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An isoenzyme marker linked to the incompatibility locus in cherry

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Abstract Analysis of two cherry progenies from semi-compatible crosses for the esterase enzyme system showed extremely distorted segregation ratios for *Est-5*. Analysis of two progenies from compatible crosses for esterase and for stylar ribonuclease proved that *Est-5* is linked with the incompatibility locus *S*. The recombination fraction is 4%. About a fifth of some 50 cultivars or selections genotyped for *Est-5* were heterozygous. The various heterozygotes could provide 'testers' for the presence in cultivars of unknown genotype of 8 of the 11 known *S* alleles. A seedling suitable for testing S_9 has been identified and crosses have been made to raise testers for S_{10} and S_{11}. Isoenzyme analysis of the four progenies for glutamate oxaloacetate transaminase, and of one of them for isocitrate dehydrogenase, showed no evidence for the linkage of *Got-1* or *Idh-2* with *S*, contrary to a previous report. Estimation of linkage with *S* in semi-compatible crosses is discussed.

Key words Cherry · Incompatibility · Linkage · Marker · *Prunus avium*

Introduction

Most cultivars and selections of cherry, *Prunus avium*, are self-incompatible and many are cross-incompatible. Incompatibility in cherry is controlled by a multi-allelic locus *S*, with gametophytic expression (Crane and Lawrence 1929). Matthews and Dow (1969) published a list giving the genotypes of some 140 cultivars with various pairs of six *S* alleles. Recently, five more *S* alleles have been proposed (Bošković et al. 1997a) and additional cultivars have been genotyped (Bošković and Tobutt 1996; Bošković et al. 1997a).

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In their work at HRI-East Malling, Bošković and Tobutt (1996) and Bošković et al. (1997a) have shown that the different *S* alleles code for different stylar ribonucleases that can be distinguished on isoelectric-focusing (IEF) and non-equilibrium pH gradient-electrofocusing (NEPHGE) gels. Thus cultivars or selections for which flowering material is available can be genotyped without the need for a laborious series of controlled pollinations. However, this assay cannot be used on non-flowering plants such as young seedlings, although cherry cell cultures may express the stylar ribonucleases (Mau et al. 1982). A marker detectable in non-flowering plants would be useful for breeding work and genetic studies.

In recent years at HRI-East Malling we have been developing a genetic map of cherry comprising markers and agronomic genes. A map of 34 isoenzyme markers including *Got-1* and *Idh-2* has been published, based on analyses in two interspecific progenies, principally *P. avium* 'Napoleon'×*Prunus nipponica* (Bošković et al. 1997b; Bošković and Tobutt 1998), but stylar ribonucleases were not analysed and no markers were reported for *S* in that work. One locus, *Est-5*, was polymorphic in 14 cultivars but was monomorphic in the parents of the interspecific progenies and could not be mapped.

Granger (1996) reported *S* to be linked to '*Got*', and to some other loci including '*Idh*', in *P. avium*, on the basis of aberrant ratios for these isoenzymes in progenies from semi-compatible crosses or from selfing, pointing out that Manganaris and Alston (1987) reported *S* to be linked with a locus for GOT and a locus for IDH in apple.

We have several progenies of *P. avium* scored for the segregation of *S* alleles by analysis of stylar ribonucleases (Bošković et al. 1997a). We have taken the opportunity of testing the linkage to *S* of *Got-1* and *Idh-2*, which are the loci considered to correspond to '*Got*' and '*Idh*' of Granger et al. (1993) (Bošković et al. 1997b). As Batlle et al. (1995) had pointed out the great polymorphism of *Got-1*, the marker for *S* in apple, we also looked to see if *Est-5*, which is relatively polymorphic in *P. avium*, might be linked with *S*. We present evidence

that *S* in cherry is linked with *Est-5* but not with *Got-1* or *Idh-2*. We have also analysed a range of cultivars of known incompatibility genotype to see which are heterozygous for *Est-5*, and thus could be used as testers for the presence of the various *S* alleles in cultivars of unknown genotype.

In addition, we have considered the general methodology for the estimation of the recombination fraction between the *S* locus and another locus in semi-compatible crosses.

Materials and methods

The possible linkages of *Got-1* and *Est-5* with *S* were studied in progenies from two semi-compatible crosses, F1/3× 'Charger', 82 seedlings, and 'Colney'×'Gaucher', 32 seedlings, and from two fully compatible crosses, 'Bradbourne Black'×'Merton Late', 47 seedlings, and 'Inge'×'Gaucher', 27 seedlings. The possible linkage of *Idh-2* with *S* was studied only in the F1/3×'Charger' progeny, as the parents of the other three progenies were all homozygous for *Idh-2*. All four progenies had previously been scored, at least partly, for the segregation of *S* alleles by analysis of stylar ribonucleases (Bošković et al. 1997a).

