

CHAPTER

9

APPLICATIONS OF POLYPLOIDY

Various applications of duplications (polyploidy at the diploid level), autopolyploidy, and allopolyploidy will be considered in the first section of this chapter. Some of the methods used in producing them will be presented in the second section.

The studies of chromosomal interchanges, inversions, and other aberrations have shown how duplications of chromosome segments in diploids might have occurred naturally or might be produced experimentally.

Many of our important crop plants are polyploids, i. e. tetraploids or hexaploids that have become established during the course of evolution. The discovery of methods of producing new ones experimentally has expanded the opportunities greatly for breeding and other studies. For general discussions consult: Blakeslee (1939), Darrow (1949), Dermen and Emsweller (1961), Emsweller and Ruttle (1941), Fyfe (1939), Huskins (1941), Hutchinson (1958), Krythe and Wellensiek (1942), Levine (1945), MacKey (1954), Muntzing (1948, 1956), Nebel (1941), Randolph (1941a), Rudorf (1943), Essad (1959), Stebbins (1956), Yamashita et al. (1957), and Elliott (1958).

Duplication

We have seen in chapters 2 and 6 that duplicated segments, an addition of whole chromosomes, or even small deficiencies may produce phenotypic effects that are transmissible to the offspring. The effect on the individual carrying the chromosomal change cannot be predicted, since the effect depends not only on the nature of the genic material duplicated, but also on its interaction with adjacent genes ("position effect"). Also there may be physiological changes as a result of the change in gene balance which may or may not show up as a recognizable new phenotype. Changes in the chemical composition of storage products, in the ability to survive and perform differently under different environmental conditions are among the conceivable possibilities.

Duplications for segments carrying known genes offer the possibility of doubling their dosage without doubling the remainder of the genome as occurs when an autotetraploid is produced. Doubling the dosage of genes for disease resistance might produce a higher degree of resistance; or doubling the genes for different endosperm compositions, as waxy, high amylose or sugary in corn, might enhance their industrial or other uses. A genetic test for the presence of a duplication has been proposed (Burnham 1962a).

Stocks tetraploid for certain chromosome segments have been produced in Drosophila for the X chromosome and for the autosomes (Burdette 1940). All showed decreased viability and fertility when homozygous. The effect did not depend on length of segment. In species with higher chromosome numbers, the imbalance thus produced may be less detrimental.

Autopolyploidy

Several of the characteristics of autopolyploids, larger size of floral and other parts, lower fertility, later maturity may be of value or may be a detriment depending on whether the species is grown for its flowers, seed, or fruit, or for forage or root production. For example, in ornamentals, the larger size of the flowers may be desirable and the lower fertility may lessen the need for removing flowers to prolong the blooming period. In forages, the larger leaves may be an advantage, but the larger stems, slower growth, and later maturity may be disadvantages. Larger seed may result in higher yields if not discounted by lower fertility. Chemical constituents may be changed also in the tetraploid as compared with the diploid. The answers to the following questions also will influence the manner in which polyploidy might be used: Is the species self-incompatible? Can it be reproduced asexually? Is it an annual or a perennial? Is it self- or cross-pollinated? Although many autopolyploids have been produced, relatively few have been used directly. These are likely to be in species that are perennial, that can be propagated asexually, or in which seed setting is relatively unimportant.

Most autopolyploids have some characteristics which prevent their immediate commercial use. This is particularly true of those that must be sexually propagated or in those grown primarily for their seed. Some have called them "raw" polyploids, emphasizing the point that when they first occur they are not stabilized. In the early generations at least, they may not breed true for the tetraploid chromosome constitution, the numbers varying from a few more to a few less than the tetraploid number. Also they may occasionally revert all the way back to diploids (Randolph and Fischer, 1939, in maize). This may occur by a process similar to that by which haploids arise from diploids.

⁴ⁿ In ^{tissues}tomatoes, diploid¹ may occur by irregular nuclear division, as described on page 262. Usually the polyploid condition may be maintained by cytological checks of sub-lines along with careful observations on their appearance. An occasional autopolyploid is so irregular in chromosome behavior it cannot be maintained, e.g. Eruca sativa (Rajan, Hardas and Parthasarathy 1950) and Vicia villosa (Hertsch 1951).

An increase in the frequency of bivalents has accompanied the selection for higher fertility in maize. Genes may be found which prevent multivalent pairing. Such a gene has been found in T. vulgare wheat, effective against pairing of homoeologous chromosomes (Riley 1958, Sears and Okamoto 1958). If such a gene were found in an autopolyploid, true-breeding heterosis might be established, i.e. heterosis for gene pairs now located in different bivalents, formerly members of quadrivalents. Another method might be to double the chromosome number of a hybrid between two lines, one of which has numerous inversions. There is evidence that in the resulting autotetraploid identical chromosomes would show a strong tendency to pair preferentially (Doyle 1959).

