

The Amphiploids

12.1: Amphiploidy and Implications

New species can arise suddenly by interspecific hybridization and doubling of the chromosomes. Such an act in nature separates the new amphiploid, a potential species, from its parental progenitors. New amphiploid species are able to invade new habitats, an invasion not possible by either parent. A new ecological range, as well as reproductive isolation from all other species, is acquired. More data are now at hand from amphiploids produced in the laboratory, because colchicine has provided an effective method for making the polyploids after the interspecific hybridization has been made. Principles of theoretical and practical value can be developed.

Not all autopolyploids and amphiploids separate into clear-cut categories since certain of their characteristics tend to overlap.⁸² Many amphiploids produced by colchicine show autopolyploid characteristics.²¹ The genetic and cytological changes that take place in later generations of propagation among such amphiploids are difficult to interpret when there is interchange between the two parental genomes. A classification designed by Clausen, Keck, and Heisey sought to visualize how a gradual merger between autopolyploids and amphiploids obtains if a number of cases are compared. Table 12 in their paper places amphiploids in positions from the upper left-hand corner to the lower right, in a gradient from autopolyploid to amphiploid.²¹ The conclusions incorporated in this chart were made after analyzing natural and experimentally produced amphiploids.

While the limits between some autopolyploids and amphiploids are not clearly defined, the requirements for the success of an amphiploid as a new species are extremely sharp, almost to the point of being restrictive. Limits appear to be set that cannot be violated, that is, if the new plants are to succeed in nature. We should consider whether the requirements for success in agricultural situations are not equally restrictive. The requirements may be somewhat different, but new

polyploids must meet exacting demands in order to succeed as new crop species.

The diploid, interspecific hybrid, if it is to become a successful polyploid, must have good vigor, excellent growth of vegetative characters, and an all-around vegetative cycle that is in harmony with its environment.²¹ Combined with these characteristics, the two parental genomes should be incompatible in the diploid hybrid to the extent that no interchange can occur between them. There should be no gene exchange between the parental sets of chromosomes, which means no intergenomal pairing. Briefly, the diploid hybrid according to these requirements should be entirely sterile until a doubling of the chromosomes occurs. Working in almost direct opposition to these conditions, describing the source of amphiploid from diploid hybrids between species, are biological laws that tend to prevent achieving the best-suited sterile hybrid. To acquire such genome incompatibility between the parents, one immediately moves the relationships of the two species farther apart. Usually the farther apart they are, the more difficult the hybridization will be. Even after the hybrid has been made, a more distant relationship often results in plants that are weak, poor in vigor, and lacking in good growth generally. A poorly growing diploid hybrid cannot be expected to change into a vigorous, successful amphiploid by merely doubling the number of chromosomes.

If hybrids are made from species too closely related, gene exchanges between the parental sets of chromosomes occur. Then after four or five generations, segregations tend to destroy the individuality of the amphiploid from the parental type.²¹ Of course, by gene exchange the transfer of a trait from one species to another at the polyploidy level can occur. The moment gene exchanges take place, the future of the amphiploid as a distinct and isolated individual becomes endangered.⁴ Cytological mechanisms may automatically cause the plants of later generations to drift to one or the other parental type.

Experimentally produced amphiploids have been studied for enough generations to demonstrate that genetic exchanges can take place between the two parental sets of chromosomes. From a plant breeder's point of view this would seem to offer opportunity. Otherwise a strict independence between genomes, like those of *Raphanobrassica*, permits a true breeding type distinct from either parent, but further hybridization with either parental species to improve the amphiploid is ineffective.²⁶ If the amphiploid is not like the *Raphanobrassica* case and intergenomal pairing does occur, gene exchange leads to segregation in F_2 and later generations. Many segregates may be weak, sterile, and poor. Occasionally, new and vigorous combinations may arise. Certainly a series of new lines can be developed when there is exchange between genomes.⁷⁵

Suppose that lines are isolated by selection after interspecific segregation among progenies of amphiploids. One cannot expect these lines to compete in nature as successful independent amphiploids in the same rank as a distinct and differentiated species. From an agricultural standpoint these lines need not be new species, and they may or may not be valuable as new polyploids. If the transfer of genetic traits is made from one parental species to another, and the species of commercial importance is improved, the result is not a new polyploid.²⁰ For example, mosaic resistance was transferred from *N. glutinosa* to the *N. tabacum* genome.¹¹¹ The characteristics of commercial tobacco plants were not changed, but the disease resistant factor was added. Chromosome numbers were finally stabilized by selection after backcrossing at the same number as *N. tabacum* 48, and after specific selection only a few traits were transferred from *N. glutinosa*. All but the resistance to disease were eliminated. As an amphiploid then, the new *N. tabacum* with only the disease-resistance characteristic added can hardly be considered as an independent type.

Stability of a new amphiploid is proportional to the gene exchange between the two parental genomes. Lack of interchange favors relative constancy; conversely, interchange promotes instability. Experimentally produced amphiploids of all gradations from those with much interchange to others with very little, offer excellent opportunity to explore certain basic propositions controlled and observed after selection,^{103, 4} either in nature or under guidance.

Doubling of the chromosomes among sterile diploid hybrids may be done either through gametic processes, i.e., production of unreduced gametes, or by somatic doubling. The accidental doubling in nature has occurred largely by the gametic processes. On the other hand, colchicine is most effectively applied to somatic tissues. The differences between these methods of doubling the chromosomes are important and should be compared when such comparisons can be made.

12.2: Amphiploidy in the Gramineae

Economically, the grasses comprise the most important family among all plants. Polyploidy is common in many groups including agricultural species. Generally, their origin has been through hybridization and doubling of the chromosomes. Autopolyploidy is limited as a method of speciation⁵³ in grasses compared with amphiploidy.¹⁰⁵ Polyploidy among grasses presents problems^{57, 10, 11, 5, 70} that involve both theoretical and practical aspects.^{17, 23, 47, 59, 35, 86, 90, 104} The origin of hexaploid wheat¹⁰⁸ has many theoretical phases,^{86, 100} and no one can escape the practical importance attached to this one species, *Triticum aestivum* L.¹¹⁸

12.2-1: *Origin of hexaploid wheat.* Bread wheat, *Triticum aestivum* L. (*T. vulgare* Vil.) is mankind's most important single species in cultivation. Millions of people depend on the annual grain production of this plant. As an achievement in agriculture, the accession of this one species alone is man's important contribution as a plant breeder.

