CHAPTER 11 APOMIXIS

The term apomixis is applied to those modes of asexual reproduction in which there is no fusion of gametes, but the structures involved are those commonly concerned in sexual reproduction. The contrasting term is amphimixis, in which there is a fusion of two gametes. In many species or species groups apomixis occurs regularly in successive generations. Through suppression or circumvention of both meiosis and fertilization there is no alternation of chromosome number. Apomixis occurs in lower forms as well as in seed plants. In facultative apomicts, some progeny are produced by sexual reproduction also. In obligate apomicts the progeny arise almost wholly by apomixis.

Occasionally in non-apomictic species an egg develops without fertilization (parthenogenesis) into a haploid sporophyte, but it does not reproduce itself apomictically (see pp. 179 to 184). This has been termed "non-recurrent apomixis" by Maheshwari (1950).

This chapter includes brief discussions of the various types of recurrent apomixis, an experimental analysis of the modes of reproduction in an apomictic species, and methods of producing lines with new combinations of genes. For an extensive review, see Gustafsson (1946, 1947 and 1947a). A list of apomictic species is included in his 1947a paper, pages 306 to 320. For other reviews, consult Sharp (1934), Fagerlind (1940a), Stebbins (1941, 1950), Darlington and Mather (1949), Darlington (1956, 1958), and Maheshwari (1950).
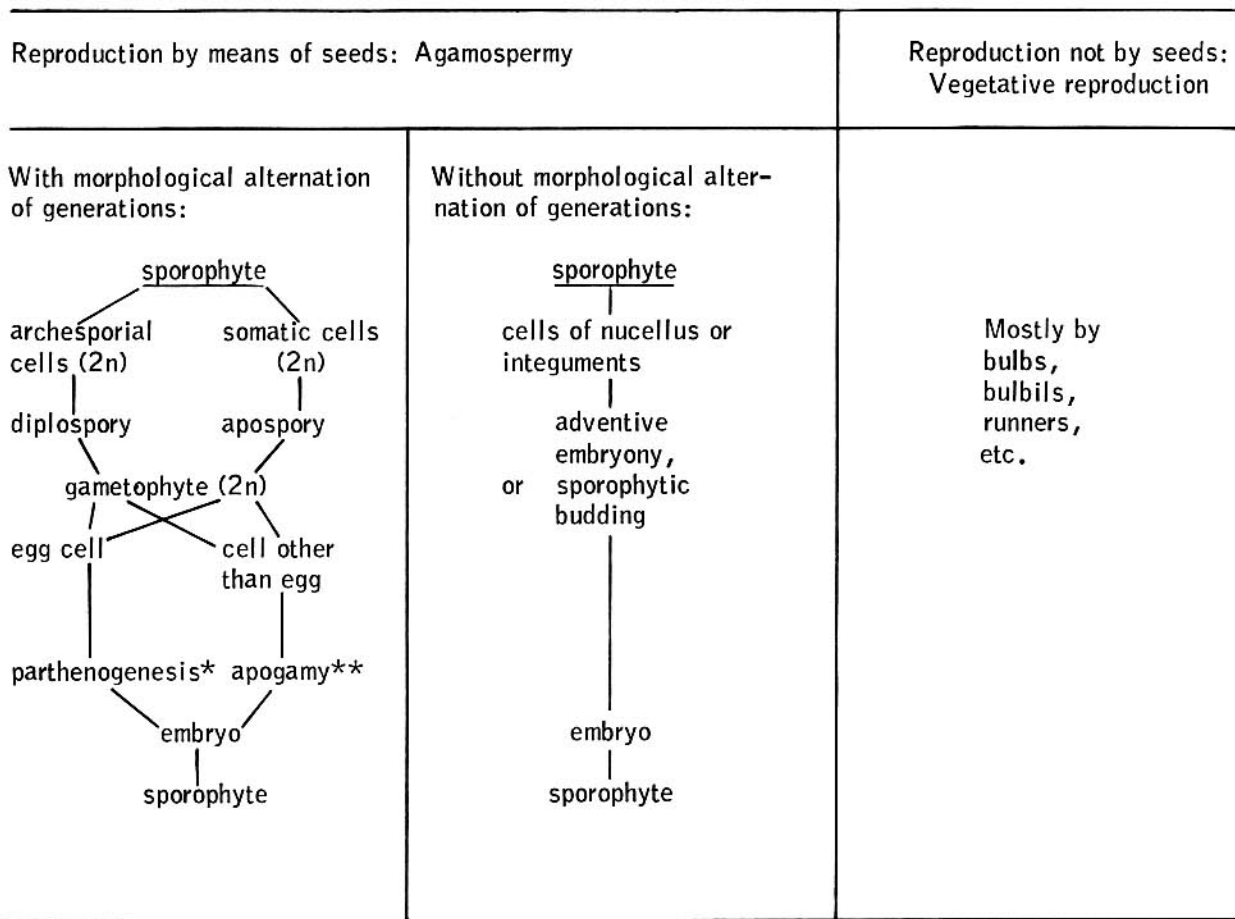
Terminology