Sacharov, Frolova and Mansurova (1944), have reported obtaining tetraploids representing 9 varieties of buckwheat. Selection for four generations produced a number of highly fertile lines whose yields were 3 to 6 times those of the normal diploids. They also reported the tetraploids were more frost resistant.

Other notable examples of increased fertility by selection are in corn (Gilles and Randolph 1951), in sugar beets (Feltz 1953), and in rye (Müntzing 1951). An autotetraploid variety of rye, Tetrapetkus, has been introduced from Germany. It is reported to give higher yields than the diploid in areas where it is sufficiently winter hardy. A tetraploid rye has been developed in Sweden that is at least equivalent in yield to the diploid commercial varieties and in some environments outyields them. Since crosses with diploids produce shriveled seeds that are triploids, tetraploid rye must not be grown

adjacent to diploid rye (Müntzing 1948). This must be taken into account in yield trials for the evaluation of tetraploids (Müntzing 1951). This is true in any tests of cross-pollinated tetraploid crops grown for seed. In rice, crosses between Indica and Japonica varieties are often partially sterile. In progenies from the doubled hybrids it was possible to select for higher fertility (Luong 1952).

In crops grown primarily for their forage, such as alsike and red clover, low seed set may not be as serious except for the greater cost of producing the seed. Tetraploid marigolds and snapdragons are being used commercially. Low seed setting resulting in the dropping of seed pods may be an advantage in ornamentals. It is a self-picking feature which may prolong blooming.

In crops that can be propagated asexually from a single clone, e.g. certain grasses, potatoes and fruit crops, the entire beneficial effect of polyploidy including heterosis is maintained. Similar results should be possible with clonally propagated tetraploid forest trees, ornamental vines, shrubs, and trees. In strawberries and raspberries, the reduced fertility results in deformed fruits (Dermen and Darrow 1939). Tetraploid apples are too large and coarse for some uses, but tetraploid grapes are a promising improvement (Dermen 1954). Some commercial varieties of apples are triploids, but they are not always an improvement over the diploids.

In cross-pollinated crops, if autopolyploids superior to their diploid counterparts could be developed, they might be introduced without fear of their being degraded by natural crossing (Doggett 1957).

In sugar beets triploids are superior to diploids and tetraploids in yield of sugar and in certain other characters. In Japan the triploid seed used for planting is produced on tetraploid plants in a crossing field in which diploids are interplanted as the pollen source. This is workable since the tetraploid is self incompatible and the pollen with n chromosomes grows more rapidly than $2n$ in $4n$ as well as in $2n$ styles (Matsumura 1953). The triploid watermelon from $4n \times 2n$ crosses is nearly seedless, and is sweeter than the seedy diploid parent. It is used commercially in Japan (Kihara 1951, 1958).

The potentialities of autopolyploidy for a given crop cannot be determined easily or quickly. If the species is cross-pollinated, it will be highly heterogeneous and a wide range of polyploid genotypes is possible. A pool of breeding stocks in the diploid such as those available for hybrid corn will be valuable starting material for breeding programs designed to test the potential value of polyploidy. Crosses that bring together in the hybrid the largest number of favorable factors from diverse sources, e.g. in complex synthetics, should be of use. Numerous autopolyploids would be needed to test the potentials of the various genotypes. Since their response to environmental conditions may be very different from that of the diploid parent, they must be evaluated under a wider variety of conditions.

In self-pollinated species, one approach might be to use crosses or multiple crosses which will produce new genotypes which can be doubled. Another might be to induce the doubling first, then cross, and select among the tetraploids. Whatever method is used, one fact must be remembered: that completely reliable predictions as to what a given combination will do at the tetraploid level cannot be based on behavior at the diploid level.

Changes in chemical composition

The possibility exists that the content of desirable chemicals, e.g. alkaloids used as drugs, vitamins, kinds or proportions of carbohydrates, proteins or their constituents, may be increased or changed by polyploidy. The effect of chromosome doubling can not be predicted but must be determined in each individual case.

In corn, Randolph and Hand (1940) reported a 43% increase of carotenoid pigment and vitamin A activity in tetraploid over diploid yellow corn. A tetraploid clone of the commercial spearmint differed in odor from that of the diploid (Ruttle and Nebel 1939). The tetraploid Ocimum basilicum (basil) differs from $2n$ possibly in quantity and quality of oil (Lapin 1939). The $4n$ marigolds derived from odorless diploids are still odorless.

