CHAPTER 8 ALLOPOLYPLOIDY

An individual whose chromosome complement is comprised of two or more different genomes, each from a distinctly different species, is an allopolyploid. For example, in an allotetraploid with two different genomes A and B from ancestral diploids, the chromosomes may be designated as $(A_1A_1, A_2A_2 - - A_y A_y) + (B_1B_1, B_2B_2 - - B_z B_z)$ where $y + z = n$, the gametic number of chromosomes; and $2y + 2z = 2n$, the somatic number (y and z are used, since the chromosome number may not be the same in the two genomes). At the time of its origin it had the somatic complements of two diploid species.

Cytologically, such an allopolyploid might be expected to form bivalents at meiosis rather than multivalents. Winge (1917) was the first to emphasize the importance of interspecific hybridization followed by chromosome doubling for the origin of new species with higher, polyploid numbers of chromosomes. Examples of fertile constant types arising from a sterile hybrid had been found earlier. For example, Rimpau at about 1890 had produced Triticale from a cross of common or vulgare wheat with rye but it was not examined cytologically until 1936 (Müntzing 1948). According to Stebbins (1950), the first example of a fertile constant type arising from a sterile hybrid was described by Janezewski (1892), a cross of Anemone silvestris (2n = 16) x A. multifida (2 n = 32).

Clausen and Goodspeed (1925) were the first to synthesize a new species and describe the cytology of the parents, the F_1, and the fertile derivative. They crossed Nicotiana glutinosa (n = 12) as ♀ with N. tabacum var. purpurea (n = 24). One of three F_1 plants was partially fertile. The progeny from selfing closely resembled the F_1 and were uniform. Cytological examination showed that the sterile F_1 had 36 chromosomes, whereas the fertile F_2's had 72, thus confirming Winge's hypothesis. These plants had the combined somatic numbers of both parents. The new species was designated N. digluta. They made the additional point that the doubled hybrid from an interspecific cross is essentially a homozygous diploid and consequently its offspring would be expected to be uniform. This is a very important point to keep in mind in breeding and other studies of polyploids. If the parental species had been heterozygous, variation between the lines from different F_1's would be expected.

Terminology

Since some degree of relationship is seemingly necessary for the original cross to have occurred, at least partial homology might be expected between chromosomes belonging to the different parental species. Chromosomes that are only partially homologous were termed homoeologous by Huskins. This partial homology should result in the formation of multivalents or of bivalents made up in different ways, either from identical homologous mates or homoeologous mates. The extent of this behavior would be expected to vary depending on the species that were crossed. At one extreme, therefore, are those allopolyploids showing no evidence of homology between genomes, and consequently only bivalent pairing. At the other extreme are the autopolyploids in which the chromosomes

of one parental species are completely homologous with those of the other. Here multivalents are expected or bivalents made up at random from the members of the tetrasomes. Between these two extremes are those with different degrees of homology between the constituent genomes. These intermediates have been termed segmental allopolyploids by Stebbins (1950), the concept being that homologous segments are interspersed with others along the length of the chromosomes. Usually this homology is not enough for the chromosomes to pair, but when they do, there is loose pairing or they may form rod instead of ring bivalents, or occasional multivalents. The term allopolyploid will be used here to include the intermediates. Multivalents are easily confused with multipartite configurations that are the result of chromosomal interchange, but may be distinguished by careful cytological study, by breeding tests, or by their frequency.

One way to avoid confusion in terminology is to consider first the possible kinds of pairing in an allopolyploid species whose chromosomes in the two genomes are designated $A_1A_1\ A_2A_2$ - - $A_yA_y\ B_1B_1\ B_2B_2$ - - B_zB_z. There are three possible types of pairing, namely:

1. Pairing of identical homologues as illustrated by the pairs:
   A_1A_1, A_2A_2, B_1B_1, B_2B_2, etc.

2. Pairing of partially homologous chromosomes belonging to different genomes, as illustrated by the pairs:
   A_1B_1 A_1B_1, A_3B_3, A_3B_3.
   They may form quadrivalents also.

3. Pairing of chromosomes belonging to the same genome, as illustrated by A_1A_4, A_1A_4. A quadrivalent may be formed here also.

