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ENZYME POLYMORPHISMS AS GENETIC MARKERS IN THE AVOCADO[1]

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ABSTRACT

Cultivated varieties (cvs) of the avocado (*Persea americana*) originate by selfing, hybridization or chance selections, and are then propagated by grafting. The mesocarps of a given cv are consequently all genetically identical. Mesocarp gene-enzyme systems were examined by starch gel electrophoresis and were found to be highly polymorphic. Alcohol dehydrogenase, phosphoglucomutase, glutamic-oxaloacetic transaminase and leucine aminopeptidase are coded by 10 genes with 20 condominant alleles. 'Seedling' populations have been produced by selfing or by hybridizing cvs. Seedlings were found to segregate in normal Mendelian fashion for any gene for which the parent or parents were heterozygous. No isozyme evidence contradicted the reported parentage of several cvs of hybrid or selfed origin. Outcrossing, as indicated by the presence of a non-parental isozyme, was detected among the seedlings of a supposed selfed population. To our knowledge, this is the first report of molecular genetics and of the genetics of any single-gene character for the avocado. Possible applications are discussed.

MULTIPLE molecular forms of enzymes (isozymes) have been found by electrophoretic methods in nearly every organism studied (Brewer, 1970; Latner and Skillen, 1968; Lewontin, 1974). The number of isozymes detectable depends on several factors such as the number of genes coding for the enzyme, the number of alleles of each gene which specify electrophoretically distinct polypeptides, the quaternary structure of the enzyme and the extent to which the subunits of polymeric enzymes can cross-multimerize as intergenic isozymes (Torres, 1974b, 1976). In addition, the zymogram may be supplemented by the presence of secondary bands caused by complexes with cofactors or other ions, conformers or even incompletely transcribed or translated polypeptides (Kaplan, 1968; Schwartz, 1975). Isozymes have been widely used as markers in systematic, genetic and evolutionary studies. Most genetic studies have, of course, employed micro-organisms, short-lived animals and annual plants. Long-lived animals and perennial plants are generally not favorable genetic tools and have been the subject of relatively few investigations.

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The avocado is a long-lived tree of tropical American origin. Three races of *P. americana* are recognized: Mexican, Guatemalan, and West Indian. They can be distinguished by combinations of various morphological characteristics (Bergh, 1975; Ruehle, 1963). The named cultivars which form the basis of the avocado industry have been developed by selection from the races and, sometimes, by chance or controlled hybridization between races and cvs. The 500-plus cvs each originated from a single tree bearing fruits of desired characteristics which was subsequently propagated vegetatively by grafting (Ruehle, 1963). Consequently, each and every tree of a given cv (excluding seed tissues), regardless of origin, should be genetically identical to every other. The identity of one or both parents of many cvs is unknown partly because of the avocado's peculiar breeding system which promotes outcrossing (synchronous dichogamy) and partly because fruit set from attempted hand pollinations is very low (Knight, 1971; Stout, 1924). Nevertheless, selfing is possible with isolation plots or by enclosing a tree and beehive within a screen structure (Bergh, 1975). The seeds resulting from self-pollination are then grown into a "selfed seedling" population. The mesocarps of the seedling trees, like the seeds which gave rise to them, are recombinant products with identical genomes; again, all mesocarps of a single tree are genetically identical. Therefore, if the selfed cv is heterozygous for any analyzable gene, the mesocarps of its seedling trees should show segregation for that gene.

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The mesocarp is not the ideal tissue for genetic studies because, although diploid, it is exclusively of maternal origin and not a sexual product. The seed would be preferred for genetic study and no doubt has enzyme systems comparable to those of others that have been examined. However, avocado seeds contain large amounts of terpenes, quinones, phenols and tannins (Loomis, 1973) which apparently interfere with enzymatic assays once the cells are ruptured to obtain extracts. Perhaps for these reasons, and because of the generation length, there have been very few genetic studies on the avocado. As recently as 1975, Bergh, in his extensive review of the avocado, wrote that "not one single-gene character is known in the avocado . . . ."