To survey the extent of heterozygosity for the *Est-5* marker, 38 self-incompatible and two self-compatible cultivars or selections (see Table 3), growing at HRI-East Malling or at the National Fruit Collections, Brogdale, were analysed for *Est-5*.

The progenies had already been largely scored for incompatibility genotype using stylar ribonucleases (Bošković et al. 1997a) but 30 additional seedlings were analysed in the F1/3×'Charger' progeny and three in the 'Bradbourne Black'×'Merton Late' progeny following the procedures described in that paper. For the analysis of *Est-5*, *Got-1*, and *Idh-2* in the progenies, and of *Est-5* in the cultivars, extracts were prepared from leaves for *Got-1* and from bark for *Est-5* and *Idh-2*, separated using PAGE and stained, following the procedures described by Bošković et al. (1997b) and Bošković and Tobutt (1998). Scoring for *Est-5*, *Got-1* and *Idh-2* was in accord with Bošković and Tobutt (1998), Santi and Lemoine (1990) and Bošković et al. (1997b) respectively. It should be noted that *Est-5* and *Got-1* appear to have a dimeric structure.

Chi-square tests were used to compare single-locus segregations to Mendelian ratios. Co-segregations were analysed using the likelihood-ratio test of linkage if both segregations were approximately Mendelian, or the less powerful but more robust contingency table χ^2 test if either segregation was aberrant (for reasons other than semi-compatibility). These computations were done using the linkage program LINKEM developed recently at HRI-East Malling (Vowden et al. 1995), with due correction the number of degrees of freedom in the case of semi-compatible crosses. The use of LINKEM for analysing semi-compatible crosses is discussed later.

Likelihood-ratio tests were employed to assess the homogeneity of the estimates of recombination between *S* and *Est*-5 from different crosses. Pooled estimates of recombination were calculated by maximising the overall likelihood function, which is the product of the likelihood functions for each separate cross. These calculations were done using the symbolic algebra program MAPLE V (Waterloo Maple Inc., Waterloo, Ontario, Canada).

Results

The segregation data for *Est-5*, *Got-1* and *Idh-2*, and the accumulated data for stylar ribonucleases in the various families, are given in Table 1. The co-segregation analyses of *Est-5*, *Got-1* and *Idh-2* with *S* are given in Table 2.

Figure 1 shows the stylar ribonucleases of F1/3, S_1S_2, and 'Charger', S_1S_7, as well as eight of their seedlings segregating for S_1S_7 versus S_2S_7. In the whole progeny

Table 1 Segregation for *Est-5*, *Got-1 Idh-2* and *RNase* in cherry progenies from two semi-compatible and two fully compatible crosses

Locus	Parental genotypes	Observed segregation	Expected segregation	χ^2
F1/3×'Charger' 82 seedlings				
Est-5	*ab ab*	1*aa*:44*ab*:37*bb*	1:2:1	32.05***
Got-1	*aa ab*	47*aa*:35*ab*	1:1	1.76
Idh-2	*cd cd*	17*cc*:46*cd*:19*dd*	1:2:1	1.32
RNase[a]	S_1S_2 S_1S_7	$44S_1S_7$:$38S_2S_7$	1:1[b]	0.44
'Colney'×'Gaucher' 32 seedlings				
Est-5	*ab ab*	0*aa*:19*ab*:13*bb*	1:2:1	11.69**
Got-1	*ab ab*	0*aa*:1*ab*:31*bb*	1:2:1	88.19***
Idh-2	*dd dd*	Non-segregating		
RNase[a]	S_5S_6 S_5S_8	$19S_5S_8$:$13S_6S_8$	1:1[b]	1.13
'Bradbourne Black'×'Merton Late' 47 seedlings				
Est-5	*ab bb*	25*ab*:22*bb*	1:1	0.19
Got-1	*aa ab*	24*aa*:23*ab*	1:1	0.02
Idh-2	*dd dd*	Non-segregating		
RNase[a]	S_3S_5 S_1S_4	$14S_1S_3$:$12S_1S_5$:$9S_3S_4$:$12S_4S_5$	1:1:1:1	1.09
'Inge'×'Gaucher' 27 seedlings				
Est-5	*bb ab*	12*ab*:15*bb*	1:1	0.33
Got-1	*ab ab*	7*aa*:20ab:0bb	1:2:1	9.89**
Idh-2	*dd dd*	Non-segregating		
RNase[a]	S_4S_9 S_5S_8	$4S_4S_5$:$7S_4S_8$:$7S_5S_9$:$9S_8S_9$	1:1:1:1	1.89

, * Observed ratios significantly different from expected at P=0.01, 0.001

[a] *RNase* data already given, at least in part, in Bošković et al. (1997a)

