Theor Appl Genet (1993) 86:458–464

THEORETICAL AND APPLIED GENETICS

© Springer-Verlag 1993

Sweet cherry cultivar identification by leaf isozyme polymorphism

A. R. Granger, G. R. Clarke, J. F. Jackson

Department of Horticulture, Viticulture and Oenology, Waite Agricultural Research Institute, University of Adelaide, Glen Osmond, South Australia 5064, Australia

Received: 9 September 1992 / Accepted: 19 November 1992

Abstract. Isozyme polymorphism can assist in the identification of cherry cultivars. Ten isozymes, each showing variation at only one locus, provide 70 unique genotype profiles from leaf extracts of 78 different sweet cherry cultivars. Polymorphism in 6PGD, G6PD, GPI, IDH, PGM, FDP, SKDH and PER is demonstrated for the first time, while observations are extended for the previously described polymorphisms in MDH and GOT. Some cultivars with identical morphological characters and previously treated as one cultivar can be separated on the basis of isozyme genotype. Irradiated mutants and parent material could also be differentiated.

Key words: Sweet cherry – Isozymes – Cultivar identification

Introduction

Cherry cultivar nomenclature in its present state shows many inconsistencies (Bowman 1937; Crane and Brown 1937). In Australia for example, bud sports and seedlings of certain cultivars, while genetically distinct, have been selected and propagated in commerical quantities under the same name as the parent material. A further complication to this situation is the gametophytic self-incompatibility system operating in sweet cherries (Crane and Brown 1937) which has resulted in like-named cultivars having different cross-pollination requirements. Hence, there are important management implications concerning the planting of incorrect pollinator cultivars that can lead to a significant reduction in commercial returns. Isozyme analysis offers a rapid and stable means of producing a genetic profile for horticultural crops (Pierce and Brewbaker 1973). Arulsekar and Parfitt (1986) used starch-gel electrophoresis to determine the banding patterns of 13 different isozyme systems in cherry. However, their results were not used to distinguish between cultivars. Malate dehydrogenase bands have been determined for sweet and sour cherries (Hancock and Iezzoni 1988) and ten enzyme systems were used to describe genetic variation and linkage amongst progeny from wild *Prunus avium* trees (Santi and Lemoine 1990) using polyacrylamide-gel electrophoresis and isoelectric focusing. The aim of the present study was to extend the number of useful isozymes for sweet cherry and to use isozyme banding patterns to discriminate between different cultivars.

Communicated by H. F. Linskens
Correspondence to: A. R. Granger

Materials and methods

Sample collection

Leaves of 78 different cherry (*P. avium* L.) cultivars were sampled from a collection held in South Australia at Lenswood Horticultural Centre (LHC) (34°57′S; 138°48′E; altitude 470 m), soil being a shallow silt, loam over yellow clay (Dy 5.21; Northcote 1979). Samples were also collected from nearby commerical orchards. Cherry cultivars in the LHC collection have been sourced as budwood from overseas germplasm repositories, breeding programs, or other government research station collections. After Australian quarantine procedures for virus testing of each budwood entry were complete, budwood was obtained directly from quarantine or after multiplication by other State Agricultural Departments. Table 1 lists those cultivars held in the LHC collection and sampled for isozyme analysis. Cultivars held on commerical orchards in close proximity to LHC and sampled for isozyme analysis had doubtful authenticity and sources of budwood were unknown. Table 2 lists those cultivars from dubious sources with their place of origin. The cultivars Beauchamps Black, Bedford Prolific, Early Rivers, Lustre, Na-

