CHAPTER VIII

Polyploidy I: Occurrence and Nature of Polyploid Types

THE PHENOMENON of polyploidy, or the existence in genetically related types of chromosome numbers which are multiples of each other, is one of the most widespread and distinctive features of the higher plants and was one of the earliest of their cytogenetic characteristics to become extensively studied and well understood. One of De Vries's original mutations of *Oenothera lamarckiana*, mut. *gigas*, was early recognized to be a tetraploid (Lutz 1907, Gates 1909), and the artificial production by Winkler (1916) of a tetraploid form of *Solanum nigrum* through decapitation and the regeneration of callus tissue was perhaps the first example of the direct production in a laboratory experiment of a new, constant genetic type. Meanwhile, explorations of chromosome numbers in various plant genera were revealing the presence of series consisting of multiples of a basic number, as in *Drosera* ($2n = 20$ and 40, $x = 10$, Rosenberg 1909)[1]; *Dahlia* ($2n = 32$, 64, $x = 16$, Ishikawa 1911); *Chrysanthemum* ($2n = 18, 36, 54, 72, 90$, $x = 9$, Tahara 1915); *Triticum* ($2n = 14$, 28, 42, $x = 7$, Sakamura 1918, Sax 1922); and *Rosa* ($2n = 14$, 28, 42, 56, $x = 7$, Täckholm 1922). Then, Digby (1912) discovered that the spontaneous occurrence of a constant fertile type, *Primula kewensis*, from a sterile interspecific hybrid, *P. verticillata × floribunda*, was associated with the doubling of the chromosome number, but she failed to grasp the significance of this phenomenon. Subsequently Winge (1917) formulated his hypothesis of hybridization followed by polyploidy as a method for the origin of

[1] In this book, the somatic number of any particular form will be designated as $2n$ and its gametic number as n, regardless of its degree of polyploidy, unless it is an unbalanced type with an uneven somatic number. The most probable basic haploid or gametic number for any genus or for any polyploid series that forms part of a genus is given the letter x, and the somatic numbers of unbalanced types like triploids, pentaploids, etc., are designated as $3x$, $5x$, $7x$, etc.

species, but he was apparently unaware of *P. kewensis*, the only example supporting it. This hypothesis was experimentally verified by Clausen and Goodspeed (1925) through the artificial synthesis of *Nicotiana digluta* from *N. tabacum × glutinosa*, and several other artificially produced allopolyploid species were reported in quick succession, the most spectacular of them being that combining the radish and the cabbage, *Raphanobrassica* (Karpechenko 1927, 1928). A few years later, Müntzing (1930b) reported the first example of the artificial synthesis by this method of a well-known Linnaean species, *Galeopsis tetrahit*, from a hybrid between two others, *G. pubescens × speciosa*. Strong circumstantial evidence was then produced by Huskins (1931) that one of the only valid wild species known to have originated in historic times under human observation, *Spartina townsendii*, is an allopolyploid derived from the cross *S. alterniflora × stricta*. Finally, the colchicine technique of doubling chromosome numbers, developed through the cytological discovery of Dustin, Havas, and Lits (1937), followed by its practical application by Blakeslee and Avery (1937), has provided cytogeneticists with a most valuable tool for experimental studies of polyploidy. For a complete list of literature on the history and application of this technique, see Eigsti 1947.

Meanwhile, the realization that many of our most valuable crop plants, such as wheat, oats, cotton, tobacco, potato, banana, coffee, and sugar cane, are polyploids was supplemented by decisive evidence concerning the actual parentage of some of them, as in the examples of *Triticum aestivum* (McFadden and Sears 1946), *Gossypium hirsutum* (Beasley 1940a, Hutchinson and Stephens 1947, Stephens 1947), and *Nicotiana tabacum* (Goodspeed and Clausen 1928, Kostoff 1938a, Greenleaf 1941, 1942). The role of polyploidy in the origin of new types being created by the plant breeder was recognized in the case of the loganberry (Crane and Darlington 1927, Crane 1940a, Thomas 1940b), nessberry (Yarnell 1936), veitchberry (Crane and Darlington 1927), the cultivated blueberries (Darrow, Camp, Fischer, and Dermen 1944, Darrow and Camp 1945), and "perennial wheats" (Wakar 1935a,b, 1937). The final step in the use of polyploids in connection with plant breeding and organic evolution, namely, the establishment as a spontaneous, natural series of populations of a new species produced artificially by this method, is also not far distant.

DISTRIBUTION OF POLYPLOIDY IN THE PLANT KINGDOM

Polyploidy is known to a greater or lesser degree in all groups of plants. Although it has been studied very little in the thallo-phytes, multiple series of chromosome numbers have been re-ported in some genera of algae, such as *Cladophora*, *Chara*, and *Lomentaria* (Tischler 1931, 1956, 1938). In the fungi, it is ap-parently rare or lacking. Among the bryophytes, mosses are classi-cal examples of polyploidy, both natural and artificially induced (Wettstein 1924, 1927). It is found in all the major divisions of the vascular plants, namely Psilopsida (*Psilotum*), Lycopsida (*Selaginella*), Sphenopsida (*Equisetum*), Filicinae (most genera), gymnosperms (*Sequoia*, *Podocarpus*, *Ephedra*), and angiosperms.

The distribution of polyploidy among the various groups of vascular plants is very irregular and shows no obvious relation-ship to the phylogenetic position of the group. The only two surviving genera of the primitive class Psilopsida, *Psilotum* and *Tmesipteris*, have such high chromosome numbers that there is a very strong probability that they represent the last relics of very ancient, highly developed polyploid series. Similar very high chromosome numbers exist in nearly all genera of the ferns, par-ticularly the Polypodiaceae. And typical polyploid series have been found within some species of this family, notably *Poly-podium vulgare* (Manton 1947). There is good reason to believe, therefore, that polyploidy has played a particularly important role in the evolution of the family Polypodiaceae and may be respon-sible for many of its taxonomic complexities.

On the other hand, polyploidy is particularly uncommon among the living gymnosperms. It is unknown in the cycads or *Ginkgo*, and the only known polyploid species of conifers are *Pseudolarix amabilis* (Sax and Sax 1933), *Sequoia sempervirens* (Dark 1932, Buchholz 1939), *Juniperus chinensis* var. *pfitzeriana* (Sax and Sax 1933), and probably some species of *Podocarpus* (Flory 1936). It is apparently more common in the Gnetales (Florin 1932). Among the angiosperms in general, the proportion of polyploid species was roughly estimated by the writer, when preparing a re-view of the frequency of polyploidy in another connection (Steb-bins 1938b), to be about 30 to 35 percent, but these species are very irregularly distributed. Widespread families which are nearly or entirely devoid of polyploidy are Fagaceae, Moraceae, Berberi-

daceae, Polemoniaceae, and Cucurbitaceae. Families characterized by a high frequency of polyploidy, often involving genera as well as species, are Polygonaceae, Crassulaceae, Rosaceae, Malvaceae, Araliaceae, Gramineae, and Iridaceae. In addition, the high aneuploid series of chromosome numbers found in the Cypera-ceae and the Juncaceae are probably a modified form of poly-ploidy. In other families, neighboring genera differ widely in the frequency of this phenomenon. In the Salicaceae, it forms the major basis of the variation pattern in *Salix*, but is very rare in *Populus*. It is highly developed in *Betula*, but almost unknown in other genera of the Betulaceae. It is common in *Dianthus* (Caryophyllaceae), but uncommon in *Silene*; nearly absent in *Aquilegia*, sporadic in *Anemone*, but predominant in *Thalictrum* (Ranunculaceae); rare in *Lactuca* and *Prenanthes*, but common in *Hieracium* (Compositae); rare in *Lilium*, but common in *Tulipa* (Liliaceae). In some genera, such as *Potentilla*, *Primula*, *Solanum*, *Pentstemon*, and *Crepis*, some species groups are strictly diploid, while others form extensive polyploid series. Most genera of angiosperms contain some polyploidy, most often in the form of tetraploid or hexaploid species, either scattered through the genus or concentrated in some of its sections.

A classification of the genera of angiosperms on the basis of the percentage of polyploid species found in them (Stebbins 1938b) revealed the fact that, on the average, the highest percentages of polyploids are found in perennial herbs, a smaller proportion in annuals, and the lowest percentages in woody plants. The genera included in this survey are mostly those of temperate climates, since very few tropical genera are well enough known for this purpose. Data on tropical groups may show different relation-ships, particularly between perennial herbs and woody plants. Some suggestions as to the explanation of these differences in the incidence of polyploidy will be offered in the following chapter.

DIRECT EFFECTS OF POLYPLOIDY

The evidence to be presented in this chapter indicates that polyploidy as it occurs in nature is most often associated with hybridization, either between species or between different sub-species or races of the same species. If this assumption is correct, any conclusions about the physiological and ecological effects of

chromosome doubling *per se* that are based on studies of naturally occurring polyploids must be considered hazardous. If, therefore, we are to understand the significance of polyploidy in nature, we must first examine the effects of artificially induced polyploidy on pure species of various types. Studies of this sort have been made by a number of workers and have been reviewed by Noggle (1946). Some of the more comprehensive studies are those of Blakeslee (1939), Randolph (1941a), Straub (1940), Pirschle (1942a,b) and Larsen (1943).

A survey of these studies brings out the fact that the only generalization which may be safely made about the morphological and physiological effects of polyploidy is that these depend greatly on the nature of the original genotype. The popular conception, that polyploidy usually produces *gigas* types, which are larger than their diploid ancestors, is now known to be true only in special instances, particularly if the original diploid is strongly heterozygous, as is true of the progenitor of the first known *gigas* tetraploid, that of *Oenothera lamarckiana*. To be sure, polyploidy increases cell size in the meristematic tissues, but the eventual size of individual organs and of the plant as a whole depends as much on the amount of cell elongation and on the number of cells produced during growth as it does on the initial size of the cells. Both of these latter processes may be adversely affected by the polyploid condition. In general, the *gigas* effects of polyploidy are seen most often and are expressed most strongly in organs with a determinate type of growth, such as sepals, petals, anthers, few-seeded fruits, and seeds. In compound, many-seeded fruits, like those of the tomato, tetraploidy may cause a reduction in size, because of the partial sterility of the tetraploids and the consequent reduction in the number of seeds produced. In addition to the occasional *gigas* effect on individual parts of the plant, and more rarely on the plant as a whole, the following effects of chromosome doubling occur often enough to deserve consideration, although none of them is universal.

An increase in size of the individual cells is perhaps the most widespread effect of polyploidy. It often makes possible the use of measurements of certain cells of the plant, particularly the guard cells of the stomata and the mature pollen grains, to suggest the diploid or the polyploid condition of plants represented only

by herbarium specimens, or in which for other reasons actual counting is not practicable. These measurements cannot be used indiscriminately and are valueless unless the sizes of stomata and pollen grains are known in individuals of the same or closely related species in which the chromosomes have actually been counted. Nevertheless, if these basic facts are known, and if the chromosomes of all of the diploid and the polyploid species of a group are of about the same size, then measurements of the size of stomata and pollen grains can often provide valuable supplementary evidence on the relative geographic distribution of closely related diploids and polyploids, and can also give suggestions of where exploration in the field is likely to yield desired diploid or polyploid members of a particular species complex.

