CHAPTER 15

Criteria for Judging Polyploidy

15.1: Sterile Hybrids Made Fertile

In the final analysis, polyploidy is determined by counting the number of chromosomes, and comparing this number with the diploid or untreated plant. Some rapid and accurate methods are available for judging polyploids indirectly.

If a sterile species hybrid begins seed production after treatment with colchicine, the evidence is good that polyploidy has been induced.[41] Geneticists knew that doubling the number of chromosomes in a sterile species hybrid was a critical test for demonstrating the effectiveness of the drug.[3, 31, 41, 44, 47] Species hybrids of *Gossypium* were treated immediately.[4] Plants that flowered, yet failed to set bolls and seed, began seed production in those sections of the plant treated with a proper concentration of colchicine. Therefore, without counting the number of chromosomes, the preliminary efficiency of a treatment could be estimated. The chance doubling that might have occurred through unreduced gametes is of such low frequency that the effects of colchicine were not obscured by natural or spontaneous doubling.

Amphiploids among *Nicotiana* were made in large numbers.[42] The list of artificially induced polyploids increased within a few years.[24] Combining the first data from *Gossypium* and *Nicotiana* proved the value of colchicine beyond doubt.

Many combinations of interspecific and intergeneric hybrids were converted into amphiploids within the Triticinae.[1, 5] From one project, 18 amphiploids involving 10 species were created within two years.[41] The production of good pollen and eventually seed in the sectors of treated plants that showed the effects of doubling was reliable criterion for amphiploidy. Estimates of how effective colchicine was upon these plants could be checked on a percentage basis. Some modifications were necessary because the monocotyledonous species had to be treated differently from the dicotyledonous types.

After the amphiploids in Triticinae were produced in such large numbers, it was demonstrated that both monocotyledons and dicotyledons were being doubled by the use of colchicine.

A barrier in plant breeding had been removed or considerably reduced by the discovery of a ready technique for making sterile hybrids fertile and estimating the effectiveness by seed production. Incompatibilities such as failure to make hybridizations must now be overcome. Some work on embryo culture has been used to excellent advantage.

15.2: Appearance of Polyploids

New leaves and stems that grow from treated sectors are usually wrinkled, thicker, and darker green, and have coarser texture, as compared with the untreated plants.[4, 7, 14] An increase in thickness of the tetraploid leaf can be judged by holding the leaves between thumb and forefinger. By such methods a rough sorting of tetraploids can be made among large populations of treated cultures. Those that have not responded can be quite accurately eliminated.

Specific marks on the leaves such as veins, hairs, and glands are valuable references for the first sorting of possible changed types. The outline of the leaves changes; they are usually shorter and more rounded than the diploid leaves.

Flowers of the tetraploid plants are larger (Fig. 15.1*B*) and more compact than the diploid (Fig. 15.1*A*). These changes were correlated with chromosomal determinations (Fig. 15.1*C, D*). Tetraploid, triploid, and diploid flowers form a decreasing series in size of flower. These proportionate changes are illustrated for watermelon strains. At the tetraploid level, optimum size is reached, and beyond that point the increase in sets of chromosomes actually reduces the size of the flower. Among the best varieties of *Iris*, polyploids are favored over diploids.[40] The increase in size of flower has been a goal for the improvement of ornamental species.

A tetraploid plant has a more rugged appearance, looks sturdier, and has certain giant-like features. Usually the rates of growth are slower, but even the final growth does not produce a plant as tall as the diploid. Among polyploid watermelons, the vine remains green over more days than among diploids, disregarding disease factors. Another difference between the stems of diploids and those of tetraploids is the shape of the apex as viewed in longitudinal section (cf. Chapter 14).

