

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

and hexaploid wheats. What might be produced in the absence of this gene remains to be seen.

The great diversity in the cultivated allopolyploid cottons should be considered at this point. As noted above, an amphidiploid arises when hybridization between two species is followed by chromosome doubling. Each initial hybrid plant represents a single genetic combination. Unless this occurs repeatedly, the immediate variation expected from the ancestral hybrid would be very limited. As discussed by Hutchinson (1947, 1959, pp. 43-45), subsequent variation in cotton must have arisen by mutation. Fisher (1930) showed that diversity in a species can be accounted for by the overall mutation rate. As pointed out above, intergenome pairing and crossing over could add to this diversity. Hutchinson points out that the rapid expansion of the area in which a crop plant is cultivated provides much more favorable conditions for newly occurring variability than do natural populations. For example, in Uganda the cotton crop expanded about 600-fold in 30 years.

It is possible that amphiploids may be directly usable as commercially useful varieties, but this depends on the nature of the interactions between the genes of the species that were combined, their interactions with the maternal cytoplasm and with the environment. However, the variation available in each parental species can be sampled only by inducing doubling in many F_1 hybrids and then intercrossing the amphiploids.

Doubling the chromosome number has produced amphidiploids and restored fertility in *Nicotiana* crosses (Smith 1939, Greenleaf 1938); in wheat (Sears 1939a, 1941, Zhebrak 1939, 1940, 1940a, b, 1941, 1944, and many others); in *Datura* crosses (Blakeslee 1939); in basil, *Ocimum canum* x *Ocimum gratissimum* (Lapin 1939); in *Mentha aquatica* x *M. rotundifolia* (Ruttle and Nebel 1939); in wheat x rye (Müntzing 1939, 1948, 1956; Navalikhina 1940); in Upland x Egyptian cotton (Kasparyan 1940, Zhebrak and Rzaev 1940) and in many other interspecific crosses. Although amphidiploids may arise naturally, as in *Primula kewensis* the ability to produce them experimentally is of great importance. Amphidiploid sectors or branches on sterile hybrids are easily recognized by their fertility. Complete lack of pairing in F_1 is no guarantee that doubling will restore fertility. There may still be interactions between genomes or between them and the cytoplasm which result in sterility.

The amphidiploids from wheat x rye crosses, the *Triticales*, will be described here. The first ones occurred in F_2 as a result of naturally occurring chromosome doubling in the F_1 . One of these was discovered by Müntzing (1948) and one probably originated in Rimpau's cultures about 1890. Crosses made by Müntzing between the different strains showed considerable variation in F_2 . Selection produced considerably improved lines. When the colchicine method of chromosome doubling was discovered, this method was used to double larger numbers of wheat x rye hybrids. In many of these *Triticales* the anthers did not dehisce properly. Crossing followed by selection produced lines without this difficulty and with great improvement in fertility, straw stiffness, and other agronomic characters. Crosses between wheat and strains of rye selected for higher self-fertility and hardness have been used to produce *Triticale* strains that are still more promising. The kernels are 40 to 50% heavier than those of diploids and their protein content is 15 to 18% (10 to 12% in diploids). Thus far, they have been of no economic value.

Transfer of characters

Amphidiploids as well as crosses between species may be used to transfer characters from one species to another. The wide range of possible crosses which might be of use in this type of breeding may be illustrated in cotton (Knight 1948, 1954). There are several different diploid species with $n=13$. These have different basic genomes, labeled

A, D, etc. Different diploid species with similar but not identical genomes are given different subscripts, e.g.: A_1 , A_2 , D_1 , D_2 etc. (Beasley 1942).

