

the evolutionary trend may not always be from lower to higher numbers, and that autopolyploids may give rise directly to fertile diploids.

Polyploidy in animals

As pointed out earlier, polyploidy in sexually reproducing animals is rare. The reason given usually is that the chromosome balance for sex determination may be upset in the progeny of autopolyploids. This need not be true. Varying doses of X chromosomes and autosomes modify the sex expression in Drosophila, but not in Bombyx mori (silkworm), see Chapter 10, pages 279-280.

Most of the polyploids that have become established in animals in nature reproduce parthenogenetically. For example in the snout-beetles (Curculionidae), of 18 parthenogenetic species, only one was diploid, the others $3n$, $4n$, or $5n$ (Suomalainen 1954).

Triploid rabbits have been produced by colchicine treatment at the moment of fertilization (Haggqvist and Bane 1950). Of 7 young, three (1 ♀, 2 ♂) lived nearly a year, four died at 8 weeks or before. They noted that the erythrocytes and sperms in the ♂ were larger in size. Melander (1950) reported on the cytology of a male. Some mitoses were triploid and regular but others appeared to be irregular. In mice, $3n$ and $4n$ zygotes were reported after abnormal temperature treatment at an early stage. Higher, but different numbers of chromosomes were observed in cells of bone marrow and testicles.

In stickleback fishes there is one report of induced triploids that were vigorous and required less oxygen (Swarup 1959). (cf. Bungenborg deJong 1957, polyploidy in animals).

Haploids and monploids

The term "haploid", as used here, refers to individuals with the gametic number, but they may have arisen from a diploid or a polyploid. The term "monoploid" is used only for those that arise from a diploid and hence possess a single genome.

Origin and occurrence

The first monploids in a flowering plant species were observed in Datura stramonium. They occurred after exposure of young buds of diploids to cold (Belling and Blakeslee 1922). They were obtained also after prolonged exposure to low temperatures (Blakeslee et al. 1927), and after temperatures of 40° - 50° C applied immediately after pollination in corn (Randolph 1932). Monploids have been found among the progeny of interspecific or wide crosses, e.g. in the cross of Solanum nigrum x S. luteum (Jørgenson, 1928), and in the cross of Datura stramonium x D. ferox (Belling and Blakeslee 1927). Five were observed in over 3,000 plants from the cross of Crepis capillaris ($n = 3$) x C. tectorum ($n = 4$) (Hollingshead 1930).

Haploids may arise through development of the egg without fertilization, either through parthenogenesis or through pseudogamy (stimulation of development of the egg by pollination without fertilization or by fertilization of the primary endosperm nucleus only). In species having endosperm in the mature seed, the importance of fertilization to produce endosperm is obvious, but it may be equally important in initiating embryo development in other species. Randolph (unpublished data cited by Einset 1943) found a frequency of about 1 in 2,000 in untreated corn, with a range from 0 to about 6 per 1,000. The frequency is higher in certain stocks than in others when crossed with the same pollen parents; also frequencies differ when the same female parent is crossed with dif-

ferent pollen parents (Chase 1949, 1952, 1952a). Chase and also Seaney (1955) found that the frequency is still higher in diploid lines established from monophloids. Coe (1959) has reported a rate of 3.23% of haploids in selfs of one inbred line. This line also induces a higher frequency of monophloids in crosses using it as a ♂.

Luther Smith (1946) reported a rate of 1 monophloid per 1,000 plants in untreated Einkorn, 20 per 1,000 in intraspecific hybrids and up to 200 per 1,000 from delayed pollination. Seaney (1955) and Gerrish (1956) were able to double or even triple the frequency of monophloids in maize by delaying pollinations by 6 to 9 days or even longer.

Haploids have been found as one or both members of twin seedlings. Cooper (1943) found that in about 1% of the ovules in seven species of Lilium a synergid had been stimulated to divide, thus forming twin embryos, one haploid and one diploid. He found the same phenomenon in young ovules from pollinations of Nicotiana glutinosa by N. tabacum. The use of plants heterozygous for genetic markers in the female parent should furnish information also as to their origin.

The frequency of monophloids is influenced also by the extent to which deleterious recessives may be present. For example, Randolph (1938) found the highest frequency among the progeny of an inbred. Also Davis (1931) was unable to find any monophloids among the progeny of Oenothera lamarkiana crossed with other species. Emerson (1929) and Catcheside (1932) reported monophloids in the progeny of Oenothera races with 7 pairs of chromosomes.