Table 1. Background of cherry cultivars of known origin

No.	Cultivar	Country of origin	Source	Reference
1	13S-18-15	Canada	Summerland Res Stn, B.C. Canada	Lane 1990, personal communication
2	13S-24-28	Canada	Summerland Res Stn, B.C. Canada	Lane 1990, personal communication
3	Basler Langstieler	Switzerland	Wädenswil, Switzerland	Krapf and Theiler 1973
4	Bedford Prolific (P)	England	Auckland, New Zealand	Grubb 1949
5	Bing 0B260	U.S.A.	IR2 Prosser, Washington U.S.A.	Hedrick 1915
6	Colt	England	Newton, Cambridge U.K.	McVittie 1984
7	Compact Lambert	Canada	IR2 Prosser, Washington U.S.A.	Lapins and Schmid 1972
8	Compact Stella	Canada	No Source Recorded	Lapins 1974a
9	Delta	Switzerland	Wädenswil, Switzerland	Schaer et al. 1975
10	Early Purple Guinge	Unknown	Auckland, New Zealand	Grubb 1949
11	Early Rivers (L)	England	Sidney, B.C. Canada	Grubb 1949
12	Hedelfingen	Germany	IR2 Prosser, Washington U.S.A.	Hedrick 1915
13	JI 10981	England	East Malling Res Stn, Maidstone U.K.	Matthews and Dow 1970
14	JI 11247	England	John Innes Res Inst, Norwich U.K.	Matthews and Dow 1974
15	JI 11376	England	John Innes Res Inst, Norwich U.K.	Matthews and Dow 1973
16	JI 11610	England	John Innes Res Inst, Norwich U.K.	Matthews and Dow 1975
17	JI 12526	England	John Innes Res Inst, Norwich U.K.	Matthews and Dow 1975
18	JI 14007	England	East Malling Res Stn, Maidstone U.K.	Matthews and Dow 1971
19	JI 11253	England	John Innes Res Inst, Norwich U.K.	Matthews and Dow 1970
20	Krasarica	Poland	Sidney, B.C. Canada	
21	Lambert	U.S.A.	No Source Recorded	Hedrick 1915
22	Lapins	Canada	Sidney, B.C. Canada	Lane and Schmid 1984
23	Larian	U.S.A.	IR2 Prosser, Washington U.S.A.	Hedrick 1915
24	Lyons	France	IR2 Prosser, Washington U.S.A.	Grubb 1949
25	Magda	Switzerland	Wädenswil, Switzerland	Schaer 1973
26	Merton Bigarreau	England	No Source Recorded	Knight 1969
27	Merton Crane	England	Brogdale, Faversham, Kent U.K.	Knight 1969
28	Merton Glory	England	Brogdale, Faversham, Kent U.K.	Knight 1969
29	Mora Di Vignola	Italy	Borgioni, Verona, Italy	
30	Noir De Guben	England	Auckland, New Zealand	Grubb 1949
31	Nordwunder	Switzerland	Wädenswil, Switzerland	Boucher 1991
32	NY3308	U.S.A.	Geneva, New York, U.S.A.	Brown et al. 1989
33	Rainier	U.S.A.	No Source Recorded	Knight 1969
34	Salmo	Canada	IR2 Prosser, Washington U.S.A.	Brooks and Olmo 1971
35	Sam	Canada	No Source Recorded	Knight 1969
36	Schauenburger	Switzerland	Wädenswil, Switzerland	Theiler-Hedtrich 1985
37	Schnieders Späte Knorpelkirsche	Germany	IR2 Prosser, Washington U.S.A.	Hedrick 1915
38	Seneca	U.S.A.	IR2 Prosser, Washington U.S.A.	Knight 1969
39	Star	U.S.A.	Wädenswil, Switzerland	Knight 1969
40	Stella	Canada	IR2 Prosser, Washington U.S.A.	Lapins 1971
41	Sue	Canada	IR2 Prosser, Washington U.S.A.	Knight 1969
42	Summit	Canada	Vineland Res Stn, Ontario, Canada	Lapins 1974
43	Sunburst	Canada	Summerland Res Stn, B.C. Canada	Lane and Schmid 1984
44	Ulster	U.S.A.	IR2 Prosser, Washington U.S.A.	Knight 1969
45	V69061	Canada	Vineland Res Stn, Ontario Canada	Button 1982
46	Van	Canada	No Source Recorded	Knight 1969
47	Vega	Canada	IR2 Prosser, Washington U.S.A.	Tehrani and Dickson 1967
48	Venus	Canada	No Source Recorded	Knight 1969
49	Vernon	Canada	Sidney, B.C. Canada	Knight 1969
50	Vic	Canada	IR2 Prosser, Washington U.S.A.	Knight 1969
51	Victor	Canada	IR2 Prosser, Washington U.S.A.	Knight 1969
52	Vista	Canada	IR2 Prosser, Washington U.S.A.	Knight 1969
53	Vittoria	Italy	Borgioni, Verona, Italy	Bargioni 1979

