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

some information. The synthetic amphidiploid was pollen-fertile but completely female-sterile. Greenleaf (1941) found complete chromosome pairing and regular meiosis in both mega- and microsporogenesis. The failure came in the stages of embryo-sac formation. When certain races of N. tomentosa were used, the amphidiploid showed some female sterility and a few fertile plants appeared in F_2 and backcross progenies, from which fertile lines were established. Greenleaf concluded that sterility factors would account for the breeding behavior. An amphidiploid sylvestris . tomentosiformis synthesized by Kostoff (1938a) was fertile.

Cytological changes played a minor role. As summarized by Clausen (1941), comparisons of cytological behavior in haploid N. tabacum with that in the F_1 of sylvestris . tomentosa; in the amphidiploid with that in N. tabacum; and in the hybrid between them showed relatively little difference in behavior. On the other hand, numerous genetic differences were segregating in F_2 and other progenies in which segregation might be expected. Genetic studies of the synthetic allopolyploid showed it to have duplicate genes for several genetic characters which in N. tabacum were monogenic (cf. Clausen 1941). The highly duplicated genetic constitution of the original allopolyploid lost some of the duplication as it evolved into the present-day N. tabacum. As Clausen stated it, "the alterations which have occurred in tabacum since its establishment as an amphidiploid have tended to transform it in the direction of making it more effectively diploid".

In many species hybrids, the number of univalents and bivalents varies from cell to cell. Also the number of "ring" and "rod" or "open" bivalents varies. Examples of this are furnished by the numerous crosses between species of wheat and Aegilops. The data for a few of those reported by Aase (1930) are summarized in Table 101.

Table 101. Frequencies of univalents and bivalents in parents and hybrids between species of wheat with different chromosome numbers. (Aase, 1930, from Table 10, p. 48, Res. Studies State College of Wash., vol. 2).

	UNIVALENTS			BIVALENTS					
				Closed Type			Open Type		
Species or hybrid	Average	mode	range	Average	mode	range	Average	mode	range
T. monococcum (2n = 14)	0	0*	0	6.8	7*	6 - 7	0.2	0*	0 - 1
T. turgidum (2n = 28)	0	0*	0	13.0	14**	11 - 14	1.0	0**	0 - 3
T. vulgare (2n = 42)	0.4	0*	0 - 2	19.6	20,19	17 - 21	1.2	1.**	0 - 4
T. durum (2n = 28) x T. monococcum	9.2	9**	7 - 13	3.2	3	2 - 5	2.7	2,3	0 - 5
T. durum x T. vulgare (2n = 42)	7.4	7*	7 - 11	10.6	9,10,11	8 - 14	3.2	2,4,5	0 - 6

* Occurs in 75% or more of the cells.
** Occurs in 50% or more of the cells.

An occasional trivalent was observed in the hybrid between T. durum and T. monococcum, and an occasional trivalent or tetravalent in the hybrid between T. durum and T. vulgare. Conclusions as to the relationships are based on the modal number of bivalents.

The pairing in interspecific hybrids may be limited to a varying number of segments, interspersed with non-pairing segments. Different species with the same chromosome number and the same or similar genomes may differ in the closeness of their relationships to each other and to species with a different chromosome number. These may

be reflected in differences in relative frequencies of closed and open bivalents as well as the number of bivalents and univalents. In cotton this has been indicated by designating similar genomes by the same letter with subscripts to indicate there are some differences (Beasley 1942). For example, among the diploid old world species (2n = 26), Gossypium herbaceum is A_1, G. arboreum is A_2, and six of the new world diploid species are asasigned genomes D_1 to D_6. The genomes in the three tetraploid new world species that are cultivated are $(AD)_1$ for G. hirsutum, $(AD)_2$ for G. barbadense and $(AD)_3$ for G. tomentosum (see Stephens 1947).

The same may be true in wheat, but has not been indicated in the letter designations of the genomes in closely related species.

The relationships in Triticum and Aegilops species have been summarized by Lilienfeld and Kihara (1951). The einkorn or 7 II group of species has genome A (einkorn or monococcum); the emmer or 14 II group has A and B; the spelt (dinkel) or 21 II group has A, B and D (D for dinkel). The T. vulgare or common bread wheats belong to the latter group.

Certain of the 14 II species, when crossed with Aegilops squarrosa (7 II), produced hybrids which resembled T. spelta. After chromosome doubling they had 21 II and when crossed with T. spelta and T. vulgare gave fertile hybrids with good chromosome pairing (McFadden and Sears 1944, 1946). Hence Aegilops squarrosa is the source of the D genome, a conclusion also arrived at by Kihara (1944).