Different terminologies and numerous terms have been applied. The relatively simple and usable classification and the charts presented by Gustafsson (1946), and Stebbins (1950) are the basis, with slight modifications, for the chart shown in Figure 58.

As shown in the chart, apomixis falls into two main classes: (1) reproduction by means of seeds, agamospermy, and (2) by means other than seeds, vegetative reproduction. A strict application of the definition given at the beginning of the chapter would exclude vegetative reproduction that did not involve structures usually concerned in sexual reproduction. Since the end result is the same genetically, no distinction is made in the chart or in the discussion that follows.

Vegetative reproduction

For a considerable number of species almost the sole mode of reproduction may be vegetative structures such as bulbs, bulbils, stolons or rhizomes, or by cuttings of various types. For example, bulbils may replace the flowers in certain Allium species. In grasses, the spikelets may be replaced by small plant-like structures which are capable

APOMIXIS

Reproduction by means of seeds: Agamospermy		Reproduction not by seeds: Vegetative reproduction
With morphological alternation of generations: sporophyte archesporial somatic cells cells (2n) (2n) diplospory apospory gametophyte (2n) egg cell cell other than egg parthenogenesis* apogamy** embryo sporophyte	Without morphological alter- nation of generations: sporophyte cells of nucellus or integuments adventive embryony, or sporophytic budding embryo sporophyte	Mostly by bulbs, bulbils, runners, etc.

* Continued development may require pollination, and is then termed pseudogamy.

** Following Sharp (1934), apogamy is used in place of apogamety.

FIG. 58. Chart showing the major types of apomixis (modified from Stebbins, 1950).

of rooting under favorable conditions. In two varieties of <u>Teosinte</u>, a structure consisting of numerous plantlike branches appeared in place of the tassel and female inflorescences under greenhouse conditions. When the branches were placed in sand, roots appeared and plants were established from them. Some species are capable of vegetative reproduction, but in addition produce seed.

Large acreages of Pangola grass (<u>Digitaria</u> <u>decumbens</u>), have been established in Florida by discing freshly cut Pangola hay into moist soil. Coastal Bermuda grass (<u>Cynodon</u> <u>dactylon</u>), a sterile hybrid, has been established on over half a million acres in southeastern United States by vegetative propagation (stolons and rhizomes), at a cost often less than that of using seed (Burton 1956). Under certain conditions of day length and temperature, certain species may remain vegetative. Oats and wheat react in this manner and probably could be reproduced by division of the clumps.

Agamospermy

Without morphological alternation of generations

Somatic cells of the nucellus or integuments may divide and develop an embryo which pushes into the embryo sac. This has been termed sporophytic budding, also adventitious or adventive embryony. Where they are known to have developed from the nucellus, it has been termed "nucellar embryony". This occurs in certain species of Poa (Tinney 1940) and in Citrus (Webber 1940). Certain of the seedlings produced in this manner in Citrus have no thorns, whereas others are thorny and similar to those produced by sexual reproduction.

