Breeding and development

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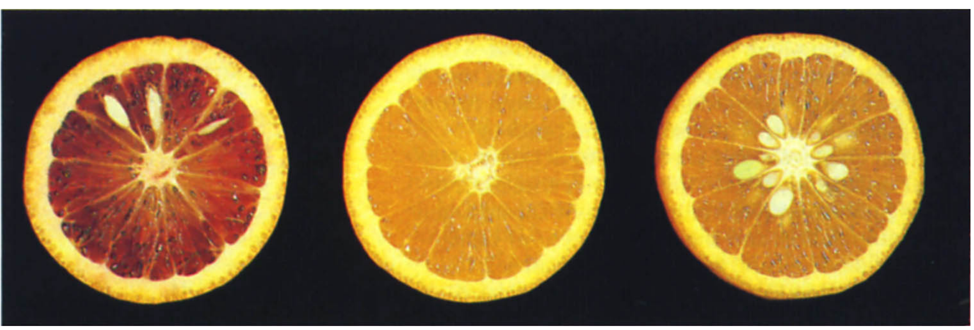
When Howard B. Frost began citrus breeding at the University of California Citrus Experiment Station in 1914, knowledge of crossing relationships in the genus was limited to earlier studies in Florida by the U.S. Department of Agriculture. Frost began a wide series of crosses among edible types, but the numbers of hybrids first obtained were limited by nucellar embryony. This is the phenomenon by which somatic cells of the nucellus (tissue in the ovule but outside the embryo sac) develop into embryos. Since these nucellar embryos develop asexually, with no male cells contributing to their formation, they are usually genetically identical with the seed parent.

Nucellar embryony has been a major obstacle to the systematic production of F_1 hybrids and self-pollinated progenies. Many cultivars produce almost entirely nucellar embryos, and there may be several in a single seed. Others form both nucellar and zygotic (sexual) embryos. Studies have identified additional cultivars that produce only sexual progeny, and these have been largely used in more recent crosses.

The occurrence of nucellar embryony enabled Dr. Frost to compare carefully the tree and fruit characters of recent nucellar seedling budlines with those of their older parental budlines. The nucellar budlines were usually more vigorous, faster growing, more thorny, and slower to fruit than parental ones. These juvenile characteristics are largely physiological, but can be long-lasting. Yields of older, established nucellar budlines have often been higher than those of their parent lines. But most importantly, many viruses and viruslike agents are eliminated at embryo formation. This has led to widespread propagation of nucellar lines and has contributed to the improvement of citrus plantings in many countries during the last two decades.



Triploid orange (with 3 sets of chromosomes) with few seeds (center) is from cross of tetraploid Ruby orange (left) by diploid King tangor (right).

Right: CES-developed seedless Chandler pummelo was grown in isolation; seedy fruit at left received other citrus pollen.

Inheritance of nucellar embryony

One problem associated with citrus breeding—as with many tree fruits—is the scarcity of characters controlled by one or a few genes. Most characters are inherited quantitatively, involving many genes. In addition, genes are apparently seldom homozygous (that is, existing in two identical copies, or alleles) within a clone. Thus, fruits showing a wide range of size, shape, color, and flavor may occur in a single progeny from two parents.

Because of the importance of nucellor embryony, U.C. researchers began to study its pattern of inheritance. By 1959, data indicated that its inheritance was rather simple within common *Citrus* species. Crosses between strictly sexual parents regularly yielded hybrids that were themselves sexually reproducing; crosses between sexual and nucellar parents usually produced hybrids of both types. Two parents that could reproduce by nucellar embryony sometimes produced offspring that were completely sexual. Tetraploid hybrids (having four sets of chromosomes) were also examined, and they showed the same kind of segregation patterns. Nucellar embryony thus appears to be dominant over sexual embryony, and very few genes may be involved.

In crosses between *Citrus* and its near relative, *Poncirus,* the inheritance is somewhat more complex. Many F_1 hybrids were sexually reproducing, even when both parents could reproduce by nucellar embryony. Yet backcrosses of these sexual hybrids to sexually reproducing *Citrus* in experiment station tests still resulted in progeny that were essentially all sexual, adding to the evidence that strictly sexual plants may be homozygous for this character.

