Theor Appl Genet (1996) 93:426–430

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Inheritance and linkage of isozymes in sweet cherry (*Prunus avium* L.)

Received: 5 February 1996 / Accepted: 9 February 1996

Abstract Eight polymorphic isozyme loci, 6PGD, G6PD, MDH, PGM, SKDH, FDP, GOT and IDH, in sweet cherry where found to be in one linkage group, with a ninth isozyme locus, GPI, being in another linkage group on a different chromosome. Isozymes were also linked to the incompatibility S locus and this explained the disturbed segregation ratios observed in the first generation from controlled hybridisations between different sweet cherry cultivars. Analysis revealed close linkage between the isozyme and S loci. The results supported a pre-existing theory that the S gene in cherry consists of three linked segments each coding for a different function. Progeny derived from selfing of Stella, the self-fertile cherry cultivar, also showed disturbed segregation ratios and an absence of homozygotes for the isozyme loci assayed. This demonstrated that co-dominant inheritance of the S alleles had not been effected by the self-fertile mutation.

Key words Sweet cherry · Isozymes · Linkage · Incompatibility

Introduction

The interpretation of isozyme banding patterns in the majority of laboratories relies on experience and on comparison with other related species which have previously undergone isozyme analysis (Kephart 1990). The interpretation and assignment of genotypes should be tested by hybridisation experiments, but this is not always possible. (Richardson et al. 1986). Tree crops are poorly suited to genetic studies because they are large, have a long generation time and an extended juvenile period. Consequently, there is limited knowledge of even simply inherited morphological traits as compared to cereal crops. Apples and peaches have been the subject of most genetic studies in tree crops, peach having more than 30 identified simply inherited characters. In comparison, Knight (1969) reports 11 plant, leaf, flower and fruit characteristics as simply inherited and likely to be under the control of a single gene in sweet chery. There is no data for the majority of tree fruit crops regarding the position of genes on chromosomes or linkage with other traits.

The quantification of isozyme inheritance through the examination of segregation ratios in the progeny of controlled crosses is necessary for verification of genotype assignment to isozyme loci. Such work will, in the future, underpin more rapid genetic gain in sweet cherry, especially if isozymes are found to be markers for commercially desirable characteristics.

Self-incompatibility results in the failure of the plants own pollen to grow through the stylar tissue of the mother plant. Crane and Brown (1937) assigned the S alleles to the self-incompatibility gene in sweet cherry. Pollen grains are haploid and carry only one S allele. If this allele is the same as one of those in the diploid tissue of the style, pollen tube growth is arrested. Disturbed segregation ratios observed in the isozyme genotypes of some species can be a result of linkage with the S locus.

Leach (1988) developed a mathematical calculation to estimate linkage between isozymes and a gametophytic incompatibility locus. With this approach, only those crosses that were 50% compatible could be used. This is because the effect of an isozyme allele being linked to the incompatibility locus is similar to that allele being linked to a recessive lethal gene in that, if a cross is fully compatible, there is no evidence or expression of self-incompatibility in the isozyme segregation ratios of the resultant progeny. If a cross is 50% incompatible and isozymes are linked to the incompatibility locus a deficiency of homozygous isozyme genotypes is observed.

Communicated by H. F. Linskens

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Materials and methods

Isozyme analysis as described by Granger et al. (1993) was conducted on the leaves and embryos of the first-generation progeny derived from a breeding program at Lenswood Horticultural Centre. Single-locus chi-square analysis for goodness-of-fit to expected segregation ratios was conducted for each cross. Contingency chi-square analyses were then used to test for independent assortment between jointly segregating loci. If significant deviation was detected, recombination fractions and their standard errors were calculated. Analyses were carried out using the computer program LINKAGE-1 (Suiter et al. 1983). The procedure was for comparison amongst the isozyme loci when parents were both fully and 50% compatible, because random assortment of genes at meiosis would not lead to the situation in which the S alleles over-rode all other linkages. Note, however, that for the analysis to detect linkage, at least one parent must be a double heterozygote for the two loci being examined; alternatively, each parent could have been heterozygous for one of the two loci.

