

## CHAPTER

# 7

# AUTOPOLYPLOIDY

## Terminology

The second class of heteroploidy is termed euploidy. In euploids the chromosome numbers are usually whole multiples of a basic number. One chromosome of each kind in a diploid constitutes a basic gametic set of chromosomes and is termed a "genome". A diploid, therefore, has its genome in duplicate. Euploidy is subdivided into auto- and allo- polyploidy. In the autopolyploid, the one basic genome has been multiplied, while in the allopolyploid two or more genomes of different phylogeny are present (sometimes termed sub-genomes) (Kihara and Ono, 1927). The degree to which these genomes differ from each other varies. The term segmental allopolyploid has been used by Stebbins for the various intermediates. The component genomes in an allopolyploid may or may not have the same chromosome number. For example, bread wheat, Triticum vulgare with 21 chromosome pairs, has three genomes (A, B and D), each with 7 chromosomes; whereas the rutabaga, Brassica napus, has 19 pairs of chromosomes, one genome with 9, the other with 10 chromosomes. In polyploids  $n$  is the gametic number of chromosomes,  $2n$  is the zygotic or somatic number, and  $x$  is the basic chromosome set or monoploid number. An autopolyploid series would include various multiples of this basic number, for example  $x$ ,  $2x$ ,  $3x$ ,  $4x$ ,  $5x$ ,  $6x$ ,  $7x$  or higher. The corresponding terms are monoploid (sometimes termed haploid, see the next paragraph), diploid, triploid, tetraploid, pentaploid, hexaploid and heptaploid, respectively. The addition of auto- or allo- as a prefix is used to distinguish between the two types of polyploids, as autotetraploid, allotetraploid. The wheat with somatic or  $2n$  numbers of 28 and 42 chromosomes are examples of allopolyploid species, in which 7 is the  $x$  or basic number. They are also referred to as tetraploid and hexaploid wheats.

The term haploid is used here for an individual with the gametic chromosome number derived from a diploid or a polyploid. A monoploid has a single genome and arises from a diploid.

## Occurrence

Polyploidy has been important in the evolution of plants. At least a third of all Angiosperm species are polyploid, but in certain groups of Angiosperms the percentage is higher. For example, about 70% of the grasses are polyploid (Müntzing 1956). Most are probably allopolyploids, but naturally occurring autopolyploids (or only slightly modified) may be more common than was thought earlier (Müntzing, 1956).

The gigas mutant in Oenothera was the first polyploid recognized in experimental culture. It was an autotetraploid (Lutz 1907, Gates 1909). At about the same time É. and Em. Marchal (1907, 1909) produced diploid gametophytes in mosses by inducing protonema (gametophyte) development from the sporophytes. These in turn produced diploid gametes whose union resulted in tetraploid sporophytes. Higher ploidy was produced in

a similar manner. Winkler (1916) produced tetraploid Solanum nigrum from shoots arising from callus tissue developing on decapitated plants. Tischler (1910) reported races of the banana, Musa esculentum, which had  $n = 8, 16$  or  $24$  chromosomes.

The preceding examples are probably all autopolyploids. Rosenberg in 1909 reported that certain species of Drosera had 20 chromosomes, others 40; and that the hybrids formed 10 II and 10 I. This is referred to as the Drosera type of pairing.

Strasburger (1910) considered that doubling of the chromosome number might occur either by longitudinal or by transverse division, but whereas longitudinal division would double the volume of the <sup>protoplast</sup>, transverse division would not change the volume. <sup>chromosome</sup> The problem of what happened to the centromeres if the increase were by transverse division was not recognized until later.

In considering the mode of origin of new species, Winge (1917) pointed out the possible importance of hybridization, followed by chromosome doubling.

### General characteristics

In general autopolyploids with higher chromosome numbers have greater nuclear and cell volumes. For example, in corn, rye, and certain dicotyledons, the stomata and guard cells are larger; while the number of stomata per unit area is decreased because of the increased cell size. Differences are often so great that they may be distinguished by examining the leaves under low power magnification. As an example, stomatal sizes in the haploid, diploid and triploid peach, Prunus persica, are progressively greater as shown in Table 70.

**Table 70.** Stomatal dimensions in  $n$ ,  $2n$  and  $3n$  peach, Prunus persica (Pratassenja 1939).

Chromosome No. ( $n$ )	Stomatal length*	Stomatal width*
8	4.54	3.61
16	5.49	4.23
24	6.1	4.45

\* Divisions of ocular micrometer.

Similar observations on the peach were reported by Dermen and Scott (1939). The occasional  $2n$  pollen grains produced by the diploid species were usually larger also.

