APOSPORIC DEVELOPMENT IN THE NORTH AMERICAN SPECIES OF CREPIS

by

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INTRODUCTION

The presence of apomictic reproduction in the North American species of *Crepis* was first suspected by HOLLINGSHEAD and BABCOCK (1930), who found in them a polyploid series of chromosome numbers based on 11, which included many triploids and pentaploids (2n = 33 and 55) — that is, „unbalanced" chromosomal types. This phenomenon was further investigated by Professor BABCOCK and the senior author, whose results form the basis of a monographic study of the group (BABCOCK and STEBBINS, 1938). In this monograph only brief reference has been made to the cytology of apomixis in the group, since the major objectives of the monograph were to determine the effect of polyploidy and apomixis on interspecific relationships. The present study was undertaken in order to provide an understanding of the cytological mechanism of apomixis in the forms which were the subject of that monograph.

Before a correct understanding can be obtained of the type of apomixis in *Crepis* a review of the classifications of different types of this phenomenon is necessary. The majority of investigators have adopted WINKLER's terminology, which is based on the morphology of the various structures. WINKLER's (1934) revised classification of the apomictic phenomena will, in the main, be used in this paper; according to which A p o m i x i s may be briefly defined as a

reproductive process in which there is no sexual fusion of gametes. Apomixis in the angiosperms may be divided into two categories: 1) Agamospermy (TACKHOLM, 1922), in which the propagative organs develop from the ovule, and in which seed formation takes place, and 2) Vegetative Propagation — the apomictic origin of a sporophyte from the cells of a sporophyte or the apomictic origin of a gametophyte from the cells of a gametophyte.

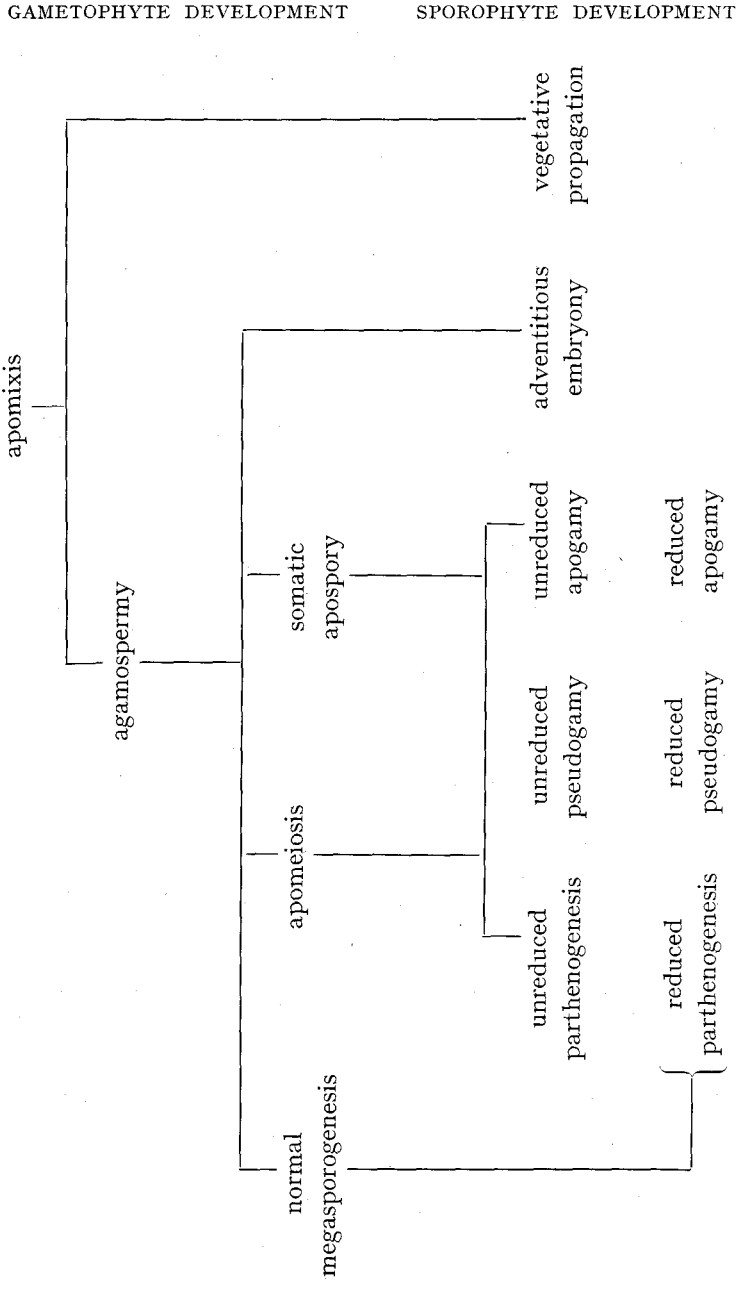
Agamospermy itself may be further divided into three categories: 1) Adventitious embryony, in which no functional gametophyte is produced, and in which the embryo arises directly from a somatic cell; 2) Somatic Apospory, in which a gametophyte arises without spore formation from a cell other than an archesporial one (EMC); 3) Apomeiosis, which WINKLER defines as the origin of a gametophyte from a spore that does not undergo reduction of the chromosome number. CHIARUGI (1926) has used the term *aposporia goniale* for this last process. However the *Taraxacum* type of development, which was included under this term by CHIARUGI, involves the formation of dyads and therefore of diploid spores; to call this process apospory is a misnomer. In *Antennaria*, on the other hand, there is no sign of tetrad or spore formation and therefore the term of generative apospory, or *aposporia goniale*, can be properly applied to the *Antennaria* scheme. Since there are so many intermediate types between the *Antennaria* and *Taraxacum* schemes (for example, *Burmannia, Hieracium, Ixeris*, etc.; ROSENBERG, 1930; GUSTAFSSON, 1935) both schemes, along with the transitional ones are properly grouped by WINKLER under the general term apomeiosis. However, a strict reading of WINKLER's definition would exclude the *Antennaria* scheme, as in this case there are no spores formed; consequently the following modification of WINKLER's definition is proposed: Apomeiosis is *a series of different processes by which an EMC gives rise to an embryosac without reduction of chromosome number.* Furthermore, the term somatic apospory is substituted for WINKLER's apospory in order that the old term generative apospory may be retained to properly define the origin of the gametophyte in *Antennaria* — that is, generative apospory is one process of the apomeiotic phenomena.