poleon, Waterloo and Williams Favourite were sampled from more than one location. Cultivars in Table 2 in all cases have unknown parentage; furthermore the source of budwood of cultivars listed in Table 2 is not documented, nor are, the details of entry into South Australia for those cultivars originating outside of the State. Since Waterloo in South Australia is a white cherry with colourless juice, whereas Grubb (1949) describes Waterloo as a dark cherry with coloured juice, it is thought that the white cherry cultivar referred to as Waterloo in South Australia is in fact Florence (W.N. Bishop, personal communication). Coincidently, Florence and Waterloo budwood were brought to South Australia at the same time and incorrect la-

Table 2. Cherry cultivars from unknown sources

No.	Cultivar	Place of origin
54	Beauchamps Black (B)	Sth. Aust., Australia
55	Beauchamps Black (G)	Sth. Aust., Australia
56	Bedford Prolific (B)	Kent, U.K.
57	Black Douglas Mother	Sth. Aust., Australia
58	Black Tartarian	Russia
59	Blackboy	N.S.W., Australia
60	Burgsdof	Victoria, Australia
61	Clements Pride	Sth. Aust., Australia
62	Early Rivers (B)	Kent, U.K.
63	Hendersons Bedford	Victoria, Australia
64	Lustre (B)	Sth. Aust., Australia
65	Lustre (L)	Sth. Aust., Australia
66	Lustre (P)	Sth. Aust., Australia
67	Napoleon	Unknown
68	Opal	Sth. Aust., Australia
69	Oregon	Unknown
70	St Margaret	Buckinghamshire, U.K.
71	Up-To-Date	Sth. Aust., Australia
72	Waterloo (B)[a]	Italy
73	Waterloo (L)	Italy
74	Williams Favourite (B)	Sth. Aust., Australia
75	Williams Favourite (G)	Sth. Aust., Australia
76	Williams Favourite (L)	Sth. Aust., Australia
77	Williams Favourite (M)	Sth. Aust., Australia
78	Williams Favourite (M) Mother	Sth. Aust., Australia

[a] Waterloo synonymous to Florence
Different locations are indicated by a letter in parentheses following the cultivar name as follows; (B); Mr W Bishop, Basket Range, S. A.; (G): Mr K Green, Lenswood, S. A.; (L): L.H.C., Lenswood, S. A.; (M): Mr R Meiglich, Lenswood, S. A.; (P): Mr David Pike, Neerim Junction, Vic. The word Mother following a cultivar name indicates that the tree sampled was the original seedling selection of that particular cultivar

belling probably occurred. It should also be noted that in no other Australian cherry-growing State is there a white cherry cultivar known as Waterloo, but Florence is widely grown.

Protein extraction

Leaves from the tips of extending shoots were collecting from one or two trees of each cultivar on cool days, with a maximum temperature not exceeding 25 °C. Leaves were placed in labelled plastic bags and immediately stowed on ice in an insulated container. Leaves were then transported to the laboratory and extracted immediately; when this was not possible, leaves were held at 2 °C for up to 7 days prior to extraction. Four hundred milligrams of leaf tissue, 150 mg of polyvinyl polypyrrolodine and 2 ml of tris-citrate extraction buffer pH 8.0 (Aruleskar and Parfitt 1986) were ground in a motar and pestle at room temperature. Samples were then centrifuged for 15 min (3,000 g) and the supernatant used for electrophoresis. Pollen, styles, fruit flesh and fruit stalks from several sweet cherry cultivars were also extracted for isozyme analysis. In addition, leaf extracts from old and young trees and those from field-grown and glasshouse-grown trees were compared.

Electrophoresis

Sheets of cellulose acetate 30 cm × 30 cm and 200 μm thick (Chemetron) were cut down to 15 cm × 30 cm gels, or smaller test strips as required. Gels were supported in perspex boxes (Richardson et al. 1986) divided into two compartments and furnished with platinum wire for electrodes. Electrophoresis was conducted at 2 °C at a constant voltage of 200 d.c. for 1.5–2 h. Extracts to be examined for 6 PGD, GOT, G6PD and PER were subject to electrophoresis in 0.02 M phosphate buffer pH 7.0. For PGM, electrophoresis was carried out in 0.025 M tris-glyceine buffer pH 8.5 and for GPI, IDH, FDP, SKDH and MDH in 0.05 M pH 7.8 tris-maleate buffer (Richardson et al. 1986).