The first type will be termed autosyndetic pairing, and the phenomenon autosyndesis. The bivalents consist of identical mates. In the other two types of pairing, the paired chromosomes are not identical. The second type, which involves the pairing of chromosomes belonging to different genomes will be termed allosyndetic pairing; and the phenomenon allosyndesis. The third type of pairing has no special term. It involves the pairing of different chromosomes belonging to the same genome, and might be expected also in the parent diploid species as the result of duplications within the genome. This usage of allo- and autosyndesis follows that of Sansome and Philp (1939), and is one of the meanings given by Darlington and Mather (1949). The terms heterogenetic and homogenetic pairing proposed by Stebbins (1950) correspond to the terms allo- and autosyndesis respectively as they are used here.

Unfortunately, allo- and auto-syndesis are not always used in this manner. The other usage distinguishes them on the basis of whether pairing occurs between chromosomes derived from the same parent (auto-) or from different parents (allo-).

Origin and general behavior

Allopolyploids have probably arisen in nature by chromosome doubling following interspecific crosses. Doubling also occurs naturally in experimental hybrids between species. The frequency of doubled sectors is low, but it may be increased by various agents (See page 264). The result is as if the zygotic numbers of both species were combined into one individual. These are known as amphipolyploids or amphidiploids if the two parental species were diploids.

Amphidiploids of essentially the same constitution may be produced by crossing

autotetraploid races of the diploid species, but the F_1's would be expected to show some variation in chromosome number resulting from irregular meiotic behavior in the tetraploid parents. The kinds of chromosome configurations at meiosis in the F_1 hybrid between two diploid species might be expected to depend on the degree of homology between the chromosomes of the two genomes. Only univalents would be expected in F_1 if there is no homology. Bivalent-formation would be expected between those that are homologous or partly homologous. Hybrids between species are usually highly sterile. This might be expected in those showing only univalents or a high frequency of univalents in F_1, but occasionally a hybrid that forms bivalents is still highly sterile. The classic example of the latter is Primula kewensis, the first clearly explained case of amphidiploidy (Newton and Pellew 1929). The hybrid between P. verticillata and P. floribunda had loosely paired bivalent chromosomes but was completely sterile until a fertile branch arose. The strain established from this fertile branch is P. kewensis. It had double the chromosome number, but the chromosomes still formed mostly bivalents since they were comprised of identical mates. Occasionally there were quadrivalents, the range being from one to three. In the original F_1 hybrid the homologies between the sets of 9 were sufficient to bring about loose pairing.

Another type of behavior is illustrated by a hybrid in Setaria (millet). A cross of the cultivated foxtail millet, S. italica with its wild relative, the green foxtail, S. viridis, showed normal pairing (9 II) but high sterility (Li et al. 1945a). About 70% of the pollen grains were devoid of starch and about 50% of the spikelets were estimated to be empty. In F_2 the sterility varied from low to high. The cross segregated for 15 gene differences governing eight qualitative characters. Of these, only two from the cultivated parent were dominant. Except for a gametophyte factor in one of the three linkage groups found, segregations were normal. They concluded that cultivated millet had originated from its progenitor, S. viridis, very recently. Based on a triplicate factor segregation for one character, they suggested that the number 9 may have originated from a lower one by duplication.

In certain cases the amphidiploid may form only bivalents at meiosis but any two of a given group that includes homoeologous chromosomes may pair, but not at random. A good example of this is the cross of autotetraploid maize with the tetraploid perennial teosinte (Emerson and Beadle 1930), see page 218.

Occasionally in interspecific hybrids only bivalents are formed in F_1 and there is no sterility. Chromosome doubling in this case would result in autopolyploid behavior.

In general, when the chromosome number of a sterile inter-species hybrid is doubled, each chromosome is again represented twice, and at the next meiosis there should be normal pairing and fertility unless there is some physiological incompatibility. There might be complications in the new species since each parental somatic complement was physiologically adequate for normal growth. Also, the set of chromosomes from the male parent must act in the foreign cytoplasm of the female parent.