WINKLER (1934) correctly limits Parthenogenesis to the origin of a sporophyte from a germ cell without fusion of nuclei or

cells, thus obviating the earlier confusion, in which two processes were included under the term: 1) the development of the egg, and 2) the production of the gametophyte, including the egg. Parthenogenesis may be divided into two categories depending upon whether the egg is haploid or diploid in respect to the sporophyte. SHARP's (1934) terms, reduced (haploid) and unreduced (diploid) parthenogenesis are self-evident and much more convenient terms than WINKLER's (1934) of gamophasic and zygophasic parthenogenesis (respectively). In order that the apomictic reproductive processes may be repeated indefinitely, unreduced parthenogenesis must be preceded by somatic apospory or apomeiosis. As mentioned above, the *Antennaria*, *Taraxacum* and *Alchemilla* schemes are classified as cases of apomeiosis followed by parthenogenesis, and not merely as cases of parthenogenesis as heretofore; while the *Pilosella* scheme will still come under somatic apospory.

P s e u d o g a m y, the stimulation of an egg by the male gamete, but without complete fertilization, may be considered as a special case of parthenogenesis. However, the term is retained here because of its usefulness and its prevalence in the literature. WINKLER's term A p o g a m y for the origin of the embryo from a cell or cells of the gametophyte other than the egg, seems to be quite firmly established in spite of its earlier usage in another sense. Both of these latter processes, pseudogamy and apogamy may, like parthenogenesis, be divided into two categories, reduced and unreduced, depending upon whether the gametophyte has the haploid or diploid chromosome number.

The following chart illustrates the authors' modification of WINKLER's terminology to describe the relationships between these phenomena, with emphasis on the two distinct processes: formation of the gametophyte, and development of the sporophyte. Androgenesis, the development of offspring with paternal chromosomes only, may also be considered as an apomictic phenomenon, which comes under WINKLER's definition of parthenogenesis; but as the exact processes involved are unknown, it has been omitted from the chart. Using the proposed terminology, the processes to be described for *Crepis* are somatic apospory followed by unreduced parthenogenesis.