For the estimation of recombination frequency, and its variance between the isozyme genes and an incompatibility gene, the methods of Leach (1988) were used. Van × Bing ($S_1S_3 \times S_3S_4$) and Stella × Stella ($S_4S_4' \times S_4S_4'$) were known to be 50% compatible and could be used in the analyses. The skewed segregation ratios of isozyme loci and the deficiency of homozygotes in the progeny of Black Douglas × Stella and Williams Favourite × Stella were also indicative of a 50% compatible cross and linkage to a gametophytic incompatibility locus (Leach 1988). While the S genotypes of Black Douglas and Williams Favourite were unknown, on the above evidence, it was assumed that a 50% cross had been made and the data was included in the analyses.

Results

In the event that the parents of a particular cross were homozygous for any of the isozyme loci, that locus was omitted from the analyses and does not appear in the results. Table 1 shows the segregation ratios found in progeny for each isozyme locus, the outcome of chi-

Table 1 Chi-square test for goodness-of-fit to expected Mendelian segregation ratios of individual loci

Locus	Parents	Parental genotypes	Progeny genotypes	Expected ratio	χ^2	*P*
G6PD	Black Douglas × Stella	ab × ab	42aa:49ab:12bb	1:2:1	17.72	0.000
GOT		bc × bc	2bb:94bc:0cc	1:2:1	88.25	0.000
6PGD		aa × ab	62aa:38ab	1:1	5.76	0.016
FDP		aa × ab	42aa:46ab	1:1	0.18	0.669
GPI		aa × ab	49aa:55ab	1:1	0.35	0.556
IDH		aa × ab	81aa:11ab	1:1	53.26	0.000
PGM		aa × ab	66aa:38ab	1:1	7.54	0.006
MDH		aa × ab	60aa:42ab	1:1	3.18	0.075
G6PD	Williams Favourite × Stella	ab × ab	46aa:80ab:40bb	1:2:1	0.65	0.722
GOT		bc × bc	0bb:83bc: 0cc	1:2:1	83.00	0.000
6PGD		ab × ab	56aa:81ab:29bb	1:2:1	8.88	0.012
FDP		aa × ab	70aa:66ab	1:1	0.12	0.731
GPI		aa × ab	103aa:64ab	1:1	9.11	0.003
IDH		aa × ab	119aa:40ab	1:1	39.25	0.000
PGM		ab × ab	86aa:83ab:0bb	1:2:1	87.58	0.000
SKDH		ab × aa	106aa:59ab	1:1	13.39	0.000
MDH		ab × ab	107aa:61ab:1bb	1:2:1	146.00	0.000
G6PD	Stella × Stella	ab × ab	51aa:139ab:0bb	1:2:1	68.14	0.000
GOT		bc × bc	2bb:185bc:0cc	1:2:1	179.13	0.000
6PGD		ab × ab	63aa:97ab:30bb	1:2:1	11.55	0.003
FDP		ab × ab	105aa:64ab:7bb	1:2:1	122.23	0.000
GPI		ab × ab	66aa:116ab:7bb	1:2:1	46.62	0.000
IDH		ab × ab	174aa:8ab:7bb	1:2:1	453.48	0.000
PGM		ab × ab	144aa:42ab:0bb	1:2:1	278.90	0.000
MDH		ab × ab	110aa:71ab:9bb	1:2:1	119.51	0.000
G6PD	Van × Bing	ab × ab	135aa:65ab:0bb	1:2:1	206.75	0.000
GOT		ac × bc	42ab:81ac:57bc:0cc	1:1:1:1	39.48	0.000
GPI		ab × ab	113aa:86ab:1bb	1:2:1	129.39	0.000
PGM		aa × ab	60aa:140ab	1:1	32.00	0.000
SKDH		ab × aa	56aa:144ab	1:1	38.72	0.000
MDH		ab × ab	18aa:182ab:0bb	1:2:1	137.72	0.000
6PGD	Van × Stella	aa × ab	149aa:11ab	1:1	119.02	0.000
G6PD		ab × ab	114aa:48ab:0bb	1:2:1	187.33	0.000
GOT		ac × bc	57ab:79ac:25bc:0cc	1:1:1:1	90.30	0.000
FDP		aa × ab	107aa:39ab	1:1	31.67	0.000
GPI		aa × ab	62aa:101ab	1:1	9.33	0.002
IDH		aa × ab	79aa:75ab	1:1	0.10	0.747
PGM		ab × ab	48aa:99ab:0bb	1:2:1	49.04	0.000
SKDH		ab × aa	50aa:63ab	1:1	1.50	0.221
MDH		ab × ab	50aa:99ab:0bb	1:2:1	49.67	0.000