The avocado fruit, particularly the pulpy mesocarp, contains an extensive array of active enzymes. Examples are those involved in the glycolytic and tricarboxylic pathways and in the formation of lipids which can constitute up to about 30% of mature mesocarp fresh weight (Biale and Young, 1971). Most enzyme work with the avocado mesocarp has concerned the physiology and biochemistry of fruit maturity and ripening. Avocado isozymes in general are poorly known although those of polyphenol oxidase have been separated by acrylamide electrophoresis for biochemical studies (Kahn, 1976). Nevertheless, the use of isozymes for genetic studies has not been exploited.

From the above, and because there are many cvs and several extant seedling tree populations available for analysis, it became apparent that formal genetic studies of mesocarp isozymes should be feasible. This paper describes our initial attempts to analyze single-gene characters in the form of isozymes by using cultivar (parental) and seedling (progeny) mesocarps. The enzyme systems examined include alcohol dehydrogenase (ADH), phosphoglucomutase (PGM), glutamic-oxaloacetic transaminase (GOT) and leucine aminopeptidase (LAP). It was not possible to resolve all of these systems for all materials available because of the seasonal nature of the crop, shipment difficulties, and the relatively perishable nature of the avocado. Nevertheless, ample data were gathered to illustrate the principles involved, to demonstrate a few applications and to indicate the potential of isozyme genetics to attack problems of evolution, racial and cv characterization and to examine the origins of cvs of questionable ancestry.

METHODS AND MATERIALS—Table 1 is a list of the cvs utilized. The number of seedlings available from each cv varied from none to 26. Avocado mesocarp plugs were taken with a 9-mm cork borer and sliced into a disk approximately 2 mm thick from about the middle of the plug. The disks were squashed onto 7 × 7-mm filter paper wicks. Very soft mesocarp was mashed and the extract taken up on wicks. The general methods used for starch gel electrophoresis, gel slicing, staining for ADH and fixing have been described (Torres, 1974a). Gel buffers (all tris-citrate) for ADH, PGM and LAP were 0.02 M pH 7.6; 7.8 for GOT. The slowest GOT isozymes which remained at the origin or even migrated into the cathodal region of the gels could probably be moved into the anodal gel section by altering the gel buffer pH and/or ionic strength. In all gels, a wick with a sunflower seed extract was inserted to provide reference isozyme markers. PGM isozymes were developed in 30 ml 0.05 M tris-HCl pH 8.0 buffer, 5.0 ml 0.05 M disodium-alpha-D-glucose-1-phosphate (Sigma grade III), 5.0 ml 0.1 M $MgCl_2$, 40 units glucose-6-phosphate dehydrogenase, 5.0 mg NADP, 5.0 mg dimethylthiazolyldiphenyl tetrazolium bromide, thiazolyl blue (MTT) and 0.3 ml 0.01 M phenazine methosulfate (PMS). GOT was stained with 10 mg pyridoxal-5-phosphate, 400 mg L-aspartic acid, 200 mg alpha-ketoglutaric acid, 300 mg fast blue BB salt; just prior to staining, 230.0 ml H_2O, then 20 ml 1 M tris-HCl buffer pH 8.8 were added. LAP was developed with a mixture of 200 mg black K salt, 80 mg L-leucyl-beta-naphthylamide HCl in 250 ml 0.04 M phosphate buffer, pH 6.0. After staining, all gels were rinsed with 7% acetic acid and about 5 ml were added to the usual 1:1 H_2O:methanol fixative.

RESULTS AND DISCUSSION—An initial survey was made of four to five fruits of each of the six Florida cvs indicated in Table 1 to verify the expected constancy of ADH zymograms of the mesocarps of each tree and each variety. As anticipated, because of the asexual propagation described, each fruit of a given tree and variety produced identical zymograms. As the Florida season was over, it was decided to examine as many California cvs as possible for variation among the ADH, PGM, GOT and LAP zymograms.

The ADH zymograms of all cvs were interpreted on the assumption that ADH is a dimeric enzyme as has been found in maize (Freeling and Schwartz, 1973), wheat (Hart, 1970) and sunflowers (Torres, 1974a), among others. In these three, dissociation-recombination experiments have confirmed the dimeric structure and have shown that intergenic isozymes are dimerization products of subunits specified by the separate *Adh* genes.