Historically, in terms of the long period of agriculture, the 42-chromosome wheats are relatively new. Certainly the tetraploid wheats antedate hexaploids, while diploid species preceded the tetraploids. No hexaploids are known out of cultivation, whereas diploids and tetraploids are represented by wild and cultivated species. Full knowledge of the origin of bread wheat probably will never be obtained, but some phases can be closely inspected by observing the experimentally produced polyploids. Colchicine has been a useful tool in tracking down certain steps in the origin of the hexaploid species, notably *Triticum spelta* and related species.¹²²

First, consideration should be given to *Triticum monococcum* L., a 14-chromosome species, to gain some idea of the oldest species of wheat in agriculture today. Another diploid, *Agropyron triticeum* Gaertn., is suspect in the hybridization with *Triticum* which created the tetraploid, or 28-chromosome, species.^{70, 100} These two parental types may be called the *A* and *B* genomes, representing *Triticum* and *Agropyron*, respectively.⁵⁸

A large group of cultivated tetraploids, having either free-threshing or invested grains, remain in cultivation as valuable economic species. The *emmer* and *durum* types play an important role in agriculture.⁹⁰ One of the most interesting tetraploids is the free-threshing *Triticum persicum*.⁵⁸

Let us return to our hypothesis that *Triticum monococcum* is the genome *A*, and that the diploid genome *B* came from *Agropyron triticeum*.¹⁰⁰ The true contribution made by *Agropyron* may now be so remote that one cannot hope to retrace these steps. Let us assume these diploids combined to make the tetraploid wheats. The evolution from tetraploid to hexaploid may be repeated more easily than that from diploid to tetraploid. By crossing tetraploid *Triticum dicoccoides*, 28-chromosomes, with diploid *Aegilops squarrosa*, a sterile triploid hybrid was obtained.^{70, 58} This plant had 21 chromosomes, was sterile, and resembled hexaploid *Triticum spelta*, or spelt wheat. Upon doubling the chromosomes, a 42-chromosome wheat was developed. This synthesized hexaploid hybridized with the natural hexaploid *T. spelta*. The selfed progenies from this hybrid did not throw segregates as one might expect from a wide cross. In fact, no segregation occurred. Pairing at meiosis among the F_1 hybrid did not indicate widely differentiated chromosomes of synthetic *T. spelta*

against natural *T. spelta*.^{100, 70} On the contrary, a close homology was suggested. There was more difference between synthetic *T. spelta* and natural *T. spelta* when amphiploids were obtained after gametic doubling⁵⁸ than those from somatic doubling.⁷⁰

Crossing with *Aegilops squarrosa* so improved the plant and the grain that one might expect a naturally occurring fertile plant like the resulting hybrid to be recognized as a new variant.⁷⁰ The geographic range of *A. squarrosa* should show in general where the original hybridization took place.⁵⁸ This species grows today in the northwestern Himalayas, the Caucasian region, and over an area where hexaploid wheats could have originated as a result of the contact of *A. squarrosa* with tetraploid species of *Triticum*. Diploid *Aegilops*, known as goat weed, is a very unpromising agricultural plant;¹⁰⁵ yet its contribution to commercial wheat by a species like *A. squarrosa* must be very specific and is apparently necessary. The genome is called the *D* genome.⁵⁷ Therefore, hexaploid wheats are now identified by genomes *A*, *B*, and *D*, each representing a genus and each sharing one-third of the 42-chromosomes.^{100, 58, 70} An isolating mechanism has been discovered in *Triticum* associated with the *D* genome.⁹⁸

Between the dawn of agriculture and some time not too long ago, the hexaploid wheat evolved. Exactly when and how many times the hexaploid species appeared remain unsolved problems. Let us say at some time between 2000 and 10,000 years ago. Or perhaps the cross between diploid *Aegilops squarrosa* and tetraploid wheat is happening today. The amphiploid *Triticum persicum* \times *Aegilops squarrosa*, which is very similar to hexaploid *Triticum*, is a species obtained from Russia.⁵⁸ If more hexaploid cases could be found in the areas where *Aegilops squarrosa* grows, such additions to our knowledge would be of great interest.⁵⁸

We know there are parts to the story that must be sketched with certain reasonable assumptions. It was remarkable that two research teams,^{58, 70} working entirely independent of each other, came so close to each other in an agreement that *Aegilops squarrosa* is suspected as one of the diploid species.

Evidence that some other diploid species of *Aegilops* contributed to wheat now becomes a burden of proof by using a cross involving other species, or else by other methods to demonstrate how the hexaploid wheats came into existence when they did. For the present at least, the independent contributions of Japanese and American geneticists that *Aegilops squarrosa* contributed genome *D* still stands.

An important character of *Triticum aestivum* is the free-threshing feature. The synthetic *T. spelta*, like natural *T. spelta*, was an invested type. How the free-threshing types such as *T. aestivum* L.

evolved remains for further study. Answering the question whether this type arose as a segregate, or directly from a diploid-tetraploid hybridization requires more data.^{70, 100} A pattern for research has been established.¹⁰⁸

Another method for converting the tetraploid species into hexaploids has been reported.⁶⁵ Planting the 28-chromosomal species in the autumn instead of spring, a regular procedure for these hard wheat types, after two, three, or four seasons the durum spring wheats, 28-chromosome species, suddenly change into the vulgare or 42-chromosomal soft wheat species. There was no evidence of hybridization, and no intergrading forms. This method obviously differs from the two explanations given by Japanese and American geneticists for the origin of hexaploid species.

12.2-2: *Other amphiploids among Triticinae.* The amphiploids made from interspecific and intergeneric hybridization among *Aegilops*, *Triticum*, and *Agropyron* have increased many fold,^{5, 9, 11, 57, 67, 74, 88, 100, 101, 118, 66, 68, 86, 90, 98, 110} since the first fertile *Triticum-Agropyron* amphiploid was produced with colchicine in 1939.⁹⁴ A wealth of material is at hand to solve the basic problems that determine the progress to be made in using amphiploids.^{10, 120} Since all the cases cannot be reviewed, a selection will be made to point out theoretical and practical problems.

Among *Aegilops*, the species have evolved by interspecific hybridization and chromosomal doubling.⁵⁷ There are diploid, tetraploid, and hexaploid species represented by haploid numbers, $n = 7$, $n = 14$, $n = 21$, respectively. Since *Aegilops* has contributed to hexaploid wheat, a knowledge of these species is important even though the group has little economic value of its own.