[b] Expected in case of semi-compatible crosses

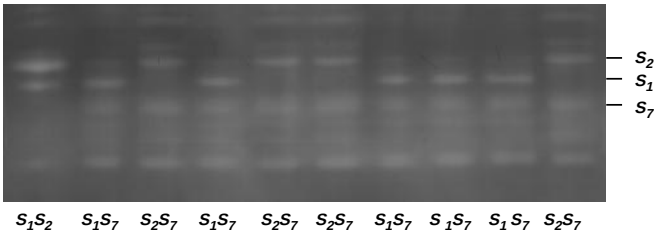

Fig. 1 Stylar ribonuclease zymograms of F1/3 (*track 1*) (S_1S_2), 'Charger' (*track 2*) (S_1S_7), and of eight seedlings segregating for S_1S_7 versus S_2S_7. The cross is semi-compatible and seedling genotypes S_1S_1 and S_1S_2 are not found

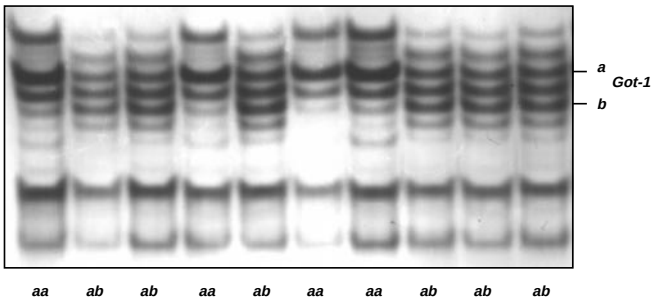

Fig. 2 GOT zymograms of F1/3 (*track 1*) (*Got-1 aa*), 'Charger' (*track 2*) (*Got-1 ab*), and of eight seedlings segregating for *aa* versus *ab*. The cross is semi-compatible but the segregation is not distorted

Table 2 Joint segregation analysis of *Est-5*, *Got-1* and *Idh-2* with *RNase* in cherry progenies from two semi-compatible and two fully compatible crosses

Locus pairs	Observed segregations				Expected segregations if no linkage	Test c or 1[a]	χ^2	% *r* (95% confidence interval)
F1/3×'Charger'								
Est-5 RNase	1 *aa* S_1S_7	41 *ab*S_1S_7	2 *bb*S_1S_7		1:1:1			
	0 *aa*S_2S_7	3 *ab*S_2S_7	35 *bb*S_2S_7		1:1:1	1[b]	119.2***	3.7 (1.5, 7.5)
Got-1 RNase	28 *aa*S_1S_7	16 *ab*S_1S_7			1:1			
	19 *aa*S_2S_7	19 *ab*S_2S_7			1:1	1	1.76	43 (32, 50)
Idh-2 RNase	6 *cc*S_1S_7	28 *cd*S_1S_7	10 *dd*S_1S_7		1:1:1			
	11 *cc*S_2S_7	18 *cd*S_2S_7	9 *dd*S_2S_7		1:1:1	1	0.69	46 (36, 50)
'Colney'×'Gaucher'								
Est-5 RNase	0 *aa*S_5S_8	19 *ab*S_5S_8	0 *bb*S_5S_8		1:1:1			
	0 *aa*S_6S_8	0 *ab*S_6S_8	13 *bb*S_6S_8		1:1:1	1[b]	62.4***	0 (0, 3.0)
Got-1 RNase	0 *aa*S_5S_8	1 *ab*S_5 S_8	18 *bb*S_5S_8		1:1:1			
	0 *aa*S_6S_8	0 *ab*S_6S_8	13 *bb*S_6S_8		1:1:1	c	0.71	– [c]
'Bradbourne Black'×'Merton Late'								
Est-5 RNase	2 *ab*S_1S_3	0 *ab*S_3S_4	11 *ab*S_1S_5	12 *ab*S_4S_5	1:1:1:1			
	12 *bb*S_1S_3	9 *bb*S_3S_4	1 *bb*S_1S_5	0 *bb*S_4S_5	1:1:1:1	1	42.8***	6.4 (1.6, 16)
Got-1 RNase	7 *aa*S_1S_3	2 *aa*S_3S_4	6 *aa*S_1S_5	9 *aa*S_4S_5	1:1:1:1			
	7 *ab*S_1S_3	7 *ab*S_3S_4	6 *ab*S_1S_5	3 *ab*S_4S_5	1:1:1:1	1	0.02	49 (35, 50)
'Inge'×'Gaucher'								
Est-5 RNase	4 *ab*S_4S_5	0 *ab*S_4S_8	6 *ab*S_5S_9	2 *ab*S_8S_9	1:1:1:1			
	0 *bb*S_4S_5	7 *bb*S_4S_8	1 *bb*S_5S_9	7 *bb*S_8S_9	1:1:1:1	1	18.6***	11 (2.9, 26)
Got-1 RNase	1 *aa*S_4S_5	2 *aa*S_4S_8	3 *aa*S_5S_9	1 *aa*S_8S_9	1:1:1:1			
	3 *ab*S_4S_5	5 *ab*S_4S_8	4 *ab*S_5S_9	8 *ab*S_8S_9	2:2:2:2			
	0 *bb*S_4S_5	0 *bb*S_4S_8	0 *bb*S_5S_9	0 *bb*S_8S_9	1:1:1:1	c	2.10	– [c]