In flax in the Redwing variety and in Redwing x Bison hybrids, the seed of the $4n$ was larger than that of the $2n$; iodine content was about the same, but oil percentage was lower in the $4n$ (Ross and Boyes 1946). Seed yields per acre were only 30 to 40% of those in diploids. Fiber production was poorer in tetraploid flax (Rudiger 1953).

The wider geographic range of polyploids has been interpreted by many as indicating that they are more hardy. There is evidence that they are not necessarily so. Tetraploid species may be more hardy, less hardy, or of the same degree of hardiness as their diploid relatives (Bowden 1940). In comparisons in freezing tests of red clover (Trifolium pratense L.) and winter rye (Secale cereale L.) the autotetraploid was in all cases much less resistant to frost damage than the diploid (Sj  seth 1957). However, the autotetraploid was more resistant when it came from a resistant diploid than when it came from a less resistant diploid.

Kostoff (1939) reported polyploids to be more variable in cell dimensions, plant size, and leaf and flower dimensions than their original diploids. This may account for their greater range in certain cases, if a similar variability in physiological characters occurs.

Based on the evidence from geographic distribution studies, Darlington (1956, page 62 ff) reaches the conclusion that "chromosome change facilitates colonization", and that these have greater ability to fit into different environments. The new areas may be at the periphery or within the original range of distribution.

Use in studies of developmental morphology

The periclinal chimeras of $2n$ and $4n$ or $2n$ and $8n$ tissue produced by colchicine treatment of seeds have furnished information on developmental morphology and histogenesis in Datura (Satina et al. 1940, 1943; Blakeslee 1941a). Chromosome numbers were determined from dividing cells, but the size of cells and of nuclei were good criteria of differences in ploidy. Various combinations of $8n$, $2n$, $2n$; $2n$, $8n$, $2n$; and $2n$, $2n$, $8n$ demonstrated the contribution of each layer to the shoot apex, to the leaves, the floral organs, and the capsule. The size of the epidermal cells and guard cells, on comparable leaves, indicated the chromosome number of the outer germ layer. Since the egg cells and pollen are formed by the second layer, pollen grain size was one criterion which could be checked by cytological examination of the pollen mother cells. In the shoot apex in Datura, each accounts for a single layer of cells. In over 200 periclinal chimeras, the cells of the inner cylinder all had the same chromosome number, and hence originated in the third germ layer. As Blakeslee (1941a) stated it "Polyploidy in periclinal chimeras may be used like tracer elements to label the different germ layers and to determine the contribution which each makes in the development of a given organ. Thus it may be of help in a study of the homologies of the floral organs." The bulk of the cells in the petal, sepal, and the leaf come from the second germ layer in Datura. In the primordium of the stamen, a single layer of cells comes from the second germ layer, the bulk of the tissue from the third germ layer, as in the shoot. In the style, the central strand of tissue through which the pollen tubes grow is derived from the outer germ layer, the same as the epidermis. A $4n$, $2n$, $2n$ chimera set capsules and seed from selfing but a $2n$, $4n$, $2n$ plant did not. Since the pollen is derived from the second germ layer, n pol-

len tubes had to grow down $4n$ tissue in the $4n$, $2n$, $2n$ chimera, and $2n$ tubes down $2n$ tissue in the $2n$, $4n$, $2n$ chimera. Since in the cross of $2n \times 4n$, the pollen tubes usually swell up and burst in the $2n$ style whereas n pollen tubes grow normally in a $4n$ style, the above observations on seed setting are explained.

Extensive studies of similar chimeras in the cranberry, peach and apple have been made by Dermen (cf. Dermen 1941, 1960). Also, chlorophyll variegation patterns in dicots and monocots have been figured and interpreted in relation to the cytological evidence from dicots.

Changes in self- and cross-incompatibility

The production of autopolyploids may be of value in changing the self- and cross-incompatibility relations. Whereas in the diploid, the pollen has only one member of the allelic series, in the tetraploid the $2n$ pollen has two members which may be the same allele or different ones. In the pollen grains, certain alleles may show dominance over others in the series or they may interact in a new way with each other or with certain genotypes of the stylar tissue.

In tobacco, certain tetraploid genotypes are self-incompatible, others are self-compatible. A similar behavior was observed in white clover, *Trifolium repens* L. (Atwood 1944). Although differences in degrees of seed-setting were observed by Atwood among different compatible crosses, the main facts appear to be explained by his proposal that genotypes which form only one kind of heterogenic gamete e.g. $S_1S_1S_2S_2$ are self-incompatible while those genotypes, e.g. $S_1S_1S_3S_4$ or $S_1S_2S_3S_4$, which form three or more kinds of heterogenic gametes are self compatible (Atwood 1940, 1944, Atwood and Brewbaker 1953, and Brewbaker 1955).