In 1913 Cook discovered a hybrid in Palestine involving the Emmer *Triticum dicoccoides* and some form of *Aegilops*. Later, Percival pointed to *Aegilops cylindrica* as a contributor of the spelt characters in the tetraploid *Triticum*. Evidence accumulated suggesting that *T. aestivum* L. arose as a segregate out of a cross between *T. dicoccoides* and *A. cylindrica*. The amphiploid ($n = 14$), *Aegilops cylindrica* ($n = 14$), was synthesized by crossing *Aegilops caudata* ($n = 7$) \times *A. squarrosa* ($n = 7$) and doubling the chromosomes with colchicine.¹⁰⁰ Now three sets of data come into focus. First, earlier taxonomic work brought tetraploid *Triticum* and the tetraploid *Aegilops cylindrica* together. Second, the tetraploid *A. cylindrica* evolved from two diploid species, one being *A. squarrosa*. Third, the synthetic amphiploid, *Triticum dicoccoides* var. *spontaneovillosum* \times *Aegilops squarrosa* is similar to natural *Triticum spelta*.^{58, 70} In 1931 a speltlike sterile hybrid between tetraploid *Triticum dicoccum* and *Aegilops squarrosa* was made by McFadden, but for want of a ready method to

convert this sterile hybrid to a fertile one, the necessary evidence remained hidden until fertile hexaploids could be made.¹⁰⁰

The *D* genome represented in hexaploid wheat and the genomes of modern diploid *Aegilops squarrosa* are probably very close in their homologies. Also, this genome is not found in any species of wheat tested that had fewer than 21 chromosomes. Tetraploid wheat lacks this genome. Finally, taxonomic characters in *Aegilops squarrosa* correspond to those traits that distinguish the hexaploid wheat from tetraploids.¹⁰⁰ These are: the square-shouldered inflorescence, hollow stem, and articulation of rachis, differentiating *Triticum spelta* from the tetraploid Emmer wheats.⁷⁰

Taxonomic characters were used to trace the probable origin of hexaploid wheat before cytogenetic evidences were at hand. The fact that diploid *Agropyron triticeum* Gaertn. has features distinguishing diploid *T. monococcum* from tetraploid wheat arouses interest.¹⁰⁰ Discovering more specifically how genome *B* was contributed and what its relation to *Agropyron* is, becomes more involved. This genus also has a polyploid series in its evolution. The base is $n = 7$ (Table 12.1).

Some intergeneric hybrids involving *Agropyron* have been made.^{5, 11, 9} Hexaploid *T. aestivum* ($n = 21$) and *Agropyron glaucum* ($n = 21$)⁸⁸ were combined to make an amphiploid with 84 chromosomes. Strong perennial tendencies arise with these high polyploids. In another case, vigorous plants with 70 chromosomes were derived by adding the hexaploid complements, 42 chromosomes, to the tetraploid *Agropyron intermedium*, 28 chromosomes. This particular 70-chromosome fertile hybrid was the first amphiploid to be reported from tests with colchicine.⁹⁴

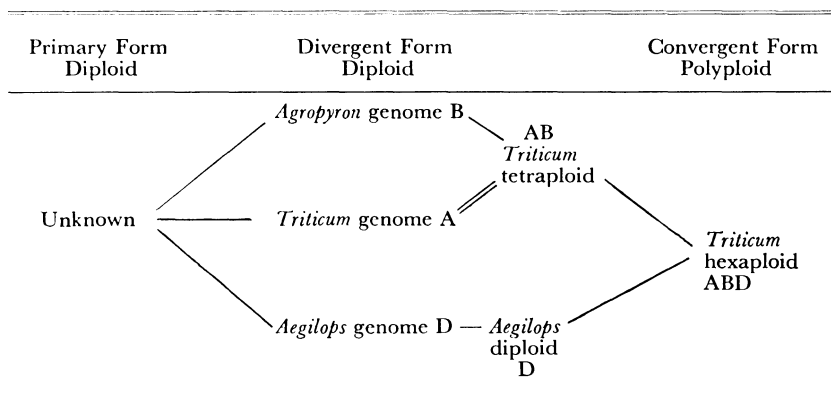
The genus *Triticum*, represented by three chromosomal levels, $n = 7$, $n = 14$, and $n = 21$, provides much material following inter-specific hybridization. A tetraploid, *T. timopheevi*, has the genome *G* not common to other well-known species.²⁶ Another free-threshing tetraploid species, *T. persicum*, produces an interesting series when crossed with *Aegilops squarrosa*.⁵⁸ Unquestionably, these amphiploids have free-threshing hexaploid bread wheat features.

Within short intervals after colchicine was discovered, more than 80 different amphiploids, involving tetraploid and hexaploid, as well as diploid species of *Triticum* were produced in Russia.¹¹⁸ Some higher numbers proved to be interesting in their hybridization characteristics in subsequent generations. Generally the sterility increased when hybrids above the hexaploid level were created. The ordinary wheat, usually self-pollinated, changed into a cross-fertilizing type as higher-level amphiploids were reached.

The complexity of sterility-fertility relationships appear in the intergeneric and interspecific hybrids among *Triticinae*.^{11, 10, 100, 70, 58}

Chromosomal pairing in the diploid hybrid, or the lack of pairing is not necessarily an index of homology. The intergeneric amphiploid *Aegilops umbellulata* \times *Haynaldia villosa* has a reduced fertility.¹⁰⁰ The particular strain made a difference in pairing; environmental and genetic factors, also, influence pairing of chromosomes. Two distantly related species may introduce physiological upsets that cause

TABLE 12.1
DIVERGENT AND CONVERGENT EVOLUTION OF HEXAPLOIDS
(Adapted from McFadden and Sears)



meiotic irregularities.¹¹ The rule cannot be established that univalency in the F_1 is predictable evidence for obtaining good fertile amphiploids.

Evolution in wheat that finally led to hexaploids may be charted as a divergence in the early period following convergent evolution giving rise to the tetraploid and hexaploid species. Some unknown diploid form evolved into three basic genera: (1) *Agropyron*, (2) *Triticum*, and (3) *Aegilops*. The first two hybridized and gave rise to a series of tetraploid species. A second step in evolution involved the combinations between tetraploid *Triticum* and *Aegilops*. A chart is used to help visualize these evolutionary patterns (Table 12.1).

