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ISOZYMES OF 'DUKE' AND ITS DERIVATIVES

Andrew M. Torres and B. O. Bergh —
Professor of Botany, University of Kansas, Lawrence 66044, and Research Geneticist, University of California, Riverside 92521.

Introduction

The cultivated avocado (*Persea americana* L.) variety 'Duke' has been of interest for about five decades because of its cold tolerance and fruit qualities. A rather complete account of the origin and history of 'Duke' has been published in the 1963 Yearbook (6).

'Duke' has been of even greater interest because of its partial resistance to *Phytophthora* root rot, and it became the first important variety in the Zent-myer program (5) for a commercial solution to root rot. In cooperation with Zentmyer, Bergh (1) has begun a breeding project toward the same end. The strategy is to attempt to enhance root rot resistance, either by combining 'Duke' genes in new ways through selfing, or by crossing 'Duke' (or its selected seedlings) with other lines also having resistance. Although in such crosses the seed parent is obvious, the pollen parent, even when great precautions are taken, may be uncertain (3). This has been true in avocado breeding generally because, until recently, there were no single gene characters available as markers to document the parentage, especially the pollen parent. Yet, it is important to identify both parents with certainty simply because, if a highly favorable combination results, the same parents may be recombined to yield even better offspring.

Two recent studies (3,4) have shown that variant forms of enzymes (isozymes) separable by starch gel electrophoresis provide excellent single gene markers for the avocado and that both fruits and leaves can be utilized. Such markers have allowed us partially to characterize each cultivar biochemically, to document parentages, and to detect outcrossing.

In the early phases of avocado isozyme research, a 'Duke' fruit had been sent to the first author's laboratory at the University of Kansas and was found (4) to have a certain isozyme/genotype,S/S, for one of the two genes that code for the enzyme alcohol dehydrogenase *(Adh-2).Upon* reexamination of a 'Duke' fruit at Riverside from a different tree, the *Adh-2* genotype was, surprisingly, *F/S.* Surprisingly, because all 'Dukes' (just as any other variety) should be genetically identical, since they are propagated by grafting and constitute a clone of genetically identical individuals, precisely as single cells may give rise by cell division to a population of cells all genetically identical to the original cell. What, then, could explain the two different genotypes? Obvious possible errors such as gel interpretation, labeling, or recording were soon eliminated. It indeed seemed that there were two 'Dukes' in the University of California plantings. One possible explanation was that a mutation had occurred, but mutation is such a rare event that it seemed unlikely. Far more likely was an error in record keeping or propagation sometime during 'Duke's' 66-year history.

A second puzzle arose concerning 'Duke 7', a supposed selfed seedling of 'Duke' which has generated considerable interest because its resistance to root rot is greater than that of 'Duke' itself. 'Duke 7' is presently being widely used by several nurseries for rootstocks, being cloned by the etiolation method (2). Because of its importance to the avocado industry, it would be desirable to know whether or not 'Duke 7' is in fact a selfed progeny of 'Duke'. If not, that is if it were an outcross, then it would be of great interest to identify the pollen parent because it apparently provided part of the resistance with which 'Duke 7' is endowed since the male ordinarily contributes half the genetic makeup of each offspring. We are assuming that the seed parent of 'Duke 7' is indeed 'Duke'. The rationale for using isozymes as genetic markers to address these sorts of problems has been reported earlier (3,4).

Materials and Methods

The sources of the 'Dukes' and 'Duke 7' used in this study are given in Table 1. All of the procedures for isozyme analysis in the avocado by starch gel elec-trophoresis have been reported (3,4).

TABLE 1. Sources of 'Duke' and its seedlings.