For brevity, numbers will be used to distinguish the genes and letters the alleles of the locus under discussion. The gene which specifies the slower (or slowest) migrating isozyme or isozyme set will be called *1*. The genes which specify

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TABLE 1. *Adh, Pgm, Got, Lap genotypes of avocado varieties*[a]

Variety	*Adh-1*	*Adh-2*	*Pgm-1*	*Got-1*	*Got-2*	*Got-4*	*Lap-2*
Florida cvs.							
Booth 7	*S/S*[b]	*F/F*	—	—	—	—	—
Brooks Late	*F/F*	*F/F*	—	*S/S*	*S/S*	*F/M*	—
Choquette	*F/F*	*F/F*	—	—	—	—	—
Itzamna	*F/F*	*F/F*	—	—	—	—	—
Lula	*F/F*	*F/F*	—	—	—	—	—
Nabal	*F/F*	*F/F*	—	—	—	—	—
California cvs.							
Alboyce	*F/F*	*F/F*	*F/S*	*F/F*	*F/F*	*M/M*	—
Anaheim	*F/F*	*F/F*	*F/F*	*S/S*	*S/S*	*F/M*	*F/F*
Bacon	*F/F*	*F/F*	*F/S*	*F/F*	*F/F*	*F/M*	*F/F*
Duke	*F/F*	*S/S*	*S/S*	*F/F*	*F/F*	*F/M*	*F/F*
Edranol	*F/F*	*F/F*	*F/S*	*F/S*	*F/S*	*F/M*	*F/S*
Ettinger	*F/F*	*F/F*	*F/S*	*F/F*	*F/F*	*F/M*	*F/S*
Fuerte	*F/F*	*F/F*	*F/S*	*F/S*	*F/S*	*M/M*	*F/F*
Hashimoto	*F/F*	*F/F*	*F/F*	*F/S*	*F/S*	*M/M*	*F/F*
Hass	*F/F*	*F/F*	*F/S*	*F/S*	*F/S*	*M/M*	—
Irving	*F/F*	*F/F*	*F/F*	*F/S*	*F/S*	*M/M*	*F/F*
Linda	*F/F*	*F/F*	*F/F*	*F/S*	*F/S*	*F/S*	*F/S*
MacArthur	*F/F*	*F/F*	*F/S*	*F/S*	*F/S*	*F/M*	*F/F*
Marshelline	*F/F*	*F/F*	*F/F*	—	*F/S*	*F/M*	*F/F*
Mayo	*F/F*	*F/S*	*F/S*	*F/S*	*F/S*	*F/M*	*F/F*
Mexicola × Guatemalan	*F/F*	*F/F*	*F/F*	*F/S*	*F/S*	—	*F/F*
Nabal	*F/F*	*F/F*	*F/F*	*F/S*	*F/S*	*M/M*	*F/F*
Nimlioh	*F/F*	*F/F*	*F/S*	*S/S*	*S/S*	—	*F/F*
Pinkerton	*F/F*	*F/F*	*F/F*	*F/S*	*F/S*	*M/M*	*F/F*
Queen	*F/F*	*F/F*	*F/F*	*S/S*	*S/S*	—	*F/F*
Reed	*F/F*	*F/F*	*F/F*	*F/S*	*F/S*	*F/M*	*F/F*
Rincon	*F/F*	*F/F*	*F/F*	*F/S*	*F/S*	*M/M*	*F/F*
Stewart	*F/F*	*F/F*	*F/F*	—	—	—	—
Taft	*F/F*	*F/F*	*F/F*	*S/S*	*S/S*	*F/M*	—
Teague	*F/F*	*F/S*	*S/S*	*F/S*	*F/S*	*M/M*	*F/F*
Thille	*F/F*	*F/F*	*F/F*	*F/S*	*F/S*	*M/M*	*F/F*
Zutano	*F/F*	*F/F*	*F/S*	*F/F*	*F/F*	*M/M*	*F/S*
151-2	*F/F*	*F/F*	*F/F*	*F/S*	*F/S*	—	*F/S*
P. nubigena	*M/F*	*F/F*	*F/F*	*F/F*	*F/F*	*M/M*	—

[a] All varieties were monomorphic for *Pgm-2* and *Got-3*. Questionable interpretations are omitted. Most Florida varieties have not been studied for other than *Adh*.
[b] Letters refer to alleles of the indicated genes.

isozymes that migrate faster toward the anode will be designated *2*, *3* or *4* as appropriate. The allelic designations are *S* (for *Slow*), *M* (for *Middle*) and *F* (for *Fast*).