The source of the 'B' genome appears to be Aegilops speltoides (Sarkar and Stebbins 1956, and Riley et al. 1958). Sarkar and Stebbins came to this conclusion by using Anderson's 1949 "Method of extrapolated correlates". This method may be described briefly as follows. If the qualitative and quantitative characters of the allo-polyploid being analyzed are intermediate and one parental species is known, the probable characteristics of the other can be predicted. For wheat, the characters of the tetraploid that are not found in the A genome source (T. monococcum) are found only in diploids in the Sitopsis section of Aegilops. The diploid that comes closest to having the required characters for the 'B' genome is Aegilops speltoides. As reported by Riley et al., in Ae. speltoides there are two chromosome pairs with satellites similar to the satellites in vulgare which have been shown to be on chromosomes one and ten in the 'B' genome. Two other diploid species studied have a much smaller satellite. Another diploid species has sub-terminal centromeres, whereas those in T. vulgare are median or submedian. Evidence from pairing in interspecific hybrids, in amphidiploids, and in hybrids between amphidiploids led them to the same conclusion, namely, that the source of the 'B' genome is Ae. speltoides.

A discriminant function, z, as devised by Fisher (1936), making use of several attributes, was used by Johnson (1962) in a study of amphiploidy and introgression in Stipa, and as an aid in determining the probable parental species.

Behavior of univalents

Occasional univalents may divide at division I of meiosis and again at division II, as for example in Saccharum hybrids (Bremer 1923), and in Raphanobrassica hybrids (Karpechenko 1927a).

Non-random segregation of the univalent chromosomes is carried to an extreme in certain species hybrids. For example, in Triticum-Haynaldia hybrids, Sears (1953a) observed a tendency for most or all univalents to pass together to one pole. In Leucopogon juniperus, (2n = 12, x = 4), there are 4 II + 4 univalents at meiosis (Smith-White 1948). In 54% of the Metaphase I figures, the 4 univalents all passed to one pole, and at Metaphase II in 15 of 29 cells, 4 - 8 was the segregation. Seed fertility was good. Good pollen ranged from 14 to 68%.

Triploid hybrids from crosses between Aster shortii (n = 18) and A. cordifolius (n = 9) were characterized by 90% pollen fertility (Avers 1954). The numbers of bivalents varied from 5 to 12, with a few trivalents and the remainder univalents. Her cytological observations showed that all univalents divided at division I and passed to the poles along with the disjoining members of the bivalents and trivalents. Counts of individual nuclei at anaphase II ranged from 13 to 19 chromosomes. On the assumption that the univalents divided at both divisions, nuclei with 9, 10 and 11 chromosomes would be missing. There was no phenotypic effect on pollen fertility of chromosome numbers between x and 2x. Since the univalents regularly divided twice, this was "believed to be a cytogenetic demonstration of a functional tertiary split in the meiotic metaphase chromosome".

The phenomenon occurs regularly in Pygaera (moth) hybrids (Federley 1931).

In the canina roses there are 7 pairs of chromosomes and 21 univalents (Täckholm 1922 and Hurst 1931). In the pollen mother cells, the bivalents and univalents lie on the equatorial plate of metaphase I. The bivalents divide first then the univalents divide. At division II, the univalents again divide but late, in fact so late that most do not reach the poles. In the megaspore-mother cell, the univalents are grouped usually at the micropylar end. The seven bivalents disjoin. Hence one cell of the dyad has seven chromosomes and is small, the other has seven plus all or most of the univalents and is large. The second division is regular for both cells. The large cell at one end of the linear quartet of megaspores is the embryo-sac-mother-cell. According to Fagerlind (1940) the univalents are absent from the functional pollen, but all are present in the functional eggs.

New characters from interspecific hybrids

Evidence of a different nature on the genetic makeup of an allopolyploid was obtained by Lammerts (1934, 1934a) in a study of genetic characters that appeared in lines derived from the hybrid between allopolyploid N. rustica pumila (24 II) and N. paniculata (12 II) backcrossed to each parent. In the progeny of the second and third backcrosses to paniculata he selected plants that had 24 chromosomes or 12 pairs; and then selfed the plants that were pollen fertile. The progeny included many distinctive plants. These were selfed to establish true-breeding lines. These were then crossed with the normal N. paniculata stock, and the segregation studied in F_2. Twelve lines proved to be simple recessives. Examples are: wide corolla lobes, elongate leaf, heterostyle, giant plant, compact habit, male-sterility, and several seedling lethals. Spurred corolla, not found in this genus, was more complex in inheritance. He had started with homozygous parents, but the progeny from the interspecific hybrid backcrossed to the parent with the low chromosome number segregated for new characters. One interpretation is that the N. rustica parent had accumulated over the years many mutations which could not appear until the covering alleles in the second genome had been removed. An entire N. rustica chromosome might have been substituted in the N. paniculata complement or segments of one or more N. rustica chromosomes might have been substituted by crossing over. In this experiment involving the selection of 24-chromosome offspring from the backcross to N. paniculata, each of the 12 N. rustica chromosomes which pair with N. paniculata chromosomes in the F_1 was given a chance to be substituted in whole or in part in the N. paniculata genome. If this N. rustica chromosome or segment carried a recessive gene, the character might appear in the self progeny of the backcrossed plant. Position effect as proposed by Lammerts may be the explanation for certain of the new characters.

