving aside the possibility that some *S* alleles have electrophoretic variants, there are several possible explanations for the discrepancies. In some cases the accessions we analysed may not have been identical with those contributing to the table in Matthews and Dow (1969) as we have explained e.g. for ‘Emperor Francis B’, ‘Knight’s Bigarreau’ and ‘Ursula Rivers’. Grubb (1949, 1955) has explained how misnaming is common in cherry and different cultivars can share synonyms, and Matthews and Dow (1969) pointed out that cross-linkings of groups in different countries by inference from bridging varieties should be treated with caution as homonyms are common. In some cases, erroneous conclusions may have been drawn from pollination tests, for example, if failure to set was due to poor quality pollen rather than incompatibility. Moreover, once a misleadingly named cultivar or misgenotyped cultivar is used as a reference in crossing tests to genotype further cultivars, additional incorrect interpretations are likely.

Table 1 provides an excellent framework for genotyping additional cultivars. The table presented by Matthews and Dow (1969) represented a great collective achievement of cherry pomologists, breeders and geneticists, but it clearly contains a number of potential traps for the unwary. For example Tao et al. (1999) cannot have developed an allele-specific test for S_5 from ‘Bigarreau Burlat’ as this cultivar does not contain this allele.

We have proposed three new alleles, bringing the number of *S* alleles known in 95 accessions of *P. avium* (92 sweet cherries, two mazzard rootstocks and one timber selection) to 13. In comparison, we have identified

23 *S* alleles in 77 cultivars of almond (Bošković et al., submitted) and 25 *S* alleles in 56 cultivars of apple (Bošković and Tobutt 1999). It would be interesting to know if further *S* alleles occur in sweet cherries, as well as the extent of polymorphism in wild cherry.

Of the five alleles reported by Bošković et al. (1997), three were not found in sweet cherry cultivars initially. S_7 occurred only in the mazzard rootstock 'Charger', and S_{10} and S_{11} only in the timber clone Orleans 171. We have now found S_7 in two sweet cherry cultivars, 'Cryall's Seedling' and 'Guigne d'Annonay', but have not found S_{10} and S_{11} in sweet cherries. With 13 *S* alleles there are potentially 78 groups; if S_{10} and S_{11} are ignored there are 55 groups. Table 2 shows the frequency of the different genotypes and alleles reported in Table 1. Twenty eight combinations are represented (29 if Orleans 171 is included). All combinations of the original alleles S_1 to S_6 are now represented. The new alleles S_7 and S_9 to S_{14} are found only in combination with the original six alleles (except for $S_{10}S_{11}$). S_1S_2, S_1S_3 and S_3S_4 are the most-frequent genotypes. S_3 is the most-frequent allele, as pointed out for a partially overlapping set of cultivars by Williams and Brown (1956). S_1 and S_2 are the next most-frequent.

The estimated pI values of cherry *S* alleles are similar to those of almond, *Prunus dulcis*, and generally lower than those of apple, *Malus pumila*, a less-closely related rosaceous species. In cherry the maximum pI value found so far is 9.6 and in almond 9.25, whereas in apple the pI values of 18 out of 25 alleles exceed 9.6 and the maximum is 10.30.

As we have explained previously (Bošković and Tobutt 1996), the genotypes we report should be regarded as predictions. Ideally, they would be proved by crossing tests, or perhaps supported by allele-specific PCR which is now being developed (Sonneveld et al. 2001). Compared with PCR-based methods (Tao et al. 1999; Sonneveld et al. 2001), the analysis of stylar ribonucleases has the disadvantage of requiring flowering material. However, it is particularly useful for detecting the newer alleles, from S_7 upwards, for which allele-specific PCR tests are not currently available.

The data presented here have helped check and tidy up the results collated by Matthews and Dow (1969), on which subsequent incompatibility tables have been based (e.g. Thompson 1996). They should be useful for planning cultivar combinations for orchards and for designing crosses in breeding programmes, though it should be recognised that many of these cultivars are becoming superseded. In addition, the data will be useful for further research in cherry on the molecular genetics of self-incompatibility, by identifying sources of new alleles for sequence analysis and studies of molecular evolution, and also on the population genetics of this exceptionally polymorphic locus.

Acknowledgements Cherry breeding and genetics at HRI-East Malling is funded by the Ministry of Agriculture, Fisheries and Food. Radovan Bošković thanks the East Malling Research Association and the Royal Society for bursaries. We are grateful to the institutes listed in the footnote to Table 1 for the provision of plant material.

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