In most species there is a progressive increase in size of leaves and flowers and in thickness of stalks and leaves as the numbers increase from  $1n$ ,  $2n$ ,  $3n$  to  $4n$ . Similar relationships occur in Datura, but the autotetraploids may be darker green, longer-flowering and somewhat slower in reaching maturity than are the diploids (Blakeslee 1941). There appears to be a limit, however, beyond which an increase in chromosome number does not increase the size of plant parts. In fact, in Datura a few very weak plants were identified as being  $6n$  or  $8n$ . Hexaploid branches that occurred on triploid plants and octoploid branches on  $4n$  plants produced small capsules that had no viable seed (Avery et al. 1959). In sugar beets, the optimum seems to be at the triploid levels. In maize, octoploids have been shorter and sturdier than tetraploids but sterile (Randolph, personal comm.). Usually the flowers on autotetraploid plants are larger than those on diploids of the same species, but they may be indistinguishable as in two species of Portulaca (Blakeslee 1939) or they may be smaller than the related diploids as in Plantago and Alyssum.

Wettstein (1937) reported that in the moss, Bryum caespiticium, from the experimentally produced diploid gametophyte ( $2n = 20$ ) one spore produced a line which in the course of 11 years gradually became more fertile and lost the gigas-form and cell size without any change in chromosome number. This occurred in the original plant as well as in the vegetatively and sexually propagated material. At the end of this period, it still could not be crossed with the original haploid ( $n = 10$ ), and hence behaved as a new species. In Datura there had been no change in size of pollen after many generations of selfing. Gigas strains in some species are not a result of chromosome doubling (Schwanitz and Pirson, 1955).

For certain genes, there may be a cumulative effect of each dominant factor; e.g. the B factor for plant color in maize; whereas for others a single dose of the dominant gives full expression of the characters, e.g. the normal (Su) allele of sugary (su) in corn.

For certain characters, the relation between dosage and phenotype is the same in the triploid as in plants trisomic for the chromosome carrying that particular locus, as for example P (smooth) vs. p (peach) and S (simple) vs. s (compound) in tomatoes. In  $2n + 1$  and  $3n$  plants, heterozygotes for both characters are intermediate. For the D<sub>1</sub>-d<sub>1</sub> genes (tall vs. dwarf) in tomato D<sub>1</sub> completely dominates d<sub>1</sub>d<sub>1</sub> in the trisomic, but only partially dominates them in the triploid and in the tetraploid (Lesley 1937). A difference in gene balance is the probable explanation. Even in individuals that are homozygous, there may be unpredictable physiological effects resulting from interactions of non-homologous loci. Although each gene has had the same increase in number, the end result may be quite different, since the additional doses may not add the same increments for different genes. For example the effect of one gene, i.e. the gene product may increase geometrically, another arithmetically with a similar increase in gene dosage. The end result is a very different balance from that in the diploid. Unexpected phenotypic effects of chromosome doubling occasionally occur. For example, in a squash in which the fruit of the  $2n$  is pear-shaped the fruit of the  $4n$  is disk-shaped. The  $2n$  Bottle Gourd has a constricted neck, but the neck is missing in the  $4n$  fruit (Blakeslee 1941, see his Fig. 6, p. 124). In the diploid, these differences may be determined by a single gene.

In dioecious species, tetraploidy is expected to produce plants with several different constitutions for X and Y: XXXX, XXXY, XYY, YYY, and YYY. In Melandrium, the Y has a strong  $\sigma^r$ -determining power, and hence XXXX plants are  $\sigma^r$  and plants with XXXY and all other dosages of Y are  $\sigma^r$  (Westergaard 1940). The same is true in spinach (Spinacia oleracea).

There appears to be a higher frequency of natural autopolyploids in perennial than in annual species (Müntzing 1956). Since autotetraploids are usually slower to reach maturity, polyploids originating in a perennial species would have a second chance if, when they first appeared, they flowered too late to mature. A self-pollinator might have a better chance to leave some tetraploid offspring than would a cross-pollinator. Since the cross-pollinator is most likely to cross with a diploid, its offspring would be mostly triploids which are usually highly sterile.

## Autotriploids

### Origin and occurrence

Triploids occur occasionally among the progeny of diploids as a result of the functioning of a  $2n$  gamete. Evidence as to their origin from diploids is furnished by cases in which the genetic constitutions of the parents are known. Two of these were reported in corn. One arose in the progeny of a testcross of a trisomic for chromosome

5: PrV2 . PrV2 . prv2 ( $2n + 1$ ) x pr pr v2 v2 ( $2n$ ). From a purple seed there was one plant with 32 chromosomes which was homozygous for v2 (Rhoades 1933). Chromosome doubling must have occurred in the ♀ parent at or after meiosis (since at least one of the chromosomes was a crossover, i. e. Pr v<sub>2</sub>), probably in an ( $n + 1$ ) megaspore at one of the nuclear divisions to form the gametophyte.