In a parallel experiment the F_1 of N. rustica pumila x N. paniculata was backcrossed to N. rustica (Lammerts 1934b). Progeny with 17 II + 7 I, 18 II + 6 I, and 19 II + 5 I were backcrossed again to N. rustica. When used as the ♀ parents, the univalents were transmitted at random, indicating little or no lagging. When crossed as the pollen parents on

rustica, transmission was very low or absent. Reversion to the parental chromosome number (48) was complete but the resulting plants "were usually quite distinct from one another as well as from N. rustica pumila." Only five of 164 F_2 plants were similar to normal. True-breeding lines that were highly fertile were established. The derivative types were usually dominant or partially dominant over normal. Some of the types were: dwarf habit, elongate flower tube, dark green cordate leaf, dark green acute leaf, luxuriant growth habit, and dark green pubescent. Seven types behaved as simple dominants, two differed by many dominant and recessive factors and two were definitely recessive. Possible explanations are (1) "substitution of whole chromosomes from those of N. paniculata for those of N. rustica, with occasional parallel mutation or (2) transfer of factors from N. paniculata to N. rustica by either (a) regular crossing over -- or (b) rather rare crossing over resulting in the substitution of segments of N. paniculata for those of N. rustica", the observed character being a result of the interaction. Loss of a suppressor gene as a result of the substitution is one possibility. Since there is evidence that mutation rates may be higher in hybrids, new mutations may have occurred also.

In allopolyploids it is possible to lose whole chromosomes or segments in some cases without detrimental effects. In other cases such a change produces a phenotypic effect. Such losses do affect transmission, especially through the pollen; e.g. in monosomics the deficient pollen is capable of functioning, but at a great handicap in competition with pollen that has the normal chromosome number.

Secondary pairing

Secondary pairing at meiosis, a cytological behavior found only for small chromosomes, involves close orientation but no chiasma formation. It has been interpreted as indicating distant relationships between different sets of chromosomes in allopolyploids, as discussed by Darlington (1931b) and by Lawrence (1931). Cytological studies of the Pomoideae have shown multivalents and secondary pairing (Darlington and Moffett 1930, Moffett 1931). The basic chromosome number in apples is 17. At meiosis of diploid varieties, 17 bivalents were usually formed. These showed a variable degree of secondary pairing:

	3 groups of 3 bivalents =	9
	4 groups of 2 bivalents =	8
Total -	7	17 bivalents

This was interpreted to mean that there were really only seven different kinds of chromosomes, four of which were tetrasomic and the remaining three hexasomic, as represented in the following scheme:

AA AA AA

BB BB BB

CC CC CC

DD DD

EE EE

FF FF

GG GG

Secondary pairing has been described in Fragaria also (Yarnell, 1931).

Heilborn (1936) doubted the significance of the results, pointing out that it had been found only in species having small chromosomes. He suggested that the pre-metaphase stage may show this as a chance behavior, or that the orientation may be a purely physical one because they are small. He did concede (1937) that the number of groups might have some significance. In corn, this writer has noted associations which had no evident relationship to the configurations at earlier or later stages.

Stebbins (1950, page 362) has stated that the evidence from secondary pairing agrees in no case with evidence on relationship based on comparative morphology and cytology. "At present... secondary association can be considered an actual phenomenon and one which in many instances suggests the polyploid nature of a species or genus, but one which may be considerably modified by segmental interchange, duplication of chromosomal segments and other phenomena not at all related to polyploidy". It is not a reliable index of the exact basic haploid number. Caution should be exercised in its interpretation. That secondary association does occur when there is partial homology is shown by results obtained by Riley (1960) in wheat (page 250).