square analysis for goodness-of-fit to expected Mendelian ratios, and its accompanying probability at the 0.05 level. Examination of Table 1 reveals that most of the chi-square *F*-tests were highly significant, leading to a rejection of the hypothesis that the observed and expected ratios were the same. For instance, in the first cross listed in Table 1, Black Douglas × Stella, both parents have the heterozygous bc genotype for the GOT isozyme and would be expected to produce progeny with bb, bc, and cc genotypes in the ratio of 1:2:1. Results show that no cc progeny were observed and only two bb homozygotes were recorded compared with 94 bc genotypes. Further examination of Table 1 shows the same result for all of the crosses listed. That is, the combination of parents heterozygous for the GOT c allele and the absence of the cc genotype in progeny. In contrast, some isozyme loci did display the expected segregation ratio; for instance, FDP in the progeny of Block Douglas × Stella and Williams Favourite × Stella crosses. Other families showed disturbed segregation ratios for FDP. Similar outcomes were observed for GPI, G6PD, IDH and SKDH. While the Van × Stella cross was completely compatible, most of the isozymes displayed disturbed segregation ratios.

Results of the chi-square test for independent assortment between jointly segregating loci have been tabulated in Table 2. While each pair of loci has been tested in contingency tables, only those showing significant departures have been included in Table 2, along with estimates of the recombination fraction and standard error.

Estimates of the recombination frequency between isozymes and the S gene of the self-incompatibility locus are shown in Table 3.

Discussion

Tests for independent assortment between the isozyme loci showed that 6PGD, G6PD, MDH, PGM, SKDH, FDP, GOT and IDH occur within one linkage group. GPI is linked to G6PD with a recombination frequency of 0.5, suggesting that it resides on another chromosome. Different families showed different recombination frequencies, which was expected because different individuals used as parents are expected to have different distances between the same genes and alleles along the DNA. In the case of PGM, which displays close linkage to most of the other isozymes (Table 2), caution needs to be exercised when interpreting these results because of the absence of the homozygous bb genotype. According to Kephart (1990) PGM phenotypes in many species are a result of the co-migration of two loci. Thus, for cherries the bb genotype may be present but indistinguishable because of banding from another PGM locus. In this situation the recombination frequency would be underestimated.

The isozymes examined are all involved in essential metabolic cycles, such as the citric-acid cycle, and so it seems reasonable that they are all inherited as one linkage group. In some instances there is an overlap between cycles where reaction products in one cycle may react in another. In this case linkage between isozymes in overlapping cycles would also be advantageous to the survival of the organism.

Most of the isozymes segregated into non-Mendelian ratios for the crosses examined. Only FDP, GPI and MDH in the progeny of Black Douglas × Stella crosses, and IDH and SKDH in the progeny of Van × Stella crosses (Table 1), showed agreement between expected and observed segregation ratios. The cross between Van and Stella was fully compatible ($S_1S_3 \times S_4S'_4$) and in this case, assuming inhibition of pollen tube growth did not occur, it was expected that all of the isozyme loci studied would exhibit the expected Mendelian ratios. This was not the case and may have been caused in part by the close linkages shown between the isozyme loci.