Diploids of *Petunia axillaris* Lam. were self-incompatible whereas tetraploid branches were self-compatible (Stout and Chandler 1941). In *Petunia inflata*, the tetraploids derived from the 10 self-incompatible heterozygous diploid hybrids between five S allele homozygotes were all self-compatible. These all formed only one kind of heterogenic pollen (Brewbaker and Shapiro 1959). In *Brassica rapa* L., *B. campestris* and *Raphanus sativus*, tetraploid branches continued to be self-incompatible (Howard 1942a). The same was true for *Oenothera organensis* (Lewis 1943).

As mentioned earlier, in maize, self- and cross-incompatibilities were found which did not exist in the diploids (Randolph 1940 and Fischer 1941).

Changes in interspecific crossability

In many cases, related species may have characteristics which would be useful if they could be transferred to the commercial varieties. Often these have a different chromosome number, and crossing is difficult. No progress can be made unless the cross can be made and the F_1 is viable. Doubling the chromosome number of the diploid has improved crossability in many cases, though not in all. For example, in cabbage, Karpechenko (1937) found that doubling the chromosome number increased its crossability, as shown by the data in Table 118.

Note that the cross of diploid *B. oleracea* on *B. chinensis* was unsuccessful, whereas the tetraploid form readily produced hybrids.

Doubling the chromosome number of certain diploid species of *Solanum* has improved their crossability with *Solanum tuberosum* (Livermore and Johnstone 1940).

Table 118. Data comparing the crossability of diploid vs. tetraploid cabbage with other species.

Cross	Flowers polli- nated	No. Seeds	No. Hybrids	No. of Hybrids per 100 Flowers
<i>B. carinata</i> (n=17) x <i>B. oleracea</i> (n=9)	334	20	14	4.2
reciprocal	271	1	1	0.4
<i>B. carinata</i> x <i>B. oleracea</i> (n=18)	408	776	740	181.4
reciprocal	118	55	32	28.0
<i>B. chinensis</i> (n=10) x <i>B. oleracea</i> (n=9)	122	0	0	0
reciprocal	70	0	0	0
<i>B. chinensis</i> x <i>B. oleracea</i> (n=18)	155	209	127	81.9
reciprocal	131	4	4	3.1

Allopolyploidy

related
 Amphiploids are more likely to be usable directly than are autopolyploids. When diploid species, to the natural allopolyploid are crossed, many of the hybrids show complete lack of pairing in F_1 and are sterile. Fertility is often restored by doubling the chromosome number. These are, in effect, new species. They may or may not resemble already existing species.

The change from a sterile hybrid to a fertile amphidiploid by chromosome doubling may be accompanied by other changes. For example, the odor of an amphidiploid mint hybrid was not the same as the odor of the sterile hybrid (Ruttle and Nebel 1939) as mentioned earlier.

Crosses of the synthetic allopolyploid with the natural one, if successful, offer another possibility of transferring useful genes from the diploid species to the natural allopolyploid. Doubling the chromosome number of the diploid species as mentioned earlier, may make it possible to produce hybrids with the natural polyploid.

In evaluating the potential of synthetic allopolyploids, it is important to recognize that an amphiploid resulting from doubling the chromosome number of an F_1 interspecific hybrid is expected to produce remarkably uniform offspring; since the F_1 carried a particular gametic combination from each parent. Chromosome doubling produces an individual comparable to a homozygous diploid obtained by doubling a monoplod. If either diploid parent showed considerable diversity, a single synthetic allopolyploid would not sample the available variation. New mutations would lead to some variation. The extreme uniformity in diploids derived from monoplods is soon lost in succeeding generations. Similar changes might be expected in the amphiploids. Only dominant changes would be expected to be visible in earlier generations, since recessive changes would most likely be covered by the normal alleles in the other genomes. In amphiploids, changes in later generations might result also from intergenome pairing and crossing over. This behavior depends on the degree of homology between chromosomes in the different genomes and the consequent opportunities for crossing over between chromosomes belonging to different genomes. Pairing between chromosomes in different genomes depends not only on the degree of homology but may be determined genetically as in *T. vulgare* wheat. The absence of one arm of chromosome 5 from the genome (the nullisomic or the nullisomic + telocentric 5R) allows chromosomes that are partially homologous to pair and supposedly cross over and produce new gene combinations (Riley 1958, Sears and Okamoto 1958). Seemingly a gene (or genes) carried in chromosome 5 normally suppresses the pairing of partially homologous chromosomes in the tetraploid