GAMETOPHYTE DEVELOPMENT
SPOROPHYTE DEVELOPMENT
apomixis
agamospermy
vegetative propagation
adventitious embryony
somatic apospory
unreduced apogamy
reduced apogamy
apomeiosis
unreduced pseudogamy
reduced pseudogamy
unreduced parthenogenesis
reduced parthenogenesis
normal megasporogenesis

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MATERIALS AND METHODS

Buds of *Crepis* were fixed in the field during the summers of 1936, 1937, and 1938. Table 1 gives the locality at which each collection was made. Herbarium specimens representing each collection have been placed in the Herbarium of the University of California; these bear the collection numbers listed in the second column. The collections of 1936 (1600–2000) were made by E. B. BABCOCK and the senior author, those of 1937 (2100–2480) by the two authors, and that of 1938 (2499) by the senior author. The forms were identified according to the recent systematic revision of the group (BABCOCK and STEBBINS, 1938). Somatic chromosome numbers were based partly on counts from sections of root tips, partly on counts from various tissues of the sectioned buds, and partly on counts from aceto-carmine smears.

Buds were pretreated for 2 to 4 minutes in a 3 to 1 alcohol-acetic mixture, then fixed for 24 hours in RANDOLPH's (1935) modification of chrom-acetic-formalin, and embedded in paraffin according to his schedule, except that the larger and harder ones were treated for 15 hours before embedding in a 2 per cent. solution of hydrofluoric acid in 70 per cent. alcohol. Sections were cut from 20 to 25 micra and stained with STOCKWELL's (1934) modification of FLEMMING's triple stain.

Drawings were made with the aid of a camera lucida and a Zeiss microscope (30 × compensating ocular, 8 mm and 16 mm apochromatic objectives, the latter giving a magnification of 550 × and the former 1100 ×). All drawings were reduced one-half in reproduction.

TABLE 1. IDENTITY AND SOURCE OF MATERIAL

Species	Herbarium Number	Somatic Chromosome Number	Locality [1]
acuminata	1864	22	Lakeview, Oregon
acuminata	1869	22	Near Beatty, Oregon
acuminata	2163	*ca.* 33	East of Loyalton
intermedia apm. *Grayi*	2173	44	Truckee River, South of Verdi, Nevada
intermedia	1700	*ca.* 44	Sierra Valley
intermedia apm. *lacustrensis*	2499	*ca.* 55	Near Clear Lake, Lake County
modocensis subsp. *subacaulis*	2158	55 or 66	East of Loyalton
modocensis subsp. *subacaulis* apm. *setosissima*	2159	44	East of Loyalton
monticola	1973	22	Mt. Shasta
monticola apm. *australis*	—	44	Mt. Hamilton
occidentalis subsp. *typica*	2170	22	Reno, Nevada
occidentalis subsp. *typica*	1671	22	Sierra Valley
occidentalis subsp. *typica*	2169	44	Reno, Nevada
occidentalis subsp. *typica* apm. *pinnatisecta*	2117	33	Sierra Valley
occidentalis subsp. *conjuncta*	1589	—	Truckee
occidentalis subsp. *pumila*	1686	—	Sierra Valley
occidentalis	2179	—	Verdi, Nevada

[1] All localities are in Northern California except where otherwise stated.

Species	Herbarium Number	Somatic Chromosome Number	Locality [1]
subsp. *pumila* *occidentalis*	2180	—	Verdi, Nevada
subsp. *pumila* *occidentalis*	—	77	Mt. Hamilton
subsp. *pumila* apm. *hamiltonensis* *pleurocarpa*	1975	55	Yreka
pleurocarpa	1976	*ca.* 55	Yreka

Photographs were made with a Zeiss Phoku camera, using two different magnifications. Plate 1, figures 1–10, and Plate 2, figure 7 were made with a 16 mm high power objective, and (except for Plate 1, figure 10) were enlarged from the negatives to a magnification of 1100 ×, while Plate 2, figures 1–6 and 8–11 were made with an 8 mm objective, and enlarged to 300 ×. All were reduced one-half in reproduction.