Nevertheless, the work of Tobler (1931) and Barthelmess (1941) on mosses shows how the effects of chromosome doubling on the size of mature cells can vary with the nature of the original diploid genotype. Using the well-known method of regenerating gametophytes from the stalk of the spore capsule, Tobler obtained diploid gametophytes from each of 54 segregating haploid individuals derived from a cross between two races of *Funaria hygrometrica*, one of which had cells twice as large as those of the other. The range in cell volume among the diploid plants was about the same as that among their haploid progenitors, but the mean volume for the diploids was about 3.5 times that for the haploids. There was, however, no significant correlation between cell size in the haploid individuals and in their diploid derivatives. In some genotypes, doubling had almost no effect, while in others it increased the volume to eight times that found in the haploid. Furthermore, there was a significant negative correlation (—0.65) between the initial size of the cells and the degree to which this was increased by doubling. Genotypes with initially small cells were affected much more than those with large ones. The results of Barthelmess (1941) on *Physcomitrium piriforme* were essentially similar.

A number of secondary effects are associated with the primary effect of polyploidy on cell size. The first of these is on water content. Since in most mature plant cells the cytoplasm is appressed to the wall, and the central part of the cell is occupied by the vacuole, the relation of water to protoplasm depends partly

on the ratio between volume and surface in the cell. Furthermore, since any increase in cell size causes a corresponding increase in the ratio of volume to surface area, such increases are likely to increase the water content of the cell relative to the amount of protoplasm, and therefore to lower the osmotic tension. This tendency was demonstrated experimentally by Becker (1931) in mosses, by Schlösser (1936) in a strain of tomato, and by Hesse (1938) in *Petunia*. But Fabergé (1936) found no significant difference in water content between the cells of diploid and tetraploid tomatoes, while Fischer and Schwantz (1936) record that in polyploids of *Anthoceros* cell size is reduced and osmotic tension is increased. Since many factors other than size affect the osmotic properties of cells, the tendency for polyploidy to reduce osmotic tension can be expected to be less general than the effect on cell size. The effect of polyploidy on cell size and cell elongation is probably responsible for the differences between diploids and autotetraploids in the content of various substances, such as protein, chlorophyll, cellulose, auxin, and various vitamins, as discussed in the reviews of Randolph (1941a) and Noggle (1946).

Another series of effects of chromosome doubling depends on changes in growth rate. In general, the growth rate of autopolyploids is slower than that of their diploid progenitors, although the degree of retardation may vary greatly with the nature of the original genotype. This causes autotetraploids to flower relatively later and in some instances to flower over a longer period of time. Another effect on growth is the reduction in the amount of branching, which occurs very frequently, but not universally (Hesse 1938, Pirschle 1942a,b, Levan 1942b). In many grasses this effect results indirectly in a reduction of the size of the plant as a whole, since the number of basal shoots or tillers per plant is significantly reduced, as in *Stipa lepida* (Stebbins 1941b).

The least consistent of the effects of chromosome doubling are those on the shape of the individual organs of the plant. This is as expected, since these shapes are the product of the interaction of tendencies affecting growth rate as well as cell size, and the individual processes may be differentially affected by polyploidy. The most consistent effect, since it is the one most directly affected by cell size, is on the thickness of leaves and other appendages. Randolph, Abbe, and Einset (1944) have found that the leaves

of tetraploid maize are consistently thicker than those of the diploid, and that this increase is about the same for each variety tested. Similar differences in leaf thickness have been reported by most other workers on autotetraploids. The leaves and other organs are usually shorter and broader in autotetraploids, but this effect is by no means universal (Pirschle 1942a). In *Stipa lepida* (Stebbins 1941b) progenies of sister diploid plants differed in this respect, the autotetraploids having in one instance significantly broader leaves and in another significantly narrower leaves than their diploid ancestors.

Of equal and perhaps greater significance from the evolutionary point of view than those on the morphology and physiology of the plants are the effects of polyploidy on fertility and genetic behavior. The most conspicuous and universal of these is the reduction of pollen and seed fertility in autopolyploids as compared with their diploid ancestors. Like other effects of chromosome doubling, this varies greatly with the genotype of the diploid. Some tetraploid varieties of maize set 80 to 95 percent of seed (Randolph 1935, 1941a), and 75 to 80 percent fertility is found in colchicine-produced autotetraploids of the grass species *Ehrharta erecta* (Stebbins 1949b). At the other extreme, Einset (1944, 1947a) found only 5 to 15 percent of seed setting in autotetraploids of cultivated lettuce, while Beasley (1940b) found almost complete sterility in an autotetraploid of *Gossypium herbaceum*. Most autotetraploids lie somewhere between these extremes.

The opinion originally held about the cause of sterility in autopolyploids, namely, that it is due to the formation and irregular segregation of multivalent associations of chromosomes (Darlington 1937), has had to be revised in recent years. Randolph (1941a) concluded that sterility in autotetraploid maize is largely controlled by specific genes or gene combinations and is chiefly physiological in nature. Sparrow, Ruttle, and Nebel (1942) found that differences between the fertility of autotetraploids derived from different varieties of the snapdragon *Antirrhinum majus* were not correlated with the differences in the frequency of multivalents at meiotic prophase and metaphase, but did show a positive correlation with the frequency of lagging chromosomes and other abnormalities seen at later stages of meiosis. Similar results were obtained by Myers and Hill (1942) and Myers (1943) in com-

parisons of meiotic abnormality and fertility in different strains of *Dactylis glomerata*, a wild species which behaves cytologically like an autotetraploid, and by Myers (1945) in autotetraploid *Lolium perenne*. Einset (1944, 1947a) found that a small proportion of the high sterility in autotetraploid lettuce is due to abortion of pollen grains and ovules, but that most of it is caused by the failure of pollen grains to complete growth down the styles and by the inhibition of fertilization. Westergaard (1948) found in the species complex of *Solanum nigrum* great differences in seed production between artificially produced autopolyploids of different diploid species, as well as between allopolyploids from different interspecific hybrids. Since these differences could not be explained on the basis of the meiotic behavior of the chromosomes, he concluded that fertility and sterility in allopolyploids, as well as in autopolyploids, are to be explained in genetic terms rather than on a cytological basis.

These data all suggest that the principal causes of sterility in autotetraploids, as well as in some allopolyploids, are a series of disharmonies produced at various stages of the sexual cycle, of which disturbances of the spindle and other external features of meiosis are among the most important. The effects of abnormal pairing between groups of three and four chromosomes completely homologous to each other are of relatively minor importance. This point of view is strengthened by the fact that in autogamous plants crossing between races tends to increase the fertility of the tetraploids, as in *Antirrhinum* (Sparrow, Ruttle, and Nebel 1942) and *Lactuca* (Einset 1947), and that similar increases can be obtained in allogamous plants by selection within the heterozygous material, as in *Fagopyrum* (Sacharov, Frolova, and Mansurova 1944).

Polyploidy also affects the incompatibility relationships of some self-incompatible ("self-sterile") species. If the diploid is strongly self-incompatible, as in *Brassica* and *Raphanus* (Howard 1942), *Oenothera organensis* (Lewis 1943), and *Taraxacum kok-saghyz* (Warmke 1945), the autotetraploid is likely to be equally so. But if some self-incompatibility is present at the diploid level, this may be greatly increased in the polyploid, as in *Petunia* (Stout and Chandler 1941), *Allium nutans* (Levan 1937a), *Pyrus* spp. (Crane and Lewis 1942, Lewis and Modlibowska 1942), and *Trifolium*

repens (Atwood 1944). Lewis (1943, 1947) has described carefully the effect of polyploidy on genetic mechanisms for self-incompatibility and has concluded that competition between pairs of different self-sterility (S) alleles is chiefly responsible for the increased compatibility.

The evidence from natural polyploids suggests that in many instances self-incompatibility is maintained at the polyploid levels, at least in the flowering plants. In the Gramineae, which have been the subject of many studies on self-incompatibility (Beddows 1931, Smith 1944), there are many polyploid species which are self-incompatible to at least such a degree that they are rarely or never inbred in nature. Well-known examples are *Bromus inermis*, *Festuca arundinacea*, *F. ovina*, and *F. rubra*, *Dactylis glomerata*, *Agropyron repens*, and *A. cristatum*. On the other hand, most of the polyploid species in this family which are self-compatible or strictly autogamous, such as *Bromus secalinus*, *B. mollis*, *B. rigidus*, *Hordeum nodosum*, *Avena fatua*, and *A. barbata*, appear to have arisen from self-compatible or autogamous diploids. Lewis and Modlibowska (1942), however, record that tetraploid species of *Tulipa* and *Hyacinthus* are self-compatible, while corresponding diploid species are self-incompatible. Natural polyploidy has probably had some effect on self-incompatibility, but this has certainly been much less than the alterations of this condition which have been produced by gene mutations.

Another effect of polyploidy is on the genetics of segregation, due to the presence of duplicated genes. The early study of this subject by Haldane (1930) has now been followed by several others, which are reviewed by Little (1945). The principal effect in all cross-bred populations is, of course, the increase of the proportion of individuals heterozygous at any locus and the decrease in frequency of homozygosity, so that the effects of recessive genes, and particularly gene combinations, are rarely realized in the phenotype. This is due to the fact that homozygotes must possess four similar genes at any locus rather than only two, and three degrees of heterozygosity are possible with respect to each gene locus. Segregation ratios are further complicated by the degree to which the chromosomes form multivalent associations, their chiasma frequencies, and the way in which the multivalents segregate. The details of these segregations are significant chiefly to plant breeders, who can work with genetically pure material.

The final and most important effect of polyploidy is the genetic barrier which is immediately erected between a polyploid and its diploid progenitor. Autopolyploids are usually rather difficult to cross with related diploids, and the F_1 progeny of such matings are highly sterile triploids. Polyploidy, therefore, is one way, and perhaps the only way, in which an interspecific barrier can arise at one step, and thus give an opportunity for a new line to evolve independently and to diverge from the parental type.

POLYPLOIDY AND HYBRIDIZATION

The fact that polyploidy is the best way of producing constant, fertile species from sterile interspecific hybrids has been recognized ever since the experimental confirmation of Winge's hypothesis through the synthesis of *Nicotiana digluta*. Typical allopolyploids or amphiploids, which resemble diploids in the regularity of chromosome pairing at meiosis and in the constancy of their genetic behavior, have now been synthesized in forty or more instances, and the actual or probable allopolyploid character of scores of wild species has been determined. At least half of the naturally occurring polyploids are probably strict allopolyploids, in that they have originated from F_1 hybrids between two ancestral species which were so distantly related to each other that their chromosomes were essentially nonhomologous. This does not include species which are diploid with respect to the basic number of their genus, but of which this generic number may be of polyploid derivation, as in the subfamily Pomoideae of the Rosaceae.

The remainder of natural polyploids, including between fifty and a hundred species which have been analyzed to date, and probably several hundred or even several thousand yet to be investigated, have certain cytogenetical properties which are often associated with autopolyploidy. In particular, they resemble related diploid species more or less closely in external morphology, and their chromosomes form a greater or lesser number of multivalent associations at meiosis, indicating some duplication of chromosomal material. We cannot conclude from this evidence, however, that such polyploids have all been derived from pure, fertile diploid species rather than from interspecific hybrids. Both the morphological and the cytological evidence on polyploids of known origin warns us against such inferences.