Variety	*Field Identification*	*Source and Notes*
'Duke'	UCLA	UCLA 'Duke' grafted 1933 from budwood of the second Oroville 'Duke'
'Duke'	R14T9[1] (CSDA 3497) R15T7 (CSDA 3439)	From budwood of UCLA 'Duke' grafted in 1950 on 'Duke' seedlings, growing on Santa Barbara Hospital grounds
'Duke'	19/22/8[2]	From budwood of UCLA 'Duke' grafted in 1943
'Duke'	11/15/2	From budwood of R15T7 'Duke,' Santa Barbara
'Duke'	23/26/6	From budwood of Field 19 UCR 'Duke'
'Duke'	3/2/3	From budwood of UCLA parent 'Duke,' 1958
'Duke' sdlg	3/18/1	Grafted seedling of Field 19 UCR 'Duke,' 1964
'Duke' sdlg	3/6/13	Seedling of unknown 'Duke,' 1960
'Duke' sdlg	3/18/6	Seedling of 'Duke' from Mclntyre Ranch, 1964
'Duke' sdlg	3/18/8	Seedling of 'Duke' #2 from Statom Ranch, 1964
'Duke' sdlg	3/18/9	Seedling of 'Duke'#2 from Statom Ranch, 1964
'Duke' sdlg	3/18/19	Seedling of 'Duke' from Poister Ranch, 1964
'Duke 7'	Field 46	'Duke' seed from CALAVO planted 1960

[1] We are grateful to Mr. George Goodall, Farm Advisor, Santa Barbara, for providing the CSDA materials.

[2]Field/Row/Tree of UC plantings at Riverside (UCR— Fields 11,19) or at South Coast Field Station (Fields 3, 23, 46).

Results and Discussion

The 'Duke' problem—In 1977, when the first author was preparing a listing of the gene/isozyme characteristics of several cultivars, a 'Duke' specimen was found to have the genotype S/S for one of the two genes which specify the enzyme alcohol

dehydrogenase *(Adh-2)* (3). The sample used was a fruit from a tree growing in the University of California South Coast Field Station, Field 3, Row 6, Tree 13 (abbreviated as 3/6/13). In 1978, a second 'Duke', from 3/18/19, was surprisingly found to be *F/S* for this same gene. It would indeed be very rare if the changed genotype were due to mutation, an alteration of the DNA constituting the gene. Far more likely, an error had been made in the Torres laboratory, in recording field data, or in propagation—for example, mixing up of budwood. When these two samples were reexamined, the same differences were again found, raising several questions: which is the real 'Duke', are there yet other kinds of 'Duke', and what is their origin? To answer these questions as fully as possible, samples of every available 'Duke' were assembled for reanalyses of all earlier gene/enzyme systems in addition to new ones that have subsequently been developed from both fruits and leaves (3). The formal genetics of the earlier systems have been reported (4), and the new ones will be reported elsewhere. For the present, we need only indicate that the new systems are peroxidase (PX) from leaves and malate dehydrogenase (MDH) from both fruits and leaves. For brevity, the genes will be named after the corresponding enzyme (e. g., *Px* and *Mdh)* and will further be given a number if more than one gene codes for (that is, specifies) the same enzyme. Letters, generally *F* (for *Fast), M* (for *Medium),* and S (for *Slow),* in terms of relative mobilities during electrophoresis, will designate the forms or alleles of the genes as well as their corresponding products, the isozymes. A fuller explanation of our gene/enzyme nomenclatorial system is presented in a companion paper in this issue (3).