*** Observed ratios significantly different at P=0.0001
[a] c=contingency table χ^2 test, used if one or both ratios aberrant; l=likelihood ratio test, used if both ratios approximately Mendelian
[b] The distorted segregation of *Est-5* is thought to be a consequence of its linkage with *RNase*
[c] Recombination fraction cannot be estimated reliably because of the distorted segregation of *Got-1*

there were 44 seedlings with the former genotype and 38 with the latter. This approximates to the 1:1 ratio expected in a semi-compatible cross (χ^2=0.44, 1 *df*). As reported by Bošković et al. (1997a) the segregation of stylar ribonucleases in the other semi-compatible cross, 'Colney'×'Gaucher', approximated to 1:1 and the segregations in the two fully compatible crosses, 'Bradbourne Black'×'Merton Late' and 'Inge'×'Gaucher', approximated to 1:1:1:1.

Figure 2 shows the GOT zymograms of F1/3, *Got-1 aa*, and 'Charger', *Got-1 ab*, and of eight seedlings segregating for *aa* versus *ab*. The region cathodal to that ascribed to *Got-1*, in accord with Santi and Lemoine (1990), showed variation parallel to *Got-1*, and is considered a "shadow". In the progeny as a whole, the segregation was 47 *aa* to 35 *ab*, approximating to 1:1 (χ^2=1.76, 1 *df*). Figure 3 shows the IDH zymograms of F1/3 and 'Charger', both *Idh-2 cd*, and of eight seedlings

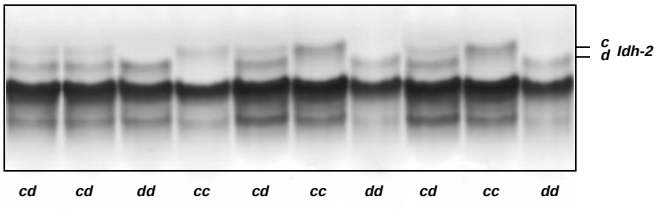

Fig. 3 IDH zymograms of F1/3 (*track 1*) and 'Charger' (*track 2*) (both *Idh-2 cd*), and of eight seedlings segregating for *cc*, *cd* and *dd* . The cross is semi-compatible but the segregation is not distorted

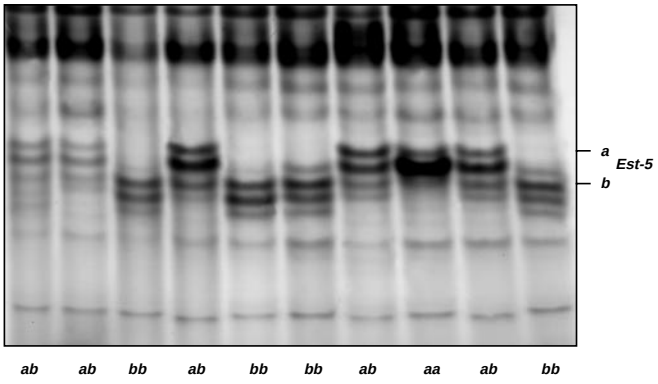

Fig. 4 EST zymograms of F1/3 (*track 1*) and 'Charger' (*track 2*) (both *Est-5 ab*), and of eight seedlings segregating for *aa*, *ab* and *bb*. The segregation in this semi-compatible cross is distorted

segregating for *cc*, *cd* and *dd*. The seedling segregation, 17 *cc*, 46 *cd* and 19 *dd*, approximated to 1:2:1 (χ^2=1.32, 2 *df*). These undistorted ratios indicated that neither *Got-1* nor *Idh-2* is linked to the incompatibility locus *S*. *Got-1* in the other semi-compatible cross, 'Colney' (*ab*)×'Gaucher' (*ab*), segregated aberrantly, 0 *aa*: 1 *ab*: 31 *bb* (χ^2=88.19, 2 *df*). In the fully compatible crosses, the segregation in 'Bradbourne Black' (*aa*)×'Merton Late' (*ab*) was 24 *aa*: 23 *ab* (χ^2=0.02, 1 *df*), and that in 'Inge' (*ab*)×'Gaucher' (*ab*) was 7 *aa*:20 *ab*: 0 *bb* (χ^2=9.89, 2 *df*) and thus was aberrant.