A haploid having the set of chromosomes from the male parent was found among the progeny of interspecific crosses in Nicotiana by Clausen and Lammerts (1929) and one by Kostoff (1929). These are androgenic haploids. In Crepis tectorum there was one among the progeny of X-rayed flowers pollinated by normal pollen (Gerassimova 1936). In maize, Seaney (1955) found four out of 916,789 seedlings, and Gerrish (1956) found two in his studies.

Diploids having the characteristics of the male parent (androgenic diploids) occur rarely among the progeny of interspecific crosses. The first was reported by Collins and Kempton (1916). Among the progeny of the cross Tripsacum dactyloides x Euchlaena mexicana ♂, there was one plant with the characteristics of E. mexicana. As another example, the cross of (Nicotiana sylvestris x N. tabacum) x N. sylvestris produced one plant that was identical with sylvestris. It was the only one among the progeny (21 plants) that had 12 chromosome pairs.

Monophloids will be discussed in detail here. Haploids from polyploids will be discussed in the next chapter.

General description

In general, monophloid plants are much weaker and smaller than diploids. The leaves, flowers and other parts, also the nuclei and cells are smaller. Hence the number of stomata per unit area is usually higher than in a diploid. In maize, Randolph (1932) found by examination with a hand lens at the seedling stage that the stomata were visibly smaller and closer together than in diploids. Older monophloid plants from dent corn hybrids were often leafy and upright in appearance as if the stalk were telescoped or they had prolific ear production (Gerrish 1956). They were characterized by high pollen abortion and shriveled anthers. The tassels were either completely sterile or showed occasional sectors with normal anthers.

Cytology

A study of meiosis in the microspore mother cells of monoploid *Datura* was reported by Belling and Blakeslee (1927). They found that at metaphase I whole chromosomes moved to the poles at random, then divided at metaphase II as in a normal second metaphase division. The distribution to the two poles at metaphase I was at random as shown in Table 81.

Table 81. Distribution of chromosomes to the two poles at metaphase I of meiosis in monoploid *Datura* (Belling and Blakeslee, 1927, p. 358, LaCellule 37).

	<u>11-1</u>	<u>10-2</u>	<u>9-3</u>	<u>8-4</u>	<u>7-5</u>	<u>6-6</u>	<u>Total</u>
Observed No.	1	3	12	19	38	27	100
Calc. if random	1	3	11	24	39	23	

They also found up to 12% of non-reduction in which all the chromosomes split at the first division forming a dyad, each cell of which had the normal number of chromosomes. Twelve percent of full pollen grains was the average of several counts on different dates.

These monoploids gave rise to diploid offspring which theoretically should have been completely homozygous. However, of 173 diploids which were selfed four produced progeny segregating 3 : 1 for normal vs. recessive seedling characters (Blakeslee et al. 1927). At least three of these were new mutants (tricarpe, curled, and pale). Of 363 selfed diploids obtained later from the haploid, none segregated. Even those that segregated would be homozygous for most of the genes they carried. In a haploid tomato, Lindstrom and Koos (1931) and Humphrey (1934), reported that the chromosomes were segregated at random at division I, these then divided at division II. The spores formed were of unequal size. The occasional normal pollen grains probably came from dyads resulting from a restitution nucleus containing the 12 chromosomes, this nucleus then dividing once.

Each of the 5 monoploids found by Hollingshead (1930) among the progeny of the cross *Crepis capillaris* ($n=3$) x *C. tectorum* ($n=4$) had three chromosomes. In 110 root tips from monoploid plants, 28 had at least one diploid metaphase, while 42 had only diploid metaphases. Certain plants had diploid, fertile branches. At meiosis in the haploid, a spindle was formed, but rarely a metaphase plate. The chromosomes which did not lie on the spindle did not reach either pole but formed micronuclei. Whole chromosomes on the spindle might move to the poles without division, or they divided; but only in a small percent of the cases did the halves separate and pass to opposite poles. Rarely all three chromosomes divided, the halves passing to opposite poles to form normal spores. One to 5 telophase nuclei per P. M. C. were observed. The various types of sporads and their frequencies were:

	<u>Quartet</u>	<u>Triad</u>	<u>Dyad</u>	<u>Monad</u>
No. of sporads	19	57	195	12

The quartets probably came from diploid tissue. Part of the dyads were normal also. Normal pollen was rare, and there was no seed set on haploid portions. Diploid tissue should have been homozygous, but lack of pairing was frequent as shown by the following observations:

85 P. M. C.	---	3 pairs
99 "	---	2 pairs + 2 univ.
41 "	---	1 pair + 4 univ.
8 "	---	6 univ.

Univalents would not be expected if the chromosome pairs were completely homologous. However the diploid plants of this original strain of C. capillaris frequently showed univalents (Hollingshead 1930), suggesting that the lack of pairing may have been due to a genic constitution for low chiasma frequency.