For any of the genes having certain functions in one parent species, there might be ones with similar function in the other parent species. Or a particular biological synthesis may have been arrived at by different pathways in the two parents. Differences of this sort might account for the high frequency of abnormal and weak types in F_2 of interspecific crosses in cotton (Harland 1935). This might be expected to vary in different genera or families. For example, this behavior has not been noted in wheat hybrids.

The loss of a whole chromosome or of segments without serious consequences other than a possible upsetting of the genic balance might be expected in the subsequent evolution of the amphidiploid. Probable examples of doubling of the number followed by loss of a pair of chromosomes are cited by Darlington (1956, page 85). He has termed this the "polyploid drop".

The cytoplasm-nucleus interaction must be considered also. Evidence from diploids indicates that for certain characters there is maternal transmission generation after generation, apparently not modified by genes located in chromosomes. To this portion of the hereditary material the term plasmon has been applied. For other characters there is maternal transmission to the next generation, but there it may be modified by nuclear genes. A balance must be struck between the two somatic complements of chromosomal genes in an amphidiploid and between them and a cytoplasmic complement or plasmon derived from the maternal parent. An amphidiploid from A x B may differ from one produced from the reciprocal cross, B x A. Some attention must be paid to this aspect in the breeding of polyploids since reciprocal hybrids may differ in important economic characters. Good examples of cytoplasmic differences in diploids have been found by Michaelis (1954) in Epilobium; and there are many others.

Evidence of homologies between chromosomes

That there are homologies between chromosomes of different genomes in an allopolyploid is shown by several kinds of evidence:

1. multivalent pairing in the allopolyploid and bivalent pairing in the haploid derivative,
2. frequencies of visible mutants in a polyploid series,
3. degree of sterility in structural heterozygotes,
4. compensating effect of specific tetrasomic plus nullisomic combinations, and
5. duplicate and triplicate segregation ratios.

1. Multivalent pairing

Multivalent pairing in allopolyploids is usually infrequent and variable, for example in Triticum vulgare. The associations as bivalents and multivalents observed in haploids derived from an allopolyploid are also an indication of these homologies. Rod bivalents are interpreted as indicating less homology than do ring bivalents. For a general review see Kostoff (1941).

A summary of the observations in several such haploids is in Table 99.

Table 99. Observations on chromosome pairing at meiosis in haploids from polyploid species.

Species	Parent somatic No.	No. in haploid	Pairing	Reference
Triticum compactum	42 (6x)	21	0-3 II	Gaines and Aase 1926
Triticum vulgare	42 (6x)	21	1.6 II (avg.)	Sears and Okamoto 1957
Aegilotriticum	56 (8x)	28	0-3 II	Katayama 1935
Nicotiana tabacum	48 (4x)	24	0-3 II	Chipman & Goodspeed 1927
Nicotiana tabacum	48 (4x)	24	1 II	Lammerts 1934b
Digitalis mertonensis	112 (16x)	56	5-12 II	Buxton & Darlington 1932
Solanum nigrum	72 (6x)	36	5-12 II	Jorgensen 1928
Parthenium argentatum	72 (4x)	36	18 II	Bergner 1946
Medicago sativa	32 (4x)	16	8 II	Stanford & Clement 1958
Solanum tuberosum	48 (4x)	24	12 II*	Peloquin and Hougas 1958
Agropyrum desertorum	28 (4x)	14	5-7 II	Dewey 1961

* One haploid had 12 II in 2/3 of the metaphase I figures, another only in 10%.

In the first few species listed, the pairing in the haploids indicates partial homology for only a few of the chromosomes. Difficulties in the cytological observations are the inability to determine if the same chromosomes are involved in the associations, and if they belong to different genomes. Usually the number of multivalents observed in the allopolyploid is less than indicated by the associations in the corresponding haploid, as might be expected. Evidence as to which chromosomes are associated in haploids of Triticum vulgare has been obtained from identification of the chromosomes involved in interchanges carried by offspring obtained from haploids, (Sears and Okamoto 1958). The exchanges between two of the three chromosomes belonging to the same group of homoeologs are much higher than would be expected by chance, and much higher than those between chromosomes belonging to different groups (see page 237 for a listing of the seven groups) (page 239 for the chromosomes involved in these interchanges).