Figure 4 shows the EST zymograms of F1/3 and 'Charger', both *Est-5 ab*, and of eight seedlings segregating for *aa*, *ab* and *bb*. The segregation of *Est-5* in this semi-compatible cross, 1 *aa* (the only individual with such a genotype found in this work), 44 *ab* and 37 *bb*, was distorted (χ^2=32.05, 2 *df*), as was the segregation in the other semi-compatible cross, 'Colney' (*ab*)×'Gaucher' (*ab*), 0 *aa*, 19 *ab* and 13 *bb* (χ^2=11.69, 2 *df*). Instead of 1:2:1, both ratios were approximately 0:1:1 (χ^2=0.56, 2 *df* and χ^2=1.25, 2 *df* respectively); this is consistent with a linkage of *Est-5* with *S*. In the fully compatible cross 'Bradbourne Black' (*ab*)×'Merton Late' (*bb*), the segregation was undistorted, 25 *ab* to 22 *bb*, and approximated to a 1:1 ratio (χ^2=0.19, 1 *df*). In the other fully compatible cross, 'Inge' (*bb*)×'Gaucher' (*ab*), again there was no sign of distortion, and the ratio, 12 *ab* and 15 *bb*, approximated 1:1 (χ^2=0.33, 1 *df*).

When the co-segregation of *Idh-2* and *RNase* was considered in F1/3×'Charger', a semi-compatible cross, there was no evidence of linkage (χ^2=0.69, 1 *df*). Nor was there evidence of linkage between *Got-1* and *RNase* in this progeny (χ^2=1.76, 1 *df*). In the other semi-compatible cross, 'Colney'×'Gaucher', although the segregation of *Got-1* was distorted there was no evidence of linkage with *RNase* (χ^2=0.71, 2 *df*), based on the contingency table χ^2 test. Nor was there any evidence of linkage of *Got-1* with *RNase* in the fully compatible crosses 'Bradbourne Black'×'Merton Late' (χ^2=0.02, 1 *df*) and 'Inge'×'Gaucher' (χ^2=2.10, 6 *df*), the latter test again being a contingency table χ^2 test, due to the distorted segregation of *Got-1*.

There was strong evidence of linkage from the co-segregations of *Est-5* and *RNase* in the fully compatible crosses 'Bradbourne Black'×'Merton Late' (χ^2=42.8, 1 *df*) and 'Inge'×'Gaucher' (χ^2=18.6, 1 *df*). The estimated recombination fractions were 6% and 11% respectively. There was also strong evidence of linkage between *Est-5* and *RNase* in the semi-compatible crosses F1/3×'Charger' (χ^2=119.2, 1 *df*) and 'Colney'×'Gaucher' (χ^2=42.9, 1 *df*). The estimated recombination fractions were 4% and 0% respectively.

Combining the estimates from all four progenies gave a pooled estimate of 4%. However, there was some evidence of heterogeneity amongst the estimates (χ^2=8.21, 3 *df*). Excluding the data from the semi-compatible cross 'Colney'×'Gaucher' gave a pooled estimate of 5%, with no evidence of heterogeneity (χ^2=2.34, 2 *df*).

Table 3 shows the *Est-5* genotypes of the 39 cultivars analysed and of 12 cultivars analysed earlier (Bošković and Tobutt 1998), together with their known *S* allele genotypes. Forty one are homozygous *bb* and ten are heterozygous *ab*.

Discussion

The non-distorted segregation of *Got-1* and *Idh-2* in the semi-compatible cross F1/3×'Charger' indicated that these loci are not linked with *S*. Confirmation was provided by the various co-segregation analyses.

Interestingly, the segregation of *Got-1* was aberrant in the semi-compatible cross 'Colney'×'Gaucher' and in the fully compatible cross 'Inge'×'Gaucher'. In both progenies 1:2:1 ratios were expected. However, in the former progeny the homozygous *aa* seedlings were missing along with most of the heterozygotes, whereas in the latter it was the homozygous *bb* seedlings that were missing. It is difficult to propose a simple explanation for these two different types of distortion.

Our conclusion that *Got-1* and *Idh-2* are not linked with *S* in cherry differs from that of Granger (1996). One of the five progenies on which he based his conclusions was derived from selfing the self-compatible cultivar 'Stella'. Granger (1996) reported 'Stella' to be heterozygous for *Got* and *Idh*, and the absence from the 'Stella'

Table 3 Genotypes for *Est-5* of 51 cherry cultivars of known incompatibility genotype