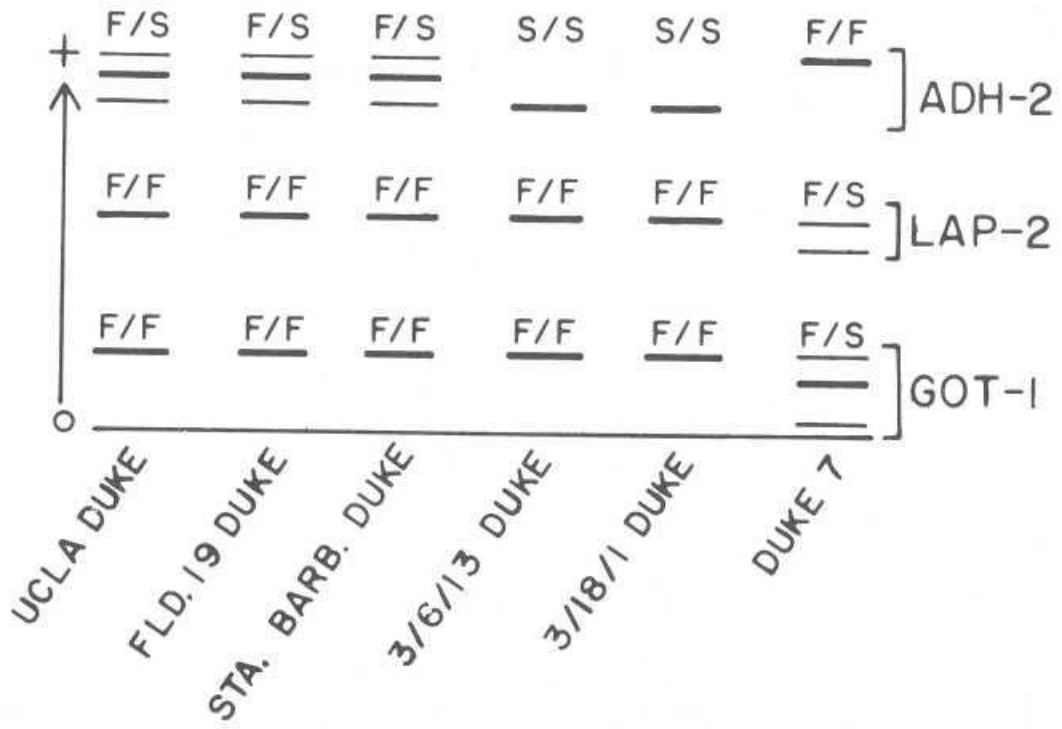


Figure 1. Schematic illustration of *Adh,* Lap and Gof zymograms of various 'Dukes'.'The origin of insertion of the sample in the gel is indicated by 0, the anode by +. Gene/enzyme systems are shown along the right. Relative migration distances of the isozymes are not represented.

TABLE 2. Genotypes of various 'Dukes.'

		Genotypes							
Variety	*Field Identif.*	Adh-2	Got-1, 2	Lap-2	Mdh[3]	Pgm-1	Px-C	Px-1	Px-2[4]
'DUKE'	UCLA	FS	FF	FF	D	SS	MS	MS	AC
'DUKE'	R14T9[1]	FS	FF	FF	D	SS	MS	MS	AC
'Duke'	R15T7[1]	FS	FF	FF	D	SS	MS	MS	AC
'Duke'	19/22/8[2]	FS	FF	FF	D	SS	MS	MS	AC
'Duke'	11/15/2	FS	FF	FF	D	SS	MS	MS	AC
'Duke'	23/26/6	FS	FF	FF	D	SS	MS	MS	AC
'Duke'	3/2/3	FS	FF	FF	D	SS	MS	MS	AC
'Duke' sdlg	3/6/13	SS[5]	FF	FF	D	SS	MM?	MS	AC
'Duke' sdlg	3/18/1	SS[5]	FF	FF	D	SS	SS[5]	SS[5]	AA[5]
'Duke' sdlg	3/18/6	FS	FF	FF	D	SS	MS	MS	AA[5]
'Duke' sdlg	3/18/8	SS[5]	FF	FF	D	SS	MS	MS	AC
'Duke' sdlg	3/18/9	SS[5]	FF	FF	D	SS	MS	MS	AC
'Duke' sdlg	3/18/19"	FS	FF	FF	D	SS	MS	MS	AC
'Duke 7'	Field 46	FF	FS[7]	FS[7]	D	SS	MM	SS	AA