Self-incompatibility in citrus

Hybridization in citrus can also be complicated by self- and cross-incompatibility, which occurs in a number of cultivars. Self-incompatible forms set little or no seed, and often very little fruit, after self-pollination. Climatic factors, however, sometimes favor fruit set even in the absence of seeds; this condition is called parthenocarpy.

Self-incompatible cultivars may produce many fruits and seeds after cross-pollination by particular pollen parents, indicating cross-compatibility. Reactions of this sort occur in a number of plant families and are considered to be caused by one or more series of self-incompatibility alleles. Studies reported by Dr. Soost in 1965 indicated that such alleles are present in several citrus taxa, including some pummelos and their hybrids, certain grapefruit hybrids such as Minneola and Orlando, and the Clementine mandarin. In such cases, cultivars that can serve as pollinators are often needed to produce good crops of fruit.

The use of polyploidy

Most common citrus is diploid, with two sets of chromosomes, but tetraploids often occur spontaneously among seedling populations. Dr. Frost was one of the first to identify and collect these forms, among a series of citrus cultivars. Although not themselves commercially useful, tetraploids have been important in breeding few-seeded triploids (with three sets of chromosomes). Fruits with few seeds are desirable for eating out of hand, but most diploid hybrids are seedy. Triploids can be produced by crossing diploids with tetraploids, and by 1938 Frost had obtained limited numbers of such hybrids. Recently, large populations have been produced and are under study.

When a partly sexual tetraploid is used as seed parent and a diploid as pollen parent, triploids are usually obtained, but the reciprocal cross results in few triploids and numerous hybrid tetraploids. U.C., Riverside, studies showed that the chromosomes occasionally doubled in the diploid seed parent, followed by selective survival of the resulting tetraploid embryos. An unfavorable balance between chromosome numbers in the young embryo and endosperm (the nutritive tissue) is responsible for this unusual behavior. However, such new tetraploid plants, some of which are strictly sexual, have already been used as additional seed parents to produce more triploids.

The triploid embryos that arise from pollination of tetraploid seed parents by diploids also have a certain developmental disadvantage, since they sometimes tend to mature earlier and produce smaller seeds. They can usually be germinated satisfactorily by use of *in vitro* techniques.

Mutations and chimeras

Somatic mutations occur frequently in citrus; they are often unfavorable, but occasionally highly valuable. In Japan, mutations in the satsuma mandarin have resulted in closely related clones differing in characteristics such as color and ripening time. At Riverside, Dr. Frost found that similar mutations had produced satsuma seedling lines that varied in ripening time, productivity, and fruit shape. One of these nucellar lines has been used commercially as an improved cultivar.

Mutations that arise in single somatic cells are sometimes perpetuated in only a part of the tissues of a plant or animal, resulting in a chimera. In 1942, chimeral citrus trees were identified at Riverside in which certain histogenic, or tissue-producing, cell layers were diploid, whereas others had become tetraploid. This caused differences in the height, shape, and leaf texture of these plants.

We have studied an interesting case of chimerism among pigmented grapefruits, in cooperation with the U.S. Department of Agriculture. It is known that the Thompson and Foster pink grapefruits arose as limb sports on white grapefruit trees early in this century. When nucellar seedling trees of these cultivars were grown in about 1960, those of Thompson had no pink in their fruits, whereas those of Foster had deeper color than their parent. Various tissues in many plants arise from two or three basic histogenic layers, but a nucellar embryo stems originally from a single seed parent cell. The evidence indicates that the pink grapefruits carry genetic factors for color in only one or two of their histogenic layers. Thus nucellar embryos (believed to arise from layer II) can either lack a color factor or, conversely, can carry it in all their tissues.

Chemical factors as markers

Citrus taxonomy has long been controversial, and the origin and relationships of many forms are uncertain. Intensive studies of a range of chemical and physical characters have been directed toward solving these problems. Under the leadership of R.W. Scora, U.C. scientists have investigated terpenes of leaf and rind oils, leaf hydrocarbons, protein and isozyme patterns, oxidase browning, virus resistance, and morphological characters. The overall results suggest that there are four primary species, namely, the subtropical *Citrus reticulata* and *C. medica*, the tropical *C. grandis* and the recently discovered *C. halimii*. The latter is somewhat intermediate between the genera *Citrus* and *Fortunella*. Other forms that have often been classified as species can, in fact, be identified as members of complexes involving asexual reproduction. In studies of closely related biotypes, relationships were found between diploid and tetraploid forms, among identical twin individuals, and among F_1 hybrids of *Citrus* and *Poncirus*.