Cultivar	Genotype for *Est-5*	Incompatibility genotype	Reference for incompatibility genotype[a]
'Alman Gulrod'	*bb*	S_2S_3	M & D 69
'Baumann May B'	*bb*	S_1S_2	M & D 69
'Bedford Prolific A'	*bb*	S_1S_2	M & D 69
'Bigarreau de Schrecken'	*bb*	S_1S_3	M & D 69
'Bing'	*bb*	S_3S_4	M & D 69, B & T 96
'Black Downton'	*bb*	S_1S_2	M & D 69
'Bradbourne Black'	*ab*[b]	S_3S_5	B et al. 97
'Carnation C'	*bb*	S_1S_2	M & D 69
'Caroon B'	*bb*	S_1S_3	M & D 69
'Charger'	*ab*[b]	S_1S_7	B et al. 97
'Colney'	*ab*[b]	S_5S_6	B et al. 97
'Early Rivers'	*bb*[b]	S_1S_2	M & D 69, B & T 96
'Elton Heart'	*bb*	S_3S_6	M & D 69
F1/3a	*ab*[b]	S_1S_2	B et al. 97
'Frogmore Early'	*bb*	S_1S_3	M & D 69
'Gaucher'	*ab*[b]	S_5S_8	B et al. 97
'Governor Wood'	*bb*	S_3S_6	M & D 69, B & T 96
'Hedelfinger'	*ab*	S_3S_5	B et al. 97
'Hookers Black'	*ab*	S_3S_5	B et al. 97
'Inge'	*bb*[b]	S_4S_9	B et al. 97
'Kentish Bigarreau'	*bb*	S_2S_3	M & D 69
'Knights Early Black A'	*bb*	S_1S_2	M & D 69
'Lambert'	*bb*	S_3S_4	M & D 69
'Lapins'	*bb*	S_1S_4'	B & T 96
'Late Amber'	*bb*	S_2S_3	M & D 69
'Late Black Bigarreau'	*ab*	S_4S_5	B et al. 97
'Ludwigs Bigarreau'	*bb*	S_2S_3	M & D 69
'Merton Crane'	*bb*	S_1S_3	B & T 96
'Merton Heart'	*bb*[b]	S_3S_6	B & T 96
'Merton Late'	*bb*[b]	S_1S_4	B & T 96
'Merton Premier'	*bb*	S_2S_3	M & D 69
'Merton Reward'	*bb*	S_1S_4	M & D 69
'Napoleon'	*bb*[b]	S_3S_4	B & T 96
Orleans 171	*bb*	$S_{10}S_{11}$	B et al. 97
'Peggy Rivers'	*bb*	S_2S_4	B et al. 97
'Rainier'	*bb*	S_1S_4	M & D 69
'Ronald's Heart'	*bb*	S_1S_2	M & D 69
'Roundel'	*bb*	S_1S_2'	B & T 96
'Stella'	*bb*[b]	S_3S_4'	B & T 96
'Sunburst'	*bb*	S_3S_4'	B & T 96
'Summit'	*bb*	S_1S_2	B & T 96
'Turkey Heart'	*ab*	S_4S_5	B et al. 97
'Ulster'	*bb*	S_3S_4	B & T 96
'Van'	*bb*[b]	S_1S_3	M & D 69, B & T 96
'Velvet'	*ab*	S_2S_3	M & D 69
'Vernon'	*bb*	S_3S_4	M & D 69
'Vic'	*bb*	S_2S_4	M & D 69, B & T 96
'Victor'	*bb*	S_2S_3	M & D 69, B & T 96
'Victoria Black A'	*bb*	S_1S_3	M & D 69
'Waterloo'	*bb*	S_1S_3	M & D 69
'Windsor A'	*bb*	S_1S_3	M & D 69

[a] M & D 69=Matthews and Dow (1969), B & T 96=Bošković and Tobutt (1996), B et al. 97=Bošković et al. (1997a)
[b] genotype given by Bošković and Tobutt (1998)

selfs of both classes of homozygotes in the case of *Got* and of one class of homozygotes and the heterozygotes in the case of *Idh* he attributed to linkage with *S*. When we analysed 'Stella' for *Got-1* and *Idh-2* we found it to be homozygous for both loci (Bošković et al. 1997b) and so we would not expect a self progeny to segregate for either locus.

Furthermore, we consider that *Got-1* in cherry, which is the same locus as the one Granger (1996) reported to be linked with *S*, is not homologous to *Got-1* in apple, which Manganaris and Alston (1987) found to be linked with *S*. *Got-1* in cherry is linked to *Gpi-2* and *Lap-1* (Bošković et al. 1997b) and, when almond is considered, *Pgm-2* can be added to this linkage group (Arus et al. 1992). As pointed out by Bošković and Tobutt (1998), a similar linkage group occurs in apple (Hemmat et al. 1994), but the GOT (=AAT) locus in this apple group is not the one linked with *S*.

The segregation data presented for *Est-5* in the two fully compatible crosses establish the existence of this locus. Previously this locus was considered to be only putative (Bošković and Tobutt 1998).

The distortion of the *Est-5* segregations in the two semi-compatible crosses, but not in the two fully compatible crosses, suggested linkage of this locus with *S*. The co-segregations of *Est-5* and stylar ribonuclease in the fully compatible crosses allowed the recombination fractions to be estimated as 6% and 11%. Estimation of the recombination fractions in the semi-compatible crosses gave 4% and 0%. The pooled estimate was 4%, increasing to 5% if the estimate from the cross 'Colney'×'Gaucher', which was slightly inconsistent with other estimates, was excluded.

The linkage confirmed our suspicion that the relatively high polymorphism of *Est-5* in *P. avium*, as the only locus polymorphic in cultivars but not in interspecific progenies (Bošković and Tobutt 1998), might be due to linkage with *S*. Batlle et al. (1995) suggested that the high polymorphism of *Got-1* in apple might be a consequence of its linkage with *S*.