The occurrence of diploid sectors in monoploid plants probably accounts for the occasional sectors that have normal pollen, normal anthers, or normal seeds.

In monoploids for certain species, two or more of the chromosomes may pair. Usually the association is between short segments only, and should indicate partial homology, e.g. a duplication in diploids which would normally not be revealed by pairing in the diploid. Alexander (1953) has found occasional pairing at meiosis in monoploids in corn, and that some of the $2n$ plants from them are heterozygous for an interchange. These are probably the result of crossing over between duplicated segments in different chromosomes. If true, the same interchange should recur. Identifications of the chromosomes involved in the interchanges have not been completed. Walters (1954) suggested that some of the apparent associations in Bromus may not be from true pairing, and referred to them as pseudobivalents.

Monoploids and the production of homozygous diploids

Monoploids are of special interest because their diploid offspring should be homozygous. Diploid cells occur occasionally, probably by the formation of a restitution nucleus. From these probably arise the occasional diploid sectors that have normal pollen, normal anthers or normal seeds. If the doubling occurs later, or at meiosis, occasional normal spores may be produced.

The possibility of producing homozygous diploids in this manner was recognized by East (1930) soon after the first monoploids were produced in Datura. Their use for this purpose has been discussed by Beasley (1942), Kostoff (1941), and many others. Preliminary tests were made by Emerson, Randolph and Stadler, but extensive trials were not made until the method of using pollen with dominant markers came into use. The method was suggested by Burnham (1946a) but no trials were made. The results from experiments by Chase (1949, 1952a), and by Gerrish (1956) will be summarized in more detail. It can be thought of as a method of applying gamete selection for breeding.

By making crosses using pollen from stocks carrying the dominant alleles of characters that are expressed in the embryo or in the germinating seedling, it has been possible to screen thousands of seedlings for the occasional haploids. One of the stocks used for this has brown plants and is aa BB P1P1 CC RR. The seedling roots of the hybrids are all purple (Bb P1 p1), whereas the monoploids are green (b pl). The seedlings having green roots in the germination pans are transplanted and checked for chromosome number by root tip count, stomatal size and number, growth habit or pollen sterility. To establish a homozygous diploid line, each monoploid must be self pollinated.

The monoploids produce diploid fertile sectors occasionally, but there may not be a doubled sector in both ear and tassel. If chromosome doubling occurs early enough, the same sector may include both. The main difficulty in establishing a diploid line from each of the monoploids is the low frequency with which fertile sectors occur. Their

frequency appears to have been considerably increased by colchicine treatment but there was lower survival (Chase 1952a, Gerrish 1956). The lowest concentration used, .025%, was effective whether introduced into the coleoptile sheath under vacuum or through a cut first leaf. The work with monoploids is best carried on in the greenhouse where the plants can be watched more closely and not be subjected to storms. Sectors so small that only a single normal anther is produced can be found and used in self-pollination. Chase (1951) found 43 monoploids among 38,684 seedlings, a rate of 1.11 per 1,000. A summary of what happened to the haploids in extensive tests by Chase (1951) illustrates the problems. Of 1,327 haploid seedlings isolated,

283 died before maturity
 44 used for cytology and other observations
 1,001 matured
 453 could be selfed
 178 yielded self seed
 105 diploid lines were established.

Hence only about 8% of the haploids were represented in established diploid lines. About half were sweet corn and half were dent lines.

In the studies reported by Gerrish (1956), single cross hybrids were used as the female parents. All plants that had green roots as seedlings were grown to maturity. Seeds with colorless aleurone were discarded as probable contaminations. Some of these should have been seeds in which the endosperm nuclei developed without fertilization, but only occasionally would their embryos have been monoploid. A summary of the results obtained from the green seedlings selected as putative haploids is in Table 82.