Since such valuable species have arisen through combinations of genomes, this approach was suggested as a "radical" method of wheat breeding. Desirable characters would be transferred to *T. aestivum* L. by using specific series of synthesized amphiploids. Four were suggested. The first series involves the *D* genome from *Aegilops squarrosa* added to various tetraploids because the hybrids are more fertile than crosses between tetraploids and hexaploids within *Triticum*. A second series involves combinations between tetraploid wheat and

Aegilops other than *A. squarrosa*. Third, the combined genomes *A* and *D* united with various species of *Agropyron* would lead to ways for introducing genes from the latter genes to the present *B* genome of hexaploid wheat. Fourth, the synthesized *B* and *D* genomes added to diploid *Triticum* would allow transfer of einkorn characters to the hexaploid wheat. Such a program is exceedingly involved; however, it merits serious attention. (cf. Chapter 11. Ref. No. 49).

12.2-3: *Triticum aestivum* L. \times *Secale cereale* L.—*Triticale*. In 1876 the first hybridization between wheat and rye was made. About 4 per cent of hybridizations between wheat and rye give some idea of the success to be expected. Under unusual circumstances a fertile 56-chromosome F_2 can be obtained. An unreduced gamete most likely explains the mode of doubling. Since colchicine became available, new methods¹¹⁴ have been developed to increase the production of *Triticales*.^{18, 35, 77}

There are five well-known strains,²¹ (1) Rimpau 1891, (2) Meister 1928, (3) Lebedeff 1934, (4) Taylor 1935, and (5) Müntzing 1936. Since 1936 many more have been made. Actually no accurate record can be given because of the number of unpublished cases.

Biologically the 56-chromosome plant is of interest because the constant number has been maintained in the Rimpau strain after more than fifty generations. Backcrosses to wheat give some index of the stability that *Triticales* can maintain. The 56-chromosome plants survive better, are taller, and maintain a stable genetic mechanism in spite of some meiotic irregularities.²¹ At meiosis in the F_1 very little pairing has been observed, 0-3 pairs; and upon doubling, mostly bivalents are seen with as high as 6 unpaired chromosomes in some strains. There is practically no homology between the wheat and rye chromosomes.²¹

Among backcross progenies a pair of rye chromosomes have been substituted for one pair of wheat chromosomes (cf. Chapter 14, Ref. No. 37), so there would appear to be slight possibility for gene exchange under selection. In nature the *Triticale* could evolve as a new species because there is some degree of difference between the strains regarding fertility and segregations in the subsequent generations. However, the *Triticale* would remain at the octoploid level, and consequently, a group of new species could evolve with 56 chromosomes²¹ (cf. Chapter 14, Ref. No. 37, 27, 46, 51).

Economically these species bring into one plant two of the world's important bread-producing species, wheat and rye. Since doubling the chromosomes can be done with colchicine, a serious attempt to improve *Triticale* on a large scale should have possibilities.

An all-out attack on this problem was begun in 1939 in Holland; it involved the processing of hundreds and even thousands of combinations.¹¹⁴ A new method of clonal division and vegetative propa-

gation of the F_1 plant was devised so that several hundred plants could be obtained in one season. These were treated by soaking the roots in colchicine.¹¹⁴ Fertile spikes indicated 56-chromosome plants. The work was progressing satisfactorily until in 1944 the research plot became the scene for World War II. Because of considerable loss of material and change in personnel, the original plan had to be modified radically.

It is encouraging from the viewpoint of polyploidy that *Triticales* are now regarded as potential breeding material instead of a genetical curiosity, as it was for a good many years.

12.2-4: *Artificial and natural polyplids in Gramineae.* Large-scale synthesis of polyplids by colchicine can be of use theoretically and practically.¹⁰⁵ Newly created polyplids in grasses were placed for testing on range, pasture, and untended habitats. Following such an introduction, continuing records will show up the potentialities for adaptation of the new species, for the competitive success or failure would become evident after several generations. To a degree, principles governing success apply to polyploidy among intensively cultivated situations, as well as in pastures or wild habitats.¹⁰⁵

Among *Triticales* we mentioned the maintenance of constant 56-chromosomal plants after fifty generations of culture. Backcrosses to wheat always favored the more vigorous 56-chromosomal plants. Apparently a stabilizing mechanism operates in the *Triticales* complex. Undoubtedly this is true for many polyplids among grasses where 70 per cent of the species are natural polyplids. Therefore, new polyplids with high numbers and complex genomic additions should bring important facts to our attention.²¹

Such projects involving artificial and natural polyplids carried out by Stebbins and his associates have already added important information.^{105, 52} Further research based on long-range objectives will surely advance our knowledge of polyploidy.

In the valleys and foothill regions of California, agricultural practices have created three ecological situations into which natural and artificial polyplids should show differences in adaptation. First, the once native grasslands that have been there are heavily grazed and are now covered with annual species from Europe. Second, ungrazed fields nearby are filled with introduced species. Third, there are pastures suitable for reseeded forage crops or grasses and for controlled grazing. Obviously this is a unique situation representing three unstable plant associations. Into these habitats artificial as well as natural polyplids can be introduced by seed and/or vegetative starts.¹⁰⁵

Large populations of artificial polyplids, both autopoloid and allopoloid, were made by colchicine methods.¹⁰⁵ One successful autopoloid, *Ehrharta erecta*, will be discussed in the next chapter. Here

general outline of the amphiploids will be sketched. Polyploids from 24 interspecific crosses involved six genera: *Bromus*, *Agropyron*, *Elymus*, *Sitanion*, *Melica*, and *Stipa*. Major emphasis was given to *Bromus* because thirteen combinations were taken from this genus. Considerable cytogenetical information has already accumulated for three out of five recognized sections. Representative species are native to the American continents; perennials and annuals and natural polyploidy series exist.¹⁰⁵

A polyploid with 112 somatic chromosomes involving *Bromus carinatus* and *B. marginatus* exceeds the 84-chromosome level, highest known for the genus under natural conditions. The artificial polyploid into the C_4 generation was vigorous, apparently more than the F_1 hybrid as shown by considerable vegetative growth that occurred in the garden. A successful allopolyploid with 112 chromosomes was a remarkable new case testifying to an effective use of colchicine when combined with an appropriate hybridization.¹⁰⁵

Even more notable were the polyploids *B. carinatus-trinii* and *B. maritimus-trinii*, which apparently combine the genomes from seven different ancestral diploid species, thereby being 14-ploid, containing 98 somatic chromosomes. The immediate success demonstrated by these polyploids is of exceptional interest when viewed together with the implications about amphiploidy mentioned in the first section of this chapter. The hybrids were very vigorous and meiotic processes were irregular after doubling; plants in the C_3 and C_4 generation showed seed fertility in the range from 70 to 94 per cent. In all probability this is a successful polyploid.¹⁰⁵

As shown by this work and an increasing number of other cases, sterility-fertility relationships cannot be predicted in advance. Of all the problems that confront polyploidy breeders, sterility-fertility status among the newly created polyploids may well be the most significant.⁵² The lowered fertility in autopolloids has been confirmed again and again. A conclusion that amphiploids necessarily have higher fertility can be very misleading. A breeder using artificial polyploidy must face the problems of sterility. Accordingly, two factors stand out as deserving primary consideration: vigor and fertility.