In Digitalis and Solanum nigrum haploids the pairing shown in Table 99 suggests that these species are autopolyploid for at least part of their chromosome complement.

The haploids of guayule (Parthenium), alfalfa (Medicago sativa) and potato (Solanum tuberosum) show mostly pairs at meiosis, as might be expected from diploids. These species, therefore, probably originated as autopolyploids, but have undergone subsequent modification, as will be shown below.

When the tetraploid potato (Solanum tuberosum, 2n = 48) is crossed as ♀ with diploid species (2n = 24), haploids of tuberosum occur in the progeny. From 6,041 pollinations (a potential of 150 to 300,000 seeds) in matings among ten selections of Solanum tuberosum and selections from four diploid species with dominant characters such as pigmented flower, stem and tuber, 806 seeds were obtained that germinated. Of these, 28 were haploids, Hougas and Peloquin (1958). Some diploids are more effective as haploid inducers than others (Peloquin, et al. 1959). Most of the embryos that develop from this cross are in seeds with the hexaploid chromosome number in the endosperm rather than the expected pentaploid number. Hence the embryos that develop are most likely to be in endosperms fertilized by sperms with double the chromosome number (Wangenheim, et al. 1960). This offers a possible clue to differences in effectiveness of different diploid species as inducers of haploid offspring in crosses on tetraploids.

The haploids from tetraploid Solanum tuberosum vary greatly in fertility (Peloquin and Hougas, 1958). One from Katahdin was highly pollen fertile. At meiosis there were 12 bivalents in over 2/3 of the pollen mother cells. One from another source was only slightly pollen fertile, but did set some seed in crosses.

One of the haploids in Medicago sativa is described as slower growing, completely self and male sterile, but partially fertile in crosses (Lesins 1957). At meiosis some cells had 8 pairs, but 25 to 30% of the divisions were abnormal. In crosses with diploids a few 8-chromosome gametes from the haploid functioned, but in the progeny from selfing only spores with close to n = 16 chromosomes functioned (Stanford and Clement 1958).

In a study of height and yield in a cross between two widely different selections of tetraploid alfalfa "variances calculated from the model assuming tetrasomic inheritance more closely approximated the variance based on regression and analysis of variance components than the variances calculated from the disomic model" (Pergament and Davis 1961).

2. *Mutation frequencies*

A comparison of the mutation rates in members of a polyploid series indicates genetic homologies not revealed by multivalent frequencies. Examples are furnished by

Stadler's 1929 data on the rate of mutation per r unit of X-rays in n = 7-, 14- and 21-chromosome species of oats and wheat, summarized in Table 100.

Table 100. Mutation rate per r unit of X-rays in polyploid series in oats and wheat.

	n chrom. number		
	n = 7	n = 14	n = 21
oats	4.1 ± 1.2		0
wheat	10.4 ± 3.4	2.0 ± 1.3	0

The lower rates of observable recessive mutations in species with 14 and 21 chromosome pairs are explainable if normal alleles in the other genomes suppress their expression. If two genomes are present, the same mutation must occur in the second genome before the character can appear if "dominance" is complete. If there are three genomes, a third allele is possible. To restate it, in an allopolyploid species, the different genomes may be related to such an extent that the same recessive mutation would need to occur and be present in each different genome before the character could appear. The rate of mutation to recessives might or might not be the same for each genome but in the polyploids many or most of the mutants would be covered up. In a diploid, a single recessive occurrence would show up in the next generation. The absence of mutations in 21-paired wheat is explainable if a high percentage of the loci are present in triplicate.

Additional evidence for this interpretation is being obtained in oats and wheat in experiments in which the material is exposed to radiation (X-rays or neutrons) in successive generations. Seeds from fertile heads in each generation are selected for treatment in the next generation. By the end of the third cycle, chlorophyll mutations have appeared (Caldecott 1961). The expectation is that as mutants are produced at specific loci and become homozygous, there is a chance that an allele in the second genome may mutate. After it becomes homozygous, a mutation for the allele in the third generation will segregate for the mutant phenotype, and in a 3:1 ratio as in a diploid. This is described as a diploidization process. The final check for the interpretation will come when the final mutant is crossed with the original non-X-rayed material and segregation studied in F_2 and F_3.