In the second case in corn, the cross  $gl_1 gl_1 \times ws_3 ws_3$  produced a triploid which was tested with both  $gl_1$  and  $ws_3$  (Rhoades 1936a). The cross:  $3n \times gl_1 gl_1$  produced 89  $Gl_1:20gl_1$ ; while the cross:  $ws_3 ws_3 \times 3n$  produced 42  $Ws_3:90 ws_3$ . These are trisomic ratios and would be expected from a triploid of the constitution:  $Gl_1 Gl_1 gl_1 Ws_3 ws_3 ws_3$ . This indicates that the  $2n$  gamete came from the male parent, its genotype being the one underlined  $Gl_1 Gl_1 ws_3 ws_3$ . The simplest explanation is the one suggested by Rhoades, i. e. that the egg was fertilized by two sperm nuclei. There are other possibilities, however. It probably did not come from a diploid ♂ gametophyte, since the  $2n$  pollen tube would have had to compete with tubes having  $n$  chromosomes. It could have resulted from nondisjunction at one of the post-meiotic divisions to form the male gametophyte, probably at the division of the generative nucleus. Such a pollen grain would have a haploid tube nucleus and would probably behave as any other normal pollen grain. The one diploid sperm would be carried down to the embryo sac and might fertilize the egg. In this case the primary endosperm nucleus would need to be fertilized by a sperm from another pollen tube. Either method is possible, since Virginia H. Rhoades (cited in the same paper) found 11% of the embryo-sacs contained more than one pollen tube, and also since Sprague (1932) has shown that hetero-fertilization does occur.

Triploids occur regularly in the progeny of tetraploids crossed with diploids. In some species, e. g. in barley, this cross is difficult to make, but embryo culture has been used to obtain  $F_1$  plants. The results obtained in maize by Randolph (1935) are summarized in Table 71.

**Table 71.** Results from reciprocal crosses between tetraploid and diploid maize.

Cross	Hybrid seeds		% of filled seeds that germinated	Number of plants	
	Well-filled	Aborted		3n	4n
2n x 4n	427	20,180	10	48	4
4n x 2n	90	10,170	most	438	

The crosses using the tetraploid as the female parent were the most successful. Most of the well-filled grains and many of the aborted ones germinated. In the reciprocal cross, only about 10% of the well-filled grains germinated, many were germless. The  $4n$  offspring from the  $2n \times 4n$  cross may have come from a  $4n$  sector or from unreduced gametes in the diploid ♀ parent. The behavior in the  $2n \times 4n$  vs.  $4n \times 2n$  crosses in corn agrees with the general experience that interspecific crosses are usually more successful when the higher polyploid is the ♀ parent (Thompson, 1930). Since there are exceptions, any attempted interspecific crosses should be made reciprocally.

### Cytology

At meiosis, each trisomic group of chromosomes in an autotriploid may form a trivalent or a bivalent plus a univalent. The configurations at diplotene vary, depending on the number and positions of the chiasmata, as shown in Fig. 48. Only two chromosomes are associated at any one point. In corn, the longer chromosomes form trivalents more frequently than the shorter ones. If crossovers between the three chromosomes are necessary to hold the three together in a trivalent, such a relationship would be expected, since the longer chromosomes would have a greater chance of having such crossovers. This is not a direct relation, since in many diploid species the shorter chromosomes appear to have a higher frequency of chiasmata relative to length than do the longer ones.



McClintock (1929a) reported that at diakinesis in triploid maize 9 trivalents plus a bivalent and a univalent were most frequent. No case was observed with more than four univalents. At metaphase I, 10 trivalents were found frequently but commonly there were fewer trivalents with a resulting increase in bivalents and univalents. Univalents were in or near the equatorial plate, and seemed to divide occasionally at division I. They were also found off the plate, either in the spindle or in the cytoplasm. The second division showed less irregularity. The resulting spore-containing cells (sporads) contained from two to many microspores, some of which had micronuclei or were multinucleate. Pollen grains varied greatly in size. Some indication of the chromosome behavior in the trivalents in triploids may be gained from the behavior of trisomics. McClintock and Hill (1931) reported in the trisomic for chromosome 10 that a trivalent was formed in about two-thirds of the sporocytes, one-third being a bivalent and univalent. The trivalent regularly showed 2-1 disjunction; but in meiocytes with the bivalent + univalent the univalent behaved irregularly, often not passing to either pole. Einset (1943) reported that for the trisomic for chromosome 3, 2 to 4% of the sporocytes at pachytene showed a univalent, 7-11% at diplotene, 30% at diakinesis and 32% at metaphase I. For chromosome 2, the percentage of univalents at diakinesis was 28%, at metaphase 30%. For chromosome 9, the figures were 32 and 44%; for chromosome 10, 33 and 37% in one cross, and 49% at metaphase in another cross.