Table 82. Numbers of the different types among surviving green seedlings from single cross $\frac{O}{+} \times a B Pl C R$ (Brown plant), Gerrish 1956.

Type	Number (1953)	Number (1955)
<u>dilute sun red (green seedling roots)</u>	247	116
Maternal monoploids	212	71
Heterozygous diploids	33	
Homozygous diploids (maternal)	2	45*
<u>colored plant (with B or Pl)</u>	266	41
Monoploid, brown	0	2
Diploid, purple (intense)	200	31
Diploid, sun red (intense)	64	7
Diploid, brown	2	1
Total survivors	513	157

* progeny not grown to distinguish the two types.

A high proportion of the plants from seedlings with green roots proved to be monoploids. Of the 35 that were diploids, two segregated for plant color, possibly from mutations of B to b. Progeny of the others were grown in the field; 31 of them were segregating for characters of the female parent, possibly by apogamous development of a diploid cell. The other two were homozygous and are of interest because they probably originated as haploids and then at a subsequent somatic division doubled their chromosome number. Plants similar to the female parent and true breeding were reported first by East (1930) in species crosses in *Nicotiana* where as high as 100 of this type per capsule of 400 seeds were observed. First generation progeny from these maternals

were extremely uniform, but succeeding generations were less and less uniform, probably as the result of mutations. As a check on their mode of origin, East used hybrids of *Fragaria vesca* which were heterozygous for two pairs of genes, R vs. r for red vs. white fruit and P vs. p for pink vs. white flower. These were pollinated by *F. chiloensis* or *F. virginiana* ($2n=56$). Maternal of the RP, Rp, rP and rp phenotypes were obtained, all with the diploid chromosome number and homozygous. This is indisputable evidence that their origin is from haploid cells followed by chromosome doubling. In corn, the frequency appears to be very low, but if it could be increased in some manner, the problem of the low fertility of haploid plants would be circumvented. East pointed out the potential value of such behavior to agriculture.

The two brown (androgenic) monoploids listed in Table 82 were from a seed with twin embryos (Gerrish 1956). These are of interest because they have the ♀ cytoplasm along with the complete set of genes from the male parent. Conversion of inbred lines to cytoplasmic sterile lines could be accomplished in one step (Chase 1951a).

The intense sun red diploids were probably the result of mutations of Pl to pl. The purple diploids were probably the result of misclassification for green vs. purple roots in the seedlings. The brown diploids may have been mutants of A to a; or androgenic diploids (page 180).

Of the 328 monoploids that reached tasseling stage, 91 were selfed, of which 18 set seed (1 to 10 seeds per ear); 165 were tassel sterile and were crossed as the ♀ parent. Forty-four of these 165 pollinations set seed with 1 to 46 seeds per ear. Of the 67 open pollinated monoploids, 42 set seed with 1 to 27 seeds per ear.

From the eight monoploids having self seed in one experiment, five homozygous diploids were established. These represent about 2% of the original seedlings with green roots. The plants were grown in the field. This may account for the low rate.

One might expect the survival of only those monoploids having gene combinations which result in the greatest vigor. As a result of this natural selection, one might expect diploid lines established from haploids to be superior to inbreds established from the same material by conventional methods. In extensive tests of homozygous diploids in sweet and field corn established by Chase, and of unselected inbreds from the same material, Thompson (1954) found no significant differences in topcross yields, also the homozygous diploids were not superior in root or stalk characters. No comparative tests have been made on haploids and inbreds derived from open pollinated material.

The production of haploids and of diploids from haploids has applications to basic problems as well. In tomatoes and in *Datura*, diploid lines have been produced from haploids, and tetraploids in turn from those diploid lines. As Chase has stated it "As tools for experimental research monoploids offer many possibilities: in the cytological field for studies of the meiotic distributions of unpaired chromosomes, non-homologous synaptic relations of the chromosomes and mechanics of mutational effects, measurement of mutation rates, studies of cytoplasmic effects, and biochemical investigations; in the agronomic field for the production of diploid, homozygous stocks directly from the monoploids." A study of the progressive change from great uniformity to greater and greater variability should be possible utilizing diploid lines established in this manner. Multiple interchange stocks might be an aid in analyzing the changes.

Theoretical genetic ratios for a single locus

As noted in Chapter 6, the additional homologous chromosomes in trisomics and tetrasomics increase the number of possible heterozygous genotypes and also affect the