The commercial upland cotton is an allotetraploid with $n=26$ chromosomes and the basic genomes A and D. The somatic constitution of a diploid might be A_1A_1 , A_2A_2 , or D_1D_1 , that of an allotetraploid might be $(AD)_2(AD)_2$. An amphidiploid from the cross of $A_1A_1 \times D_2D_2$ would be designated $A_1A_1 D_2D_2$. Several possible methods of utilizing the various species available are the following:

Method 1. Cross two different diploid species, e.g. $A_2A_2 \times D_1D_1$ to produce an $F_1 A_2 D_1$. Double the chromosome number of the F_1 and cross it with the allotetraploid, this cross being: $A_2A_2 D_1D_1 \times (AD)_2(AD)_2$. The F_1 from this is $A_2D_1 (AD)_2$, i.e. single sets of A_2 , D_1 and $(AD)_2$ chromosomes are present from each of the original parents involved. Or the two different species may be doubled; then crossed; and this F_1 then crossed with the allotetraploid.

Method 2. Double the chromosome number of the diploid species (e.g. A_2A_2) and cross it with the allotetraploid: $A_2A_2A_2A_2 \times (AD)_2(AD)_2$. The F_1 from this is $A_2A_2 (AD)_2$. Darlington (1956) has called this one-sided doubling. The functioning of female gametes with doubled chromosome number occurs naturally and frequently in species crosses in sugar cane (Bremer 1959, 1961) and in certain grasses.

Method 3. Direct transference through the triploid. Cross the diploid with the allotetraploid; i.e. $A_2A_2 \times (AD)_2(AD)_2$, the F_1 being $A_2(AD)_2$.

In each method, attempts might be made to produce offspring by selfing, or by backcrossing once to the commercial cotton followed by selfing and selection. The procedure might vary depending on whether the characters sought were dominant or recessive, simply inherited or multigenic.

Also, if the original diploids used were heterozygous and heterogeneous, many hybrids between them, or between them and the allopolyploid would be needed to sample adequately the available genes.

To eliminate individuals with deviating chromosome numbers when dealing with crosses between species differing in chromosome number the hybrid or the selection may be used as the male rather than the female parent; thus making use of the selective advantage of pollen with the normal chromosome number. This has been used in interspecific crosses in wheat (Allard 1949).

In cotton, the transfer of characters from diploids to the new world cultivated allopolyploid species (Gossypium hirsutum and G. barbadense), or the transfer between G. hirsutum and G. barbadense has not been as simple in practice as the above listing of methods might suggest. For example, the attempt to transfer genes for high fiber strength from the diploid G. thurberi to the new world cottons was unsuccessful after 15 generations (Kerr 1951, Hutchinson 1959). The same was true of attempts to combine the high productivity of an Upland cotton with the long-staple character of Sea Island by inbreeding the descendants of the (G. hirsutum race latifolium \times G. barbadense) cross. Harland (1933, 1936) showed that in F_2 and later generations of this cross, the great majority of the plants were unstable, "odd-ball" types low in vigor. His proposal to explain this behavior was that the genotype of a species is an integrated balanced system built up gradually over the years. When two different species are crossed, there is a disintegration following recombination. The stabilized types that can be established from crosses between G. hirsutum and G. barbadense are close to one parent with a limited number of genes from the other parent. Resistance to blight has been added to the Egyptian types of G. barbadense (Knight 1954).

Among the progeny of Vicia hybrids, lines with entirely new characters appeared, as described by Sveshnikova (1936). Interactions with the cytoplasm are probably important also, since some recombinants may be incompatible in their new cytoplasmic background.

In species which show a considerable degree of cross-pollination, there has been a greater opportunity for integrating the mutations that have occurred into a balanced genotype. As Hutchinson (1951) has pointed out, Vavilov's principle that in the later stages of the development of crop plants, there is often great diversity at the center of origin applies best to crop plants that are self-pollinated.

The aberrant segregants in cotton mentioned above have not been described in wheat. Rust resistance has been transferred from emmer and durum ($n=14$) wheats to T. vulgare ($n=21$) wheats. Iumillo, a durum variety with mature-plant resistance to stem rust was crossed with Marquis to produce Marquillo, a T. vulgare type (Hayes, Parker and Kurzweil 1920). This resistance is recessive in crosses and has been of great practical value. Thatcher, widely grown in the U.S.A. and Canada for many years, was produced in Minnesota from crosses of Marquillo x (Marquis x Kanred). It has the mature plant resistance from Iumillo and physiologic resistance from Kanred.