In the case of crosses with 50% compatibility, such as Stella × Stella ($S_4S'_4 \times S_4S'_4$) and Van × Bing ($S_1S_3 \times S_3S_4$), disturbed segregation ratios were both expected and observed, and as previously mentioned the basis of this is linkage to the self-compatibility locus. Linkage analysis as described by Leach (1988) showed that GOT, 6PGD, IDH, G6PD, GPI, FDP, PGM and MDH are all linked to the self-incompatibility gene (Table 3). In some cases the recombination frequency was estimated at zero suggesting that the isozymes were a functional part of the S gene, particularly since both characteristics show co-dominant inheritance. If this were so the radiation treatment of pollen to generate the S'_4 mutant should have produced a point mutation resulting in at least a single base change in the DNA sequence. This would have led to a change in the charge of the encoded isozyme which would have subsequently changed its migration distance in an electric field. The absence of changes in any of the isozyme phenotypes in samples from self-fertile cultivars tended not to support the role of isozymes as a functional part of the S gene; although it is possible in the case of a point mutation that a change in molecular charge did not occur. What the evidence does suggest is that the S gene occurs in at least two parts, because it is linked by an estimated recombination fraction of 0 and 0.096 to the G6PD and GPI loci, respectively, while those two loci were linked by a recombination fraction of 0.5. de Nettancourt (1977) describes a theory which was developed by Lewis in the 1960s and suggests that the incompatibility gene as found in monofactorial gametophytic systems, such as in cherries, consists of three linked segments. The three segments are thought to involve a specificity part of recognition for pollen and style S genes. The other two segments have been attributed to regulatory genes for pollen and stylar activity. The linkage relationships between isozymes and the S gene found here in sweet cherry support the multi-segment locus theory for the gametophytic self-incompatibility gene.

In apple, GOT and IDH have been identified as markers for the S gene and combined S and isozyme

Table 2 Probability and recombination values between loci observed to be linked in progeny from various controlled hybridisations within sweet cherry