MEIOSIS IN THE POLLEN MOTHER CELLS

Pollen-mother-cell meiosis was studied in two diploid sexual forms and in three apomictic forms. The two diploid forms, *C. acuminata* (1864) and *C. occidentalis* (1671),were normal throughout; 11 bivalents regularly appeared at the first metaphase, followed by normal disjunction at anaphase. In another diploid species, *C. monticola* (1973), an examination of a large number of microspore tetrads indicated complete regularity of meiosis in this form also.

Two of the apomictic forms, *C. occidentalis* subsp. *typica* (2169) and *C. intermedia* apm. *Grayi* (2173), are tetraploids (2n = 44). In its external morphology the former is very similar to the diploid, and on this basis it could be considered as nearly autopolyploid. In any case, it must have a preponderance of genes and chromosomes derive from diploid *C. occidentalis*. On the other hand, the *intermedia* form

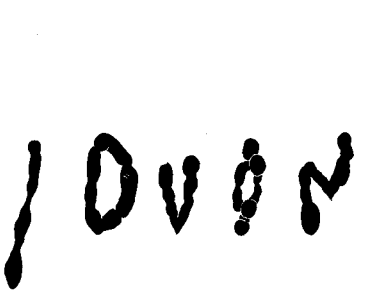
[1] All localities are in Northern California except where otherwise stated.

is intermediate between diploid sexual *C. occidentalis* and diploid sexual *C. acuminata* and is unquestionably of allopolyploid origin.

Unfortunately this material is not well suited to a careful study of meiosis; the chromosomes are large and numerous, particularly in the polyploids. This results in a very crowded condition at all stages of meiosis. Consequently, complete analysis at diakinesis or first metaphase was possible only in a few cases; and for the most part, only rough estimates of the number of bivalents, univalents, and multivalents, were possible.

The most striking fact is that meiosis is fairly regular in both of these apomictic forms; and furthermore, it is quite similar in the two. In both of them most of the chromosomes form bivalents, but a few univalents (from 1 to 4) and multivalents are always present. In *C. occidentalis* (2169) the number of multivalents ranged from 2 to 6, and in *C. intermedia* (2173) from 4 to 7. However, the number of cells observed was insufficient to determine whether or not there is a significant difference between the two in respect to the frequency of multivalents — in any case the difference is small.

The multivalents were mostly of the chain type with a minimum number of chiasmata but closed rings were occasionally found (fig. 1). No cases of triple chiasmata were observed. This latter observa-



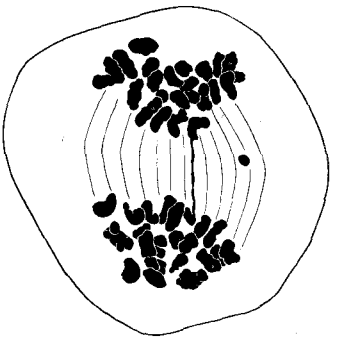


Fig. 1. Fig. 2.

Crepis intermedia (2173), PMC Meiosis

Fig. 1. First metaphase multivalent configurations; × 2500.

Fig. 2. First anaphase, showing bridge-fragment configurations; × 2500.

tion is quite in keeping with the usual behavior of *Crepis* chromosomes at meiosis; where, in spite of their great length, rarely more than

two chiasmata per bivalent are formed, and frequently only one.

The chromosomes, for the most part, disjoin reguarly at the first anaphase, and lagging univalents are comparatively rare. In both apomicts bridge-fragment configurations (fig. 2) — indicating chiasma formation in an inverted segment — were frequently found. Out of 100 first anaphase figures in tetraploid *C. occidentalis* (2169), 63 were normal, 32 had one bridge, and 5, two bridges. Evidence of lagging chromosomes or bridgefragment configurations was found in 35 per cent, of the cells at interkinesis; while 31 per cent, of the microspore tetrads show extra nuclei or microcytes. The proportion of „normal" to abnormal tetrads is about the same in *C. intermedia* (2173); an accurate estimate was not possible due to the presence of many dark-staining bodies in the cytoplasm which were difficult to distinguish from the smaller micronuclei. A careful study of more than 200 first anaphase, interkinesis, and second division figures in diploid sexual *C. occidentalis* failed to show any bridge-fragment configurations.