Stem and leaf rust resistance from Yaroslav Emmer, a T. dicoccum ($n=14$) variety, were incorporated in the Hope and H44 T. vulgare types by means of selection in crosses with Marquis (McFadden 1930). Hope and H44 carry an "adult plant" type of stem rust resistance. They are resistant to nearly all races except 15B. Several commercial varieties carry this type of resistance.

Triticum timopheevi ($2n=28$) is another source of resistance to stem and leaf rust. Selections from a cross between T. timopheevi and Steinwedel, a hexaploid wheat, and from crosses of Bobin² x Gaza (vulgare² x durum) were introduced from Australia by MacIndoe. The variety Lee, a selection from Hope x Timstein, obtained its resistance to leaf and stem rust from Timstein, Ausemus and Reitz (1959). Timstein, thought to be a selection from T. timopheevi x Steinwedel, was probably a selection from Bobin² x Gaza from which came a related selection named Gabo (Watson and Stewart 1956).

Use of addition or substitution lines (also see p. 236)

It has been impossible to transfer the winter hardiness and disease resistance from rye, Secale cereale ($n=7$), to hexaploid wheat by ordinary breeding methods. There is no pairing between the chromosomes of the two species. One approach to the problem has been to produce what R. E. Clausen termed "alien addition" lines, lines with one pair of chromosomes from one species added to the full complement of the other. By crossing the wheat-rye amphidiploid with wheat, O'Mara (1940) produced a line in which the chromosome 1 pair with the hairy-neck gene or genes was added to hexaploid wheat. Lines for four of the seven chromosomes, including 1, were reported by Riley and Chapman (1958a). Each rye pair modified the phenotype of the recipient wheat variety. Most of the changes were in quantitative characters, but certain chromosomes contributed resistance to disease, e.g. mildew and yellow rust. As they pointed out, the addition lines enabled them to compare the genetical effects of the individual rye chromosomes and their cytological appearance and behavior on a uniform wheat background. They also made it possible to locate genes for which only one allele is known, as in monosomics. Univalents were more frequent in the addition lines than in the wheat variety, and fertility was usually somewhat lower, but the lines varied in these respects (Riley 1960a).

Five lines with a Haynaldia villosa chromosome added to hexaploid wheat have been produced by Hyde (1953).

The transfer of leaf rust resistance from Aegilops umbellulata to hexaploid wheat, reported by Sears (1956), illustrates one method of utilizing the addition lines and certain of the problems encountered in attempts to transfer desirable genes from one species to another.

The cross of hexaploid wheat with Ae. umbellulata produced only inviable seeds. The amphidiploid T. dicoccoides x Ae. umbellulata was used as a bridge to obtain plants carrying the Ae. umbellulata and hexaploid wheat genomes. The cross of Chinese Spring with the amphiploid, followed by two backcrosses on Chinese Spring and selection for resistance at each step produced a 43-chromosome plant in which the extra chromosome was from Ae. umbellulata and carried the gene or genes for rust resistance. Undesirable characters had been transferred as well. Since the added chromosome did not pair with a wheat chromosome, X-rays were used as a substitute for crossing over. Plants with a derived isochromosome for the arm carrying resistance were given a heavy dose of X-rays before meiosis and the pollen applied to normal Chinese Spring. The pollen was expected to act as a screen against transmission of the intact extra chromosome and thus favor intercalary translocations. Among 6091 offspring, 132 were resistant including 40 with a translocation involving the Ae. umbellulata chromosome. One intercalary translocation was obtained which showed essentially normal pollen transmission. Homozygous plants were like Chinese Spring except for rust resistance and slightly later maturity.

The utilization of this source of leaf rust resistance in a soft red winter wheat breeding program is in the final testing stages before possible release (Caldwell et al. 1961).