15.3: Fruit and Seed

The development of larger seeds from tetraploid lines is a consistent macroscopic characteristic that has been confirmed for hun-

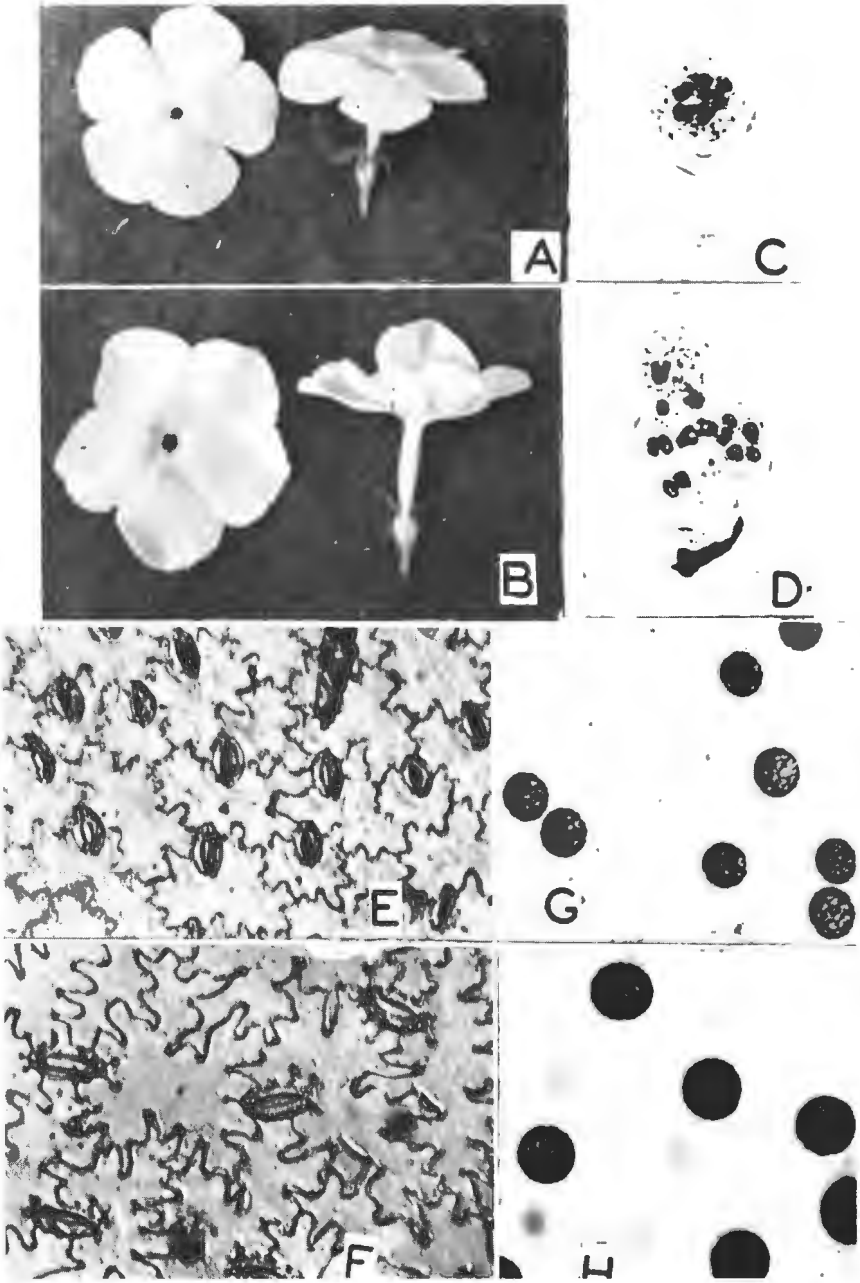

Fig. 15.1—Flower, pollen, stomata, pollen mother cells of diploid and tetraploid strains of **Phlox drummondii**. **A, B.** Diploid and tetraploid flowers, respectively. **C.** Pollen mother cell with 7 bivalents. **D.** Tetraploid pollen mother cell, n—14. Note quadrivalency. **E, F.** Stomata of diploid and tetraploid respectively. **G, H.** Pollen grains of diploid and tetraploid, respectively. (After Eigsti and Taylor)

dreds of cases.[26] The sizes can be judged by volumetric measurement, weights, or length and width measurements. As a sorting method for choosing the tetraploid rye plants in the treated generation, size of seed is a reliable feature.[32] The grain weights of tetraploid rye were distinctly separated from diploids. Table 15.1 shows the increase based on thousand-grain weights for diploid and tetraploids. A mean weight of 30.34 was obtained for diploid and 46.50 for tetraploid.[32]

Increasing the size of seed has been used as an argument to improve the crop yield of diploids through polyploidy. The fallacy lies in the fact that the seeds of tetraploids may be larger and heavier, but the reduced number of seeds per plant prevents complete use of the increase. Reduced fertility in autoploids is the most common cause of decreased yield in number of seeds. Decreased seed production in watermelon brought out this relation. A comparison of ten fruits, diploid and tetraploid, showed avarages of 290.0 and 92.7 per fruit, respectively.[21] Since cultivation was similar and the varieties were strictly comparable, the reduction was directly correlated with tetraploidy. For reasons discussed in the previous chapter, triploids are without seeds.