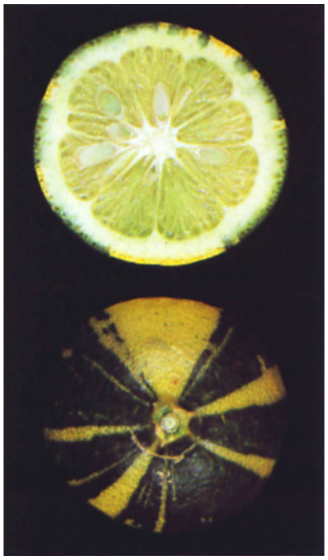
U.C. researchers, in cooperation with the University of Kansas, have recently identified enzyme characters that are simply inherited and useful for both breeding and taxonomy. The browning reaction in young shoot tissue results from oxidation of phenolic substrates. Presence of the substrate is under single-gene control, which often permits the separation of nucellar from zygotic seedling plants. Another reaction causes coagulation of shoot tissue in some forms. Coagulation is recessive to non-coagulation so that, again, separation of different genetic types is often possible. Analyses of several other leaf enzymes, also inherited in single-gene fashion, have been especially rewarding. The presence or absence of as many as eight of these markers, taken together, can permit early and rapid separation of nucellar from zygotic plants in a great many crosses, and can assist in determining parentage of some long-established cultivars.

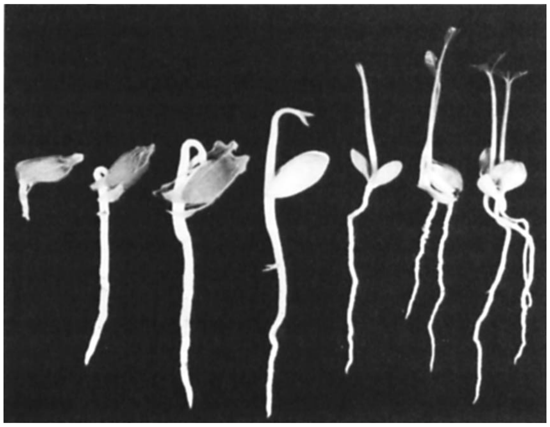
New citrus cultivars

Efforts to produce early-maturing citrus fruits of moderate acidity have been aided by the use of a clone of pummelo (*Citrus grandis*) that has essentially no titratable acid. When U.C. plant breeders crossed this pummelo with cultivars of medium acidity, the resulting hybrids had acid levels averaging well within the edible range at early mid-season. Compared hybrids with a medium- or high-acid pummelo as a parent were usually too sour. One of the acidless pummelo hybrids, when selfed, produced acidless individuals in proportions that suggest single-gene action. These individuals may serve as new acidless parents.

Oroblanco triploid grapefruit hybrid, developed at U.C. in 1980, is nearly seedless and earlier ripening than the Marsh grapefruit.

Below: Immature fruit of variegated sour orange. Yellow rind sectors have normal growth; green sectors are depressed.





Two germinating seeds on right show polyembryony (production of more than one embryo). Others are monoembryonic.

Two U.C.-developed hybrids involving the acidless pummelo have been introduced: the Chandler pummelo (1961) and the Oroblanco grapefruit hybrid (1980). Both are of good flavor and are usually edible by December in southern California plantings. The Oroblanco has very few seeds, because it is a triploid. Studies of seed number in the Chandler indicate that it is self-incompatible and, when planted in isolation, can produce good yields of seedless fruit. As with other such cultivars, this characteristic varies with climatic conditions and by years. Fruits with few seeds can sometimes be smaller in size.