With morphological alternation of generations

This may occur in several ways. One or more cells in the archesporial tissue (with the somatic chromosome number) may function as a spore (embryo-sac-mother cell) to form a gametophyte, thus avoiding meiosis. This is known as diplospory. Or the cell that functions as an embryo-sac-mother cell may be from non-archesporial tissue in the nucellus, referred to as apospory. There are numerous ways of producing this embryo-sac-mother cell, in some of which an abortive meiosis may have provided an opportunity for pairing and crossing over. For details of the various modifications, see Gustafsson (1947) and Maheshwari (1950). In certain tribes the unreduced embryo sacs usually have four nuclei, as in the aposporous apomicts in the Gramineae, subfamily Panicoideae, including certain species of Panicum, Paspalum, and Eragrostis (Brown and Emery 1958). In many of these, megaspores are formed, but they do not develop into embryo sacs, as described by Warmke (1954) and Snyder (1957). In other tribes of the Gramineae the unreduced embryo sacs may have 8 nuclei, as in the apomicts in the subfamily Festucoideae, including Poa (Tinney 1940). In certain populations of Agropyron scabrum, reduction at meiosis is suppressed, but crossing over may occur (Hair 1956). In many species, more than one embryo sac per ovule is formed. In their survey of the Gramineae, Brown and Emery (1958) found aposporic apomixis in 43 of the 153 species examined. The diploid species had 8-celled embryo sacs and reproduced sexually. The egg cell of the embryo sac may develop into an embryo (parthenogenesis) or some other cell of the embryo sac may develop into an embryo (apogamy). The initiation of the divisions of these 2n cells may require pollination with or without fertilization of the polar nuclei, termed pseudogamy. In some apomicts the embryo starts to develop but the endosperm nuclei must be fertilized for the continued development of the embryo, as in citrus (cf. Frost 1948). In others development of endosperm and embryo are autonomous, no pollination is needed, as in Alchornea illicifolia, and Euphorbia dulcis. At no stage in the cycle was there a change in chromosome number. See Gustafsson (1947) and Maheshwari (1950) for other examples.

There are many variants from these main types. Also in many species, normal meiosis and fertilization may occur occasionally. The progeny then are a mixture derived apomictically and by a sexual process, as for example in Poa and Parthenium.

In three species of Hieracium, the megaspore mother cell undergoes meiosis; but a somatic cell in the chalazal region enlarges greatly and its nucleus undergoes three somatic divisions to form an 8-nucleate embryo sac. The megaspores produced by the normal meiotic divisions are crushed. (cf. also Mendel's letters to Nägeli).* This behavior has been reported in Malus, Crepis, Hypericum, Ranunculus and Poa. The original cell which functioned as the embryo-sac-mother cell may have been in archesporial tissue, in the nucellus, or in the integuments.

* Mendel's attempts to apply his methods to Hieracium

Methods of determining the mode of reproduction

For genetic studies and breeding work the mode of pollination and the mode of reproduction must be known. Powers and Rollins (1945) have reported the results of extensive experiments in guayule (<u>Parthenium</u> <u>argentatum</u> and <u>P</u>. <u>incanum</u>), family Compositae. Individual plants were selected for the studies. The crosses were made reciprocally, the cross and its reciprocal being between the same two plants. The following six treatments were made, one for each treatment or a multiple on the same plant, using heads of similar maturity:

Treatment	Information furnished
1. Florets not treated, but labeled.	1. check for others
2. Florets brushed with camel's hair brush during anthesis, then stigmas examined for adhering pollen, bagged and unbagged.	2. does assured pollination increase % viable seed? does self-pollination produce seed under bags?
3. Emasculated but not covered with a bag.	3. do emasculated heads receive pollen from surrounding unemasculated ones?
4. Emasculated, no pollen applied; all heads of the branch covered with one bag (two kinds of bags used).	4. comparison with 5, 6 shows if pseudogamy is occurring. does seed set if not pollinated?
5. Emasculated, covered with a bag, pollen from the same plant applied daily until florets are brown.	5. effectiveness of self-pollination, also effect of emasculation and bagging
6. Emasculated, covered with a bag, pollen from ♂ plant applied daily.	6. checks effectiveness of pollen of the ♂ used, progeny test will determine method or methods of reproduction.

In guayule the results and conclusions were as follows:

Treatment 2, assured pollination did increase the % of viable seed.
Treatment 3, pollination of emasculated, uncovered heads did occur.
Treatment 4, emasculated, bagged but unpollinated heads produced no viable seed.
     Apomixis without pollination did not occur. Both kinds of bags, paper and percale, were equally effective.
Treatment 5, self-pollination was effective. There was no adverse effect of bagging or from emasculation.
Treatment 6, cross-pollination was effective, recognizable if the parents had contrasting characters.