Loci	Parents[a]	Parental genotype	Progeny	genotypes								P	r + SE
G6PD/MDH	B.D. × Stella	ab/aa × ab/ab	34aa/aa	8aa/ab	24ab/ab	1bb/aa	10bb/ab					0.000	0.1698 ± 0.0528
6PGD/FDP	B.D. × Stella	aa/aa × ab/ab	20aa/aa	31aa/ab	22ab/aa	11ab/ab						0.014	0.3691 ± 0.0527
FDP/PGM		aa/aa × ab/ab	31aa/aa	11aa/ab	24ab/aa	22ab/ab						0.036	0.3977 ± 0.0522
GPI/PGM		aa/aa × ab/ab	26aa/aa	23aa/ab	40ab/aa	15ab/ab						0.038	0.3944 ± 0.0479
IDH/MDH		aa/aa × ab/ab	52aa/aa	28aa/ab	3ab/aa	7ab/ab						0.032	0.3440 ± 0.0501
G6PD/FDP	W.F. × Stella	ab/aa × ab/ab	21aa/aa	14aa/ab	36ab/aa	28ab/ab	10bb/aa	24bb/ab				0.017	0.3477 ± 0.0584
G6PD/GPI		ab/aa × ab/ab	32aa/aa	14aa/ab	42ab/aa	38ab/ab	29bb/aa	11bb/ab				0.048	0.5000 ± 0.0549
G6PD/IDH		ab/aa × ab/ab	38aa/aa	4aa/ab	56ab/aa	20ab/ab	23bb/aa	15bb/ab				0.008	0.3375 ± 0.0535
6PGD/SKDH		ab/ab × ab/aa	36aa/aa	18aa/ab	58ab/aa	22ab/ab	12bb/aa	16bb/ab				0.017	0.3658 ± 0.0535
6PGD/MDH		ab/ab × ab/aa	55aa/aa	14aa/ab	1aa/bb	35ab/aa	31ab/ab	0ab/bb				0.003	0.3955 ± 0.0593
PGM/SKDH		ab/ab × ab/ab	64aa/aa	21aa/ab	42ab/aa	38ab/ab	0bb/aa	0bb/ab				0.009	0.2470 ± 0.0475
PGM/MDH		ab/ab × ab/ab	66aa/aa	19aa/ab	1aa/bb	41ab/aa	42ab/ab	0ab/bb	0bb/aa	0bb/ab	0bb/bb	0.004	0.1978 ± 0.0247
G6PD/FDP	Stella × Stella	ab/ab × ab/ab	34aa/aa	7aa/ab	0aa/bb	71ab/aa	57ab/ab	7ab/bb	0bb/aa	0bb/aa	0bb/bb	0.014	0.2865 ± 0.0298
G6PD/MDH		ab/ab × ab/ab	44aa/aa	7aa/ab	0aa/bb	66ab/aa	64ab/ab	9ab/bb	0bb/aa	0bb/ab	0bb/bb	0.000	0.2493 ± 0.0265
6PGD/IDH		ab/ab × ab/ab	53aa/aa	7aa/ab	3aa/bb	93ab/aa	1ab/bb	2ab/aa	28bb/aa	0bb/ab	2bb/bb	0.012	0.4360 ± 0.0355
FDP/PGM		ab/ab × ab/ab	88aa/aa	0aa/bb	41ab/aa	22ab/ab	0ab/bb	3bb/aa	4bb/ab	0bb/bb		0.009	0.1982 ± 0.0245
FDP/MDH		ab/ab × ab/ab	79aa/aa	25aa/ab	1aa/bb	19ab/aa	37ab/ab	8ab/bb	2bb/aa	5bb/ab	0bb/bb	0.000	0.1901 ± 0.0237
IDH/PGM		ab/ab × ab/ab	141aa/aa	29aa/ab	0aa/bb	0ab/aa	8ab/ab	0ab/bb	2bb/aa	5bb/ab	0bb/bb	0.000	0.1034 ± 0.0168
IDH/MDH		ab/ab × ab/ab	104aa/aa	63aa/ab	7aa/bb	1ab/aa	5ab/ab	2ab/bb	5bb/aa	2bb/ab	0bb/bb	0.016	0.2461 ± 0.0264
PGM/MDH		ab/ab × ab/ab	98aa/aa	41aa/ab	5aa/bb	12ab/aa	26ab/ab	4ab/bb	0bb/aa	0bb/ab	0bb/bb	0.000	0.1872 ± 0.0229
G6PD/GOT	Van × Bing	ab/ac × ab/bc	42aa/ab 46aa/ac	31aa/ac 16aa/ac	0ab/ab 35ab/ac	26ab/bc	4ab/cc	0bb/ab	0bb/ac	0bb/bc	0bb/cc	0.000	–[b]
GOT/PGM		ac/aa × bc/ab	6ab/aa	36ab/ab	25ac/aa	56ac/ab	15bc/aa	42bc/ab	14cc/aa	6cc/ab		0.000	–