The zymograms of four of the six Florida varieties were basically the same; two about equally intense bands which are assumed to represent homodimers specified by two genes, *Adh-1* (coding for the slower migrating band) and *Adh-2* (specifying the more anodal or faster moving band; Fig. 1). Faint bands were sometimes observed trailing the *Adh-1* band. This basic zymogram is called the 'Nabal' pattern (see channel 2, Fig. 1). The 'Itzamna' zymogram was like that of 'Nabal' (Fig. 1, channel 3) but had a lighter staining but distinct band intermediate between the two 'Nabal' bands. Because of the light staining intensity and electrophoretic position of this third band, it was assumed to be the intergenic product of *Adh-1* and *Adh-2*. It is now widely recognized that two isozymes may have the same electrophoretic mobilities but different primary structures which could result in differential abilities to multimerize (e.g., Singh, Lewontin, and Felton, 1976). Apparently the subunits coded by the 'Nabal' genes are unable to form an intergenic isozyme and therefore may have one or more *Adh-1* and/or *Adh-2* alleles different from those of 'Itzamna'. This hypothesis could probably be tested with a series of dissociation-recombination experiments.

'Booth 7' yielded three isozymes but the lowest was slower migrating than the slow of 'Nabal' and 'Itzamna' (channel 6, Fig. 1). The intermediate isozyme, again because of spacing and intensity, suggested it was intergenic; it migrated to a position below the middle 'Itzamna' band, but above the lowest 'Itzamna' isozyme band.

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Apparently, in all Florida varieties examined both genes are homozygous, but *Adh-1* in 'Booth 7' is homozygous for different alleles than those in 'Nabal' or 'Itzamna' which may in turn be different from each other. We detected no evidence of *Adh* heterozygosity in any of the Florida varieties, but the potential seemingly exists—e.g., in a cross of 'Booth 7' with 'Lula', 'Itzamna' or 'Choquette'. If we designate the 'Nabal' and 'Itzamna' genotypes for *Adh* as *1F/1F, 2F/2F*, 'Booth 7' is *1S/1S, 2F/2F*—a genotype which to date is unique (Table 1).

'Booth 7' originated as a "seedling of an unknown Guatemalan parent in mixed planting with West Indian avocados" (Ruehle, 1963). Since 'Nabal' is of Guatemalan origin and all other Guatemalan cvs examined have the 'Nabal' or 'Itzamna' pattern, it is possible that the $Adh\text{-}1^S$ alleles of 'Booth 7' are of West Indian origin. Florida West Indian cvs will be studied when in season.

Among the California cvs examined, most produced 'Nabal' ADH patterns. 'Teague' and 'Mayo', however, contained a variant which reinforced the two-gene interpretation. The variation consisted of three bands in the *Adh-2* region of the 'Nabal' pattern (channel 4, Fig. 1) with the extra two just below the upper 'Nabal' band. The pattern suggested, of course, that *Adh-2* in these cvs is heterozygous resulting in three isozymes in the *Adh-2* region. According to this interpretation, 'Teague' and 'Mayo' are of genotype *1F/1F, 2F/2S*.

'Teague' is a hybrid of 'Fuerte' × 'Duke'. 'Fuerte' is of genotype *1F/1F, 2F/2F* and 'Teague' is *1F/1F, 2F/2S*, but early in the study 'Duke' had not been examined; according to our hypothesis, it must however be either *2F/2S* or *2S/2S* with *1F/1F* as in 'Fuerte' and 'Teague'. Only an $Adh\text{-}2^S$ allele contributed by 'Duke' could account for the 'Teague' pattern. This prediction was tested and 'Duke' was found to be homozygous *Slow* for *Adh-2*. It is the only variety examined to date that is of *Adh* genotype *1F/1F, 2S/2S*.