Chromosome studies showed that some plants had about 36 chromosomes, others 54 $\pm$, and others 72 $\pm$. A study of the morphological characters of the progeny of representatives of these different types, combined with chromosome number determinations of a sample of the progeny, from selfs and from the various intercrosses showed what types of reproduction in 72-chromosome guayule occurred (Powers 1945, Powers and Rollins 1945). Rollins and Catcheside (1951) have summarized the behavior as com-

prising three types of egg formation which may or may not be followed by fertilization resulting in a total of six types of offspring as follows:

1, 2: apomixis, vegetatively produced unreduced eggs-

    a. if unfertilized give rise to maternal 72-chromosome plants,
    b. if fertilized give rise to 108-chromosome triploids;

3, 4: apomixis, unreduced eggs (72-chromosmes) formed by ameiosis in which the second meiotic division has been omitted, but crossing over might have occurred in the first division;

    a. if unfertilized give rise to "deviating maternal" 72-chromosome plants. This varied from 1 to 22%,
    b. if fertilized give rise to "triploids" with some variation;

5, 6: normal sexuality, reduced eggs produced by normal meiosis,

    a. if unfertilized give rise to 36-chromosome "haploids";
    b. if fertilized give rise to 72-chromosome hybrids.

When emasculation is difficult, removal of stigmas at various times, e.g. from 36 hours before anthesis, then at 1/2 or 1 hour intervals after anthesis may serve to determine the "role of pollination and fertilization in caryopsis development" (Warmke, 1954, in Panicum; Snyder, et al. 1955, in Pennisetum).

Cytological studies to determine the origin and mode of development of the embryo sacs is helpful also. For Brown and Emery's study of species of the Gramineae, pistils gathered when some of the spikelets were in anthesis were sectioned and the number of nuclei in the embryo sacs determined. Chromosome numbers in endosperm tissue were determined also. The presence of four nucleate embryo sacs was considered as evidence of aposporic apomixis. As mentioned earlier, this type is characteristic of certain tribes of Gramineae. Cytological study combined with a study of the breeding behavior from selfs and crosses should distinguish between the various types of apomixis. A qualitative marker is a useful aid in studying the origin of progeny from crosses. Burton's use of the red vs. white stigma character in Bahia grass is a good example (unpublished).

Apomixis and plant breeding

For the practical breeder, it is desirable to develop superior strains and retain apomixis; since any heterosis obtained in hybrids theoretically could be maintained. Extensive studies of Poa pratensis (Kentucky bluegrass) (reviewed by Myers, 1947) have shown "a range in type of reproduction from forms that are almost completely apomictic to forms that are completely, or nearly completely, sexual." Brown and Emery (1958) found that one collection of a species might be apomictic, another sexual. Crosses between lines and between species were made in which reduced eggs had been fertilized.

Methods of producing strains of guayule (Parthenium argentatum Gray) with new characters have been discussed by Rollins and Catcheside (1941); and Burton (1959) has produced improved strains of apomictic grasses.

The results and proposals of Rollins and Catcheside with guayule will be discussed first. The "deviating maternal" 72-chromosome plants resulting from unfertilized unreduced eggs following an abortive meiosis in which crossing over might have occurred

furnish one possible source of variable material for selection. In guayule, microsporogenesis in the 72-chromosome apomict proceeds normally, and the pollen furnishes spores with the reduced chromosome number, 36.

When an apomictic strain is crossed with a sexual one, the F_1 and most of the F_2 are sexual. It is difficult to obtain apomictic segregates. Frequently, other irregularities appear also. One method where there is a low percentage of sexual reproduction is to cross such strains; utilizing the segregation afforded by this small percent of sexual reproduction. This requires recognition of the segregating lines within which selection is to be made.

Another method which Rollins and Catcheside proposed as still more promising was the use of 36-chromosome "haploids" which result from parthenogenetic development of reduced eggs, and appear occasionally among the progeny of the 72-chromosome plants. Different "haploids" will contain different combinations of genes, and might be propagated vegetatively. They are apomictic also and all the functional eggs in these haploids are unreduced. Pollen with the reduced chromosome number from different 72-chromosome sources produce 72-chromosome hybrids which are expected to be apomictic. Many should be new gene combinations which might be used in a selection program. In guayule, certain of the hybrids produced in this manner had much higher percentages of rubber than either the "haploids" or the 72-chromosome plants.

Variant sectors might also appear as a result of somatic crossing over in a heterozygous apomict. Since apomicts are usually polyploids the same crossover would have to recur in the other genomes in tissue resulting from the first cross-over before any recessive characters would appear. Large scale testing of vegetatively propagated lines would be needed. Repeated exposure to radiation might uncover recessive characters also.