GOT/SKDH		ac/ab × bc/aa	17ab/aa	25ab/ab	18ac/aa	63ac/ab	10bc/aa	47bc/ab	11cc/aa	9cc/ab		0.002	–
GPI/MDH		ab/ab × ab/ab	12aa/aa	101aa/ab	0aa/bb	5ab/aa	81ab/ab	0ab/bb	1bb/aa	0bb/ab	0bb/bb	0.021	0.3820 ± 0.0327
PGM/SKDH		aa/ab × ab/aa	32aa/aa	28aa/ab	24ab/aa	116ab/ab						0.000	0.2600 ± 0.031
PGM/MDH		aa/ab × ab/ab	11aa/aa	49aa/ab	0aa/ab	7ab/aa	133ab/ab	0ab/bb				0.010	0.3888 ± 0.0488
6PGD/G6PD	Van × Stella	aa/ab × ab/ab	113aa/aa	36aa/ab	0aa/bb	1ab/aa	10ab/ab	0ab/bb				0.000	0.0088 ± 0.0104
6PGD/GOT		aa/ac × ab/bc	48aa/ab	76aa/ac	25aa/bc	0aa/cc	8ab/ab	3ab/ac	0ab/ac	0ab/cc		0.05	–
6PGD/IDH		aa/aa × ab/ab	77aa/aa	66aa/ab	2ab/aa	9ab/ab						0.023	0.4415 ± 0.0400
G6PD/GOT		ab/ac × ab/bc	23aa/ab 72aa/ac	19aa/bc 0aa/cc	34ab/ab 7ab/ac	6ab/bc	0ab/cc	0bb/ab	0bb/ac	0bb/bc	0bb/cc	0.000	–
G6PD/FDP		ab/aa × ab/ab	61aa/aa	37aa/ab	45ab/aa	2ab/ab	0bb/aa	0bb/aa	0bb/ab			0.000	0.3775 ± 0.0569
G6PD/IDH		ab/aa × ab/ab	68aa/aa	40aa/ab	11ab/aa	35ab/ab	0bb/aa	0bb/ab				0.000	0.3703 ± 0.0550
G6PD/MDH		ab/ab × ab/ab	46aa/aa	55aa/ab	0aa/ab	4ab/aa	44ab/ab	0ab/bb	0bb/aa	0bb/ab	0bb/bb	0.000	0.2198 ± 0.0279
GOT/FDP		ac/aa × bc/ab	48ab/aa	7ab/ab	39ac/aa	29ac/ab	18bc/aa	3bc/ab	0cc/aa	0cc/ab		0.001	–
GOT/GPI		ac/aa × bc/ab	30ab/aa	27ab/ab	28ac/aa	51ac/ab	4bc/aa	21bc/ab	0cc/aa	0cc/ab		0.015	–
GOT/IDH		ac/aa × bc/ab	15ab/aa	39ab/ab	44ac/aa	33ac/ab	20bc/aa	3bc/ab	0cc/aa	0cc/ab		0.000	–
GOT/MDH		ac/ab × bc/ab	8ab/aa 44ab/ab	0ab/bb 25ac/aa	48ac/ab 0ac/bb	17bc/aa	6bc/ab	0bc/bb	0cc/aa	0cc/ab	0cc/bb	0.000	–
FDP/PGM		aa/ab × ab/ab	43aa/aa	48aa/ab	0aa/bb	3ab/aa	36ab/ab	0ab/bb				0.000	0.652 ± 0.0306
FDP/SKDH		aa/ab × ab/aa	8aa/aa	49aa/ab	33ab/aa	6ab/ab						0.000	0.1459 ± 0.0360
FDP/MDH		aa/ab × ab/ab	45aa/aa	54aa/ab	0aa/ab	1ab/aa	33ab/ab	0ab/bb				0.000	0.0217 ± 0.0179
IDH/PGM		aa/ab × ab/ab	40aa/aa	39aa/ab	0aa/bb	7ab/aa	55ab/ab	0ab/bb				0.000	0.1489 ± 0.0424
IDH/SKDH		aa/ab × ab/aa	22aa/aa	53aa/ab	27ab/aa	5ab/ab						0.000	0.2524 ± 0.042
IDH/MDH		aa/ab × ab/ab	50aa/aa	22aa/ab	0aa/bb	0ab/aa	75ab/ab	0ab/bb				0.000	0.000 ± n.d.
PGM/SKDH		ab/ab × ab/aa	5aa/aa	36aa/ab	45ab/aa	27ab/aa	0bb/aa	0bb/ab				0.000	0.1220 ± 0.0435
PGM/MDH		ab/ab × ab/ab	34aa/aa	7aa/ab	0aa/bb	16ab/aa	77ab/ab	0ab/bb	0bb/aa	0bb/ab	0bb/bb	0.000	0.0917 ± 0.0917
SKDH/MDH		ab/ab × aa/ab	0aa/aa	45aa/ab	0aa/ab	50ab/aa	5ab/ab	0ab/bb				0.000	0.0000 ± n.d.