Data on the frequency of trivalents in triploids of several species having a haploid number of 12 chromosomes, in Lycopersicon (Upcott 1935), Lilium (Chandler et al. 1937), and Solanum (Lamm 1945) are shown in Table 72.

**Table 72.** Frequencies of trivalents at metaphase I of meiosis in triploids of various species having  $n = 12$  chromosomes.

Species	0	1	2	3	4	5	6	7	8	9	10	11	12	Total cells	Mean no. of III per cell	References
<u>Lycopersicon</u> <u>esculentum</u>				5	13	17	10	5						50	4.9	Upcott 1935
<u>Lilium</u> <u>tigrinum</u>								5	10	25	27	29	2	98	9.7	Chandler et al. 1937
<u>Solanum</u> <u>chaucha</u>			1		1	2	3	1	7	2	6	2		25	7.1	Lamm 1945

The chromosomes of Lycopersicon are relatively short, those of Lilium are long. This probably accounts at least in part for the higher frequency of trivalents in Lilium.

The most complete study of chromosome disjunction in triploids is that of Satina and Blakeslee (1937, 1937a), Satina et al. (1938) for triploid Datura stramonium. They reported data on chromosome segregation in the meiotic and postmeiotic divisions in ♀ and ♂, and also the final chromosome numbers in the viable progeny. These have been reported in detail in three papers, a summary of which follows.

First are the numbers of spore mother cells with different segregations of the 36 chromosomes at meiotic divisions I and II as shown in Table 73.

Table 73 includes only those cells in which the total at the two poles was 36. The values in the last line are calculated on the assumption that each of the 12 chromosomes in excess of the diploid number passes at random to either pole. Hence  $(\frac{1}{2})^{12}$  is the frequency with which the 12 univalents should pass to one pole, etc. The observed values show that the frequencies of cells in which 6, 7 and 8 of the 12 extra chromosomes passed to one pole (18-18, 17-19, 16-20) are lower, while those in which 9, 10, 11, and 12 passed to one pole (15-21, 14-22, 13-23 and 12-24), underlined in Table 73, are higher than ex-

**Table 73.** Frequencies of the various disjunctions in triploid *Datura* at meiosis, divisions I and II in the ♂ and division I in the ♀ (from Satina and Blakeslee, 1937, Table 1, p. 520, Table 1, p. 622, Amer. Jour. Bot. 24).

	Percent of cells							Total cells	Total of first 4 classes
	12-24	13-23	14-22	15-21	16-20	17-19	18-18		
I div. in ♂	<u>0.8</u>	<u>4.5</u>	<u>8.5</u>	<u>14.5</u>	22.9	30.8	18.0	1000	28.3
II div. in ♂	<u>3.0</u>	<u>5.5</u>	<u>11.0</u>	<u>16.5</u>	20.0	30.5	13.5	200	36.0
I div. in ♀	0	<u>3.5</u>	<u>9.0</u>	<u>14.0</u>	21.5	34.5	17.5	200	26.5
Calculated if random ( $\frac{1}{2} + \frac{1}{2}$ ) <sup>12</sup>	0.05	0.6	3.2	10.7	24.2	38.7	22.6	100%	14.55

pected from random distribution. As shown in the last column, there is a greater excess in division II than in division I. This indicates a strong tendency for the chromosomes to pass together in groups to one pole, rather than at random.

Lagging chromosomes at meiosis are also frequent in triploids. A summary of counts made by Satina et al. is in Table 74.

**Table 74.** Frequency of nuclei with lagging chromosomes and the number of chromosomes lost at divisions I and II in ♂ and ♀ of triploid *Datura* (from Satina and Blakeslee, 1937, 1937a).

♂ Div. I,	89 nuclei in 2,000 or 4.5%	lost 109 chrom., i.e. 0.05 chrom. per nucleus
♂ " II,	20 " " 800 or 2.5%	" 27 " " 0.03 " " "
♀ " I,	105 " " 200 or 52.5%	" 218 " " 1.1 " " "
♀ " II, ca. 52	" " 100 or 52 %	" 128 " " 1.3 " " "

The number of nuclei with lagging chromosomes was extremely high in the female (over 50%), but was much lower in the male (3 to 5%). Also in the nuclei with laggards more chromosomes were lost in the ♀ than in the ♂. These data indicate that the conclusion, so often implied if not stated, that chromosome behavior in the ♀ can be assumed to be similar to that observed in the microsporocyte divisions may not be warranted.