There are now several examples which show that even extreme allopolyploids, derived from hybridization between species having widely different chromosomes, may resemble one or the other of their parental species so closely that they have not been recognized as distinct by systematists. One of the most striking of these is *Nasturtium microphyllum*. This species was originally regarded by Manton (1935) as an autotetraploid form of the ordinary watercress, *N. officinale*, chiefly on the grounds of its external morphology. When, however, the true autotetraploid of the latter species was produced artificially, it was found not only to be entirely different in appearance from the wild plant but in addition to form sterile hybrids with it (Howard and Manton 1940). Furthermore, the hybrid between the natural tetraploid and typical *N. officinale* was found to possess 16 bivalent and 16 univalent chromosomes, indicating that one of the two genomes present in the former is completely nonhomologous with that of the latter. The natural tetraploid was therefore described as a new species, *Nasturtium uniseriatum* (Howard and Manton 1946), and its authors now believe it to be an allotetraploid derived from a hybrid between *N. officinale* and some species of a different but related genus, *Cardamine*. Airy-Shaw (1947) has shown that the tetraploid species had already been recognized as *Nasturtium microphyllum*.

A similar example is *Madia citrigracilis* (Clausen, Keck, and Hiesey 1945a). On morphological grounds, this species was first thought to be a form of *M. gracilis*, but later its haploid chromosome number was found to be $n = 24$, while that of *M. gracilis* is $n = 16$. Finally, *M. citrigracilis* was synthesized artificially via the triploid hybrid between *M. gracilis* and the very different diploid species *M. citriodora* ($n = 8$). Since the triploid F_1 has practically no chromosome pairing, and *M. citrigracilis* regularly forms 24 bivalents, the latter ranks as a true allopolyploid, as does also *M. gracilis*. Furthermore, there is another hexaploid, even more similar to *M. gracilis*, as well as a third species, *M. subspicata*, which is diploid, but is nevertheless so similar to *M. gracilis* in external appearance that the two were thought to belong to the same species until they were analyzed cytologically. Typical *M. gracilis* may be an allotetraploid of *M. subspicata* and some other species. In the complex of *M. gracilis*, therefore, we have a

diploid, a tetraploid, and two hexaploid forms which on the basis of external morphology were judged by experienced systematists thoroughly familiar with the genus to belong to one species, but which actually include allopolyploids derived from hybridization between at least three, and perhaps four, original diploid species so distantly related that their chromosomes were almost completely nonhomologous with each other (see Fig. 32).
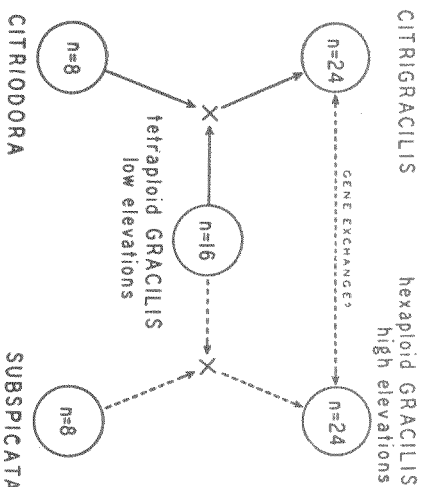
The two preceding examples show that even typical allopolyploids may be placed by systematists in the same taxonomic species as one of their parents if their cytogenetic condition has not been analyzed. Such polyploids, however, nearly always possess morphological characteristics by which they may be recognized, once these are made clear. This is definitely true of the two examples mentioned above. If, however, a polyploid has originated from hybridization between two closely related species, with chromosomes partly homologous to each other, some derivatives of this polyploid may be hardly distinguishable from autopolyploids of one or the other parental species. And if, through backcrossing, such a polyploid acquires a preponderance of genes derived from one or the other of the parental species, it may fall entirely within the range of variation of the latter.

CITRIGRACILIS

n=24   n=8   n=16   n=24   n=8

tetraploid GRACILIS low elevations   hexaploid GRACILIS high elevations

GENE EXCHANGE?

CITRIODORA   SUBSPICATA

FIG. 32. *Madia citrigracilis* and allies. Relationships arrived at by experiment indicated by solid lines; those by circumstantial evidence, by broken lines. From Clausen, Keck, and Hiesey 1945a.

A striking example of such a situation was produced artificially by Mehlquist (1945) in the genus *Dianthus*. He pollinated the tetraploid *D. chinensis* ($2n = 60$), the garden pink, with pollen from the very different-looking diploid species *D. caryophyllus* ($2n = 30$), the ordinary greenhouse carnation. From this he obtained, in addition to a large number of sterile triploid hybrids, a partly fertile tetraploid hybrid containing a set of 30 chromosomes apparently derived from an unreduced gamete of *D. caryophyllus*. Such a tetraploid is essentially an allotetraploid and produces gametes containing one genome from *D. chinensis* and one from *D. caryophyllus*. Repeated backcrossing for two generations of this tetraploid to *D. caryophyllus* produced a series of plants which were vigorous and fertile and appeared like autotetraploid plants of the carnation. They had thick, stiff leaves, larger flowers, and other similar characteristics, but the influence of genes from *D. chinensis*, which, as shown in the pedigree, Fig. 33, must have been

P_1 D. CHINENSIS $\frac{c}{c}\frac{c'}{c'}$ D. CARYOPHYLLUS $\frac{P}{P}$

F_1 693 TRIPLOIDS $\frac{c}{c}\frac{c'}{P}$ 10 TRIPLOIDS $\frac{(c)c}{c}\frac{P}{P}$ 6 TETRAPLOIDS $\frac{c}{c}\frac{c'}{c'}\frac{P}{P}$ 38131-1 TETRAPLOID $\frac{P}{P}$ 39147-4 D. CARYOPHYLLUS

BC_1 $\frac{(c)c}{c}\frac{P}{P}$ $\frac{(c)c,c'}{c}\frac{P}{P}$ $\frac{(c)c}{c}\frac{P}{P}$ 41516-3 $\frac{P}{P}$ SPECTRUM SUPREME

BC_2 41621-1 $\frac{Pcc'}{P}\frac{P}{P}$ 33 $\frac{Pcc'}{Pcc'}\frac{P}{P}$ 25 $\frac{Pcc'}{Pcc'}\frac{P}{P}$

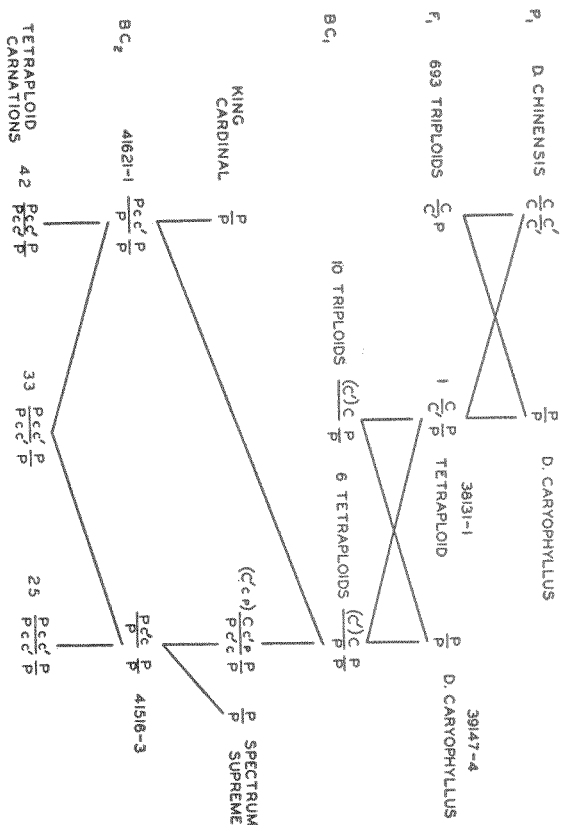TETRAPLOID CARNATIONS   KING CARDINAL   42 $\frac{Pcc'}{Pcc'}\frac{P}{P}$

FIG. 33. Diagram showing the pedigree of a tetraploid carnation which, though morphologically similar to *Dianthus caryophyllus*, must contain some genes derived from *D. chinensis*. Further explanation in the text. From Mehlquist 1945.

present, was not detectable. As in the case of many individuals which are the product of introgressive hybridization, their origin could not have been discovered by studying their external appearance.

That this type of polyploid is often produced in nature seems inevitable. The examples of introgressive hybridization in *Tradescantia* discussed by Anderson, Riley, and others (see Chapter VII) frequently involve tetraploids. It is not improbable that many of the apparently autopolyploid individuals of *T. canaliculata*, *T. occidentalis*, and other species of this genus (Anderson and Sax 1936, Anderson 1937b) are actually derived from such introgression and contain genes from one of the other species. This possibility is strengthened by the observation of Giles (1941) that the triploid hybrid between the diploid *T. paludosa* and tetraploid *T. canaliculata* is morphologically very similar to the latter. On theoretical grounds, one would expect that genotypes having morphological and physiological characteristics similar to a well-established wild species would usually have a higher selective value than entirely new hybrid combinations, and therefore that natural selection would tend to favor backcross derivatives from allopolyploids whenever these could be produced and had a reasonably high fertility. This seems to be true in the genus *Galium*. Fagerlind (1937) records that autopolyploids belonging to different species of this genus frequently hybridize when they grow together in the same locality, but that truly intermediate types are much less common than forms which closely resemble one species, but possess a few characteristics of another.

The foregoing discussion shows that in many instances one cannot decide on the basis of external morphology alone whether a given polyploid contains only genes derived from a single interfertile species population, or whether it has obtained through hybridization genes from two or more species. For such decisions, the external morphology of the chromosomes, as well as their behavior at meiosis, often provides valuable evidence, but this also is by no means infallible. Some authors, as Bergman (1935a) in *Hieracium*, and Sørensen and Gudjónsson (1946) in *Taraxacum*, have suggested that if three or more morphologically similar sets of chromosomes can be recognized in the somatic divisions of

the root tips, this provides strong evidence in favor of the autopolyploid nature of the species concerned. But studies of meiosis in interspecific hybrids of *Paeonia* (Stebbins 1938a) and many other genera have shown that chromosome sets which look almost identical with each other in the ordinary root tip preparations may actually differ by large and numerous structural rearrangements. In genera like *Taraxacum*, in which species that form completely sterile hybrids may have chromosomes so similar to each other that they can pair perfectly in the F_1 (Poddubnaja-Arnoldi 1939a), conclusions based on the external morphology of the chromosomes may be particularly misleading.

Similar difficulties accompany the interpretation of the nature of polyploidy on the basis of chromosome association at meiosis. Müntzing and Prakken (1940) have pointed out that in some polyploids of experimental origin and known to contain three or more identical chromosome sets or genomes, the chromosomes form mostly pairs at meiosis. Furthermore, the number of trivalent and quadrivalent configurations formed may differ between autopolyploids produced from different strains of the same species, as in the snapdragon *Antirrhinum majus* (Sparrow, Ruttle, and Nebel 1942). In polyploids derived from undoubted interspecific hybrids, such as *Primula kewensis* (Upcott 1939), *Lycopersicum peruvianum-esculentum* (Lesley and Lesley 1943), and several other examples listed elsewhere by the writer (Stebbins 1947a), a smaller or larger number of multivalent associations may be formed. This is to be expected from the fact that, as stated in Chapter VI, most F_1 diploid hybrids between closely related, but distinct, species have a high degree of chromosome pairing in spite of their sterility. Furthermore, since this pairing indicates that such hybrids possess a considerable number of homologous segments in common, one would expect polyploids produced from them to have many gene loci present four times. Therefore, as Dawson (1941) has pointed out, tetrasomic ratios, which sometimes have been assumed to be a diagnostic of autopolyploids, may be expected in polyploids derived from some types of sterile interspecific hybrids.