[a] + Abbreviations for cultivar names: B.D. = Black Douglas, W.F. = Williams Favourite; [b] *Possible multiple parent linkage and unknown phase of alleles precludes estimation of r

Table 3 Crosses as defined by Leach (1988) between cultivars of known S and isozyme genotypes, observed ratios of isozymes, and the estimated recombination fraction and its variance

Cross[a]	Parents (♀ × ♂)	Isozyme	Progeny aa	Progeny ab	Progeny bb	Estimation of recombination frequency (r)	Variance (r)
Type 1	Van × Bing	PGM	60	140	–	0.3	0.001
Type 2[b]	Van × Bing	SKDH	56	144	–	–	–
Type 3	Van × Bing	G6PD	135	65	0	0.0	0.0
		GPI	113	86	1	0.009	0.0
		MDH	18	182	0	0.0	0.0
	Stella × Stella	G6PD	51	139	0	0.0	0.0
		GOT	2	185	0	0.0	0.0
		6PGD	63	97	30	0.32	0.002
		FDP	105	64	7	0.0625	0.007
		GPI	66	116	7	0.0959	0.0009
		IDH	174	8	7	0.0387	0.0004
		PGM	144	42	0	0.0	0.0
		MDH	110	71	9	0.0756	0.0007

[a] Refer to Table 1 Leach (1988) for a description of type of cross

[b] Note, for cross type 2 it is not possible to calculate estimates of the recombination frequency

genotypes have been proposed (Managanaris and Alston 1987). Data presented in the present paper has established close linkage between the GOT locus and the S locus in sweet cherry. Closer examination of segregation ratios in Table 1 for GOT shows an almost complete absence of homozygotes. This is expected with close linkage to the incompatibility locus because like-S alleles cannot combine to form homozygotes. In the case of Stella, which carries the self-fertile allele S'_4, it was expected that selfing would produce homozygous self-fertile ($S'_4 S'_4$) progeny and that this would be reflected by a greater number of progeny with homozygous GOT genotypes. The results in Table 1 show this was not so; that is, progeny from Stella × Stella crosses had an absence of homozygotes in the GOT isozyme data. Thus, just as S_4S_4 homozygotes were not formed under the gametophytic incompatibility system of cherry neither were $S'_4S'_4$ homozygotes. That is, the co-dominant inheritance of the S alleles has not been altered by the self-fertile mutation. Given this, the linkage genotypes of the S and GOT alleles are proposed as S_4b/S'_4c. There were a small number of GOT, bb homozygotes recorded (Table 1) and these are probably a result of crossing over between chromatids of homologous chromosomes resulting in the rearrangement of the linked alleles to give S_4b/S'_4b.

Following this proposal of linked genotypes it should be possible to use the GOT genotypes to determine the S genotypes of cherry cultivars. This can be tested by comparing the GOT genotypes of cultivars with known S alleles. For instance, in Table 1 Van is listed as having the ac GOT genotype and Bing the bc genotype. According to the initial proposal both Van and Bing should be carrying the S'_4 allele. In fact the S allele they have in common is S_3. This suggests that S'_4 is a mutation of the S_3 allele. To prove this beyond doubt, the S genotypes of the parents and progeny from controlled hybridisations would have to be determined by pollinating flowers with pollen of known S genotypes. Analyses to test for independent assortment between jointly segregating S and isozyme loci could then be conducted to determine linkage relationships.

References

Crane MB, Brown AG (1937) Incompatibility and sterility in the sweet cherry, (*Prunus avium L.*) J Pomol Hort Sci 15:86–116

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

Kephart SR (1990) Starch-gel electrophoresis of plant isozymes: a comparative analysis of techniques. Am J Bot 77:693–712

Knight RL (1969) Abstract bibliography of fruit breeding and genetics to 1965. *Prunus* technical communication No. 31. Commonwealth Agricultural Bureau, Bucks, England

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

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

Nettancourt D de (1977) Incompatibility in angiosperms. In: Frenkel R, Gall GAE, Linskens HF (eds) Monographs in theoretical and applied genetics vol 3. Springer-Verlag, Berlin Heidelberg New York

Richardson BJ, Baverstock PR, Adams M (1986) Allozyme electrophoresis. A handbook for animal systematics and population studies. Academic Press, Sydney, pp–144

Suiter KA, Wendel JF and Case SJ (1983) LINKAGE-1: a PASCAL computer program for the detection and analysis of genetic linkage. J Hered 74:203–204