Staining

Staining protocols for 6PGD, GOT, G6PD, GPI, IDH, PGM and MDH were according to Richardson et al. (1986), FDP and SKDH activities were revealed using a protocol adapted from starch-gel electrophoresis described by Soltis et al. (1983), PER was also adapted from starch-gel methodology (Ashari et al. 1989) as was LAP (Arulsekar and Parfitt 1986). Stained gels were incubated in an oven at 37 °C and photocopies made as the isozymes developed. Scoring the resultant banding patterns was carried out by adopting the basic principles established for vertebrate animals (Richardson et al. 1986) and using the nomenclature developed for almond genotypes (Jackson and Clarke 1991; Jackson 1992).

Results

Twenty eight different isozyme staining protocols were examined for activity, resolution, and variability, in cherry leaf extracts. The ten selected which showed useful polymorphism were: 6-phosphogluconate dehydrogenase (6PGD), glutamate oxaloacetate transminase (GOT), glucose-6-phosphate dehydrogenase (G6PD) glucose phosphate isomerase (GPI), isocitrate deyhdrogenase (IDH), phosphoglucomutase (PGM), fructose-1, 6-diphosphatase (FDP), shikimate dehydrogenase (SKDH), peroxidase (PER) and malate deyhdrogenase (MDH). Pollen extracts showed no activity for GPI, IDH, SKDH or PER but did show activity for two additional isozymes, Xanthine Oxidase (XO) and Adenylate Kinase (AK). XO was not detected in leaves, while testing for AK produced weak staining and uninterpretable bands for some leaf extracts. Style extracts did not show activity for IDH, PER or FDP. Fruit and fruit stalk extracts did not produce resolvable isozyme activity. Leaf age and time of year were critical in obtaining adequate isozyme patterns. Youngest leaves at the apex of actively growing shoots were best, while older, fully expanded, leaves, which had much higher protein contents, produced poor results due primarily we believe to the presence of polyphenols. Time of year was critical because as fruit maturity advanced, zymograms of leaf extracts showed adequate activity, but were smeared. All observed isozyme patterns and their genetic segregation for the ten enyzme systems studied are represented diagrammatically in Fig. 1.

The ten isozymes analysed resulted in the assignment of 70 different cherry genotypes within the 78 cultivars sampled (Table 3). Genotyps have been computer-sorted