A very careful examination, involving hundreds of cells, of these two and all other apomictic forms in which microspore tetrads were found, with the exception of *C. occidentalis* subsp. *pumila* apm. *hamiltonensis*, which is described below, failed to reveal the presence of a single dyad. Consequently, the formation of restitution nuclei following a semi-heterotypic division in the PMCs of these *Crepis* species must be an exceedingly rare phenomenon. An examination of the tetrads in the other apomicts showed about the same proportion of normal-appearing tetrads — namely, about two thirds. For example, in *C. occidentalis* apm. *pinnatisecta* (2117), the aposporic development of which is described below, 65 per cent. of the tetrads were normal in appearance, while the majority of the remainder contained only one micronucleus or microcyte.

In one apomict, *C. occidentalis* subsp. *pumila* apm. *hamiltonensis*, meiosis is considerably more irregular. From counts of root tip cells, its somatic number was definitely established as 77. At first metaphase 5 to 8 univalents are always found scattered about the spindle. No associations of more than four chromosomes were found, but in this difficult material their presence would not necessarily be detected. At first anaphase chromatin bridges are about as common as in *C, intermedia* (2173), and these often extend from one plate to the

other at second metaphase. The first division is completed normally, except that in 60 to 70 per cent, of the cells a few of the univalents lag and are left in the cytoplasm. The second division is normal except for one important deviation. At the second metaphase or the second anaphase, two adjacent groups of chromosomes, belonging to different spindles, become joined into one (figs. 3–6). Reunion of two groups belonging to the same spindle, that is, failure of the second division to be completed, has not been observed. The union is caused partly by the presence of chromatin bridges connecting the groups,

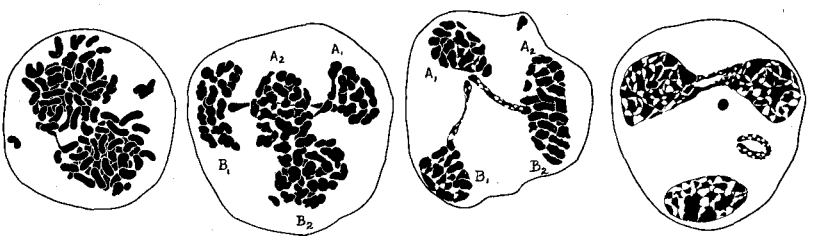


Fig. 3. Fig. 4. Fig. 5. Fig. 6.

Crepis occidentalis subsp. pumila apm. hamiltonensis, PMC Meiosis

Fig. 3. Polar view of a second metaphase, showing fusion of plates; × 1000.

Fig. 4. Polar view of a second telophase, „tripolar spindle", which has resulted from the fusion of adjacent nuclei from two different spindles; nuclei A_1 and A_2 belong to one spindle, B_1 and B_2 to the other; × 1000.

Fig. 5. Side view of the same configuration as in figure 4; the chromatin bridge A_1 and A_2 was formed in the second division while that between A_1 and B_1 was formed in the first; × 1000.

Fig. 6. Late second telophase, the nucleus at the bottom arose from the fusion of two, those at the top are connected by a first division bridge; × 1000.

which have persisted from first anaphase. It is also made easier by the large number of the chromosomes and the relatively small size of the sporocytes, which makes the spindles closely crowded together. The union of two anaphase groups frequently gives the appearance of a tripolar spindle (figure 5). The fusion results in the formation of triads or dyads, depending on whether one or both sets of anaphase plates become united. Both dyads and triads are frequent in apm. *hamiltonensis*.

Fusion at the second division of chromosome groups belonging to different spindles has been reported many times, and is listed by

BERG (1934) as one of three causes of non-reduction (cf. BERG, p. 119 for a list of cases). In several cases, including two not mentioned by BERG, a *Papaver* hybrid (YASUI, 1921) and *Petunia* (MATSUDA, 1928) —chromatin bridges were found at first anaphase, which according to our present knowledge, would be interpreted as dicentric chromatids derived from inversions. Hence the persistence of these bridges in the second division may likewise be a cause of these cases of fusion as well as those reported in the present paper.