Amphiploids do not show the same consistent increase in seed weight or size compared with the parental species. A comparison between amphiploids and parental types was made among species of *Bromus* of the Gramineae. On the basis of weight for 200 seeds, the amphiploid increased as much as 75 per cent, while other increases were not more than 17 per cent[44] (Table 15.2). Genetic factors and the contributions by each parent have a greater influence than merely doubling the number of chromosomes.

A given kind of plant may regularly show specific marks among the tetraploid seeds. Close inspection of the tetraploid seed of watermelon showed that fissures developed in the seed coat upon drying.[21] A rupture of the outer layers of ovules creates this condition. These marks as well as size of seed are good criteria for making preliminary sorting of the tetraploid. Another distinction was the thickness of "triploid" seeds and tetraploid. Seed from tetraploid fruit pollinated by diploids are called "triploid" seed and are thinner than the seed from tetraploid fruits pollinated by tetraploids.[21] Other marks such as coarseness, special spines, ridges, and color differences, once noted can be reliably used as guides in selection among treated plants and the tetraploid generations.[4, 11, 15, 16, 17, 18]

Fruits of tetraploids are not necessarily larger than those of diploids. Nevertheless, distinguishing marks can be found among tetraploid fruits. The external marking, shape, and attachment to plant are some of the features that have been used. Parthenocarpic fruits, such as the triploid, may be somewhat triangular.[21] The fleshy por-

TABLE 15.1

KERNEL WEIGHT IN DIPLOID AND TETRAPLOID RYE VARIETIES

(After A. Müentzing[32])

Variety	Thousand-Grain Weight (*grams*)																Number of Trials	Mean Weight (*grams*)
	24	26	28	30	32	34	36	38	40	42	44	46	48	50	52	54		
Steel rye, diploid	1	2	4	4	2	2											15	30.34
Steel rye, tetraploid									2	1	2	6	1	3			15	46.60
Wasa II, diploid	2	...	4	3	2												11	29.55
Wasa II, tetraploid									2	1	1	3	3	1			11	46.27

tion of tetraploid tomatoes may be coarser, and for that reason the polyploids are less desirable than the diploid. Many fruited plants of horticultural importance show direct correlation between fruit size and polyploidy within certain limits. Valuable tetraploid varieties of grapes are larger and superior to diploids. Tetraploid muskmelon

TABLE 15.2

SEED WEIGHTS OF SPECIES AND ALLOPOLYPLOID DERIVATIVES OF THE HEXAPLOID SPECIES OF *Bromus* (*Ceratochloa*)

Species of Polyploid	Strain	Weight of 200 Seeds (*grams*)	Increase Over Arithmetic Mean Between Parts (*per cent*)
B. catharticus	Waite	3.1	.....
B. catharticus	Berkeley	3.3	.....
B. catharticus	San Antonio	2.5	.....
B. haenkeanus	Carmel	1.5	.....
B. haenkeanus	Sparks	1.2	.....
B. stamineus	Berkeley	1.9	.....
B. catharticus-haenkeanus	Waite-Carmel	3.5	52
B. catharticus-haenkeanus	Berkeley-Sparks	2.7	17
B. catharticus-haenkeanus	San Antonio-Carmel	2.5	25
B. haenkeanus-stamineus	Carmel-Berkeley	2.4	41
B. haenkeanus-stamineus	Sparks-Berkeley	2.8	75
B. haenkeanus-stamineus	San Antonio-Berkeley	3.6	64

fruits were more promising than the diploids according to sampling methods made in one study.