12.3: *Gossypium*

Special methods were devised for treating interspecific, sterile hybrids of *Gossypium* with colchicine.^{3, 7, 27, 34, 54, 69, 106, 119} Since fertile amphiploids would be produced at once upon doubling the number of chromosomes, a theory of the origin of tetraploid species could be tested. Skovsted proposed that the American tetraploids involved genomes from an Asiatic diploid and an American wild diploid species. By hybridization between the Asiatic and American

diploids, and doubling of chromosomes, a tetraploid species like *G. hirsutum* arose in nature. Now the test could be repeated experimentally, and those investigators who had been studying species hybrids at the time promptly applied colchicine. The synthesis was announced independently from two laboratories.^{7, 43} *G. arboreum* ($n = 13$, Asiatic diploid) \times *G. thurberi* ($n = 13$, American diploid) was changed from a 26-chromosome hybrid to a 52-chromosome amphiploid. The plants were cytologically similar to *G. hirsutum*. The synthetic amphiploid hybridized with natural tetraploids, and surprisingly good pairing at metaphase was obtained. A conclusive experiment had been performed. The hypothesis of Asiatic-American origin of tetraploid cotton was confirmed.^{7, 43}

A useful classification⁷ was formulated to bring together data about geographical distribution, morphology, chromosomal pairing, numbers, and chromosomal structure differences. The genomes from each region were given letters as follows: (1) Asiatic species, A_1 and A_2 ; (2) African diploids, B ; (3) Australian species, C ; (4) American diploid species, D_1 to D_6 ; and (5) Arabian-India diploids, E . The Asiatic species represent a central position with affinities to American, Australian, and Arabian-Indian species. They are closer in relationship to African species than the other groups. Arabian-Indian species are distant to all and particularly farther from the American diploids. One advantage of this system is the code that can be used for describing amphiploids.⁷ If the American tetraploids were derived from an Asiatic and an American source, the amphiploid should read $2(AD)$ with an appropriate subscript to indicate the species of tetraploid. Accordingly the *G. hirsutum* would be $2(AD)_1$. Table 12.2 illustrates the use of genomes and some of the important species with their geographical distribution.

Experimentally produced amphiploids are potentially new species because the duplications made by hybridization of diploids and doubling the chromosomes do not exactly replicate the natural one.¹⁵ Some kind of differentiation occurred after the first amphiploids arose. A spontaneously occurring amphiploid,¹⁵ *G. davidsonii* \times *G. anomalum*, showed how a new species might have arisen in nature and become isolated from other types. A counterpart of this spontaneously occurring cotton was made by colchicine. The data for these cases were similar.¹⁰⁶

Problems in polyploidy among species of *Gossypium* were well known before colchicine was discovered.⁴⁸ Gene systems were conceived to account for the way in which diploid and tetraploid species became differentiated. By the use of experimentally produced amphiploids, relations between genomes and the problem of speciation could be studied more extensively. Specialists in *Gossypium* began to realize more specifically that problems remained unsolved.¹⁰⁶

Interspecific hybrids between the two tetraploid species are vigorous and fully fertile in the first generation. These species, *G. hirsutum* and *G. barbadense*, both carry desirable qualities.¹⁵ Attempts to combine the best features of each in a new variety have not been as successful as one might wish.¹⁰⁶ The second generation and subsequent ones give rise to weak, sterile, and undesirable types. Backcrossing to either parent has not led to new levels of improvement. One might well ask if the combining of characters from other species, which are

TABLE 12.2
 GENOMES OF *Gossypium*
 (After Brown and Beasley, and Menzel)

Natural Species and Tetraploid Tri-species Hybrid	Descriptions	Genome Formula
<i>Gossypium herbaceum</i> L.	Asiatic 13-chromosome	2A ₁
<i>G. arboreum</i> L.	Asiatic 13-chromosome	2A ₂
<i>G. anomalum</i> Wawra. and Peyr.	African 13-chromosome	2B ₁
<i>G. sturtii</i> F. Muell.	Australian 13-chromosome	2C ₁
<i>G. thurberi</i> Tod.	American 13-chromosome	2D ₁
<i>G. armourianum</i> Kearney	American 13-chromosome	2D ₂₋₁
<i>G. harknessii</i> T. S. Brandeg.	American 13-chromosome	2D ₂₋₂
<i>G. davidsonii</i> Kellogg	American 13-chromosome	2D ₃
<i>G. klotzchianum</i> Anderss.	American 13-chromosome	2D ₃
<i>G. aridum</i> (Rose and Standley) Skovsted	American 13-chromosome	2D ₄
<i>G. raimondii</i>	American 13-chromosome	2D ₅
<i>G. stocksii</i> M. Mast	Arabian-Indian 13-chromosome.	2E ₁
<i>G. hirsutum</i> L.	American 26-chromosome	2(AD) ₁
<i>G. barbadense</i> L.	American 26-chromosome	2(AD) ₂
Hexaploid <i>G. hirsutum</i> × <i>herbaceum</i> × <i>G. harknessii</i> 2(AD) ₁ A ₁ × 2D ₂₋₂		
Hexaploid <i>G. hirsutum</i> × <i>arboreum</i> × <i>G. harknessii</i> 2(AD) ₁ A ₂ × 2D ₂₋₂		
Hexaploid <i>G. hirsutum</i> × <i>anomalum</i> × <i>G. harknessii</i> 2(AD) ₁ B ₁ × 2D ₂₋₂		
Hexaploid <i>G. hirsutum</i> × <i>stocksii</i> × <i>G. armourianum</i> 2(AD) ₁ E ₁ × 2D ₂₋₁ × 2D ₂₋₂ <i>G. harknessii</i>		
Hexaploid <i>G. hirsutum</i> × <i>stocksii</i> × <i>G. raimondii</i> 2(AD) ₁ E ₁ × 2D ₅		

possible now that many fertile amphiploids can be produced, will not face the same difficulties confronting a breeder who tries to combine the characters of the already well-known Upland and Sea Island cottons.