Two distinct types of semi-compatible cross occurred. The first type was of the form $S_1a/S_2a \times S_1a/S_3b$. This leads to the following co-segregation probabilities

	aa	*ab*
S_1S_3	$r/2$	$(1-r)/2$
S_2S_3	$r/2$	$(1-r)/2$

,

where r is the recombination fraction. Estimation of r is therefore based on the single-factor segregation of *aa*:*ab*, using the formula given in the first row of Table 1 of Leach (1988). The second type of semi-compatible cross was of the form $S_1a/S_2b \times S_1a/S_3b$. This gives the following cosegregation probabilities

	aa	*ab*	*bb*
S_1S_3	$r(1-r)/2$	$[r^2+(1-r)^2]/2$	$r(1-r)/2$
S_2S_3	$r^2/2$	$r(1-r)$	$(1-r)^2/2$

.

The maximum-likelihood estimator of r is the solution to a cubic equation that does not have a simple explicit solution. This estimator differs from the single-factor segregation estimate given in the third row of Table 1 of Leach (1988).

The relative efficiency (*RE*) of the co-segregation method to the single-factor method for the second type of semi-compatible cross is defined as the ratio of their information functions. This may be shown to give

$$RE = 4\frac{3r^2 - 3r + 1}{2r^2 - 2r + 1}.$$

This function decreases, roughly linearly, from $RE=4$ at $r=0$ to $RE=2$ at $r=0.5$. Thus the co-segregation method is two to four times more efficient than the single-factor method, implying that the latter method would require a sample size two to four times as large to achieve the same precision for estimating r. One might expect that the *RE* would be at least two, because individuals that are heterozygous at the second locus carry no information about linkage in the single-factor analysis. However, the calculations show that much greater efficiency gains arise when there is close linkage. The co-segregation analysis should therefore always be used when the genotypes at the incompatibility locus are known. Examples of studies in which the co-segregation method of analysis might have been useful in mapping *S* are those of Ballester et al. (1998) and Jacobs et al. (1995) who scored *S* allele segregation in semi-compatible crosses of almond and diploid potato respectively.

Although LINKEM does not have any special facilities for incompatibility, it can be used to analyse both types of semi-compatible cross. The likelihood-ratio test for linkage and the maximum-likelihood estimate and likelihood-based confidence limits for the recombination fraction are all calculated correctly. The contingency table chi-square statistic is calculated correctly, but has the wrong degrees of freedom. The goodness-of-fit statistic (the deviance) is calculated incorrectly and has the wrong degrees of freedom, but this statistic is not presented in this paper.

It is tempting to specify the male parental genotype at the *S* locus as S_3S_3 rather than S_1S_3 in order to recover the correct degrees of freedom for these latter statistics, but this does not give the correct estimate of the recombination fraction.

Of the 51 cultivars scored for *Est-5*, ten were heterozygous *ab* and the remaining 41 were *bb*. None was *aa*. This may reflect not only the low frequency of the *a* allele but also its frequent association with one particular *S* allele; out of eight sweet cherry cultivars heterozygous for *Est-5*, seven carry the S_5 allele, probably in coupling with the *a* allele. A single seedling homozygous *aa* for *Est-5* was detected in the wild cherry progeny F1/3×'Charger'. With a view to creating more seedlings homozygous *aa*, we made a fully compatible cross between two heterozygotes, 'Bradbourne Black' (*Est-5 ab* S_3S_5) ×C89–19 (*Est-5 ab* S_8S_9) from the 'Inge'×'Gaucher' progeny.

A heterozygote could be a useful 'tester' for the presence of one or other of its two alleles in a cultivar of unknown genotype. For example, 'Velvet,' *Est-5 ab* S_2S_3, could be used as a pollinator for an unknown. If the unknown is homozygous for *Est-5* but all the seedlings are heterozygous, or all are homozygous, or if the unknown is heterozygous for *Est-5* but one class of homozygotes is missing from the seedlings, then the unknown must have S_2 or S_3. The heterozygous cultivars encompass all alleles from S_1 to S_8 and, with appropriate testers, it should be possible to identify any of the eight alleles in unknowns. If the phase of coupling of *S* alleles and *Est-5* alleles in the tester is known, as in the case of the five parents of the crosses analysed that were heterozygous for *Est-5*, i.e. 'Bradbourne Black', 'Charger', 'Colney', F1/3 and 'Gaucher', then one of the *S* alleles of the unknown can be deduced directly if the cross is semi-compatible; S_1 to S_3 and S_5 to S_8 can be so determined. Though *Est-5* cannot be detected in cotyledons, it can be

detected in bark samples, available perhaps 2 or 3 years before the seedlings flower.