Table 3. Leaf isozyme genotypes of cherry cultivars

Isozyme	Genotype	Cultivars with genotype[a]
6PGD	aa	4,5,9,13,14,18,20,24,25,26,28,29,30,31,32,33,37,45,46,47,49,55,56,57,58,59,61,63,64,66,68,69,71,73
	ab	1,2,3,6,7,8,10,11,12,15,16,17,19,22,23,27,34,36,38,39,40,42,43,50,51,52,53,54,60,62,67,70,72,74,75,76,77,78
	bb	21,35,41,44,48,65
GOT	ab	11,15,19,26,32
	ac	2,6,13,14,16,17,22,24,25,27,28,30,31,34,37,38,39,43,46,49,51,52,53,54,58,59,60,61,62,63,69,70,71,72,74,75,76,77,78
	bb	10
	bc	1,3,4,5,7,8,9,12,18,20,21,23,29,33,35,36,40,41,42,44,45,47,48,50,54,55,56,57,64,66,67,68,73
G6PD	aa	8,12,16,17,43,52,58,59,70
	ab	1,2,3,4,5,6,7,9,10,11,13,14,15,18,19,20,21,22,23,24,25,26,27,28,29,30,32,33,34,35,36,37,38,39,40,41,42,44,45,46,47, 48,49,50,51,53,54,55,56,57,60,61,62,63,64,65,66,67,68,69,71,72,73,75,77,78
	bb	31,74,76
GPI	aa	3,4,6,13,14,17,18,19,20,24,25,26,27,29,30,31,32,33,35,36,37,38,41,42,44,45,46,47,49,50,51,53,54,55,56,57,58,60,61, 62,63,64,65,66,68,70,71,72,73,74,75,76,77,78
	ab	1,2,5,7,8,9,10,11,12,15,16,21,22,23,28,34,39,40,43,48,52,59,67,69
IDH	aa	1,2,3,4,5,7,8,9,10,11,12,13,14,15,16,17,18,19,21,22,23,26,27,28,29,30,31,32,33,34,35,36,37,39,41,42,43,44,45,46,47, 48,49,50,51,52,53,54,55,56,57,58,59,60,64,65,67,68,69,71,72,73,74,75,76,77,78
	ab	25,38,40,62,63,66
	fast ab	6
	bc	20,24,61,70
PGM	aa	7,8,12,16,17,20,25,30,36,39,31,45,51,52,54,55,57,64,65,69,70,72,74,75,78
	aa	1,2,3,4,5,6,9,10,11,13,14,15,18,19,21,22,23,24,26,27,28,29,31,32,33,34,35,37,38,40,42,43,44,46,47,48,49,50,53,56,58, 59,60,61,62,63,66,67,68,71,73,76,77
FDP	aa	4,5,6,8,12,17,21,23,33,34,46,50,52,54,55,57,58,59,63,64,72,75,77,78
	ab	7,9,11,13,16,18,19,22,26,27,29,30,32,35,37,39,40,41,43,44,45,47,48,51,53,56,60,61,62,65,66,67,69,71,73,74,76
	bb	1,2,3,10,14,15,20,24,25,28,31,36,38,42,49,68,70
SKDH	aa	2,5,8,9,12,13,14,15,16,17,18,19,20,22,23,26,27,28,29,33,34,36,37,38,40,41,44,45,47,49,50,51,52,53,54,55,57,60,61, 62,63,64,66,67,68,69,70,71,72,73,74,75,76,77,78
	ab	1,3,4,7,10,11,21,24,30,32,35,39,43,46,48,58,59,65
	ac	6
	bb	25,31,56
PER	aa	2,3,4,6,9,11,12,16,21,24,32,34,35,40,41,42,43,53,54,55,57,59,61,64,68,69,71,75,77
	ab	1,5,7,8,10,13,14,15,17,18,19,20,22,23,25,26,27,28,30,31,33,36,37,38,39,44,45,46,47,48,49,51,52,56,58,60,62,63,66, 67,70,72,73,74,76,78
	ac	29,50
MDH	aa	7,12,16,17,20,30,36,45,48,54,57,59,68,69,70,72,75,78
	ab	1,2,3,4,5,6,8,9,10,11,13,14,15,18,19,21,22,23,24,25,26,27,28,29,31,32,33,34,35,37,38,39,40,41,42,43,44,46,47,49,50, 51,52,53,55,56,58,60,61,63,64,65,66,67,71,73,76,77
	bb	62,74

[a] Numbers correspond to those assigned to cultivars in Tables 1 and 2

in descending order, one isozyme at a time. Thus for 6PGD there are three main groups, those cultivars falling into aa, ab or bb genotypes. Next, each group was sorted for GOT genotypes and so on. The overall results from this data manipulation in terms of separate and identifiable cultivar groups was 6PGD = 3 groups; + GOT = 8 groups; + G6PD = 14 groups; + GPI = 21 groups; + IDH = 29 groups; + PGM = 41 groups; + FDP = 58 groups + SKDH = 65 groups; + PER = 69 groups; + MDH = 70 groups. Those cultivars which shared the same isozyme genotype at all ten loci were JI 10981 and Schneiders Späte Knopelkirsche; JI 11247 and Vernon; Beauchamps Black (G) and Lustre (B); JI 14007, Waterloo (L) and Vega; Beauchamps Black (B) and Williams Favourite (G); Waterloo (B) and Williams Favourite (M) Mother; Burgsdorf and Merton Crane. Those cultivars sampled