The only conclusions which can be drawn from the preceding facts are as follows. In the first place, no single criterion can be used to decide whether a given natural polyploid has arisen from

a fertile species or a sterile interspecific hybrid. Before even any hypothesis can be erected concerning the nature of polyploidy in a given species, it must be thoroughly studied from the cytogenetic as well as the morphological point of view, and all of its immediate relatives must be equally well understood. Before any final decisions can be made, the form in question should be hybridized with its putative diploid ancestor or ancestors, or, better yet, it should be resynthesized. In the second place, no classification of naturally occurring polyploids which recognizes only two contrasting categories, autopolyploids and allopolyploids, can hope to express even roughly the pattern of cytogenetic variation which polyploidy produces, no matter on what criteria these categories are based. Such an oversimplification does more to hide the complexities of origin and relationship which exist in such groups than to clarify them.

The difficulty resides not only in the fact that various intermediate conditions connect typical autopolyploidy with typical allopolyploidy. In addition, the four criteria which are generally used to distinguish these two types, namely, morphological resemblance, chromosome behavior, presence or absence of tetrasomic segregation, and the fertility or sterility of the diploid from which the polyploid was derived, do not run parallel to each other. It is quite possible for a polyploid to be judged an autopolyploid on the basis of one or more of these criteria, and an allopolyploid on the basis of the remaining ones. For this reason, the writer has presented elsewhere (Stebbins 1947a) an amplified classification of polyploid types, in which four principal categories have been recognized. There is no doubt that these categories are not sharply distinct from each other and are connected by a series of borderline cases. This is inconvenient to those whose primary purpose is classification, but nevertheless the categories recognized represent modal types, with characteristics of their own, so that their recognition provides a firmer basis for understanding the true character and origin of the various types of natural polyploids. A summary of this classification will therefore be presented in the following section.

TYPES OF POLYPLOIDS AND THEIR CHARACTERISTICS

The four types of polyploids recognized by the writer (1947a)

are *autopolyploids, segmental allopolyploids, true* or *genomic allopolyploids,* and *autoallopolyploids.* The first two occur in nature predominantly or entirely at the level of triploidy or tetraploidy; true allopolyploidy can occur at any level from tetraploidy upwards, while autoallopolyploidy is confined to hexaploidy and higher levels of polyploidy. The term amphiploid, coined by Clausen, Keck, and Hiesey (1945a), is suggested as a collective term to cover all types of polyploids which have arisen after hybridization between two or more diploid species separated by barriers of hybrid sterility. It therefore includes segmental allopolyploids, true allopolyploids, and autoallopolyploids plus aneuploids which have arisen from hybridization between two species belonging to an aneuploid series with lower numbers, as in *Brassica* (Nagaharu U, 1935, Frandsen 1943) and *Erophila* (Winge 1940). A diagram showing the interrelationships of these categories is presented in Fig. 34.

Typical autopolyploids have now been produced artificially from a large number of species, mostly of cultivated plants. Their morphological and physiological properties have already been
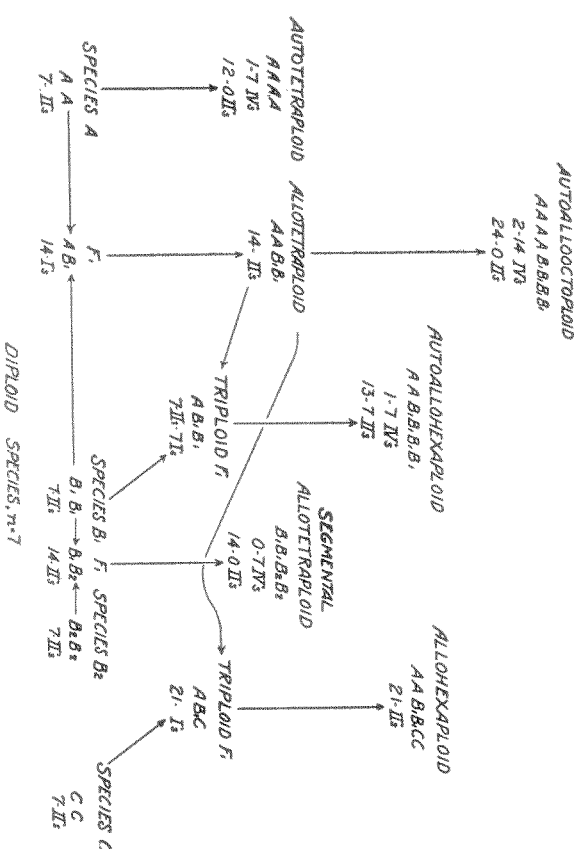

FIG. 34. Idealized diagram showing the interrelationships, genome constitution, and mode of origin of typical autopolyploids, allopolyploids, segmental allopolyploids, and autoallopolyploids. Original.

discussed. In general, the resemblance between such polyploids and their diploid progenitors is remarkably close. Such differences as exist in size and shape are considerably less than those ordinarily found between different subspecies or ecotypes of the same species. The physiological differences, likewise, although they would permit autopolyploids to grow in different situations from those occupied by their diploid progenitors, are relatively small compared to those between many ecotypes having the same chromosome number.

As has been emphasized by Clausen, Keck, and Hiesey (1945a), as well as by the present writer (Stebbins 1947a), many examples of natural polyploids once thought to be autopolyploids are now known or suspected to be of hybrid origin, and the number of polyploids which appear to have arisen in nature from a single diploid type is relatively small. One of the clearest examples is *Galax aphylla* (Baldwin 1941). This species belongs to a monotypic genus of the eastern United States which has no close relatives. The tetraploid differs in little besides its more sturdy character and thicker leaves. It has about the same geographic distribution as the diploid, but its range is somewhat wider. Similar examples are *Sedum ternatum* (Baldwin 1942a) and *S. pulchellum* (Baldwin 1943; Smith 1946). A probable autotriploid of this character is that of *Fritillaria camschatcensis* (Matsuura 1935). This plant is completely sterile, reproducing only by offsets from its bulbs. It occurs in lowlands throughout the island of Hokkaido, Japan, while its fertile diploid ancestor is confined to mountain summits. *F. camschatcensis* occurs also in Kamchatka, the Aleutian Islands, and the Pacific coast of North America south to the state of Washington, and is the only member of the section Liliorhiza found in the Old World (Beetle 1944). The chromosome number of the plants growing outside of Japan is not known. A related species of California, *F. lanceolata*, also has autotriploid and autotetraploid forms (Beetle 1944). These likewise are characterized by their robust stature, but so far as is known they occur only within the range of the diploid form.

In a number of instances, natural tetraploids are related to a series of diploid forms which differ morphologically from each other and have different geographical distributions and ecological preferences but form fertile hybrids on the diploid level, and

therefore, unless other types of isolating mechanisms are present, may be considered ecotypes or subspecies of the same species rather than separate specific entities. Such tetraploids are essentially autopolyploids, but may deviate in morphological characteristics, as well as ecological preferences, from any single wild diploid form. These differences are due to the presence of new gene combinations, as well as to the tetraploid condition. The best-known example of such a situation is *Biscutella laevigata* (Manton 1934, 1937). This tetraploid is widespread in the glaciated regions of the central Alps, but it has a series of diploid relatives which are scattered through central, southern, and southwestern Europe, from Austria to central France, mostly in lowland sites or in mountain chains not covered by the Pleistocene glaciation. Many of these diploids have been recognized as species, but the more conservative systematic treatments group them with *B. laevigata*, usually as varieties or subspecies. Clausen, Keck, and Hiesey have suggested that the isolating barrier produced by the difference in chromosome number should be recognized, and that all the diploids should be placed in one species, for which the oldest name is *B. coronopifolia*. Manton obtained some evidence indicating that typical *B. laevigata* is rather similar to a diploid form known as *B. minor*, from the unglaciated portion of the Austrian Alps, but that it possesses the ability to produce stolons or root buds, a character found in certain diploid forms of western Europe, particularly *B. arvernensis* and *B. lamottii*. It is likely, therefore, that the present tetraploid contains genes derived from several of the different diploid forms, and that this fact, as well as its tetraploid condition, is responsible for its wide distribution and range of ecological tolerance. Whether it originated from ancient hybrids between two or more different diploid forms, which may have grown together during one of the interglacial periods of the Pleistocene, or whether its present condition is the result of hybridization between tetraploids which were derived from different ones of the modern diploid species or subspecies cannot be told, and is perhaps not of great importance.

A similar case is *Dactylis glomerata*. This tetraploid pasture grass has long been thought to be derived by autopolyploidy from

the forest-loving diploid of central and northern Europe, *D. aschersoniana*, and Müntzing (1937) suggested that both the morphological and the ecological differences between the two species are due solely to the effects of doubling the chromosome number in *D. glomerata*. But Myers (1948) obtained a diploid plant from Iran which in its narrow, glaucous leaves, contracted inflorescences, and strongly ciliate glumes possesses in an extreme form the characteristics of *D. glomerata* which distinguish it from *D. aschersoniana*. It resembles forms identified as *D. hispanica*, *D. juncinella*, and *D. woronowii*. Hybrids between this diploid and *D. aschersoniana* are fully fertile and resemble *D. glomerata* in size, date of maturity, and other characters.

Intervarietal autopolyploids of this sort may be found to be not uncommon when more polyploids are analyzed with this possibility in mind. Suggested ones are *Allium schoenoprasum* (Levan 1936b), *A. paniculatum-oleraceum* (Levan 1937b), *Cuthbertia graminea* (Eigsti 1942, Ownbey 1944), *Polygonatum commutatum* (Eigsti 1942, Ownbey 1944), *Eriogonum fasciculatum* (Stebbins 1942c), and some of the various polyploids of *Vaccinium* (Camp 1945, Darrow and Camp 1945). One would expect this type of autopolyploid to segregate in the direction of its ancestral diploid subspecies, except in habitats which favored new combinations of genes. Randolph (1941a) has pointed out that the smaller amount of segregation in intervarietal tetraploids than in diploid hybrids of similar origin should favor the persistence of hybrid vigor in all combinations in which this phenomenon appears. For several reasons, therefore, we should expect intervarietal or intersubspecific polyploids to be the commonest type of autopolyploids in nature.