Pistillate flowers of tetraploids pollinated with pollen from diploid strains may reduce the size of grain in such a plant as rye. Normally these species are cross-fertilized, so planting side by side gives the diploid pollinator an advantage over the slower-growing pollen from tetraploid flowers.[17] Yield is at once reduced. The effect of diploid pollen upon fruit development in watermelon is quite the opposite. The triploid plants must be interplanted with diploids to insure pollination, for the diploid pollen stimulates parthenocarpic or seedless fruit formation. The number of fruits produced by triploids may be double the number for a representative diploid. Yield trials showed that this feature favors the polyploid.

15.4: Physiological Differences

Excellent reviews have been made to differentiate the diploid and tetraploid plants.[34] An ever-increasing number of autotetraploids adds more material for such study, including physiology, incompatibility,[2, 48] morphology, and anatomy. Final superiority of the

tetraploid depends upon the physiology of the particular strains.[6, 43, 206] Advantages such as protein content,[37] vitamins,[34] yield of sucrose,[38] and other valuable characters[25, 28, 36] are products of the functioning plant.

A superior baking flour was produced by the tetraploid rye varieties. Bread with better texture and color, as well as a larger volume of bread per sample of flour was made from the tetraploid flour. The value for tetraploid was 279 in contrast to the value 260 for a diploid, or an increase of 10 per cent in favor of the tetraploid. Higher protein content was correlated with the improved baking properties and these were in turn correlated with the tetraploid varieties.

Increased sugar content in triploid watermelon and tetraploid muskmelon improved the eating quality. Increases from 8 to 9 per cent for diploids were shown to be raised to 12 per cent in the triploid. The quality and final test of any variety depends upon the genetic nature of the diploid or the hybrid, so that variation exists between tetraploids quite as much as between diploids. The induction of polyploidy does not automatically guarantee improved fruit quality.

In a previous chapter, reference was made to the significant increase in amount of sugar produced in the larger sizes of triploid roots compared with the diploid. Tetraploid sugar beets are generally lower in yield of sucrose per unit weight of root. Other plant products, such as latex produced by *Taraxacum kohsaghyz* and translated into rubber production, gave the tetraploids an increase of three times the diploid. Drug production in *Datura stramonium* showed increased atropin in the tetraploid. Another species, *Cannabis sativa*, showed increased potency of the marihuana content when additional sets of chromosomes are built into a variety. Environment influences potency of drug production as noted in Chapter 5, but the addition of chromosomes also causes changes in production of special plant products.

The superiority of tetraploid red clover and alsike clover may be correlated with an increase in forage production. The amount improves in the second year over the first. Enough tests have been made with these forage crops, and on a sufficiently large scale, that the conclusion of increased leafage is reliable.[1]

15.5: Microscopic Characteristics

Pollen size may be used for preliminary sorting of polyploids before the final chromosomal counts are made for a particular plant. This microscopic classification permits one to handle large numbers of individuals. After the macroscopic identifications are completed, a logical step is to measure the pollen grains.

True autotetraploids have larger grains than the diploid (Fig. 15.1*H, G*). Microscopes are equipped with measuring oculars that make this procedure routine. The correlation between the size of the pollen grain and the number of sets of chromosomes has been so well established that no further discussion need be made on this point. Triploid pollen grains are notable for their irregular dimensions and are useful in separating triploid and tetraploid plants on a field scale basis.

The mean diameters for the diploid and tetraploid watermelon varieties were 57.3 and 67.5, respectively. The smaller grains in triploids averaged 62.1 and the larger sizes, 67.5. Similar size comparisons have been made for the guard cells of epidermal cells. A photomicrograph (Fig. 15.1 *E, F*) gives a visual picture of the differences between the larger tetraploid and smaller diploid. Also the distribution of guard cells varies; the diploid cells are closer together than the tetraploid.

The relation between the size of pollen grains and guard cells of a given plant are important for the reasons discussed in the previous chapter under the subject of periclinal chimeras. If the pollen is tetraploid and the guard cells are diploid, treatment with colchicine has produced a chimera in which the deeper layer that produced the pollen was made tetraploid and the outer layer remained diploid. A reverse situation may occur. In these instances the guard cell would show tetraploid characteristics and the pollen, diploid. The breeding behavior of such a plant would be that of a diploid. Seed from this plant would not lead to the expected tetraploid types, according to information based on the guard cell sizes. Sometimes, a mixture of diploid and tetraploid pollen exists in the same anther, or mixtures of diploid and tetraploid guard cells appear on the same leaf. These cases are a result of mixoploidy, a direct action of colchicine.