3. *Degree of sterility in structural heterozygotes*

In comparisons of degrees of sterility induced in a polyploid series by X-rays, Tascher (1929) reported as follows:

> "sterility induced by X-raying dormant seeds was also found in Triticum monococcum and T. durum, but not in T. vulgare. Sterile plants were common in T. monococcum, but comparatively rare in T. durum. Sterility increased with dosage. A wide range in the percent of sterility indicates that they probably result from different chromosome aberrancies. It is assumed that this behavior is related to the reduplication of the chromosomes."

Sears (1939) found plants with a ⊙4′ chromosomes in the 2n progeny of a haploid (n = 21) vulgare wheat and pointed out that deficiency-duplication gametes could function and bring about no great reduction in ♀ or ♂ fertility.

4. Compensating effect of specific tetrasomic plus nullisomic combinations

Studies of aneuploids in vulgare wheat have shown that the characteristic phenotype of a particular nullisomic plant is restored to normal when certain specific chromosomes are present as tetrasomes. This compensation is only among the three chromosomes that belong to the same homoeologous group, one chromosome from each genome. There is little if any compensation between chromosomes belonging to different groups, see page 238 (Sears 1954, Sears and Okamoto 1958).

5. Duplicate and triplicate genetic ratios

Duplicate and triplicate factor ratios corresponding to heterozygosity at 2 or 3 loci, are common in allopolyploid species. One example is the red grain color in T. vulgare wheat, first studied by Nilsson-Ehle (1911a). Varieties of red wheat may have one, two or three factors for red.

In Nicotiana tabacum the hairy filament and yellow burley characters are determined by duplicate factors. For each character, the two genes have been shown to belong to different genomes (Clausen and Cameron 1944). The gradual establishment of homozygosity for recessives at homologous loci should transform polyploid segregation into segregation that appears to be diploid. Duplication within a genome may also be present, and may account for occasional duplicate factor segregations in allopolyploids, specific examples are not known, but these ratios do occur in diploids.

Identification of the probable parents of allopolyploids

Studies of chromosome pairing in crosses between the putative parents of an allopolyploid, and between them and the allopolyploid usually furnish decisive information on the relationships. Good examples of this are the studies of tobacco, Nicotiana tabacum (Clausen 1928), wheat, Triticum vulgare (Sax 1922, Kihara 1944, Lilienfeld and Kihara 1951), and cotton, Gossypium hirsutum (Skovsted 1937, Webber 1939, Beasley 1942 and Hutchinson 1959). As an example, the analysis of N. tabacum will be described.

The F_1 of Nicotiana tabacum x N. sylvestris was described as never having more than 24 chromosomes at diakinesis or metaphase I, i.e., 12 large ones (bivalents) and 12 smaller ones (univalents) (Goodspeed and Clausen 1927). The N. tabacum x N. tomentosa hybrid showed a similar behavior, 12 II + 12 I, referred to as the Drosera type of pairing. Hence in each cross, the 12 chromosomes from the diploid parent were homologues of 12 in N. tabacum. That the chromosomes of the two diploid parents belonged to different genomes was shown by studying F_1 hybrids of N. sylvestris x N. tomentosa. These usually had 24 I or only an occasional bivalent (Goodspeed and Clausen 1928). Hence the 12 I in the two crosses of N. tabacum x diploid also belonged to different genomes. If the 24 pairs of chromosomes in N. tabacum are assumed to be comprised of 12 pairs from sylvestris (s) and 12 pairs from tomentosa (t), the F_1 with sylvestris would be $\frac{12\ s}{12\ s}$ + 12 t, and the F_1 with tomentosa would be 12 s + $\frac{12\ t}{12\ t}$. Further evidence supporting this was obtained by doubling the chromosome number of the N. sylvestris x N. tomentosa hybrid. This synthetic allopolyploid was similar in many respects to the natural allopolyploid, N. tabacum.

One goal of subsequent studies was to determine the nature of the alterations that transformed the original amphidiploid into the later-day allopolyploid N. tabacum. The cytogenetic behavior of the synthetic allopolyploid and its behavior in crosses furnishes