The second type of polyploid is that termed by the writer (Stebbins 1947a) a *segmental allopolyploid*. It may be defined as a polyploid containing two pairs of genomes which possess in common a considerable number of homologous chromosomal segments or even whole chromosomes, but differ from each other in respect to a sufficiently large number of genes or chromosome segments, so that the different genomes produce sterility when present together at the diploid level. This definition includes a great range of forms. At one extreme are types like *Zauschneria californica* (Clausen, Keck, and Hiesey 1940), which is derived from two species (*Z. cana* and *Z. septentrionalis*) capable of crossing to

form a fertile F_1 hybrid, but one which produces many inviable offspring in the F_2 generation. Near the other extreme are *Primula kewensis* (*P. floribunda-verticillata*, Newton and Pellew 1929, Upcott 1939), *Saxifraga adscendens-tridactylites*[2] (Drygalski 1935), and probably *Triticum durum-timopheevi* (Zhebrak 1944a,b, 1946). These are descended from diploid hybrids with a certain amount of pairing at meiosis, but with considerable meiotic irregularities. The polyploids form mostly bivalents, and, though somewhat more variable, behave not very differently from typical allopolyploids.

The writer (Stebbins 1947a) has listed more than twenty-five allopolyploids of this type which have been produced artificially in recent years. Many of these, like *Crepis foetida-rubra* (Poole 1931), *Layia pentachaeta-platyglossa* (Clausen, Keck, and Hiesey 1945a), and *Allium cepa-fistulosum* (Jones and Clarke 1942), have been so sterile in both the original and later generations that they would have been complete failures under natural conditions. But others, like *Primula kewensis*, *Tradescantia canaliculata-humilis* (Skirm 1942), and *Nicotiana glauca-langsdorffii* (Kostoff 1938b), have either been fertile from the start or have yielded highly fertile and in some instances constant types after a number of generations of selection.

Natural polyploids of this type have not as yet been recognized in many instances. Both in their close morphological resemblance to diploid forms and in the presence of multivalent configurations of chromosomes at meiosis, they simulate autopolyploids and have most often been confused with them. The actual nature of a segmental allopolyploid is uncertain until both of its parents have been identified by appropriate hybridization experiments, although external morphology may in some instances provide strong clues. The clearest examples of natural segmental allopolyploids, in addition to *Zauschneria*, are *Galium mollugo-verum* (*G. ochroleucum*), which originated through hybridization between two autopolyploids (Fagerlind 1937), and the cultivated species *Aesculus carnea* (*A. hippocastanea-pavia*, Upcott 1936). Another well-known polyploid which probably is of this type is

[2] For the sake of uniformity, artificial allopolyploids, except those whose names are well established in the literature, will be designated by the names of their two parental species, in alphabetical order and separated by a hyphen.

Solanum tuberosum, the common potato. Its somatic number is $2n = 48$, which makes it tetraploid, since the basic number for *Solanum*, as well as a large number of other genera in the Solanaceae, is $x = 12$. Both multivalent configurations and tetrasomic ratios of gene segregation have been found in this species (Cadman 1943, Lamm 1945). But Ivanovskaya (1939) found that a haploid plant was not only completely sterile, but in addition formed occasional univalents and 7 percent of bridge-fragment configurations at meiosis, which phenomena are much less common in typical diploid plants. She therefore concluded that *S. tuberosum* is a polyploid derived from a hybrid between two species having chromosomes similar enough to pair, but not enough to permit free interchange of genetic materials. Propach (1940) found that hybrids between diploid species of the section *Tuberarium* of *Solanum* are rather difficult to obtain, but that all of the F₁ plants secured, whether fertile or sterile, had good chromosome pairing. Lamm (1945) found that the number of multivalents in *S. tuberosum* is significantly less than that in an artificial autotetraploid of the diploid *S. rybinii*, and that the number of chromosome pairs with satellites seen at somatic mitosis is two, just as in diploid species of this section. He concluded that "polyploidy in *S. tuberosum* is mainly autopolyploidy with slight differentiations in the allopolyploid direction." Cadman (1943) found associations of six and eight chromosomes in strains of *S. tuberosum*. Since only bivalents are formed in its diploid relatives, these higher associations may be due to heterozygosity for segmental interchanges. *S. tuberosum*, therefore, has the cytological and genetic properties to be expected in a segmental allopolyploid, but the case cannot be considered definite until one or both of its diploid ancestors have been identified.

One of the most definite examples of a natural segmental allopolyploid is the tetraploid *Delphinium gypsophilum* (Lewis and Epling 1946, Epling 1947a). As was described in Chapter VII, this species exists in both a diploid and a tetraploid form, and the diploid is both morphologically and cytogenetically very similar to the F₁ hybrid between *D. hesperium* and *D. recurvatum*. This hybrid is highly sterile, although it produces some seed, but chromosome pairing at meiosis is nearly normal. It is likely, therefore, that tetraploid *D. gypsophilum* was produced directly from

the F₁ or F₂ hybrid between *D. hesperium* and *D. recurvatum* and therefore meets all the requirements for a segmental allopolyploid. It is somewhat more widespread in geographic distribution than its diploid counterpart.

Another group of probable segmental allopolyploids are the tetraploid species of *Paeonia*. All of them form a greater or lesser number of multivalents at meiosis (Dark 1936, Stebbins 1938a and unpublished) and on these grounds, as well as their superficial resemblance to certain diploid species, Barber (1941) and Stern (1946, 1947) concluded that they are autopolyploids. But, as pointed out elsewhere by the writer (Stebbins 1948), the various tetraploids show morphological resemblances to more than one of the diploid species, and Stern in some of his species descriptions recognizes the presence of intergrading between tetraploids which he relates to different diploid species. Furthermore, some of the tetraploids, like *P. peregrina* and *P. officinalis*, have only one or two multivalents at meiosis, and in some sporocytes may form ten bivalents. In plants with large chromosomes like those of *Paeonia* and with random chiasma formation, the presence in a plant of four completely homologous genomes should cause it to form a high proportion of quadrivalents. In fact, even some triploid hybrids between widely different species, like *P. albiflora* × *tomentosa*, may form as many as four or five trivalents. The cytological evidence, therefore, agrees with that from external morphology in suggesting that the tetraploids of *Paeonia* are mostly segmental allopolyploids.

Another probable example of a segmental allopolyploid is *Lotus corniculatus*. Dawson (1941) found that this tetraploid forms, almost entirely, bivalents at meiosis, but because he found tetrasomic inheritance for certain genetic characters he concluded that this species is an autopolyploid of the closely similar diploid species *L. tenuis*. Tome and Johnson (1945), however, secured an artificial autotetraploid of *L. tenuis* and found that it was morphologically different from *L. corniculatus* and would not hybridize with it. Careful analysis of other tetraploids believed to be autopolyploids may very well show that many of them are also segmental allopolyploids.

The most important genetic difference between segmental allopolyploids and typical or genomic ones is the ability of the

former to segregate in respect to some of the characteristics by which their ancestral species differ from each other. This results directly from their ability to form multivalents at meiosis. Such multivalents represent two types of chromosome pairing. Each quadrivalent in a newly formed segmental allotetraploid contains two completely homologous chromosomes, derived from the same species or even the same diploid individuals, and two partly homologous ones, derived from different species, but possessing in common a greater or lesser number of homologous segments. The genomes of this type of polyploid may be designated as $A_1A_1A_2A_2$, and the individual chromosomes as $1A_11A_11A_21A_2$, $2A_12A_12A_22A_2$ . . . $nA_1nA_1nA_2nA_2$, where n is the basic haploid number, A_1 and A_2 being the parental genomes. Pairing between two $1A_1$ chromosomes is designated as *homogenetic association*, and between $1A_1$ and $1A_2$ as *heterogenetic association*. The writer (Stebbins 1947a) has discussed elsewhere the derivation of these terms and their relationship to the parallel, but by no means synonymous, terms auto- and allosyndesis. Autosyndesis refers to the pairing of chromosomes derived from the same parental gamete of a particular plant, regardless of the similarity or difference from each other, while allosyndesis refers similarly to pairing between chromosomes derived from different parental gametes, such as normally occurs both in diploids and in polyploids. Depending on whether the plant is a newly formed polyploid, a hybrid between two autopolyploids, or a long-established polyploid, autosyndesis may be either homo- or heterogenetic association.

The frequency of heterogenetic association as compared to homogenetic association depends on the relative degree of affinity between the completely homologous as compared to the partly homologous chromosomes, or their *differential affinity* (Darlington 1937, pp. 198–200). This degree of affinity is determined largely by the size of the homologous segments as compared to the nonhomologous segments in the partly homologous chromosomes, but the absolute chromosome size, the genetically determined chiasma frequency, and the distribution of chiasmata, whether at random or localized, all play important roles. In some plants with small chromosomes, like *Primula kewensis* and *Lotus corniculatus*, differential affinity is marked, and the homologous chromosomes usually exhibit preferential pairing, so that most of the sporocytes

contain largely or exclusively bivalents. But in *Paeonia* and in *Crepis foetida-rubra* (Poole 1931), both of which have considerably larger chromosomes, multivalents are regularly formed, and preferential pairing is at a minimum.

On the basis of their results in *Layia* and *Madia*, Clausen, Keck, and Hiesey (1945a) have concluded that the heterogenetic association in segmental allopolyploids, which they characterize as (p. 72, Table 12) inter-ecospecific amphiploids with the identity of the parental genomes lost in recombination, will always lead to segregation of disharmonious gene combinations, and thus to the eventual failure of the line. They cite as examples of this failure *Layia pentachaeta-platyglossa*, *Aquilegia chrysantha-flabellata* (Skalinska 1935), the examples of Poole and of Hollingshead (1930b) in *Crepis*, and *Primula kewensis*. They recognize that in *P. kewensis* there is as yet little evidence of increasing sterility, but they cite the amount of genetic variation in different lines of this species reported by Newton and Pellew (1929) and Upcott (1939) as a sign that it would not be likely to remain as a stable species in nature. On the other hand, they do not refer to the earliest example of a segmental allopolyploid which eventually yielded, after six to seven generations, relatively stable and fertile lines, namely, *Nicotiana glauca-langsdorffii* (Kostoff 1938b). A more recent series of examples is the large number of allopolyploids produced by Zhebrak (1944a,b, 1946) between the various tetraploid wheats of the emmer series (*Triticum durum*, *T. dicoccum*, etc.) and the tetraploid *T. timopheevi*. The cytology of these polyploids was not investigated, but since Lilienfeld and Kihara (1934) found 12 out of a possible 14 bivalents to be the most common amount of pairing in diploid hybrids of this combination, and since the chromosomes are large and have random chiasma distribution with a normal frequency of more than two chiasmata per bivalent, multivalents would be expected in these tetraploids. Zhebrak found a certain amount of segregation in respect to the differences between the parental species, although no forms closely resembling either of the parental species were recovered after several generations of selfing. On the other hand, these polyploids were fertile when first formed, and have remained so.

Gajewski (1946) obtained evidence on the relation between

chromosome homology and fertility in a segmental allopolyploid between *Anemone silvestris* and *A. multifida*. The original production of this allopolyploid by Janczewski, in 1892, was probably the first example ever observed of a fertile, constant type obtained from a sterile interspecific hybrid, although the cytological basis for the increase in fertility was, of course, not recognized by Janczewski. The F_1 hybrid between *A. silvestris* ($2n = 16$) and *A. multifida* ($2n = 32$) forms three to seven pairs of chromosomes at meiosis and is highly sterile, but its allopolyploid forms one quadrivalent in 50 percent of the sporocytes, in addition to bivalents and occasional univalents, and in the second generation varied in fertility from 22 to 70 percent of normal seed setting. This fertility was somewhat increased in the following generation, and the data indicate that fully fertile lines will eventually be produced by selection. Gajewski presents a chart based on the relation between chromosome pairing in the F_1 and fertility of the derived allopolyploid of 19 different interspecific combinations recorded in the literature, and this indicates that there is a negative correlation between the amount of pairing in the F_1 hybrid and the degree of fertility in the derived allopolyploid. But in his study of 20 hybrids and their derived allopolyploids in *Aegilops* and in *Triticum*, Sears (1941) found no such correlation, and if Sears's allopolyploids are plotted on Gajewski's chart, all evidence of correlation disappears. Chromosome pairing is only one factor affecting the fertility or sterility of allopolyploids; the degree of similarity of the pairing chromosomes, their size, their chiasma frequency, and various genically or plasmatically controlled sterility factors must also be considered (see page 306).