Meiosis in these *Crepis* species is fairly regular as compared with most other apomicts. It parallels the situation found in *Hieracium* subg. *Pilosella*, for example, *H. Pilosella* and *H. aurantiacum* (ROSENBERG, 1917), *H. flagellare* and *H. pratense* (ALTON GUSTAFSON, 1933), and some species of *Antennaria* (STEBBINS, 1932) sich as *A. fallax*. On the other hand, the striking abnormalities of meiosis found in *Hieracium* subg. *Archieracium* (ROSENBERG, 1917; GUSTAFSON, 1933), *Taraxacum* (ÅKE GUSTAFSSON, 1935), *Chondrilla* (Poddubnaja-Arnoldi, 1933), and *Antennaria canadensis* (STEBBINS, 1932) are all absent. The number of univalents, even in the „unbalanced" types with 55 and 77 chromosomes, is always less than 11. This indicates that none of the apomicts contains a set of 11 (a genom) which is not at least partly homologous with one of the other sets that it contains. Since trivalents are frequent, the univalents may in some cases be homologous to the members of a trivalent.

The most conspicuous abnormality in these apomicts is the formation of chromatin bridges and fragments due to pairing in inverted segments. This phenomenon, the significance of which was first pointed out by MCCLINTOCK (1933), is now known to be of frequent occurrence in the higher plants, both in hybrids and in pure species (DARLINGTON, 1937 p. 274). Nevertheless, since diploid *C. occidentalis* does not form any bridges, their frequent presence (in 37 per cent. of the cells) in the tetraploid *C. occidentalis* (2169), indicates that in the latter pairing is taking place between chromosome sets that are partly different from those of diploid *C. occidentalis*. SAX (1937) has shown that an auto-triploid *Tradescantia* has a higher percentage of bridge-fragment configurations (dicentric chromatids) than the related diploid. This was explained by the fact that chiasmata are more frequent per unit of pairing length in trivalents than in bivalents. In the tetraploid *Crepis occidentalis*, however, the proportion

of multivalents formed is much smaller than in *Tradescantia*, while the increase in frequenct of dicentric chromatids is considerably greater. In *C. occidentalis* (2169), therefore, there probably are inversions that are not present in the diploid.

This last fact bears directly on the problem of whether the polyploids under discussion are auto- or allopolyploids. As a basis for this discussion, we may use the three most authoritative definitions of auto- and allopolyploidy that have appeared in recent years. The first, that of LILIENFELD (1936, p. 121) is as follows: „Sind diese [the genomes] alle homolog, so liegt Autopolyploidie vor; sind sie so weit differenziert, das sie nichthomolog zu gelten haben, so haben wir es mit Allopoloploidie zu tun. Unter zwei homologen Genomen sind solche zu verstehen, deren Chromosomen in der Meiosis stets paarweise zu echten („festen") Gemini zusammentreten und — ohne Beeinträchtigung der Lebensfähigkeit der Gameten bzw. der aus ihrer Verbindung resultierenden Zygoten — austauschbar sind". Under this definition, as explained through the parenthetical phrase, an allopolyploid may be derived from a diploid hybrid showing a high degree of pairing, if the chromosomes that pair are dissimilar enough so that gametic sterility results. Hence a relatively high number of multivalents, such as is found in these *Crepis* apomicts, is not incompatible with the assumption that they are allopolyploids. An additional fact favors this assumption. The complete absence of fertile diploid hybrids in nature, suggests that they cannot be formed.

The second definition of auto- and allopolyploidy, that of MÜNTZING (1936, p. 311), maintains that „allopolyploidy.... involves structural differences between some of the genomes". The weakness of this definition is that structural differences, that is, inversions and translocations, are often found in diploid species, and do not necessarily lead to much sterility (for example, *Paeonia*; STEBBINS, 1938). On this basis, however, the *Crepis* apomicts, since their genomes must differ by numerous inversions, should be classified as allopolyploids.