The number of satellites or of nucleoli is not a reliable indication either. Nawaschin (1934) found that in hybrids between species of Crepis, an organizer from one species might suppress that of another. Also that the organizers of different species could be arranged in order, based on their ability to compete in the formation of nucleolae. For example, the nucleolus of C. parviflora was dominant over C. capillaris but C. capillaris was dominant over those in three other species. It is not surprising, therefore, that in allopolyploids certain of the organizers in the different genomes may not be expressed.

Polyploidy and apomixis

Polyploidy is important as one source of apomictic forms, (Stebbins 1941, 1950, Darlington 1956). Whenever sexual reproduction fails in an individual for genetic or cytogenetic reasons, an opportunity of survival is offered if the plant can reproduce itself asexually or by some form of apomixis. An unreduced egg or a vegetative cell in the ovule may develop an embryo without fertilization. Triploids occur occasionally in most diploid species. Since meiosis results in complete or nearly complete sterility reproduction could be accomplished by apomixis as a substitute. Triploids in Solanum, Tulipa, and Fritillaria reproduce asexually.

In Taraxacum, triploids (24 chromosomes) that are apomictic have been derived at various times from sexually reproducing diploids (Sørensen and Gudjonsson 1946). In general these triploids breed true, but the embryo-sac-mother cell occasionally undergoes a modified meiosis and plants with 23 chromosomes are produced (3n-1). They were able to recognize 8 different phenotypes corresponding to the 8 different chromosomes. If any of the chromosomes are paired during the modified meiosis, crossing over might occur followed by segregation.

Hybrids between diploid species, haploids from allopolyploids, or hybrids between allopolyploids may be sterile and if so apomixis might occur and become established. It would replace sexual reproduction. In some apomicts sexual reproduction may still occur occasionally. Also meiosis may not be completely suppressed, chromosomes may pair and cross over followed by failure of the first or second meiotic division. This seems to be the situation in Rubus and in Poa. As Darlington (1956, page 44) states it, "a great range of haploids and triploids and sexual and asexual diploids are sometimes produced within the same fruit. Their proportions, the result of competition among dif-

ferent possible types of embryos, depend on whether the flower has been self- or cross-pollinated".

Apomixis will be discussed in greater detail in Chapter 11.

Theoretical genetic ratios

The ratios which might be expected in an allopolyploid depend on the behavior of the chromosomes that carry the genes responsible for the character being studied, and on the presence or absence of more alleles for that character than the two in a particular pair of homologous chromosomes. The extremes as to chromosome pairing in a 4n allotetraploid are: (1) complete synaptic homology between corresponding chromosomes in the different genomes and therefore association as multivalents or in some cases only bivalents but at random in all possible combinations, and (2) no synaptic homology between chromosomes in the different genomes and therefore autosyndetic pairing.

With reference to any point at which the chromosomes are paired, e.g. the centromeres, pairing as multivalents may be considered as consisting of allo- as well as auto-

Table 102. Genic constitutions of gametes formed by $A_1^A A_1^a B_1^A B_1^A$ with autosyndetic and with random pairing, first with no crossing over between the gene locus and the centromere*, then with a frequency of such crossing over that will result in maximum equational segregation. The expected ratios from backcrosses and in F_2 are included.

Crossing over and kind of pairing	Gametes	Back cross	F_2
1. No interstitial crossing over			
a. bivalents, autosyndesis	AA + Aa	∞:0	∞:0
" , random	AA + Aa	∞:0	∞:0**
b. multivalents (random seg.)			
1 autosyndetic***	1(AA + Aa)		
2 allosyndetic***	2(AA + Aa)		
Total (1 auto- + 2 allo-)	AA + Aa	∞:0	∞:0**
2. Maximum interstitial crossing over			
a. bivalents, autosyndesis	AA + Aa	∞:0	∞:0
" , random	AA + Aa	∞:0	∞:0**
b. multivalents (random seg.)			
1 autosyndetic	1(13AA + 10 Aa + 1aa)		
2 allosyndetic	2(13AA + 10 Aa + 1aa)		
Total	13AA + 10 Aa + 1aa	23:1	575:1

* The term "interstitial crossing over" is suggested, in the same sense as it is used for chromosomal interchanges.

** Certain F_3 lines will segregate, those that came from AAaa F_2 plants.

*** For multivalents "auto-" and "allosyndetic" refer to the chromosomes associated at the centromere, i.e.

$$\frac{A_1^A}{A_1^a}\ \frac{B_1^A}{B_1^A} \text{ and } \frac{A_1^A}{B_1^A}\ \frac{A_1^a}{B_1^A} \text{ respectively.}$$

In multivalent cross-shaped configurations of the type shown in Figure 52, p. 187, the distal arms would be associated allo- and auto- syndetically respectively.