Another *Adh* variant was found in *P. nubigena* which produced three bands at the *Adh-1* region and is apparently heterozygous. A comparison of the electrophoretic migration rates of the slowest *P. nubigena* band and the slowest 'Booth 7' band relative to a sunflower marker isozyme indicated the two are at different positions as shown in Fig. 1. There are therefore at least three alleles for *Adh-1*—*S*, *M* and *F*, and two, *F* and *S*, for *Adh-2* in avocados examined to date. *Persea nubigena* has, according to this scheme, genotype *1F/1M, 2F/2F*.

PGM (Fig. 1) in avocado mesocarp seems to be controlled by two genes—*Pgm-1* specifying the slower migrating bands and *Pgm-2* the faster one. Resolution of this enzyme system with our electrophoretic conditions was not as clear as the others, but certain preliminary interpretations are warranted. PGM is monomeric in such diverse organisms as man (McAlpine, Mohandas, and Hamerton, 1975), pines (Hiebert and Hamrick, pers. comm.), *Phlox* and *Oenothera* (Levin, pers. comm.), and no doubt others. Seventeen cvs produced a zymogram consisting of only two bands representing two apparently monomeric isozymes (Table 1). The *Pgm-2* isozyme was monomorphic in all cvs, but greater separation might reveal some *Pgm-2* polymorphism. For *Pgm-1*, evidence for the monomeric structure and for two alleles, *F* and *S*, was clear. Although most varieties were homozygous *Fast* for this gene, nine, including 'Fuerte' and 'Hass', were *1F/1S* and two, 'Duke' and 'Teague' were *1S/1S*. Among nine 'Hass' seedlings examined, two were *1S/1S*, four were *1F/1S* and three were *1F/1F* (χ^2 = .334, P >.80 for goodness of fit to the expected 1:2:1 ratio). 'Thille' was homozygous *Fast* and all its seedlings should have been the same, but two of 26 were heterozygous. The most likely explanation of this deviation from expected is that they were products of outcrossing. We shall see this again with 'Thille' GOT.

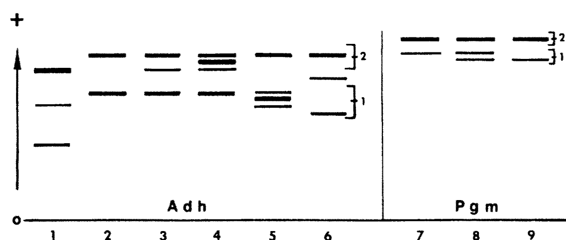

Fig. 1. Schematic representation of ADH and PGM isozymes in avocado mesocarp. O = origin, + = anode. The numbers 1 and 2 at the right of each group refer to the genes which specify the isozymes. Channels are indicated along the bottom: 1, sunflower ADH control for reference markers; 2, zymogram of genotype *1F/1F, 2F/2F*, constituting the 'Nabal' pattern; 3, the 'Itzamna' zymogram with the intergenic isozymes; 4, genotype *1F/1F, 2S/2F* of 'Teague' and 'Mayo'; 5, genotype *1M/1F, 2F/2F* of *P. nubigena;* 6, genotype *1S/1S, 2F/2F* of 'Booth 7'. Channel 7, zymogram of *Pgm* double homozygote; 8, *1F/1S* and 9, *1S/1S* for *Pgm-1*. The LAP zymograms are analogous to those of PGM but most polymorphism was found in *Lap-2*.

The most unusual and interesting results were found with GOT. The zymograms suggested that the enzyme system is controlled by four genes, *Got-1* (the slowest set), *Got-2*, *Got-3*, and *Got-4* (the fastest set—Fig. 2). The first two specify dimeric enzymes, the nature of the *Got-3* isozyme is unknown because all varieties examined had but one band, and *Got-4* apparently specifies a monomeric system since individuals produced either one or two isozymes. If the *Got-4* isozymes are multimeric, the different