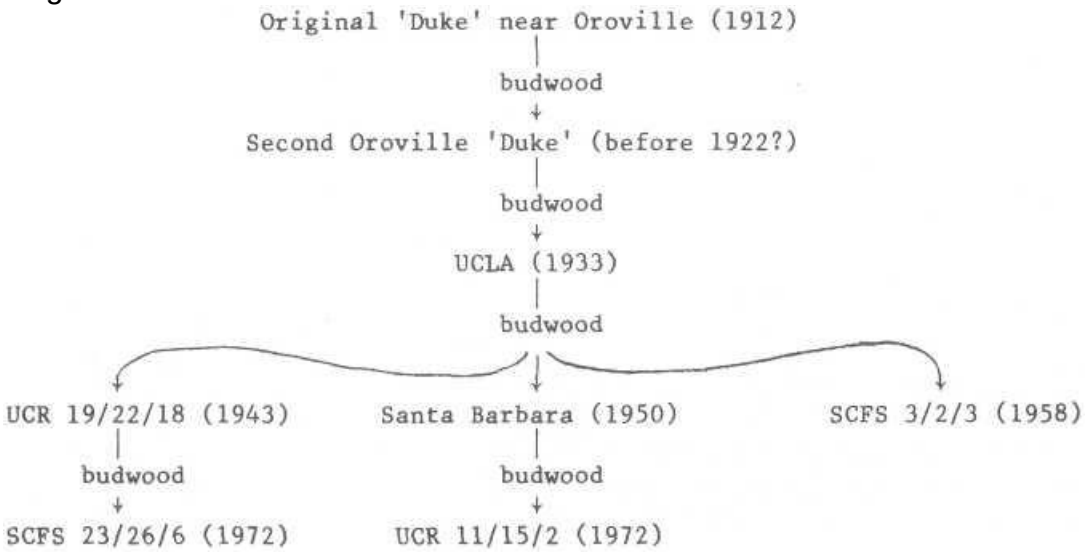
'Santa Barbara 'Duke.'
[2]Field/Row/Tree of UC plantings at Riverside or at SCFS.
[3]For the present, zymogram designations rather than genotypes will be used pending a more complete understanding of the genetic control of Mdh.
[4]Since five different alleles have been observed, letters additional to the usual *F, M* and S had to be used.
[5]This genotype shows that the plant is not a true 'Duke,' but a seedling or an outcross.
[8]Even though it is a seedling, it happens to have the same genotypes as parent 'Duke.'
[7]This genotype shows 'Duke 7' to be an outcross because 'Duke' does not have the S allele.

Table 2 is a listing of the relevant gene/enzyme profiles of the 'Dukes' listed in Table 1, and Figure 1 illustrates some of these. In Table 2 it may be seen that, *for Adh-2,* four 'Dukes' or 'Duke' seedlings are S/S, all others are *F/S,* and 'Duke 7' is *F/F.* Based on the available field records plus morphological similarities, it has been assumed that all were clonal members of parent 'Duke'. It is clear, however, that the *Adh-2* genotypes indicate otherwise. Because of the possibility that one or the other *Adh-2* class of 'Dukes' could be products of selfing or outcrossing, we may note as a first clue to the solution that the *F/S* type could, through recombination, give rise to the *S/S* type but not the reverse. That is, *F/S x F/S* could yield S/S, but S/S X S/S could not yield *F/S* since neither egg nor pollen would have the F allele. For this reason alone, it could reasonably be inferred that the real 'Duke' is a line with F/S for *Adh-2.*

Tracking down the "real" 'Duke' involves a little history. The variety originated in 1912 near Oroville in northern California's Butte County (6). The original tree was removed, apparently in the 1920's, but a graft from it was established nearby and survived for decades. This second 'Duke' tree is also now apparently gone, but a graft from it in turn was made at the University of California, Los Angeles as early as 1933.

The UCLA parent tree in turn provided grafts for 'Duke' trees at Riverside, 19/22/8 (1943), and on the Santa Barbara Hospital grounds (1950).

The UCLA tree is, therefore, the oldest 'Duke' known to us. On a weekend visit to that campus, Torres located the tree and scaled the chain-link fence surrounding it (suffering a few abrasions and contusions in the process). No fruit remained on the tree, but he found a partly rotted one on the ground—isozyme testing showed it to be in fact F/S for *Adh*-2! As Table 2 indicates, this is also the genotype of the trees derived from the UCLA 'Duke': UCR 19/22/8 (and the SCFS 23/26/6 grafted from it); Santa Barbara CSDA's (and the grafted UCR 11/15/2); and SCFS 3/2/3. The relationships may be diagrammed as follows:



All the 'Dukes' in the above lineage should be identical not only for *Adh-2,* but also for all other gene/enzyme systems. We have examined them for glutamate oxaloacetate transaminase (GOT), leucine aminopeptidase (LAP), MDH, phosphoglucose mutase (PGM), and PX. These now-assumed true 'Dukes' are all F/F forgot-1, *Got-2 and Lap-2, S/S forPgm-1* and have pattern D for *Mdh.* They are also all heterozygous for three *Px* genes (see Table 2).