In cross section the leaf of the diploid is not as thick as that of the tetraploid. Usually extra layers of cells of the mesophyll are present.

Pollen mother cells undergoing meiosis are universally used for counting chromosomes and determining the associations between chromosomes during pairing. Acetocarmine stains have speeded up such cytological work. Photomicrographs in Figure 15.1 show the differences in numbers of chromosomes and some difference in the association. Section *D* shows the multivalents in contrast to the one in *C* (Fig. 15.1).[10]

Other cells, such as the generative cells in pollen tube cultures, root tips, and leaf cells, may be used for counting the number of chromosomes. At the second meiotic division and the division of the microspore, chromosomal counting may be easier than at the first meiotic metaphase.

Comparisons at meiotic metaphase of diploid sterile hybrids and the amphiploid are important for an understanding of the possible associations that form between chromosomes of opposite genomes. While this evidence is not infallible, correlations may be obtained between pollen fertility, possible intergenomal exchange between chromosomes, and reasons for the failure in seed setting of the polyploid.

15.6: Ecological Considerations

The success of a polyploid in nature or in agriculture depends upon how closely the new variety meets the requirements for each situation. Productivity or adaptation are measured in terms of the responses such as yield, disease resistance, drought resistance, and cold tolerance. The elimination in nature occurs through competition and in agriculture at the hands of the agronomist. Wide differences exist between diploid varieties, and considerable improvement can be done at the diploid level without stepping up to the tetraploid. Adaptation problems increase, rather than decrease, with the use of tetraploids. Autotetraploid rye clearly showed that the kind of plant used to make the diploid may be as important as any other feature.

Trying to measure the rates at which artificial polyploids become established under natural conditions strikes at some basic problems in polyploidy. Already differences have been recorded for the success of the tetraploid over the diploid, or vice versa. An unusually high seed production, about 75 per cent, in autoploid *Ehrahata erecta* played some part in the establishment of the new type under natural conditions. This situation held for ungrazed conditions, but where grazing occurred, the low-growing habit of the diploid assured survival better since the flowers, being closer to the soil level, were not destroyed as readily. This is one example of the critical differences that determine success or failure of the tetraploid.[44]

Wilt diseases are devastating to watermelons in Japan. Appreciable resistance to *Fusarium niveum* was exhibited by the triploid and tetraploid varieties. By selection, notable progress can be made for insect and disease resistance if an initial advantage is provided through the production of tetraploids. Autotetraploid radishes were more resistant to the common club root disease, yielded more, and had greater vigor than diploids.

The succulence of water cress leaves was improved by increasing the number of chromosomes, but the growth rates being slower among the tetraploid reduced the yield. Fewer cuttings can be made per season with tetraploids. The slower growth and prolonged flowering period for ornamental species is advantageous. No single trait can be

established as a rule that will hold for all polyploids. In the above cases a few instances are cited which indicate that each problem must be dealt with independently according to the requirements.

15.7: Fertility

Two general methods are used to judge the fertility level of a specific polyploid: (1) percentage of good pollen as demonstrated by microscopic test, and (2) the amount of seed set. Fertility differences and chromosomal phenomena at meiosis have been correlated, but no general rule that explains the total possibility has been established.[33] Unequal distributions of chromosomes in the meiotic stages from first metaphase do cause unbalance in chromosomes in the pollen, and ultimately in the gamete. Triploids are notoriously bad with respect to chromosomal balance.[21] When the percentage of pollen that appears to be good is used to express the fertility ultimately judged by seed production, some reservations must be made.[8]

Female sterility in the ovule arises at meiosis and may or may not be the same as for pollen. Some polyploids are female-sterile and pollen-fertile, and vice versa. The embryo-sac stages are difficult to study because an involved cytological technique is required.[8]

Among progenies of amphiploids the first generation may be quite fertile, while later generations may segregate due to weak and low fertility. By successive selection the fertility level may be raised, or there may be mechanisms for improving fertility by elimination of those genotypes that are deficient or have no survival value.

Perhaps no other aspect of polyploidy is more controversial than this subject of fertility in the immediate product of doubling and in the subsequent generations. Practically and theoretically the problems are unsolved at this point.

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