The third set of definitions, those of DARLINGTON (1937), are difficult to interpret. An autopolyploid (p. 138) is defined as an organism with „four *identical* sets of chromosomes", while an allopolyploid is defined first (p. 183) as „the product of doubling in

hybrids", and later in the glossary (p. 572) as „a polyploid whose chromosomes do not usually form multivalents at meiosis. . . .". The first, rather vague definition of allopolyploidy would probably cover the *Crepis* apomicts under discussion. The second definitely would not since they usually form some multivalents. Since on page 184 (*op. cit.*) the polyploid derivative of *Crepis rubra* × *C. foetida* is classified as an allopolyploid, even though it usually forms multivalents, and in some cells all of the chromosomes are associated in this manner, it is safe to assume that Darlington's definition in his glossary need not be applied in many cases, and the *Crepis* apomicts under consideration can be regarded as allopolyploids under his criteria also.

Based on these considerations, the assumption made elsewhere by the senior author (BABCOCK and STEBBINS, 1938), that all but a few of the *Crepis* apomicts are allopolyploids, is supported by the cytological evidence. In dividing the polyploid apomicts of *C. occidentalis* into auto- and allopolyploid types on the basis of their external morphology, the plant 2169 was placed by the senior author (BABCOCK and STEBBINS, 1938, p. 123) among the allopolyploids, but it was considered to resemble the supposed autopolyploid forms as closely as any of the apomicts classified as allopolyploid. The present cytological evidence confirms the allopolyploid nature of this plant. Consequently, the other phenotypic allopolyploids, which in their external morphology are more divergent from the supposed autopolyploid form than is plant 2169, could also be expected to fulfil the cytological requirements of allopolyploidy. Two important points about the allopolyploids of *Crepis* must be emphasized; first, for reasons discussed elsewhere (BABCOCK and STEBBINS, 1938, p. 66), may of them are recognized as intraspecific polyploids in spite of their allopolyploid origin; second, they regularly form some multivalents. These two points should be borne in mind in future discussions of auto- and allopolyploidy.

DEGENERATION OF THE POLLEN CELLS

Many of the *Crepis* apomicts bear no pollen at all in their anthers (BABCOCK and STEBBINS, 1938). One such form, *C. acuminata* (2163), showed the most striking deviation from normal microspore development. The PMCs appear normal until zygotene or early pachytene

then degenerate, becoming quickly and completely crushed by the anther walls. In anthers of the age when meiosis normally occurs the loculi have completely disappeared. Parallel with this degeneration is an abnormal behaviour of the tapetal cells.

In those *Crepis* forms, in which the meiotic process is carried through to completion, the tapetal nuclei begin to divide when the pollen-mother-cells are in the early leptotene stage; from then on they continue to divide and fuse, forming nuclei with many times the somatic number of chromosomes, until PMC meiosis is finished (Plate 1, fig. 1). Development is closely similar to that described by CHIARUGI (1927) in *Chrysanthemum alpinum*. Throughout meiosis the cytoplasm is densely granular and not conspicuously vacuolate; and the nucleus is rich in chromatin.

In *C. acuminata* (2163), on the other hand, tapetal nuclei never divide at all. While the contents of the PMCs are differentiating into leptotene threads, the tapetal nuclei become much enlarged, weakly staining, and finely granular; their cytoplasm also becomes thin and granular (Plate 1, fig. 2). They remain in this condition until the PMCs reach zygotene or early pachytene, at which time they gradually become shrunken and crushed (Plate 1, fig. 3). The PMCs also become crushed, so that in anthers only slightly older than those represented in Plate 1 figure 3 all traces of the loculi are gone, except for a few dark staining remnants of the tapetum and the PMCs. The degeneration of the PMCs is thus closely correlated with the degeneration of the tapetum; and the immediate cause of the PMC degeneration may be attributed to the failure of the tapetum to keep them nourished.

As is brought out elsewhere (BABCOCK and STEBBINS, 1938), this phenomenon of PMC degeneration is found in many apomicts related to diploid *C. acuminata, C. modocensis* subsp. *typica*, and *C. exilis*; but is not regular in its distribution, since closely related apomicts may differ in this respect. The present study emphasizes the fact that the presence or absence of pollen degeneration is not correlated with the type of development that takes place in the ovules. The *C. acuminata* (2163) apomict discussed above has an aposporic development similar to that of the majority of the pollen-bearing apomicts (see below, table 2); while the two apomicts, *C. intermedia* (2173) and *C. occidentalis* subsp. *pumila* apm. *hamiltonensis*, which are more

abnormal in their aposporic development than are the other apomicts, nevertheless form pollen. This phenomenon of pollen degeneration is probably controlled genetically, perhaps through complementary factors. At any rate, the genetical mechanism controlling it is independent of that which governs apomictic development.