Lines in which all or a part of chromosome 1 of rye was substituted for one of those in wheat have been reported by O'Mara (1940). The phenotypic effects were less extreme than in the addition line for the same chromosome. It would be interesting to determine if a particular chromosome is always substituted for the same chromosome in wheat. If not the same, different phenotypes might be expected from different substitutions of the same chromosome from the donor variety.

The method of transferring a particular chromosome from one variety of hexaploid wheat to another using the monosomics has been described in the previous chapter (pages 246-249). It might be usable in interspecific transfers.

The first attempts to transfer the necrotic type of mosaic resistance from Nicotiana glutinosa ($n=12$) to N. tabacum ($n=24$) failed because the hybrid was completely sterile. The amphidiploid was highly fertile. By crossing it with N. tabacum and subsequent selection for resistance in the successive backcrosses to N. tabacum, the resistance was transferred to N. tabacum (Holmes 1938). An analysis of this Holmes Samson variety showed that a pair of N. glutinosa chromosomes had been substituted for a pair of N. tabacum chromosomes (Gerstel 1943). In hybrids with N. tabacum, the one from N. glutinosa invariably failed to pair with the one from N. tabacum, although they appeared to be physiologically equivalent. Crosses with the monosomics showed that H was the replaced chromosome (Gerstel 1945, 1948).

Although the transfer was accomplished by crossing the amphidiploid N. digluta with N. tabacum, Clausen (1941) pointed out that it might have been accomplished by crossing N. glutinosa with tetraploid N. tabacum ($2n=96$), since the F_1 would have the same composition as the cross used by Holmes. In a subsequent experiment, Gerstel (1945) made the cross in this manner, but produced chromosome addition lines, each with 24 pairs of chromosomes. One of them had the gene for mosaic resistance.

Haploidy

The use of haploids as a gamete selection technique has been described earlier (p. 182). Studies in corn show no marked beneficial effect of natural selection among haploids on the breeding potentialities of the derived diploid lines (Thompson 1954).

In cotton some doubled haploids have been as good as commercial varieties. Tests of doubled haploids and their crosses showed that those from the highest yielding haploids gave the highest-yielding hybrids (Harland 1955).

The use of "haploids" in the common potato (*Solanum tuberosum* L.), an autotetraploid species, has been suggested as a more satisfactory approach to genetic, cytogenetic, and breeding studies (Hougas et al. 1957, 1958). The essentially diploid behavior of the "haploids" results in simpler genetic ratios, and probably greater ease of synthesizing new gene combinations. They are of the opinion that the diploids may be usable directly, although lines and hybrids could be converted to the tetraploid level if desired.

These "haploids" also may be crossed with the related diploid species and the doubled hybrid used to effect a transfer of desirable genes to the common potato.

Similar possibilities exist in alfalfa which is at least a near autotetraploid ($2n=32$), since a number of "haploids" with 16 chromosomes and essentially diploid behavior at meiosis have been reported (Bolton and Greenshields 1950, Stanford and Clement 1955, 1958 and Lesins 1957). Among the progeny of haploids crossed with tetraploids, about half had 32 chromosomes.

Colchicine effects other than induction of polyploidy

The use of colchicine in bringing about chromosome doubling will be described in the subsequent section. Other effects also occur which appear to be related to the haploidy just discussed. When colchicine was used to double chromosome numbers of diploids, mutations and other unexpected breeding behaviors in the diploid progeny were found in *Sorghum* by Franzke and Ross (1952, 1957) and in flax by Dirks et al. (1956).

In *Sorghum*, seven seedlings of a true-breeding variety, Experimental-3, were treated with colchicine, eight left as checks. The checks were uniform, but the treated plants differed in foliage color, number of tillers, leaf width, and thickness of stems. Bagged heads were used to produce seed from selfing. Each progeny from the treated plants differed greatly from the checks and from each other in height and in several other characters, but the plants were uniform within each family. Thus new, true-breeding lines with new characters were obtained immediately. One of these was crossed on an untreated line. The F_1 was extremely vigorous. A line obtained from a complex series of crosses involving lines from four colchicine treatments applied in different generations had much larger seeds, 4.07 grams, as compared with any of the parents (1.77, 3.08, 2.84). There was no change in chromosome number and no obvious change in structure in any of the mutant lines (Harpstead et al. 1954). Further studies showed that certain of the mutants segregated 3:1 in crosses, others were dependent on several mutations which had occurred at random on the different chromosomes (Foster et al. 1961).