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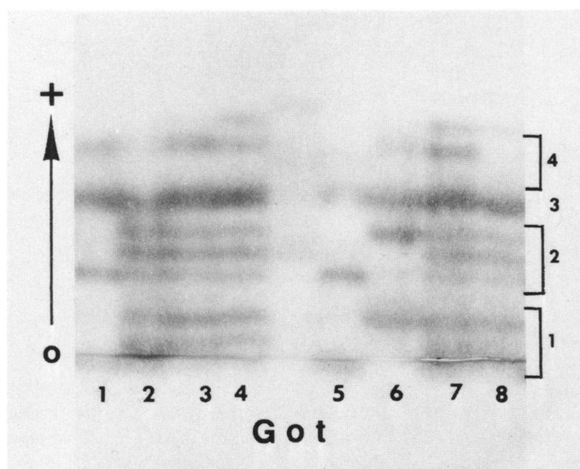

Fig. 2. Photograph of GOT isozymes. Symbols as in Fig. 1. Channel 1, 'Nabal' seedling segregant with *1S/1S*, *2S/2S*, and *4M/4M;* 2 and 3, 'Nabal' seedling and 'Thille' cultivar showing *1S/1F*, *2S/2F*, and *4M/4M;* 4, 'Thille' outcrossed as shown by *4M/4F*. Unnumbered channel is a sunflower control. Channel 5, 'Thille' seedling with *1S/1S*, *2S/2S*, *Got-4* is equivocal; 6, 'Thille' seedling with *1F/1F*, *2F/2F*, *4M/4M;* 7 and 8, 'MacArthur' seedlings with *1S/1F*, *2S/2F* and with *4M/4F* and *4F/4F*, respectively.

subunits apparently cannot multimerize. For the present we are assuming that *Got-4* is a monomeric system.

'Nabal', 'Hass' and 'Thille', among others, are heterozygous *F/S* for both *Got-1* and *Got-2*. For *Got-1*, among seven 'Nabal' seedlings, one was *1F/1F*, five *1F/1S* and one *1S/1S* ($\chi^2 = 1.28$, $P > 0.50$). Among six 'Hass' seedlings examined, two were *1S/1S*, four *1F/1S* and none *1F/1F* ($P > 0.30$). Among 25 'Thille' seedlings eight were *1S/1S*, 13 *1F/1S* and four *1F/1F* ($P > 0.50$). The results for *Got-2* were identical in all cases because there was a perfect correspondence between *Got-1* and *Got-2* phenotypes. None of the expected six possible recombinant types was found—e.g., *1F/1F* with *2F/2S* or *2S/2S*. The most likely explanation for this correlation is that the two genes are linked. How tightly could not be estimated since no exceptions were found. We have no reason to believe that *Got-1* or *Got-2* isozymes are artifacts of the other gene's products since multiple *Got* genes are known in other organisms (Roose and Gottlieb, 1976). Another possibility to account for these observations is that the *Got-2* isozymes are intergenic combinations of *Got-1* isozyme subunits with those of *Got-3* or *4*. This possibility was rejected because of the relative mobilities and the staining intensity relationships among the bands.

Evidence was found for three alleles of *Got-4*—*S*, *M* and *F*. *Got-4*^{S} was found only in 'Linda' as *4F/4S*. 'MacArthur' was *4F/4M* for this gene, and its five seedlings were 1:3:1, *F/F:F/M:M/M* ($P > 0.90$). The remainder of the cvs were either homozygous or of equivocal phenotype (Table 2). *Got-4* provides another example of the use of isozymes to detect outcrossing. The 'Thille' cultivar is homozygous *4M/4M*, thus all of its seedlings should also have been homozygous; one of 26, however, was heterozygous *4F/4M* with two isozymes (Fig. 2, channel 4). Barring mutation, a rare event, outcrossing is again the most reasonable explanation.

TABLE 2. *Genotypes of Fuerte × Duke = Teague*[a]

Gene	Duke	Fuerte	Teague
Adh-2	(*S/S* or F/S)	F/F	F/S
Pgm-1	(*S/S* or F/S)	F/S	S/S
Got-1, -2	F/F	(S/S or *F/S*)	F/S
Got-4	(M/M or *F/M*)	M/M	M/M
Lap-2	F/F	F/F	(*F/F*)

[a] Parentheses indicate predicted possible genotypes. Italics indicate actual genotype.