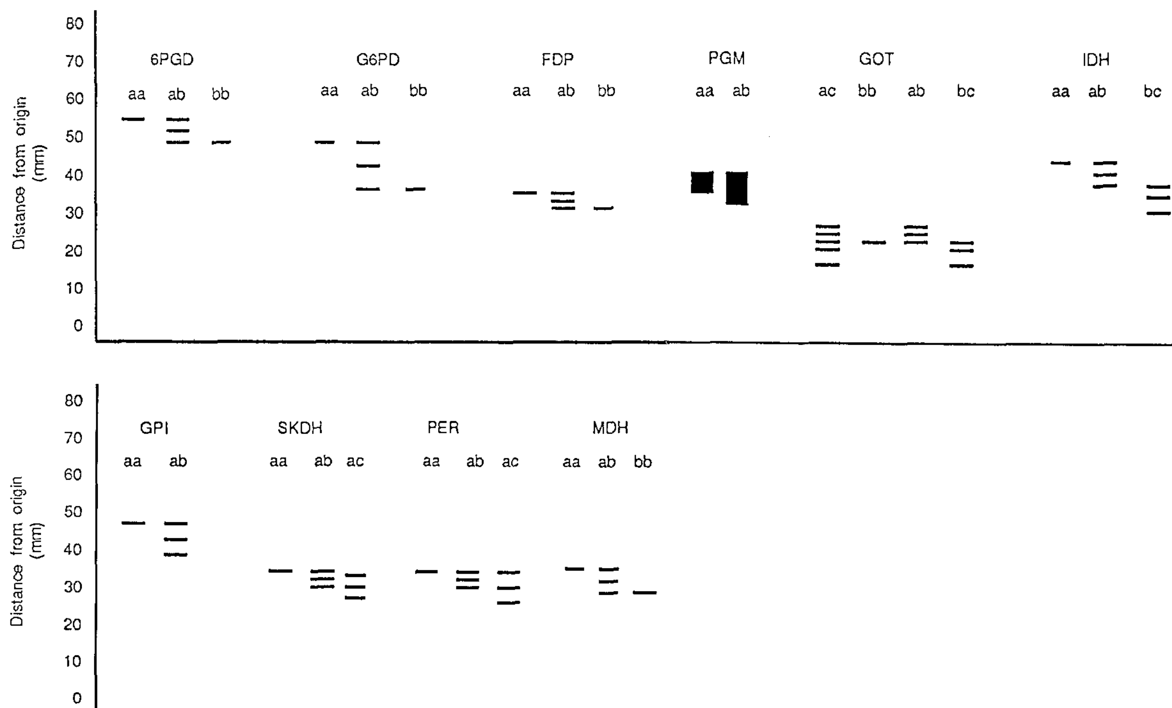

Fig. 1. Diagrammatic representation of isozyme banding patterns. *Capital letters* denote isozyme. *Lower case letters* represent genotypes

from more than one source and having more than one genotype as determined from banding patterns were Williams Favourite, Lustre, Beauchamps Black, Bedford Prolific and Early Rivers. Early Rivers (B) and (L) were separated by differences at six loci. Samples of Bedford Prolific and Beauchamps Black were separated by differences at three loci.

For some of the cultivars investigated, the parentage is fully documented; these present us with an opportunity to test the genotype assignments further. Thus Lapins, bred from Stella × Van (Lane and Schmid 1984), was found to have a genotype at all ten loci which is fully consistent with the expected outcome of a cross between these two parents (see Table 3 for genotype assignment). Similarly, Rainer (Knight 1969), Salmo (Brooks and Almo 1971), Summit (Lapins 1974b), Sunburst (Lane and Schmid 1984), Vega (Tehrani and Dickson 1967) and V69061 (Button 1982) all show genotypes at the ten loci which are fully consistent with the known parentage.

Discussion

In the majority of cases identification of a cultivar requires the use of all ten isozymes, although there were instances of a single cultivar having a unique genotype. Early Purple Guigne was the only cultivar to show a bb banding pattern for GOT. Colt samples produced two unique banding patterns, being assigned fast ab for IDH and ac for SKDH (Table 3). Colt is an interspecific hybrid between *P. avium* and *P. pseudocerasus* used as a rootstock for sweet cherries (McVittie 1984). The two unique genotypes it produced were probably incorporated from the *P. pseudocerasus* genome because they were not detected in any other *P. avium* sampled. This means a single test could be used to identify Early Purple Guigne and Colt within this sample group. Similarly, other genotypes associated with only 2–4 cultivars can reduce the number of tests required. For example, Mora di Vignola and Vic were the only cultivars to show the ac banding pattern for PER. Additional isozymes showing polymorphism at more than one locus would be advantageous and could reduce the need to run 8–10 isozyme systems. Banding patterns for GOT (Fig. 1) at one polymorphic locus were consistent with those observed by Santi and Lemoine (1990) on polyacrylamide gels. In particular, these authors also resolved five-banded heterozygotes and postulated that this was probably a result of duplication at that locus. Kephart (1990) reports GOT as having a dimeric structure in plants. This suggests that GOT, in cherries, is a dimer with three different sub-units each coded for by a different allele at one gene locus. MDH was studied in detail by Hancock and Iezzoni (1988) using starch-gel electrophoresis. They found two loci operating in sweet cherry leaves producing monomorphic three-banded patterns. Pollen extracts displayed an additional four bands, probably controlled by one locus and cathodal to the same three-banded pattern found in leaves, and corre-