There was evidence also of a varietal difference in response. Experimental-3 produced diploid mutants, rarely polyploids, whereas the Norghum variety rarely produced diploid mutants, but more polyploids (Atkinson et al. 1957). Also treatments of Experimental-3 failed at times.

The treatments were so effective in many experiments in producing heritable variations of economic value that it is highly important to determine the factors that determine success or failure. In one series of trials with Experimental-3, pure-breeding diploid mutants were obtained only in sand cultures grown under exposure to light from an infra-red bulb immediately after colchicine treatment (Franzke et al. 1960). As in previous trials, no tetraploids were obtained. When grown in agar with full nutrient, a few polyploids were obtained but no true-breeding mutants. Hence genotype, light conditions, quality or type of light, type of medium, and growing conditions all seem to affect the type of reaction to colchicine.

In flax the growing points of F_1 hybrids between varieties that differed in a number of characters were treated with colchicine and the branches harvested separately (Dirks et al. 1956). From one plant heterozygous for yellow and brown seeds, three branches produced only brown seed, two had only yellow seeds, and one had a mixture. Seeds from those having either brown or yellow seeds were grown separately. Each bred true for flower and seed color, general plant type and rust reaction. About 5% of the treated plants showed changes of this type. Some of the sectors bred true for new characters, not shown by either parent, e.g. semi-dehiscent bolls. For another 25% of the hybrids there were anomalous segregations for certain of the characters. As Dirks et al. suggest, the results are explainable if colchicine effects include changes that involve mutation, or mutation followed by somatic reduction and doubling. In root tips of treated seedlings of *Sorghum*, reductional groupings of the chromosomes were observed in about 8% of the dividing cells, groupings that varied from 4:16 to 10:10. Supposedly deviants with less than a complete genome might not be functional, but numbers greater than 20 should occur.

A cytological study in tomatoes of polyploid plants obtained by colchicine treatment revealed irregular meiotic behavior in occasional cells. These have appeared in plants grown from seeds germinated on colchicine-agar (Gottschalk 1958, 1958a). In one plant from treated tetraploid seeds, there were some P. M. C. 's with the diploid number. The reduction must have occurred in pre-meiotic mitoses. Changes to numbers lower than the diploid number also occurred during the meiotic divisions. Normally in tetraploid tomatoes, by the time metaphase is reached bivalents are mostly formed. In one type of division it was as if one member of each bivalent passed to one pole forming a $2n$ group, but the others formed two groups with about the same n number of chromosomes in each. In certain cases the first division produced two groups, one with about $3n$ and the other n chromosomes. Triploid P. M. C. 's were observed also, but they never showed the characteristic behavior of a triploid. They formed exclusively $n - 2n$ separations at division I. Gottschalk (1959) was of the opinion that many were complete sets of n chromosomes. By selfing, it is possible that diploid offspring could be obtained.

The frequency of the "downward regulating behavior" was a maximum of 1.5% of the P.M.C.'s, but only a part of these led to haploid germ cells. Similar types of behavior were observed in treated auto-octoploid tomato plants, and in other plants among the progeny of treated tetraploids (Gottschalk 1958a).

It appears that in tomatoes occasional plants have undergone as a result of colchicine treatment some type of change which in occasional cells modifies the normal course of chromosome behavior during division toward something that resembles somatic reduction.

The discovery of methods by which polyploids may be produced experimentally has provided a tool by which many new problems may be attacked. The full realization of the possibilities of practical applications will require many more years. The full value of polyploidy breeding for a given crop species will be obtained only if extensive and carefully planned work is done.