On theoretical grounds, the hypothesis presented in Chapter VI, that chromosomal sterility is due to heterozygosity for translocations and inversions of small chromosomal segments, and that these segments are inherited independently of the morphological and physiological differences between the parental species, leads to the postulate that segregation and selection over a long enough period of time should eventually lead to the production of fertile, genetically stable types derived from segmental allopolyploids. The length of time needed for this to happen could be estimated only on the basis of some knowledge of the number of chromosomal differences between the parental species. Since this is

not available at present for any example, no estimates of this nature can be made.

Segmental allopolyploids, therefore, have two distinctive properties. The first is the ability for genetic segregation, in respect to both the morphological differences between the parental species and the chromosomal differences which formed the sterility barrier between these parents. Selection pressure in favor of increasing fertility will affect the segregation of these chromosomal differences in two ways. If the chromosomes of the parental species were largely similar and differed by only one or two small nonhomologous segments, selection would favor the elimination of these segments and evolution in the direction of autopolyploidy. But if the chromosomes differed initially by numerous or large segments, but nevertheless possessed enough homology so that they could occasionally form multivalents in the original, "raw" allopolyploid, selection would favor the further differentiation of these chromosomes by means of mutation and further chromosomal rearrangements and the elimination of multivalent formation. Thus, segmental allopolyploidy is an unstable condition, which through segregation and chromosomal alteration, guided by selection for fertility, will evolve in the direction of either auto- or typical allopolyploidy. However, the direction of this evolutionary trend will be according to the constitution of individual chromosomes, not of the entire complement of the polyploid. In many segmental allopolyploids, some of the parental chromosomes may be so similar that selection will favor elimination of structural differences and the formation of four similar chromosomes, while others of the initial parental sets may have been so strongly differentiated that further differentiation and the elimination of multivalent formation will be favored.

The stable derivatives eventually produced from the raw segmental allopolyploid may therefore be expected to have one of three different types of constitution. First, they may be completely autopolyploid in respect to the structural make-up of their chromosomes, although still intermediate between their original parental species in external morphology and in ecological preferences. Second, they may become cytologically allopolyploid, so that they rarely or never form multivalents or exhibit heterogenetic association. Finally, they may come to possess some chro-

mosomal types present in the form of four complete structural homologues, and others of the same original complement so strongly differentiated that they form only bivalents. This third type will be permanent, stable segmental allopolyploids. They will regularly form a small number of multivalents and will segregate for some of the morphological differences between the parental species, but they will nevertheless be highly fertile and able to maintain themselves under natural conditions.

The second property of segmental allopolyploids is that they can be expected to form partly fertile hybrids through backcrossing with autopolyploid derivatives from either of their parental species. By means of hybridization and introgression they may greatly obscure or completely obliterate the morphological and genetic barrier which existed between these parental autopolyploids. This fact is probably largely responsible for the usual treatment of this type of polyploid in the systematic literature; they are usually placed in the same taxonomic species as one of their diploid ancestors, and their existence may cause conservative botanists to combine both of their ancestral diploids into the same species.

The third type of polyploid, the typical or genomic allopolyploid, is a much better known type of plant. Well-known artificial examples are *Nicotiana glutinosa-tabacum* (*N. digluta*, Clausen and Goodspeed 1925), *Brassica oleracea-Raphanus sativus* (*Raphanobrassica*, Karpechenko 1927, 1928), and *Secale cereale-Triticum aestivum* (*Triticale*, Müntzing 1939). Natural allopolyploids among wild species whose parentage has been proven by synthesis of the same or a similar polyploid from diploid ancestors are *Galeopsis tetrahit* (*G. pubescens-speciosa*, Müntzing 1930a,b), *Rubus maximus* (*R. idaeus-caesius*, Rozanova 1934, 1938, 1940), *Nicotiana rustica* (*N. paniculata-undulata*, Goodspeed 1934, Eghis 1940), *Brassica napus* (*B. campestris-oleracea*, Nagahuru U, 1935), *B. juncea* (*B. campestris-nigra*, Frandsen 1943), *Madia citrigracilis* (Clausen, Keck, and Hiesey 1945a), and *Bromus arizonicus* (*B. haenkeanus-trinii*, Stebbins, Tobgy, and Harlan 1944, Stebbins 1949b). Cultivated species of which the allopolyploid origin has been similarly demonstrated are *Nicotiana tabacum*, *Gossypium hirsutum*, and *Triticum aestivum* (see page 372). In addition, more or less convincing indirect evi-

dence has been obtained for the parentage of the following natural allopolyploids: *Spartina townsendi* (*S. alterniflora-stricta*, Huskins 1931), *Penstemon neotericus* (*P. azureus-laetus*, Clausen 1933), *Iris versicolor* (*I. setosa-virginica*, Anderson 1936b), *Prunus domestica* (*P. divaricata-spinosa*, Rybin 1936), *Poa annua* (*P. exilis-supina*, Nannfeldt 1937, Litardière 1939), *Nicotiana arentsi* (*N. undulata-wigandioides*, Goodspeed 1944), *Artemisia douglasiana* (*A. ludoviciana-suksdorffii*, Clausen, Keck, and Hiesey 1945a, Keck 1946), *Oryzopsis asperifolia* and *O. racemosa* (*O. holciformis vel aff.-O. pungens vel. aff.*, Johnson 1945), and *Bromus marginatus* (*B. aff. laevipes-stamineus*, Stebbins and Tobgy 1944, Stebbins 1947c). In many other examples one of the parental species of an allopolyploid has been identified, and a still larger number of species has been shown to have the chromosome number and behavior which one would expect in a typical allopolyploid.

Typical allopolyploids are derived from hybridization between two or more distantly related species, of which the chromosomes are so different that they are unable to pair in the diploid hybrid, or form only a small number of loosely associated bivalents. The hybrids which are capable of giving rise to allopolyploids are usually completely unable to give diploid progeny, so that interchange of genes between their parental species is impossible. The species ancestral to allopolyploids, therefore, usually belong to different cenospecies, according to the definition of Clausen, Keck, and Hiesey (1939, 1945a). In their systematic position as based on characteristics of external morphology, they may belong to the same section of a genus, as with some examples in the genera *Nicotiana* and *Madia*, but more often they belong to different sections, subgenera, or even genera.

A typical allopolyploid, therefore, contains two or more sets of very different genomes, and may be given the formula AABB, AABBCC, and so on, where each letter represents a set of chromosomes of the basic haploid number for the genus. The only type of pairing which normally occurs is that between similar chromosomes of the same genome, or homogenetic association. This causes allopolyploids to breed true to their intermediate condition and to segregate relatively little. Furthermore, hybrids formed by backcrossing an allopolyploid to either diploid parent

or to their autopolyploid derivatives are usually partly and sometimes completely sterile. A typical allopolyploid, therefore, in contrast to a segmental allopolyploid or an autopolyploid, not only is often fully fertile and constant from the start; in addition it is strongly isolated from and as a rule morphologically discontinuous with its nearest relatives. The result of this condition is that most of the polyploid species complexes which are difficult problems for the systematist contain segmental allopolyploids, autopolyploids, or both; typical allopolyploids most often stand out as clearly marked species.

Nevertheless, such polyploids, although they resemble diploids in many respects, still possess a number of qualities connected with their polyploid origin. The most significant of these is the presence of duplicated genetic material, which enables them to withstand losses of chromosomes or chromosome segments which would be fatal to diploid organisms. This ability is most strikingly manifest in wheat (*Triticum aestivum*), in which Sears (1944b) has been able to produce 21 different viable nullisomics, each lacking a different one of the 21 chromosome pairs present in normal *T. aestivum*. Sears showed, furthermore, that genetic material could be duplicated in chromosomes completely incapable of pairing with each other. Plants monosomic for chromosome II are morphologically very similar to those monosomic for chromosome XX and in both cases are definitely weaker than normal plants. But a plant which has 42 chromosomes and is monosomic or nullisomic for chromosome II and trisomic or tetrasomic for chromosome XX is nearly normal in every respect. Nevertheless, although they compensate each other in their influence on viability and fertility, chromosomes II and XX are completely unable to pair at meiosis, even when they have no other mates.

In another allopolyploid which has been carefully analyzed, *Nicotiana tabacum*, Clausen and Cameron (1944) have produced and studied the phenotypic characteristics of monosomics lacking one member of each of the 24 chromosome pairs normally present in the species. Each of these monosomics has its recognizable distinctive features, and the viability of gametes lacking a chromosome differs greatly depending on the particular chromosome concerned. In *Gossypium hirsutum*, on the other hand, the

amount of duplication of genetic material appears to be so slight that monosomics are difficult to obtain and rarely perpetuate themselves even in artificial cultures (M. S. Brown, oral communication). Allopolyploids, therefore, may differ considerably from each other in the amount of genic duplication they possess, even though they are much alike in their normal genetic and cytological behavior.

Another property of many allopolyploids which results from the presence of some duplicated genetic material is the ability of their chromosomes to undergo occasional heterogenetic association, and so to segregate with respect to some of the characteristics which differentiated their parental species, or for characteristics which have originated by mutation since the origin of the polyploid. The progeny resulting from such segregation appear like sudden mutants. They have been most carefully studied in certain cereals, where they are responsible for the "fatuoid" variants in cultivated oats (*Avena sativa*) and the "speltoids" in wheat (*Triticum aestivum*). Huskins (1946) has reviewed carefully the extensive literature on these forms and has marshaled an abundance of evidence to show that they have actually resulted from this type of pairing, rather than from hybridization or from the addition or subtraction of whole chromosomes, as other authors have believed. The detection of variants of this nature is very difficult except in well-known and genetically constant species like wheat and oats, but there is little doubt that occasional heterogenetic association is an important source of variation in many wild allopolyploids. Huskins (1941) has made the apt suggestion that in most polyploid species chromosomal changes are a more important source of variation than gene mutation.

In an allopolyploid which is well adapted to its environment, chromosomal variation is likely to lead to deleterious variants, and selection will favor mutations which make the chromosomes more different from each other. The original, or "raw," allopolyploid is likely to become progressively "diploidized," until its behavior more nearly resembles that of a diploid species. R. E. Clausen (1941) has determined accurately the nature of this diploidization for certain chromosomes of *Nicotiana tabacum*, by comparing cultivated strains of this species with allopolyploids newly produced from its putative parents, *N. sylvestris* and *N.*

tomentosiformis. These "raw" allopolyploids have in duplicate certain factors, such as the dominant allele to the mammoth factor, the normal allele to a factor responsible for asynapsis, and another to a recessive white-seedling character, which are all present only singly in *N. tabacum.* Since the chromosomes of the "raw" allopolyploid pair perfectly with those of *N. tabacum* in the F_1 hybrid, the elimination of duplicate alleles during the evolution of *N. tabacum* appears to have been either by mutation or by the loss of very small chromosomal segments.