If some chromosomal mechanism prevents the recombinations of genes contributed by each parent, then merely growing large progenies and exercising selection can hardly be expected to yield results.¹⁰⁶ The evolution of the tetraploid from diploids can be explained by the hybridization and doubling of chromosomes. This does not explain the differentiation of the tetraploid species after they once originated as an amphiploid. An argument supported by considerable data¹⁰⁶ asserts that a structural differentiation of chromosomes was basic to speciation and this was of the cryptic type, that is, in very small segments, so that a differentiation could not be observed by pairing or irregularly arranged chromosomes at meiotic metaphase. Therefore, a genetic hybridity and a hybridity caused by the differentiation of small chromosomal segments could not be detected by the ordinary genetic and cytological methods. The nature and extent of chromosomal differentiation may be measured by tracing marked genes in subsequent generations and recording the rates at which the genes are lost by successive backcrossing. Such chromosomal differentiation may be important in *Gossypium*.¹⁰⁶ At least, the suggestion has led to reflection on these problems in polyploidy.

Among the second generations of the interspecific hybrid between *G. hirsutum* and *G. barbadense*, asynaptic genes account for the sterility observed, notably when certain parents are used.⁷ Genes for asynapsis have been found in both genomes *A* and *D*. By the use of trisomics, additional data about these asynaptic types have been collected. The fully sterile plants eliminate the completely asynaptic types, but partial asynaptic types are carried along.¹⁵ Some of the phenomena attributed to a cryptic structural hybridity might be explained on the basis of asynaptic and partially asynaptic genes.¹⁵

Sterility resulting from asynaptic genes is a kind of genic²⁶ sterility and may well be important in such sterility that causes failure in chromosomal pairing. The extreme sterility at the diploid hybrid level can be overcome by doubling the chromosomes. But a sterility due to asynaptic genes is not cured through doubling the number of chromosomes. Later generations introduce new problems in maintaining the fertility level as well as the characters brought together in the hybrid. If by selection some desirable characters contributed into the hybrid are eliminated and undesirable ones retained, polyploid breeding is faced with a difficult task. To incorporate into commercial varieties the desirable characters found in other species can be put

down on paper more easily than producing the plants. One step is hybridization and the doubling of chromosomes; the next procedure requires some new approaches.

Certain species are totally incompatible.¹⁵ The tri-species¹⁶ hybrids have overcome these difficulties, for some genomes can be brought together in a tri-species hybrid not possible in a regular hybridization. *Gossypium arboreum* and *G. harknessii* have not been brought together except when the hexaploid $G. hirsutum \times G. arboreum$ was crossed with *G. harknessii*. In this manner a tetraploid brought together genomes $(AD)_1 A_1 D_2$ representing *G. hirsutum*, *G. arboreum*, and *G. harknessii*, respectively. Six new tetraploid tri-species hybrids were developed by this method¹⁶ (Table 12.2).

From a plant-breeding standpoint, amphiploids incorporating genomes of *G. anomalum*, *G. raimondii*, and *G. harknessii* with the commercial strains of *hirsutum* are promising and represent a new attack on the problem of cotton improvement.⁹⁵ Increases in fiber strength are possible; however, a problem arises when one tries to gain in fiber strength and also maintain the good qualities necessary for commercial varieties of *hirsutum*. Much cytological work is needed; integrating the theoretical knowledge with practical testing appears to be the outstanding problem at the moment. A final practical contribution resulting from the incorporation of characters from other species is promising. Numerous amphiploids have been made in a short time. Much has been done with colchicine as a preliminary to the larger work of sorting out, by polyploid breeding, gains from accumulated knowledge.

Among polygenomic hybrids, mosaics in flower and leaf appeared.⁷² Increasing the number of chromosomes shows some increasing tendency toward mosaicism, but number alone does not determine the degree. This is a side problem with no specific explanation except that the polyploids exhibit such characters.^{72, 16} Another side problem is the somatic reduction in numbers of chromosomes within a hexaploid species hybrid. An original plant with 78 chromosomes developed sectors that were triploid, having 39 chromosomes. Perhaps the method offers a way to extract useful components from a complex hybrid.^{16, 72}

Aneuploids in *Gossypium* are readily developed because the triploids and pentaploids are unbalanced types. Backcrossing and selection for trisomics and tetrasomics are possible among the synthetic polyploids. Resultant aneuploid types have their effects upon leaf texture, color, and structure. New lines with an extra pair of chromosomes, 54 instead of 52, may include Asiatic or American chromosomes placed into the opposite germ plasm.¹⁵ Intraspecific and interspecific trisomics and tetrasomics were obtained. Such lines may be partially stable, fertile, and morphologically distinguishable.¹⁵

12.4: *Nicotiana*

A theory of evolution was experimentally verified when *N. digluta* was made in 1925. The parental species, *N. tabacum*, a natural tetraploid with 48 chromosomes ($n = 12$), and the diploid *N. glutinosa* were hybridized to make the sterile triploid with 36 chromosomes. A fertile hexaploid was isolated that had 72 chromosomes. This number was a new and high one for the genus. Previous to the development of *N. digluta*, 48 chromosomes was the highest number.^{12, 40, 41} Using colchicine, *N. digluta* was resynthesized. Since then numbers higher than hexaploid have been built into polyploids of *Nicotiana*.⁵⁶ These polyploids were made by bringing together the proper species in hybridizations and doubling the chromosomes of the hybrids. A combination of three natural tetraploids included 144 chromosomes in one plant.⁵⁶ Another report of 176 chromosomes has been made.⁶⁰

The development of plants with high numbers is not the sole objective. Of particular significance is the combining of widely diverse genomes in order to establish higher polyploid-amphiploids that are fertile, vigorous, and relatively stable in later generations of propagation.⁵⁶ The changes that take place in subsequent generations of these polyploids show what mechanisms might operate genetically when new species at new levels of chromosomal numbers become established. Furthermore, the effects of selection upon these types are of basic importance.^{103, 4}

An important development that resulted from the synthesis of *N. digluta* was the eventual transfer of mosaic resistance to the commercial varieties of tobacco.¹¹¹ The necrotic factor from *N. glutinosa* was transferred to the *N. tabacum* genome.^{20, 38} An example of polyploid breeding is illustrated by this program. After full review of the work necessary to make the transfer, one becomes convinced that these methods are not short cuts.