As we saw above, selfed progeny cannot have alleles not possessed by the parent. If any new allele/isozyme is detected for *Got, Lap-2, Pgm-1 or Mdh,* it would clearly indicate outcrossing to a pollen parent having the different allele. On the other hand, although 'Dukes' with S/S for *Adh-2* are different from true 'Duke', this could arise by recombination, there is no evidence of outcrossing, and we may assume that they are selfed seedlings. In attempting to trace the origin of these so-called 'Dukes', we met with Mr. Fred Guillemet and Dr. George Zentmyer to review field records. At this meeting, the solution to the problem was finally realized. The Field 3 plants, as indicated by old records of Mr. Bill Thorn, were seedlings of 'Duke' grafted onto rootstocks. They are not 'Dukes' at all, but rather 'Duke' x 'Duke' recombinational products to judge from their fruit and leaf morphology. At last the mystery was solved.

The 'Duke 7' problem—Seeds from an unknown 'Duke' source were obtained by Dr. Zentmyer from CALAVO about 1960 for his resistance studies. One of these developed into 'Duke 7' and it had been assumed that 'Duke 7', because of its high degree of root rot resistance, was a 'Duke' x 'Duke' (selfed) seedling. It differs somewhat in morphology from 'Duke' in having a darker green skin, a larger leaf, and in

being more resistant to root rot, more vigorous, and more resistant to boron toxicity. Yet, all of these characteristics would be expected to be within the range of 'Duke' genetic variability. In short, there was no reason to suspect that 'Duke 7' was anything but a 'Duke' self progeny.

However, perusal of Table 2 shows that 'Duke 7' has two alleles not present in 'Duke'. These are S for *Lap-2* and S for *Got-1* and *Got-2.* This shows clearly, by the reasoning given above, the 'Duke 7' may have had 'Duke' as a seed parent, but could not have 'Duke' as the pollen parent as well. It could not be a 'Duke' self, but could be a 'Duke' outcross. If we assume that the CALAVO fruit bearing the 'Duke 7' seed was indeed 'Duke', and this seems reasonable since CALAVO considered it such, we are then faced with the question of the pollen parent. This is of more than passing intellectual interest because it is quite possible that the pollen parent passed on to 'Duke 7' some desirable gene or genes for *Phytophthora* resistance. If that pollen parent could be identified, a new source of resistance might be investigated and exploited.

Unfortunately, we will probably never be able to identify the pollen parent with certainty, but we can approach the problem by searching for cultivars which have the right combination of *Got* and *Lap-2* genes. We would be looking for a variety that is S/S or F/S for both genes; that is, the pollen parent has to possess an S allele to be able to pass it on to a 'Duke' egg.

We have now examined over 100 varieties for these genes in connection with a general cataloging of avocado germ plasm and find very few reasonable candidates. The most likely ones are 'Hasten' (F/S for both genes), 'Harms' (S/S and F/S for *Got* and *Lap,* respectively), and 'Clifton' (F/S and S/G). 'Hasten' was eliminated as a candidate because it was not yet introduced. 'Harms' can be eliminated on morphological grounds—it is a thick-skinned, late-maturing

Guatemalan. 'Clifton' was grown more widely than the others, and a 'Duke' x 'Clifton' cross would probably fit the morphology of 'Duke 7'. Thus, 'Clifton' may be worthy of trial as a source of root rot resistance. As is apparent, knowledge of additional gene/enzyme systems would possibly be very valuable in helping us identify the actual pollen parent.

A disclaimer is very much in order. It is perfectly possible that we have not yet examined enough cultivars to pinpoint the real pollen parent, and that the one we are seeking may no longer exist. When seed sources are a packing house, it is virtually impossible to trace the origin of the 'Duke' fruits, to examine ranch maps to identify the particular 'Duke' and know which varieties grew near it. Thus, we are only guessing and deducing from available information knowing full well we may be wrong.

Summary

Isozyme data show that there are several genetically distinct types, all referred to in the records as 'Duke'. Our studies strongly indicate that true 'Duke' is F/S for *Adh-2, F/F* for *Got-1,2, F/F* for *Lap-2, M/S* for *Px-C, M/S* for *Px-1* and A/C for *Px-2,* Types differing from this in any respect are 'Duke' selfs or outcrosses. 'Duke 7' is evidently an outcross, as shown by its isozyme profile. The pollen parent, which apparently contributed its own genes for *Phytophthora* resistance, may have been the 'Clifton' variety.

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