Although typical allopolyploids can be formed only through hybridization between species the chromosomes of which are so different from each other as to be nearly or quite incapable of pairing, by no means all polyploids from such hybrids are successful allopolyploids. If the parental species are separated by factors for genic as well as for chromosomal sterility, the allopolyploid combining their genomes will be as sterile as their diploid hybrid. For instance, *Nicotiana sylvestris-tomentosiformis,* although it has normal chromosome pairing and good pollen, is completely female sterile because of abortion of the embryo sac at the two- to four-celled stage (Greenleaf 1941, 1942). Nearly all of the allopolyploids between *N. sylvestris* and members of the *N. tomentosa* complex, if they are produced through doubling of the somatic tissue of the diploid F_1 hybrid, have the same type of sterility (Clausen 1941). But Kostoff (1938a) was able to produce a fertile allopolyploid of this combination by using unreduced gametes of the F_1 hybrid, and through the intermediate stage of a triploid. On the basis of evidence obtained from hybrids between Kostoff's allopolyploid and the one produced by somatic doubling, Greenleaf (1942) concluded that during one of the stages in the formation of Kostoff's allopolyploid, the female sterility factor was eliminated by heterogenetic association and crossing over.

An allopolyploid exhibiting a very different type of genic sterility is the one produced by Sears (1941b) between *Aegilops umbellulata* and *Haynaldia villosa.* The F_1 hybrid of this combination had a mean value of only 0.3 pairs at meiosis and was completely sterile, as might be expected from the remoteness of the relationship between its parents. But the allopolyploid, produced by somatic doubling through colchicine treatment of the F_1 hybrid, was also highly sterile and had 6 to 26 out of a possible

28 unpaired univalent chromosomes, in spite of the fact that each chromosome possessed a potential mate with which it was completely homologous. Li and Tu (1947) have found a similar situation in the colchicine-produced allopolyploid, *Aegilops bicornis-Triticum timopheevi.* The best explanation of this situation is, as Li and Tu have suggested, that the parental species differ by certain genetic factors affecting timing or some other process essential to chromosome pairing at meiosis. At present, no criteria are available by which one may tell whether such factors are at work in an F_1 hybrid, and so predict whether or not the allopolyploid will be sterile. More intensive study of various examples of artificial allopolyploids of all types will be needed before satisfactory generalizations can be made concerning the probable success or failure of the allopolyploids of any type, based on the study of parental species and the diploid hybrid.

The fact that artificially produced "raw" polyploids of all types have for the most part proved only partly fertile lends great importance to ways in which fertility may be increased in later generations. That such an increase can take place is evident from the observations of Sando (1935) on *Triticum turgidum-Haynaldia villosa,* of Katayama (1935) on *Triticum dicoccoides-Aegilops ovata,* of Oehler (1936) on *Triticum durum-Aegilops triuncialis,* of Kostoff (1938b) on *Nicotiana glauca-langsdorffii,* and of Gajewski (1946) on *Anemone multifida-sylvestris.* The two sets of factors which probably cause this increase in fertility are briefly mentioned by Kostoff and are discussed by Gajewski. These are, first, alterations in chromosomal structure which would eliminate heterogenetic association, with its consequent formation of gametes containing duplications and deficiencies of chromosomal segments; and, second, gene mutations suppressing or counteracting the genetic physiological disharmonies of meiosis which are responsible for asynapsis of completely homologous chromosomes, with the consequent formation and irregular distribution of univalents. Both of these factors are probably operating in different polyploids, and their relative importance most likely depends on the nature of the particular "raw" polyploid concerned. Fagerlind (1944a), based on the observations of Wettstein (1927) and Wettstein and Straub (1942) on the moss genus *Bryum,* has suggested that in perennial species, physiological ad-

justment leading to greater fertility can occur during the lifetime of a single plant, presumably through somatic mutations. The determination of the relative importance in polyploids of different types of these factors for increasing fertility is one of the major tasks of plant breeders making use of artificial polyploids, and obviously it is of great importance to an understanding of plant evolution.

Although the majority of allopolyploids are either tetraploids or hexaploids, and so combine the genomes of only two or three different ancestral species, a number of higher polyploids of this type are known. For instance, one of the most common species of grasses of western North America, *Bromus carinatus* and its relatives, is an allopolyploid containing four different genomes (Stebbins and Tobgy 1944). Another species belonging to a different subgenus, *B. trinii*, is an allohexaploid containing three different genomes, and the F_1 hybrid, *B. carinatus* × *trinii*, forms mostly univalents at meiosis, with 5 or 6 bivalents as the maximum amount of pairing. The allopolyploid produced from this hybrid has 98 chromosomes as the somatic number, and at meiosis it forms either 49 bivalents or 47 bivalents and one quadrivalent (Stebbins 1949b). This polyploid is vigorous and fertile, and the first stage of establishing it in nature as a spontaneous species is proving successful. Here, therefore, is an example of a probably successful allopolyploid which contains seven different genomes, acquired by a succession of hybridizations probably extending over a period of millions of years and involving seven different ancestral diploid species.

The fourth type of polyploid can exist only at the level of hexaploidy or higher, and combines the characteristics of the two preceding ones. This is the type called by Kostoff (1939c) an *autoallopolyploid*. He cites the example of *Helianthus tuberosus*, a hexaploid with $2n = 102$ chromosomes, which produces with the diploid *H. annuus* ($n = 17$) a tetraploid hybrid forming 34 bivalents at meiosis. On this basis, Kostoff has assumed that *H. tuberosus* has the genomic formula $A A A_t B_t B_t$ and *H. annuus*, the formula $B_t B_t$, the genomes designated by the letters A and B being entirely different from each other, but those with the same capital letter having enough chromosomal segments in common so that they can pair normally. Thus, in respect to the

A genome, *H. tuberosus* is either autopolyploid or segmental allopolyploid, and we should expect to find in it both multivalents and tetrasomic inheritance. But the presence in this species of the genome designated B makes it partly an allopolyploid, and at least one interspecific hybridization must have been involved in its origin.

Two other well-known polyploids which are probably of this type are *Phleum pratense* and *Solanum nigrum*. The former species was judged by Gregor and Sansome (1930) to be an allopolyploid, an opinion shared by Clausen, Keck, and Hiesey (1945a). But Nordenskiöld (1941, 1945), after intensive study, considered it to be an autopolyploid containing the diploid complement of *Phleum nodosum* trebled, while Myers (1944) noted cytological characteristics indicating autopolyploidy. But since two different haploid plants of *P. pratense*, one studied by Nordenskiöld and one by Levan (1941a), typically form 7 bivalents and 7 univalents, their genomic formula must be AAB, and that of the diploid AAAABB. The evidence of Nordenskiöld suggests that the A genome is very likely that of *P. nodosum*, and the B genome, that of the diploid *P. alpinum*. The tetraploid *P. alpinum*, which Gregor and Sansome believed to be ancestral to *P. nodosum*, was shown by Nordenskiöld (1945) to be an allopolyploid containing the B genome and a still different one, belonging to some diploid species as yet unidentified.

Solanum nigrum was considered by Jorgenson (1928) to be an allopolyploid, but Nakamura (1937) believed it to be an autopolyploid, since he found quadrivalents at meiosis in this species and noted a strong resemblance to a diploid species from southern Japan described by him as new, but actually conspecific with *S. nodiflorum* Jacq. The haploid of *S. nigrum* ($n = 36$) forms approximately 12 bivalents and 12 univalents (Jorgenson 1928), so that it is most likely an autoallopolyploid containing four genomes from *S. nodiflorum* or some other species closely related to it, and two from some diploid species as yet not identified. Both in this example and in the previous one, the resemblance of the autoallohexaploid to one of its diploid ancestors is so strong that the two have been placed in the same species by most systematists. This is likely to be true of most autoallopolyploids which contain two or more genomes derived from one species

and only one genome from another. It is likely that a considerable proportion of the polyploids at the hexaploid level or higher which are believed to be autopolyploids are actually of this constitution.

Another type of autoallopolyploid can be produced by doubling the chromosome number of a typical allotetraploid, to produce an octoploid with the genomic formula AAAABBBB. A typical example is the autopolyploid produced by Clausen (1941) from *Nicotiana tabacum*. Among wild species, *Rubus ursinus* ($2n = 56$) is probably of this nature, since the hybrid between this species and the diploid *R. idaeus* forms 14 bivalents and 7 univalents (Thomas 1940a,b). According to Fedorova (1946), *Fragaria grandiflora*, *F. chiloensis*, and *F. virginiana* are similar. Other high polyploid species are known to form multivalents and to resemble closely certain diploids or lower polyploids. Typical of these are *Agropyron elongatum*, in which a form with $2n = 70$ resembles one with $2n = 14$ (Wakar 1935; Simonet 1935); *Penstemon neotericus*, an octoploid ($2n = 64$), which is probably derived from a closely similar hexaploid, *P. azureus*, and a diploid species, *P. laetus* (Clausen 1933); and *Rubus lemurum* (Brown 1943), with $2n = 84$ chromosomes, which is closely similar to and apparently forms partly fertile hybrids with the octoploid *R. ursinus*, mentioned above. Most of the high polyploids in the genus *Chrysanthemum* (Shimotomai 1933) are probably of this nature, as are those described by Callan (1941) in *Gaultheria* and *Pernettya*. In fact, there is good reason to believe that most of the polyploids at the octoploid level or higher contain some duplicated chromosomes or chromosome segments, but the behavior of artificial autopolyploids indicates that few if any of these higher polyploids can be strict autopolyploids. It is very difficult, and perhaps not of major importance, to make the distinction between those which combine autopolyploidy with allopolyploidy and the higher polyploids which are partly segmental and partly typical allopolyploid in constitution.

When extensive duplication of chromosomal material exists, as it does in such polyploids, regular behavior of the chromosomes at meiosis is not essential to the production of viable gametes, since many different combinations of various numbers of chromosomes can function. Love and Suneson (1945) found that an

F₁ hybrid between *Triticum macha* and *Agropyron trichophorum*, both of which have $2n = 42$, gave rise to a fertile F₂ plant with 70 chromosomes. The best explanation is that an unreduced gamete with 42 chromosomes united with a partially reduced one having 28. Plants with 84 and other numbers of chromosomes might be expected to arise from this same F₁, so that it is potentially the progenitor of a number of different evolutionary lines, each with a different chromosome number and capable of becoming a different species. In the genus *Saccharum* Bremer (1928) and Grassl (1946) have found at high polyploid levels a great variety of different chromosome numbers, ranging from $2n = 60$ to $2n = 120$. Many of these numbers are found in the recently produced "noble canes," and are therefore the result of plant breeding in recent times. Most of these forms, whether euploid or aneuploid, are reasonably fertile.

At these higher levels of polyploidy, therefore, various combinations of the auto- and allopolyploid condition are probably the most common situation. In some favorable instances analysis of these polyploids and the discovery of their diploid ancestors may be possible. But in the majority of them, their origin is too complex to be analyzed in its entirety, and at least one of the diploid ancestors may be extinct.