sponding to the MDH locus of leaves described in this study.

These results also show that different genotypes do occur within what, up to now, we have regarded as a particular cultivar. Using traditional means of identification, such as fruit juice colour, fruit maturity, flowering time and other morphological characters, what we know now as genetically different plants were previously grouped under the same cultivar name. For example, Table 2 lists five Williams Favourite cultivars each of which we have found to have a different genotype at one or other of the ten loci. Ashari et al. (1989) measured genetic relatedness between mandarin types by comparing the number of loci at which the isozyme patterns differed. The greater the number of differences the weaker the genetic relationship. Applying this general rule to cherries shows that Early Rivers, Bedford Prolific, Beauchamps Black and Lustre all had differences at three or more isozyme loci. This amount of variation suggests that the origin of these cultivars could only have been through hybridisation or incorrect labelling of propagation material rather than, for example, by simple mutation. Similarly, Lustre (B) and (L) sourced from South Australia have a somewhat closer relationship to one another than with Lustre (P) imported from another state, Victoria. One morphological characteristic for which sweet cherry cultivars can be grouped without doubt is juice colour (Grubb 1949). No support has been provided by isoyzme analysis for the use of this method of horticultural grouping as a means of classifying sweet cherry cultivars into sub-species. Only a unique isozyme genotype for at least one locus, as occurred with Colt, would warrant this, Weeden and Lamb (1985) could not differentiate between sports of some apple cultivars and the original cultivar, and this may indicate that those like-named cultivars with different genotypes are more likely to be chance seedlings. The spur type cherries Compact Stella and Compact Lambert, generated by the selection of mutants from irradiated budwood of the conventional cultivars (Lapins and Schmid 1972; Lapins 1974a), have several isozyme differences between parent and progeny material. This indicates that genetic mutants or sports of sweet cherry, in contrast to apples, can be distinguished with the isozyme variation available.

Comparing the known parental genotypes with that of progeny cultivars provides a good internal check of results (Weeden and Lamb 1985). The parentage of Lapins, Rainier, Salmo, Summit, Sunburst, Vega and V69061 have been confirmed as those reported in the literature. And this supports the assumption that segregation at several codominant loci was responsible for the variation in cherry leaf isozyme banding patterns. Smearing of zymograms, observed with advancement of the season, could well have been caused by the increased production of large-molecular-weight polysaccharides during this phase. Such molecules have been detected as xylar embolisms in sweet cherry during the fruit maturation phase (Lasko, personal communication 1990).

Acknowledgements. The authors thank Messrs. W. Bishop, K. Green and R. Meiglich for supply of plant material. Also Ms. D. Shuttleworth for the provision of records regarding cherries imported through Australian quarantine.

References

Arulsekar S, Parfitt DE (1986) Isozyme analysis procedures for stone fruits, almond, grape, walnut, pistachio and fig. Hort Science 21:928–933

Ashari A, Aspinall D and Sedgley M (1989) Identification and investigation of relationships of mandarin types using isozyme analysis. Scientia Horticulturae 40, 305–315

Bargioni G (1979) La ciliegia "Vittoria" caratteristiche pomologiche e producttive informatore. Agrario 35:6289–6291

Bowman FT (1937) Cherry pollination and variety investigations in New South Wales 1930–34. Depth Agric NSW Sci Bull 55:5–49

Brown SK, Way RD, Terry DE (1989) Sweet and Tart cherry varieties: descriptions and cultural recommendations. New York Food Life Sci Bull no 127