Realizing all that was involved in the requirements for transfer and the cytological and genetic data at hand as late as 1943, there was no complete assurance that the factor for resistance in *N. glutinosa* could be incorporated in the genome of *N. tabacum*.²² Each time the transfer was tried, disadvantageous traits were carried along with the chromosome contributed by *N. glutinosa*. Therefore, the problem was one of maintaining the good features of commercial tobacco varieties and utilizing only the disease resistance of the *glutinosa* type. Fortunately, some chromosomal change occurred during generations of selection, and a true tobacco type with mosaic resistance of the kind noted for *N. glutinosa* appeared in the cultures. The plant had 48 chromosomes and possessed the resistance factor incorporated in the *tabacum* genome.³⁸ Perhaps one might call the new variety, *N. tabacum* var. *virii* after a type made by Kostoff.⁶⁰ No

doubt only a small segment of the chromosome from *N. glutinosa* was transferred to a chromosome of *N. tabacum*. If more than a small segment were involved, greater disturbance to the genotypical balance of the *tabacum* genome might be expected.⁶⁰

Evidence that parts of chromosomes were involved was given by the fact that homozygous, low-blooming, mosaic-resistant segregates¹¹¹ that were different from the Burley tobacco appeared in backcrossing *N. digluta* to *N. tabacum*. These segregates in one case appeared in the fifth backcrossing generation. Similar segregates were obtained when Gerstel's 50-chromosome "alien additional race," which had a pair of *N. glutinosa* chromosomes, was backcrossed to *N. tabacum*. The number of chromosomes during crossing was reduced to 48. In the process these homozygous, low-blooming, mosaic-resistant plants, that differed from Burley tobacco, appeared much the same as when *N. digluta* was the starting material.¹¹¹

The assumption may be made that an interchange had occurred between the two genomes. In this case a segment was transferred from one chromosome of a genome to another chromosome of the opposite genome. The exchange was small, and transfer was limited to the disease-resistance character. When whole chromosomes of *N. glutinosa* were substituted for a whole chromosome of *N. tabacum*, the differences were such that substitution races differed from regular varieties of tobacco.¹¹¹

Morphologically and genetically distinct populations were isolated among specific amphiploids as well as diploid hybrids. If the selection was directed to a particular character, the progress made toward a certain goal was faster at the diploid level than the amphiploid.¹⁰³ Generally, the amphiploid populations were less fertile. The tremendous power of selection that is possible among amphiploids can be demonstrated if the particular type has some intergenomal exchange.⁴

Among species of *Nicotiana* the genetic systems are close enough to permit hybridization, yet removed from each other and sufficiently differentiated to provide sterile hybrids between species. Upon doubling the number of chromosomes, the amphiploids are fertile and partially sterile.^{2, 6, 12, 28, 32, 33, 35, 41, 83, 102, 113} There is enough pairing at the diploid level to indicate that in some combinations of species, exchange between genomes can occur. Such exchange leads to interspecific segregation in the F_2 and subsequent generations.

Pairing of chromosomes at the diploid level of interspecific hybrids is not a true picture of pairing when the amphiploid is derived. Five cases with some bivalents at the F_1 stage had no multivalents in the polyploid.⁵⁶

By interspecific hybridizations and doubling of chromosomes, synthetic tetraploids have been made that resemble *N. tabacum*, yet lack the same genotypical balance that exists in the natural species. Even though the diploid species, *N. sylvestris*, and certain diploids of the *tomentosa* group may be combined to make a polyploid that resembles *N. tabacum*, the exact genetic duplication has not been accomplished.⁶⁰ Usually the sterile hybrids doubled somatically are female-sterile. Sterility is caused by failure at the embryo-sac stage. When a long procedure of backcrossing was involved, a fairly fertile synthetic *N. tabacum* was obtained.⁶⁰ When the synthetic was crossed with a natural species, the segregation in the second generations was like the variability found between varietal crosses.

A list of the amphiploids made with colchicine is necessarily large. There are more objectives involved than have been outlined in this section. *Nicotiana* provides some good material for the study of polyploidy both from a practical and a theoretical point of view.^{40, 41, 20, 38, 103, 28, 29, 6, 1, 2, 12, 32, 33, 81, 83, 102}

12.5: Dysploidy Combined With Amphiploidy

Within the Cruciferae a natural group called the *Brassica* comparium by Clausen, Keck, and Heisey, form a dysploid series as follows: $n = 8$, $n = 9$, $n = 10$, $n = 11$, $n = 12$, $n = 17$, $n = 18$. If the artificial amphiploids are added, the series rises to the hexaploid level, i.e., dysploid, $n = 27$ and $n = 28$. At once some fundamental problems can be predicted from what has been said before.

Some notable historical events in cytogenetics occurred with this group. The first cross between radish and cabbage was produced by Sageret in 1826. One century later, Karpechenko demonstrated fertile *Raphanobrassica* plants.²¹ After Sageret's time, the cross was repeated by others. With colchicine, autotetraploid *Raphanus* was crossed with autotetraploid *Brassica* thereby repeating the intergeneric hybrid by another method.^{37, 59, 73} Previously the sterile diploid hybrid was made, and fertile plants were selected after unreduced gametes united.⁴⁴

Fruit structure in the *Raphanobrassica* polyploids is proportionally radish or cabbage, depending on the genomes present. Accordingly, diploid, triploid, tetraploid, and pentaploid series can be obtained with different doses of whole genomes.²¹

Judging from the total lack of pairing in the F_1 hybrid at diploid levels along with the independence maintained in the amphiploid, gene exchange at diploid level is exceedingly limited. Hybridization and the synthetic amphiploids have raised the level above tetraploidy

as illustrated by amphiploids of the *Brassica comparium*.^{93, 73, 59, 44, 50, 19, 36, 37, 124, 125}

Three basic genomes are represented by diploid species of *Brassica*: *B. campestris*, $n = 10$, or a ; *B. nigra*, $n = 8$, or b ; and *B. oleracea*, $n = 9$, or c . There is some evidence of homology between a and c , but no bivalents are formed between b and either a or c . The tetraploid species *B. carinata* would have genomes $ac\ cc$; *B. juncea* $aa\ bb$; and *B. carinata* $bb\ cc$. Accordingly, the hexaploid *B. chinensis* \times *B. carinata* would have $aa\ bb\ cc$ as genomes, or 27 bivalents.⁵⁰

Economically these genera of the Cruciferae comprise one of the most important groups with world-wide distribution. The number of amphiploids made at the tetraploid level has increased with the use of colchicine. 19, 36, 37, 44, 50, 73, 93, 116, 117, 121

Synthesized amphiploids, comparable to the natural tetraploid species of *Brassica*, can be hybridized readily and show possibilities for selection in the succeeding generations. A large number of progenies are under study by Gosta Olsson at Svalof, Sweden.