None of the cultivars heterozygous for *Est-5* has the incompatibility allele S_9. One of the selections from the cross of 'Inge'×'Gaucher' that has the genotype *Est-5 ab* S_8S_9 would be a useful tester for this allele. So far no testers have yet been determined for S_{10} or S_{11}. To produce testers for these, the cross, 'Charger' (*Est-5 ab* S_1S_7) ×'Orleans' 171 (*Est-5 bb* $S_{10}S_{11}$) has been made, and 150 seedlings have been raised; from these, two seedlings will be chosen that are heterozygous for *Est-5*, one having S_{10} and the other S_{11}.

Such testers could be useful if stylar ribonucleases have been used to predict the *S* alleles of a cultivar of unknown genotype. In some cases different alleles are very similar electrophoretically, e.g. S_4, S_8 and S_9 (Bošković et al. 1997a), and crosses may be the best confirmation of function. Scoring seedlings for *Est-5* would allow useful information to be gleaned from semi-compatible crosses; otherwise these could be detected only by microscopic examination of pollen-tube growth (and no-one seems to have repeated the success of Kobel et al. (1938) in detecting semi-compatible combinations by this method) or by genotyping the seedlings when they flower.

The three self-compatible cultivars that were studied, having the allele S_4', were found to be homozygous for *Est-5*, as were 11 out of 13 cultivars with S_4. So none of these self-compatible cultivars is a suitable parent for allowing the detection of self-compatible seedlings using *Est-5* as a marker.

In another *Prunus* crop, almond, *Prunus dulcis*, DNA markers have recently been reported for *S*, along with a locus for the enzyme shikimic acid dehydrogenase, *Sdh-1* (Ballester et al. 1998). When analysed by PAGE, SDH was found to be monomorphic in 14 cultivars of *P. avium* (Bošković et al. 1997b), including the parents of the progenies in the present paper, and so linkage with *S* is not testable. It would be interesting to see if the RFLP markers for the *S* locus in almond co-segregate with the *S* locus in cherry.

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References

Arus P, Vargas FJ, Romero M (1992) Linkage analysis of isozyme genes in almond. Recueil des Communications au 8eme Colloque du GREMPA, Nimes 1990, pp 201–207

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

Batlle I, Alston FH, Evans KM (1995) The use of the isoenzymic marker gene *Got-1* in the recognition of incompatibility *S* alleles in apple. Theor Appl Genet 90:303–306

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

Bošković R, Tobutt KR (1998) Inheritance and linkage relationships of isoenzymes in two interspecific cherry progenies. Euphytica 103:273–286

Bošković R, Russell K, Tobutt KR (1997a) Inheritance of stylar ribonucleases in cherry progenies, and reassignment of incompatibility alleles to two incompatibility groups. Euphytica 95:221–228

Bošković R, Tobutt KR, Nicoll FJ (1997b) Inheritance of isoenzymes and their linkage relationships in two interspecific cherry progenies. Euphytica 93:129–143

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

Granger AR (1996) Inheritance and linkage of isozymes in sweet cherry (*Prunus avium* L.). Theor Appl Genet 93:426–430

Granger AR, Clarke GR, Jackson JF (1993) Sweet cherry cultivar identification by leaf isozyme polymorphism. Theor Appl Genet 86:458–464

Hemmat M, Weeden NF, Manganaris AG, Lawson DM (1994) Molecular marker linkage map for apple. J Hered 85:4–11

Jacobs JME, Van Eck HJ, Arens P, Verkerk-Bakker B, te Lintel Hekkert B, Bastiaanssen HJM, El-Kharbotly A, Pereira A, Jacobsen E, Stiekema WJ (1995) A genetic map of potato (*Solanum tuberosum*) integrating molecular markers, including transposons, and classical markers. Theor Appl Genet 91:289–300

Kobel F, Steinegger P, Anliker J (1938) Weitere Untersuchungen über die Befruchtungsverhältnisse der Kirschensorten. Landwirtschaftl J Schweiz 52:564–595

Leach CR (1988) Detection and estimation of linkage for a codominant structural gene locus linked to a gametophytic self-incompatibility locus. Theor Appl Genet 75:882–888

Manganaris AG, Alston FH (1987) Inheritance and linkage relationships of glutamate oxaloacetate transaminase isoenzymes in apple. 1. The gene *GOT-1*, a marker for the *S* incompatibility locus. Theor Appl Genet 74:154–161

Matthews P, Dow KP (1969) Incompatibility groups: sweet cherry (*Prunus avium*). In: Knight RL (ed), Abstract Bibliography of Fruit Breeding & Genetics to 1965. *Prunus*, Commonwealth Agricultural Bureaux, Farnham Royal, pp 540–544

Mau SL, Raff J, Clarke AE (1982) Isolation and partial characterisation of components of *Prunus avium* L. styles, including an antigenic glycoprotein associated with a self-incompatibility genotype. Planta 156:505–516

Santi F, Lemoine M (1990) Genetic markers for *Prunus avium* L: inheritance and linkage of isoenzyme loci. Ann Sci For 47:131–139

Vowden CJ, Ridout MS, Tobutt KR (1995) LINKEM: a program for genetic linkage analysis. J Hered 86:249–250