Boucher WD (1991) Sweet cherry trial results – 1990/91 growing season. Stone and Berryfruits Newslett, DPI Tasmania no 2:10–12

Brooks RM, Olmo HP (1971) Register of new fruit and nut varieties. List 26. Hort Science 5:441

Button T (1982) Sweet cherries bred for self-fertility. Can Fruit Grower Sept 1982:9–10

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

Grubb NH (1949) Cherries. Crosby Lockwood and Son Ltd, London

Hancock AM, Iezzoni AZ (1988) Malate dehydrogenase isozyme patterns in seven *Prunus* species. Hort Science 23:381–383

Hedrick UP (1915) The cherries of New York. Rep NY St Exp Stn 1914, pt 2

Jackson JF, (1992) Genotype determination in almond nuts for paternity analysis Mod Methods Plant Anal 14:1–16 Springer, Berlin Heidelberg New York Tokyo

Jackson JF, Clarke GR (1991) Gene flow in an almond orchard. Theor. Appl Genet 82:169–173

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* Tech Commun no 31. Commonwealth Agricultural Bureau, Bucks, England

Krapf B, Theiler R (1973) Basler Langstieler and excellent pollinator cultivar in sweet cherry orchards. Schweiz Z Obst Weinbau 109:608–611

Lane WD, Schmid H (1984) Lapins and Sunburst sweet cherry. Can J Plant Sci 64:211–214

Lapins KO (1971) Stella, a self-fruitful sweet cherry. Can J Plant Sci 51:252–253

Lapins KO (1974a) Compact Stella sweet cherry. Can J Plant Sci 54:849–850

Lapins KO (1974b) Summit Sweet Cherry. Can J Plant Sci 54: 851

Lapins KO, Schmid H (1972) New fruits from Summerland, British Columbia 1956–1960. Publ Can Dept Agric no 1471

Matthews P, Dow KP (1970) Breeding for resistance to bacterial canker in cherries. John Innes Inst 61st Annu Rep. F. Crowe and Sons. Norwich, UK, pp 21–23

Matthews P, Dow KP (1971) Breeding for resistance to bacterial canker in cherries. John Innes Inst 62nd Annu Rep. F. Crower and Sons Norwich, UK, pp 24–25

Matthews P, Dow KP (1973) Breeding for resistance to bacterial canker in cherries. John Innes Inst 64th Annu Rep. F. Crower and Sons Norwich, UK, pp 18–21

Matthews P, Dow KP (1974) Breeding for resistance to bacterial canker in cherries. John Innes Inst 65th Annu Rep. F. Crower and Sons Norwich, UK, p 18

Matthews P, Dow KP (1975) Breeding for resistance to bacterial canker in cherries. John Innes Inst 66th Annu Rep. F. Crower and Sons Norwich, UK, p 17

McVittie AC (1984) Cherry rootstocks Cob and Colt. Rep E. Malling Stn 1983 Maidstone, Kent. Headly Bros, Invicta Press, London

Northcote KM (1979) A factual key for the recognition of Australian soils, 4th edn. Rellim Techn Pub, Glenside, S. Australia

Pierce LC, Brewbaker JL (1973) Applications of isozyme analysis in horticultural science. Hort Science 8:17–22

Richardson BJ, Baverstock PR, Adams M (1986) Allozyme electrophoresis. A handbook for animal systematics and population studies. Academic Press, Sydney, pp 99–144

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

Schaer E (1973) The cherry variety Magda. Schweiz Z Obst-Weinbau 109:90–92

Schaer E, Krapf B, Theiler R (1975) The new processing cherry cultivars Gamma and Delta raised at the federal research station, Wädenswil. Schweiz Z Obst Weinbau 111:83–87

Soltis DE, Haufler CH, Darrow DC, Gastony GJ (1983) Starch-gel electrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers and staining schedules. Am Fern J 73:9–27

Tehrani G, Dickson GH (1967) Vega and Valera sweet and cherries. Hort Res Inst Ontario Rep 1967, p 58

Thieler-Hedtrich R (1985) Sweet cherry breeding programme at the swiss federal research station. I. Results of fruit characters and flowering period inheritance. Acta Hort 169:51–62

Weeden NF, Lamb RC (1985) Identification of apple cultivars by isozyme phenotypes. J Am Soc Hort Sci 110:509–515