12.6: Other Interspecific Hybrids and Amphiploids

Four species of *Galeopsis*, two diploid and two tetraploid, became subject to colchicine methods as soon as the drug was announced for its polyploidizing action. Since the first Linnean species *Galeopsis tetrahit* L. was produced by hybridizations with the two diploid species, following doubling by gametic non-reduction, one of the first uses for colchicine was a repetition of *Galeopsis tetrahit* L. By first inducing autotetraploid *G. pubescens* and *G. speciosa*, the amphiploid was produced with little difficulty. Within a short time much polyploid material was at hand for this genus.⁷⁵

Cross combinations between diploid and tetraploid *Galeopsis* usually fail, but genomes of diploid species can be hybridized at the tetraploid level, using induced autotetraploids with natural tetraploids.⁷⁵ These crosses succeeded. Quantitative conditions control the hybridization. More crosses were made to confirm this point.⁷⁵

The octoploid number, 64, exceeds the optimum number for these genotypes, for octoploid *G. tetrahit* and *G. bifida* are much inferior to the natural tetraploids of these species.⁷⁵ Basic cytogenetical data have been increased many fold with the use of colchicine.

Cytogenetical data from certain interspecific hybrids among *Solanum* suggested that there may be small structural differentiations between chromosomes of diploid species.⁴⁶ Such changes may have significance in the evolution of species within *Solanum*. At least, considerable data for interspecific hybrids have been accumulated already, and more can be expected.

The case presented for *Gossypium* proposing "cryptic structural differentiation" as a speciation mechanism was recalled as an inter-

pretation for problems in *Solanum*.⁴⁶ Certain species of potato carry valuable economic traits, e.g., specific resistance to phytophthora, and these would be desirable to incorporate in the present polyploid species, *S. tuberosum*.

A study of meiosis in hybrids between *S. demissum* and *S. rybinii* as well as in haploid *S. demissum* shows pairing and suggests similarities coupled with these observations; the backcrossing of F_1 *S. demissum* \times *S. tuberosum* to *S. tuberosum* showed increased seed set with each backcross.⁴⁶ One is led to recall the well-known elimination of donor parent genotypes in certain interspecific backcrosses involving *Gossypium hirsutum* and *G. barbadense*.¹⁰⁶ These species have been studied extensively, and recombinations on a gene-for-gene basis that would permit transfer from one species to another runs into serious difficulty after backcrossing. If a similar situation holds in *Solanum*, then the program of amphiploidy and species hybridization requires further analysis.⁴⁶

Enough similarity exists between genomes of *S. rybinii*, *S. tuberosum*, and *S. demissum* to produce bivalents. By multiple crosses other species like *S. antipoviczii* can be crossed to *S. tuberosum* through the amphiploid *S. antipoviczii* \times *S. chacoense*.¹⁰⁹ Another case, *S. acaule* and *S. ballsii*, can be introduced through appropriate amphiploids crossed to *S. tuberosum* when the species in question cannot be crossed alone. For practical work such an approach appears promising,¹⁰⁷ of course, dependent upon chromosomal differentiation, which may increase the difficulties considerably.^{107, 109, 63, 46}

Three amphiploids can be made within the genus *Cucurbita*.⁸⁵ These are: *C. maxima* \times *C. pepo*, *C. maxima* \times *C. mixta*, and *C. maxima* \times *C. moschata*.¹¹⁵ The first is self-sterile; the second is slightly self-fertile and segregates noticeably; the third is self-fertile and cross-sterile with parental species. A relatively stable population develops from the third amphiploid with slight segregation. The amphiploid carried insect resistance to squash vine borer (*Melittia satyriniformis* Hubner), contributed by *C. moschata*, plus flavor and fruit characteristics, contributed by *C. maxima*. Diploid varieties, Buttercup, Banana, Golden Hubbard, and Gregory, represent *C. maxima*; Butternut, Golden Cushaw, and Kentucky Field, *C. moschata*. According to tests carried out at Cheyenne, Wyoming, Burlington, Vermont, and Feeding Hills, Massachusetts, insect resistance was stabilized. The fruits compared favorably with the comparable varieties. In general, this particular combination may be regarded as a "potential new species" with prospects of becoming valuable economically (cf. Chapter 13).⁸⁵

Theoretical problems must not be disregarded.¹¹⁵ A variant like *C. pepo* appeared sporadically in the first and later generations of the Eastern material. Taxonomic similarity to *C. pepo* raises the ques-

tion of interspecific segregations. Some lack of uniformity showed up in the fifth and later generations, where the early stages were uniform and did not segregate for fruit color, shape, and size. Some intergenomal pairing may have occurred. A homology between certain chromosomes was demonstrated with some pairing in the diploid hybrid. Such amphiploids should make excellent material to test the principles basic to amphiploidy and their practical possibilities.¹¹⁵

The interspecific hybrid *Trifolium repens* × *T. nigrescens* was made by crossing two colchicine-induced polyploids of the respective species involved.¹⁴ By special culturing methods the hybrid was saved in the seedling stages. The explanation for incompatibility at the tetraploid level can be adapted from the case in diploids.¹³ Particularly interesting in the amphiploid *Trifolium* is the fact that the incompatibility applied to diploids and to autopoloids holds for the polyploid that brings the two species together. The loci of genes which determine incompatibility must be at the same place in both species; furthermore, intergenomal pairing must occur in order to explain the genetic mechanism of incompatibility through oppositional alleles.

A new species, *Ribes nigrolaria*, was created by the use of colchicine and hybridization. Two Linnean species, *Ribes nigrum*, the black currant, and *R. grossularia*, the gooseberry, were the diploid parents. Thus genomes from two important horticultural species were combined. These were developed and are under observation at the Alnarp Horticultural Station, Sweden, under the direction of Professor Fredrik Nilsson.

Among these and other cases there should come into prominent use new plant breeding materials that combine the genic composition from two or more natural and artificial species. In some instances only a specific trait such as disease resistance may be desired. The key to a new plateau for plant breeders can be found among artificial amphiploids.

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