Mode of inheritance

An hypothesis to account for the origin and evolution of apomixis was presented by Powers (1945), but it was not based on an experimental analysis of the modes of inheritance.

In guayule, an apomictic polyhaploid (37 chromosomes) was crossed with a sexual diploid (2n = 36) (Gerstel et al. 1950, 1953). Both parents and the F_1 were self-incompatible, but some F_2 seeds were obtained from plants grown in an isolated open-pollination plot. The type of reproduction was determined by crossing the F_2 plants with another species, Parthenium stramonium. Only one of 575 plants had come from an unreduced egg, all the others were from reduced eggs. The F_2 plants were tested in a similar manner. Only 53 produced some progeny (39 had 15 or more plants); but all produced only hybrid progeny. They concluded that in guayule, apomixis is recessive, and controlled by a minimum of four genes. However, they raised the question whether eggs that had the genotype for parthenogenetic development could express this potentiality in a diploid plant that undergoes normal reduction.

In guayule apomixis does not depend on polyploidy, since "haploids" with the diploid number still reproduce apomictically; also certain tetraploids reproduce sexually. Triploid F_1's reproduced apomictically. Hence two doses of the genes for apomixis appear to be dominant over one dose of the genes for sexuality.

Studies on the inheritance of apomixis in Common Bahia grass (Paspalum notatum) have been reported by Burton and Forbes (1960). Common Bahia grass is a tetraploid and is apomictic (Burton 1948). This diploid Pensacola Bahia grass and its autotetraploid reproduce sexually (Burton 1955). Part of the results from the tetraploid Pensacola Bahia grass crossed as ♀ with Common Bahia are summarized in Table 120.

Table 120. The frequencies of apomictic and sexual plants in F_1 hybrids and in F_2 of four crosses of tetraploid Pensacola (PT) X Common Bahia grass (CB) (From Burton and Forbes, 1960, Table 1, p. 69).

Cross	Types of F_1 hybrids*			Types of F_2 plants from sexual F_1's.*		
	apomictic	sexual	ratio	apomictic	sexual	ratio
4 n PT-2 x CB (WSB)	10	250	1:25	46	1546	1:33.6
4 n PT-2 x " (MHB)	13	32	1:2.5	7	279	1:39.8
4 n PT-4 x " (MHB)	4	50	1:12.5	2	456	1:228.0
4 n PT-10 x" (MHB)	17	64	1:3.8	17	516	1:30.4

* Based on progeny tests in the next generation.

As shown in Table 120 part of the F_1 hybrids in each cross were apomictic. Strain MHB of Common Bahia grass, gave about 10 times as many apomictic F_1 plants as did WSB when crossed on PT-2. The apomictic F_1 and F_2 plants bred true for apomixis. In three of the sexual F_2 progenies, the segregation ratio of sexual: apomictic plants did not deviate significantly from 35:1. This suggests tetrasomic inheritance for a single locus with only the nulliplex genotype apomictic. However, the difference in behavior in F_1 and in the other F_2 indicate that more factors are involved.

In the progeny of sexual plants, segregation for other characters was noted, among them promising new gene combinations. As stated by Burton and Forbes (1960), "the method described here places at the breeder's disposal a vast bank of genes in the naturally occurring tetraploid Bahia grasses that has heretofore been unavailable because of obligate apomixis." Also, the fixing of heterosis in the apomictic F_1's was demonstrated by comparisons of yields of sexual and apomictic F_1's and the progeny of the two types in F_2.

Hybrids between apomictic Common Bahia grass (4n and apomictic) and Pensacola Bahia (diploid and sexual) are triploid and reproduce entirely by apomixis. In the absence of a suitable pollen source they are highly sterile, but seed well when interplanted with rows of pollen-producing diploid Pensacola Bahia (Burton 1956). Seed produced in this manner is used commercially in southeastern United States.

Concluding statements

There are some species that reproduce by processes in which meiosis in the female and fertilization are suppressed almost completely. Microsporogenesis may be normal. Most apomictic species retain a low frequency of normal sexual behavior, which can account for the variation present in an apomictic population, or for the new variants which occasionally arise.

Apomixis furnishes one method of maintaining the heterosis shown in hybrids.