The first new citrus cultivars introduced at Riverside (in 1935) were those produced by Dr. Frost. They were the Kara, Kinnow, and Wilking mandarins and the Trovita orange. The Kinnow has attained considerable importance in Pakistan and in parts of North Africa; it continues to be grown on a small scale in California. The Wilking, usually too small for general use, has been a parent of several mandarin hybrids with excellent eating quality. In Japan the Trovita, which is an early, few-seeded juice orange, has been used in crosses with satsuma to produce a new tangor, the Kiyomi.

Rootstocks and rootstock breeding

Rootstocks are of major importance to citrus culture in most countries, because they help to impart disease resistance, cold hardiness, high yield and fruit quality, and other favorable characteristics to their scions. In California, clones of the sweet and sour orange were the principal stocks for many years, until the damaging effects of *Phytophthora* fungi and the tristeza virus made them unacceptable in many areas.

When tristeza began to destroy orchards of sweet orange on sour orange rootstock, use of a substitute rootstock became imperative. As part of comprehensive studies of *Citrus* and *Citrus* relatives, conducted since 1946 at U.C., Riverside by W.P. Bitters, many taxa were tested for tristeza resistance. The Troyer citrange was outstanding, because sweet orange scions grown on this rootstock were tolerant to the disease, and also had other favorable qualities. Troyer continues to be the most important rootstock for oranges in California. A genetically similar cultivar, the Carrizo, has also been successful for that purpose.

One parent of Troyer is *Poncirus*—the trifoliate orange—which as a rootstock also imparts tolerance to tristeza. Clones of this cultivar have important rootstock value, but under more limited conditions than Troyer. Studies were made on other old, established hybrids of *Poncirus*, including citremons 1448 and 1449 (hybrids with lemon) and citrumelo 1452 (a hybrid with grapefruit). The two citremons showed certain good characteristics with lemons, but overall they have not been sufficiently promising. The citrumelo behaved well in some trials with orange, but showed severe problems in a Eureka lemon trial.

Identifying satisfactory rootstocks for lemons has been especially difficult. Sweet orange is favorable but should not be used where gummosis is a problem. Eureka lemons are incompatible with Troyer, as are some semidense Lisbons. Cleopatra mandarin has been perhaps the most useful of the mandarin stocks. Several recent trials with *Citrus macrophylla* have shown that it gives high yields with small tree size, but phloem necrosis can be a limiting factor.

New crosses between *Poncirus* and *Citrus* to obtain rootstocks have been made at Riverside. Successful rootstock cultivars must be highly nucellar in their reproduction, and hybrids with sweet orange have appeared promising in this respect. Some of them show resistance to *Phytophthora*, tristeza, and nematodes, but their effect on yield and fruit quality is uncertain. One of the better ones, unfortunately, has a low yield of fruit and seeds.

Trifoliolate leaf of *Poncirus*

Leaves of *Poncirus* are typically composed of three leaflets, in contrast to the simple (monofoliolate) leaves of common citrus. It has been of interest to examine the inheritance of this trifoliolate leaf character. Nearly all of the older, long-established hybrids of *Citrus* with *Poncirus* were known to have high proportions of three-parted leaves. A new U.C. study showed that among 272 F_1 individuals, all but one had significant proportions of multifoliolate leaves, but the percentages varied from 30 percent in some hybrids to nearly 100 percent in others. When these hybrids were backcrossed to *Citrus*, most of the resulting plants had very few or no multifoliolate leaves, yet some showed proportions up to 90 percent. The conclusion was that the three-leaflet character is not completely dominant, but that it is rather simply inherited, probably dependent on only a few genes.

Future prospects

Advances in breeding and genetics by conventional methods will continue to be slow because of quantitative inheritance of most characters, long generation time, and nucellar embryony. However, breeding efforts can be directed at more specific goals than in the past because of new information and plant materials. The range of strictly sexual parents has been increased, more tetraploids are available for the production of triploids, and hybrids with improved horticultural characters have been developed.

Identification and recovery of sexual seedlings should continue to improve with the use of available biochemical and *in vitro* techniques. However, progress in *in vitro* methods of hybridization has been slow, although fusion of somatic protoplasts (cells with cell walls removed) has been achieved. Research on cell selection has been initiated. Continued research should provide a choice of methods for transferring and recombining a wider range of genetic material.