Avocado LAP is apparently a monomeric enzyme system controlled by two genes, *Lap-1* and *Lap-2*. Since an unidentified cv reportedly from Florida obtained in a local market was *1F/1S*, all California cvs, although homozygous for this locus, can be designated *1F/1F*. For *Lap-2*, 18 cvs were *2F/2F*, five were *2F/2S* and none were *2S/2S*, a genotype not yet encountered. Seedling populations were not available for segregation analysis.

Several hybrids of reasonably certain origin were examined to verify or refute reputed parentages. 'Teague' has been discussed for *Adh-1*, but will again be used to illustrate the predictions and results of tests. The parents of 'Teague' are 'Fuerte' × 'Duke'. For *Adh*, 'Fuerte' was first noted to be *2F/2F*, then 'Teague' was found *2F/2S*. 'Duke' had to be either *2F/2S* or *2S/2S*. It was *2S/2S*. For *Pgm*, 'Teague' was *1S/1S* and 'Fuerte' *1F/1S*. 'Duke' was predicted to be *1F/1S* or *1S/1S*. It was *1S/1S*. For *Got-1* and *-2*, 'Teague' was *F/S* and 'Duke' was *F/F*. 'Fuerte' was predictably either *F/S* or *S/S*. It was *F/S*. *Got-4* in 'Teague' and 'Fuerte' was *M/M;* 'Duke' could have been any genotype that included at least one *M* allele. It was *4F/4M*. For *Lap*, both 'Fuerte' and 'Duke' were *2F/2F* so 'Teague' would also have to be *2F/2F*. It was. Table 2 is a summary of the genotypes of these three cvs.

As may be seen from Table 1, no isozyme evidence contradicted the accepted origins of 151-2 from 'Anaheim' × 'Edranol'; 'Reed' from 'Anaheim' × 'Nabal'; 'Pinkerton' from 'Rincon' × 'Hass'; and 'Thille' as a 'Hass' self.

While only four enzyme systems have been examined, they are coded by 10 genes, two of which are apparently linked. For the 10 genes,

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TABLE 3. *Summary of avocado genetic markers*[a]

	Gene	Alleles	Genotypes possible	Genotypes observed
	Adh-1	3	6	3
	-2	2	3	3
	Pgm-1	2	3	3
	-2	1	1	1
	Got-1	2	3	3
	-2	2	3	3
	-3	1	1	1
	-4	3	6	3
	Lap-1	2[b]	3	2
	-2	2	3	2
TOTAL	10	20	32	24

[a] Combined possible genotypes = 26,244 or 8,748 if *Got-1* & *-2* are linked so tightly that recombinants are unlikely.
[b] Includes polymorphism found in unidentified cv reportedly from Florida.

evidence has been found for 20 codominant alleles. Of the 32 individual genotypes possible, 24 have been observed (Table 3). If the *Got* genes were not linked, there would be 26,244 possible combinations. Taking into consideration the linkage of *Got-1* and *Got-2*, there are still 8,748 possible combinations of genotypes for only these four enzyme systems. Theoretically, either figure would be more than enough to characterize all extant cvs, but this possibility has not been realized because several have common genotypes (Table 1). As more isozymes are added to this survey, the probability will increase that each cv can be characterized isozymically. We attempted to include acid phosphatase in this study, but did not for two reasons: first, there were indications that additional confounding isozymes appeared as the fruit ripened. Second, the isozymes were not as clearly resolved as we would prefer. Additional experimentation with the buffer system and the use of fruits of the same age and stage of ripening should ameliorate this problem.

Some significant avocado problems in which isozyme investigations may be useful include those of the evolution and relationships of the three races, cultivar adaptability, and rootstock identification. Even the racial composition of cultivars is often conjectural; that of the leading Florida cv, 'Lula', is in fact unknown. As implied with acid phosphatase, isozymes would also be useful to study the temporal expression of various enzyme systems in developing fruit. Further, as demonstrated, outcrossing has been detected with single enzymes, and almost certainly would be with an array of enzymes.

It would seem that these initial investigations have demonstrated the feasibility of conducting molecular genetic studies in the avocado using the mesocarp of cv and seedlings. The surprisingly large amount of polymorphism uncovered to date, if representative, is indeed encouraging and optimism for future work is warranted.

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