# TETRAPLOIDY IN CITRUS[1]

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Citrus seeds often contain adventitious embryos, produced, usually subsequent to fertilization of the egg, by proliferation of nucellar cells adjoining the embryo sac.[2] Seedlings developed from these embryos are therefore entirely "maternal" in their inheritance, and in interspecific crosses they can usually be readily distinguished, even in advance of fruiting, from the hybrids that result from fecundation. Among the Citrus progeny from cross-pollination that are now growing in the breeding orchard at the Citrus Experiment Station, the proportion of "apogamic" individuals usually ranges about from 20 to 85 per cent of the whole number of seedlings of a seed-parent variety. Besides the ordinary apogamic progeny, another form that shows no characters of the pollen parent, and therefore is obviously produced asexually, has frequently been found. This form is strikingly distinct from the ordinary apogamic progeny, and its differential characters are so similar in the various species and botanical varieties concerned that a single mode of origin of this aberrant form is strongly suggested. It is somewhat slower in growth than the normal apogamic progeny of the same parentage, and is about one or two years later in its first flowering. It has stout shoots and thorns, and broad, thick leaves. It has been designated "thick-leaved."

The haploid number of chromosomes in three horticultural varieties of Citrus, or in their apparently identical seed progeny produced apogamically, has recently been found to be 9.[3] This is illustrated by figure 1,[4] which shows the second metaphase in a pollen mother cell of normal apogamic progeny of Paper Rind (St. Michael) orange (*Citrus sinensis* Osbeck) from an interspecific cross. There are clearly 9 chromosomes in each group.

Few flowers have as yet been produced by the thick-leaved trees, but chromosome counts have been obtained from pollen mother cells of several buds of a thick-leaved sib of the diploid tree just mentioned. At the second metaphase and anaphase the number of chromosomes in each group approximates 18 (fig. 2). The number seems highly variable, and counts of 16, 17, 18, 19 and 20 have been obtained. Evidently, as in tetraploid Datura,[5] non-disjunction occurs very frequently at the first metaphase. Polar views at the first metaphase indicate that the chromosomes are paired as usual, and that part or all of the bivalents are often more or less closely associated in quadrivalent groups. In the small number of cases so far observed, the formation of quadrivalents is much less general than in tetraploid Datura, and is obviously secondary to ordinary pairing; in the formation of quadrivalents this tetraploid Citrus seems to be intermediate between tetraploid Datura and tetraploid Primula.[4] Figure 3 shows an unusually clear *lateral* view of the first metaphase, in which all of the 18 bivalents seem to be distinguishable. The pollen mother cells and pollen tetrads are considerably larger in the tetraploid form than in the diploid.

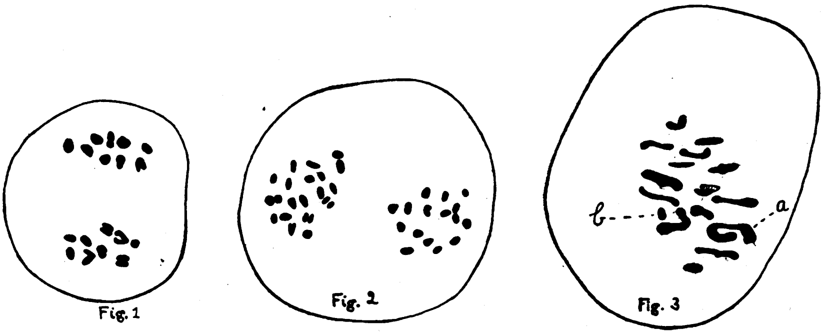


FIGURE 1

Normal (diploid) apogamic seedling progeny of Paper Rind orange. Metaphase of second meiotic division in pollen mother cell; 9 chromosomes, often distinctly split, in each group.

FIGURE 2

Tetraploid apogamic sib of the tree represented by figure 1. Second metaphase; 16 chromosomes in one group and 20 in the other; chromosomes seldom clearly split, but one widely so.

FIGURE 3

Same tree as figure 2. Lateral view of late metaphase of first meiotic division, evidently showing 18 bivalent chromosomes. At "a," two bivalents joined by a strand of chromatin; at "b," a bivalent not yet oriented on the spindle.

In the diploid tree mentioned above the pollen-tetrad stage usually appears normal, but occasionally one or two microcytes are present (for example, in 2 out of 50 random tetrads from one bud). In the tetraploid sib, on the other hand, microcytes are conspicuously numerous; counts of 50 tetrads each for two buds show 28 and 39 tetrads, respectively, with from one to four microcytes. Apparently detachment (elimination) of chromosomes is much more frequent in this tree than in tetraploid Datura.[5]

A few division figures have also been obtained for thick-leaved progeny of Lisbon lemon (*C. Limonia* Osbeck), and several approximate counts indicate that the doubled number of chromosomes is present. It may be confidently anticipated therefore, in view of the general somatic similarity of the thick-leaved forms, that they will all prove to be tetraploid.

The thick-leaved type has been produced by 12 horticultural varieties of Citrus, representing 4 species and 2 botanical varieties, and sometimes to the extent of several per cent of the whole number of progeny. It is possible that "islands" of tetraploid tissue are common in the parent trees, so that not all tetraploid seedlings represent distinct cases of doubling of chromosome number. Chimeras combining the normal and thick-leaved forms have not been found in our cultures, however, and C. S. Pomeroy states that, in the bud-variation work on Citrus directed by A. D. Shamel, the thick-leaved form has not been observed. The lack of thick-leaved bud-variation forms might, of course, be due to the slower growth of tetraploid cells, which might keep them from multiplying sufficiently to dominate any apical meristem, rather than to failure of tetraploidy to originate outside the nucellus. In many cases, however, a thick-leaved seedling has developed from a seed giving two or more non-hybrids altogether, and in these cases the other apogamic progeny from the same seeds have almost always been normals. It is therefore probable that doubling of the chromosome number occurs with especial frequency in the nucellar tissue, at least in those cells from which embryos are about to develop, or else in the very young embryos. So far as seed reproduction is concerned, tetraploidy seems to originate far more often in several Citrus species, than does either tetraploidy or triploidy in any other species in which its origin has been observed.

The thick-leaved forms of Citrus do not so far give promise of direct horticultural value. It may be feasible, however, to produce valuable new forms, triploids or modified triploids, by crossing with ordinary diploid varieties. Triploids would probably be practically seedless.

[1] Paper No. 130, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

[2] Strasburger, E., *Jenaische Zeitschr. Naturw.*, **12,** 654 (1878), and *Jahrb. wissensch. Bot.*, **44,** 482–555 (1907); Webber, H. J., *Science*, n. s., **11,** 308 (1900).

[3] Frost, H. B., *Wash. Acad. Sci. Jour.*, **15,** 1–3 (1925).

[4] The figures were drawn with the aid of a camera lucida, at a magnification of about 3050 diameters, and are reduced one-half in reproduction. The slides were prepared by Belling's method (*Amer. Nat.*, **55,** 573–574 (1921)), in the blooming season of 1925. In figure 3, part of the variation in size among the separating chromosomes may be due to variable swelling produced by acetic acid.

[5] Belling, J., and Blakeslee, A. F., *Amer. Nat.*, **58,** 60–70 (1924).