The chromosome numbers determined in the first division of the microspores and also in the divisions to form the embryo sac are shown in Table 75.

**Table 75.** Frequencies of gametophytes with different chromosome numbers in *Datura* triploids. (From Satina and Blakeslee, 1937, Table 3, p. 523 and Table 4, p. 626, Amer. Jour. Bot. 24).

	% with chromosome numbers													Total no.
	12	13	14	15	16	17	18	19	20	21	22	23	24	
Microspores:	<u>2.6</u>	<u>4.0</u>	<u>7.2</u>	<u>11.0</u>	<u>16.4</u>	16.0	11.2	10.8	9.2	5.0	<u>3.8</u>	<u>2.6</u>	<u>1.2</u>	500
Embryo sacs:	<u>7.0</u>	<u>9.0</u>	<u>5.0</u>	<u>13.0</u>	<u>17.0</u>	14.0	13.0	11.0	4.0	3.0	<u>2.0</u>	<u>1.0</u>	<u>1.0</u>	100
Expected if random:	0.02	0.3	1.6	5.4	12.1	19.3	22.6	19.3	12.1	5.4	1.6	0.3	0.02	100%

Those underlined are higher in frequency than expected if disjunction were random. Also note that the lower chromosome numbers are relatively much more frequent than are the high numbers; although both are higher than expected. Chromosome lagging and subsequent loss have caused a shift to the lower chromosome numbers, while the tendency to go together to one pole in a larger group increases the frequency of the spores with higher numbers as well as those with the lower numbers. The end result is that both are still in excess of expectation, although the excess in the lower numbers is much greater and extends over a wider range of numbers than in the higher ones.

### *Fertility*

In general, autotriploids are highly sterile, but in Spinacia oleracea they are described as having normal seed set (Janick and Stevenson 1955).

In triploid Datura, 50 to 60% of the pollen grains are filled, with considerable variation in size among them. Disintegration among the remainder begins soon after the first division of the microspore nucleus. The cells with intermediate numbers are supposedly the ones which abort, although this is probably not entirely a question of number. If the 17-, 18-, and 19- chromosome spores abort, 38% of abortion would be expected; while if the 16- and 20- chromosome classes are added, 58% would be expected. Of the full grains at maturity, 15% or less germinated; and nearly 2/3 of these pollen tubes burst at or near the stigma, leaving about 3% that behaved normally. As described previously (chapter 6), many of the  $n + 1$  grains in trisomics are normal in appearance but will not germinate or will produce pollen tubes that burst. Studies of seed development and of chromosome number in the progeny of triploids also were reported (Satina et al., 1938). In the cross of  $3n \times 2n$  Datura, 79% of the seeds were small, 21% of normal size or only a little smaller. In the normal diploid  $\times 3n$  cross, the corresponding figures were 13 and 87%. There was an average of 61 seeds per capsule in the  $3n \times 2n$  cross, the normal being 300 or more. As the seeds developed some had endosperm without an embryo, some the reverse, and others had abnormal embryos. Of 401 seedlings only 285 reached the stage at which they could be classified as to chromosome number and numbers of trivalents present. The results are in Table 76.

**Table 76.** Chromosome types in progeny of  $3n \times 2n$  Datura (from Satina et al., 1938, Table 2, pp. 599, Amer. Jour. Bot. 25).

Total No. of plants	<u><math>2n</math></u>	<u><math>2n + 1</math></u>	<u><math>2n + 1 + 1</math></u>	<u><math>2n + 1 + 1 + 1</math></u>
285	58	138	79	10

All the primary trisomics except that for chromosome 19.20 were found in the  $2n + 1$  progeny. Certain primaries showed delayed germination, the range being 3 to 258 days, whereas the diploids germinated in 3-12 days. The smaller seeds produced a higher percentage of  $2n + 1$ . In this experiment, no  $3n$  plants were found in the progeny; but in others in which  $3n$  plants were selfed over 2% of  $4n$  plants were obtained.

### **Autotetraploids**

#### *Origin and occurrence*

One of the new types which appeared in 1916 among the offspring obtained by selfing normal Datura stramonium was one with broad leaves and large flowers. This plant bred true on selfing but failed to set seed when crossed with normals (see Avery et al. 1959).