POLYPLOID PERMANENT HYBRIDS: THE *Rosa canina* COMPLEX

A most unusual type of autoallopolyploid is found in the roses of northern Europe belonging to the *Rosa canina* complex. Many years ago Blackburn and Harrison (1921), as well as Täckholm (1922), found that most forms of this group are pentaploids with 35 somatic chromosomes, though there are forms with 28 and 42, but that in any case they form at meiosis only 7 bivalents, and 14, 21, or 28 univalents. The behavior of these univalents in the meiosis leading to pollen formation is entirely different from that in megasporogenesis in the ovules. In the former divisions, the univalents are usually eliminated, so that the functional pollen grains have only 7 chromosomes, all derived from the bivalents. In megasporogenesis all of the univalents are included in the functional megaspore, so that this cell and the resulting embryo sac and egg cell have, in the *caninae* roses with 35 chromosomes as the somatic number, 28 chromosomes. The union at fertiliza-

tion of $28 + 7$ restores the normal somatic number. The constancy of individual microspecies in this complex, as well as the large number present, led Täckholm (1922) to believe that apomixis exists in this group, and some experiments on emasculation and hybridization seemed to confirm this suspicion. But Blackburn and Harrison believed these roses to be sexual and ascribed their peculiar variation pattern and cytological behavior to a type of balanced heterogamy. This opinion was shared by Darlington (1937), Fagerlind (1940b), and particularly by Gustafsson (1931a,b, 1944).

The careful cytogenetic work of Gustafsson (1944) and Gustafsson and Håkansson (1942) has provided the basis for an illuminating and highly probable hypothesis about the constitution of this group and the reasons for its anomalous and intricate variation pattern. Gustafsson found that hybrids between two common members of this complex, R. canina and R. rubiginosa, are strongly matroclinous, as are also hybrids between either species and a relatively distantly related diploid, R. rugosa. Nevertheless, the true hybrid nature of F_1 plants produced from these crossings was evident from certain features of their external morphology, and more particularly from the behavior of the chromosomes at meiosis. This is precisely the reverse of what is found in a series of hybrids between normal species. The related forms, R. canina and R. rubiginosa, yield F_1 hybrids with approximately 7 bivalents, although cells with 3 to 6 bivalents, as well as those with multivalents, are not uncommon. But the F_1 hybrids of the wide intersectional crosses between R. canina or R. rubiginosa and R. rugosa, with the latter as the pollen parent, have a much higher number of bivalents, mostly 11 to 14. The only possible explanation of this fact is that at least 4 to 7 of these bivalents are due to autosyndesis of chromosomes derived from the maternal gamete. Both R. canina and R. rubiginosa, therefore, are considered by Gustafsson to possess an "internal autotriploidy," which makes possible pairing between two sets of chromosomes derived from the same maternal gamete, provided that one of these does not have exactly similar mates contributed by the paternal gamete.

After considering several possible genomic formulae, Gustafsson has concluded that the most likely one for the somatic constitution of the pentaploid canina roses is A_1A_2CD, while

R. rugosa is CC, and the F_1 hybrids are A_1A_2CD. Since the chromosomes of A_1 and A_2 are only partly homologous and may have been derived from different original diploid or tetraploid species, the "internal autotriploidy" would be segmental allotriploidy according to the terminology used in this chapter. The degree of similarity between A_1 and A_2 varies according to the species of caninae studied. In hybrids between R. canina and either R. rugosa or R. rubiginosa the number of bivalents is somewhat smaller than in either R. rubiginosa × rugosa or R. rubiginosa × canina, indicating that a greater amount of autosyndesis is possible between chromosomes belonging to R. rubiginosa than between those of R. canina × rubiginosa and its reciprocal, which results in greater sterility of hybrids with R. canina as the maternal parent, and provides additional evidence in favor of Gustafsson's hypothesis of autosyndesis.

Fagerlind (1945), while verifying Gustafsson's hypothesis of internal auto- or segmental allopolyploidy, has shown that this condition extends beyond triploidy and, in at least some of the canina roses, may involve complete or partial homologies between all of the sets present. He crossed a tetraploid member of the canina group, R. rubrifolia, reciprocally with R. rugosa. With R. rubrifolia as mother, the tetraploid F_1 strongly resembled that species, and as in Gustafsson and Håkansson's hybrids produced 7 to 14 bivalents. But the reciprocal hybrid, R. rugosa × rubrifolia, while diploid as expected, nevertheless consistently produced 7 bivalents. This shows that the pairing or "A" set of R. rubrifolia is homologous to the haploid genome of R. rugosa. Since Fagerlind has stated from preliminary information on hybrids with R. rugosa as the maternal parent that the pairing sets of different roses of the canina group differ widely from each other in their genic content, Gustafsson's formula may yet hold for the species investigated by him, although it is obviously invalid for R. rubrifolia. On the other hand, the hypothesis of Fagerlind, that all the genomes in the canina roses are potentially capable of pairing with each other, but are usually kept from doing so by genetic factors, seems more likely at present.

Fagerlind has stated that new microspecies are formed in this group chiefly by hybridization between existing forms followed by segregation of the genes present in the pairing genomes, as well

as by mutations occurring in the nonpairing genomes. Gustafsson and Håkanson, on the other hand, emphasize the importance of heterogenetic association between chromosomes belonging to different genomes as a source of variation. This type of association would be expected most often as autosyndesis in an F_1 hybrid between two microspecies having somewhat dissimilar chromosomes. All three of these processes have probably contributed to the multitude of microspecies found in this group.

This situation in the *canina* roses provides a most interesting parallel to that in the majority of the complex-heterozygote races of *Oenothera*, discussed in Chapter XI. Both are permanent heterozygotes or hybrids and are heterogamous, in that their male gametes contain different chromosomal material from their female ones. Both probably owe their success and aggressiveness to the permanent possession either of hybrid vigor or of favorable combinations of genes. Both consist of a large number of relatively constant biotypes or microspecies, which owe their constancy partly to autogamy and partly to a great restriction of the amount of pairing and crossing over which can take place, in relation to the number of gene loci present. In both groups, new biotypes or microspecies can arise either through crossing between preexisting ones or through an exceptional type of pairing and crossing over. The chromosomes of both groups contain some elements which regularly pair and cross over and others which are normally unpaired. In *Oenothera*, the unpaired regions are the differential segments, in those portions of the chromosomes near the attachment constrictions or centromeres; while in the *canina* roses they are the univalents. There is, however, an important difference in the genic content of the pairing portions as compared to the nonpairing portions of the chromosome complements in the two groups. In *Oenothera*, the differences between races appear to lie mostly in the differential or nonpairing segments, while in the *canina* roses, if the hypothesis of Gustafsson and Håkansson is correct, differences must exist in both the pairing and the nonpairing chromosomes. That the univalents of these roses are active and with a full genic content, rather than devoid of genes like the B chromosomes of *maize* and *Sorghum*, is evident from the fact that monosomic types lacking one of them have a lowered vigor and fertility as well as differences in external morphology from their normal sibs.

Gustafsson and Håkanson have shown conclusively that the septet theory of Hurst (1925, 1928, 1932), according to which all of the polyploid species of *Rosa* contain genomes derived from only five original and sharply differentiated diploid species groups, cannot explain the constitution which they have found in the *canina* group, at least in the form in which Hurst presented it. On the other hand, they show that these tetraploid, pentaploid, and hexaploid roses could have arisen in a number of different ways from hybridization between normal diploid, tetraploid, and hexaploid species, all of which are well known in the genus. That they have had at least partly a common origin seems clear, since their peculiar method of stabilizing the results of a very irregular type of meiosis has, so far as is known, arisen nowhere else in the plant kingdom. It is not necessary, however, to assume that all the modern forms originated from a single hybridization, since new types could also arise from crosses between the permanent hybrids and other normal diploid or tetraploid species.

THE POLYPLOID COMPLEX

In many groups of plants, polyploids of some or all of the types already mentioned exist together with their diploid progenitors. And if the diploids are related closely enough to each other so that they can produce segmental allopolyploids as well as autopolyploids, exchange of genes is permitted on the tetraploid level between entities which on the diploid level are almost completely isolated from each other by chromosomal sterility. The presence of autoallopolyploids at the hexaploid or higher levels involving two or more genomes of one species and one or more of another will result in the existence of forms which are morphologically very similar to some of the segmental allopolyploids at the tetraploid level, and like them are intermediate between two or more of the original diploid species, but are isolated from these tetraploids because of the difference in chromosome number. By these means can arise the type of variation pattern designated by Babcock and Stebbins (1938) the *polyploid complex*. Such a complex may be visualized as a series of distinct pillars, representing the diploid forms, which support a great superstructure of intermediate polyploids (Fig. 34). The systematic complexity of such groups is obvious. The smaller com-

plexes containing only two or three original diploids are usually classified as a single species by conservative systematists; the larger ones, which may contain as many as ten diploid species plus various recombination types at the polyploid levels, are the most notable examples of "critical" or difficult genera, of which satisfactory classifications are difficult or impossible to make.

The example discussed principally by Babcock and Stebbins was that of the American species of *Crepis*, in which the polyploids are apomictic. This type will be discussed in Chapter X. A sexual example which the writer also had in mind (cf. Stebbins 1939) is that of the Mediterranean species of *Paeonia*. This consists of five or six distinct diploids plus a number of auto- and segmental allopolyploids which combine their characteristics, as discussed in a previous section. Two others, which are considerably smaller, are the genus *Zauschneria* (Clausen, Keck, and Hiesey 1940, 1945a) and the species complex of *Eriogonum fasciculatum* (Stebbins 1942c). In the former, there are three relatively localized diploid species, *Z. cana*, of coastal southern California, *Z. septentrionalis*, of northwestern California, and *Z. garrettii*, of Utah. The common tetraploid form of coastal California, *Z. californica*, is intermediate between *Z. cana* and *Z. septentrionalis*, but grades imperceptibly into forms which appear like autopolyploids of one or the other species. In the Sierra Nevada and the mountains of Southern California are found broad-leaved forms, *Z. californica latifolia*, which the authors believe to be autopolyploids of *Z. septentrionalis*, but some of these resemble *Z. garrettii* in both their external morphology and ecological preferences. At the tetraploid level, therefore, is found the same range of morphological characteristics and climatic adaptation as exists in the diploids. But whereas the diploids include only three relatively uniform and distinct types representing three extremes of this range of variation, the tetraploids form a continuous series throughout the whole range, with intermediate types by far the most common. The situation in *Eriogonum* is similar, except that here only two "diploid" (perhaps originally tetraploid) species are involved. A much larger and more intricate, but otherwise similar, polyploid complex is the subgenus *Cyanococcus* of *Vaccinium* (Darrow and Camp 1945, Camp 1945).

In their discussion of the polyploid complex, Babcock and Stebbins emphasized the importance of studying the distribution and the morphological characteristics of the entire complex before making any generalizations concerning its origin or its evolutionary tendencies in relation to climatic and ecological trends. The importance of such complete knowledge has become more and more evident as additional polyploid complexes have been reported in the literature. In both this and the next chapter, numerous examples are mentioned of erroneous conclusions about the effects of polyploidy in a particular group. These statements were made on the basis of a partial knowledge of the group and had to be revised when it was more completely known.