GENTSCHEFF (1937) has shown that, in the apomicts of *Hieracium* subg. *Archieracium* with very abnormal meiosis and subsequent pollen degeneration, there is also degeneration of the tapetal nuclei. The earlier development of the tapetum in these forms is not unlike that in the *Crepis* forms, both sexual and apomictic, which have normal pollen development. Degeneration sets in, however, during meiosis; whereas the tapetal cells normally function until the pollen grains are formed. As in *Crepis*, this degeneration is accompanied by vacuolization of the cytoplasm. Gentscheff has suggested that the degeneration of the dividing PMCs is caused partly by the abnormalities in the tapetum; this opinion is well supported by the parallel but more extreme case in *Crepis*.

MEGASPORE AND EMBRYOSAC DEVELOPMENT

Megasporogenesis was investigated in three diploid sexual forms, namely: *C. acuminata* (1869), *C. occidentalis* (1671), and *C. occidentalis* (2170). The last was investigated most thoroughly, and a brief description of it will serve as a model of normal sexual development in these *Crepis* species. The prophase of meiosis is initiated at about the same time in the PMC's and the EMC's, though it proceeds much more rapidly in the former: pollen grains are usually present before the completion of the prophase of the EMC. The completion of the two meiotic divisions in the EMC is followed by the formation of a linear tetrad of four megaspore nuclei, which are soon separated by walls (fig. 7). The basal or chalazal megaspore develops into an eight-celled embryosac in the usual manner. Polar fusion takes place before fertilization; and the antipodals may go through one or two additional divisions, giving six or nine antipodal cells (fig. 8).

The apomicts all show some form of somatic apospory in which a cell or cells of the integument immediately below the EMC, that is, in the chalazal region, develop into embryo sacs containing the somatic number of chromosomes. There is considerable variation in

the time when apospory is initiated and also variation in the number of aposporic embryo sacs developing in each ovule; consequently, for purposes of description three types may be established: 1) Apospory is relatively infrequent and usually takes place at the megaspore tetrad stage or later. 2) Apospory is the most common procedure and usually takes place before the megaspore tetrad stage. 3) Apospory always takes place and the EMC never develops beyond the prophase

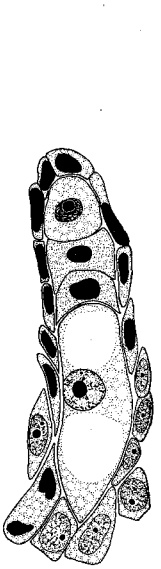


Fig. 7.

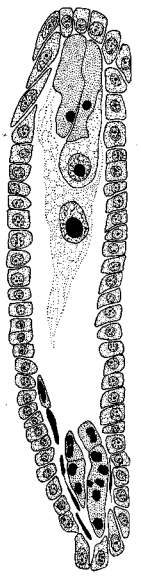


Fig. 8.

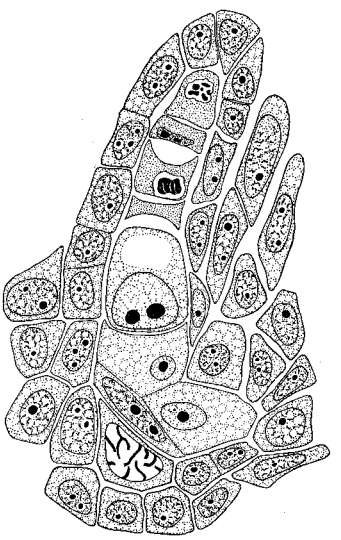


Fig. 9.

Sexual and Aposporic Embryo-sac Development

Fig 7. *C. occidentalis* (2170) — diploid sexual — normal megaspore tetrad with the basal cell enlarging; × 550.

Fig. 8. *C. occidentalis* (2170) — diploid sexual — mature embryo sac with synergids and multiple antipodals already showing degeneration; × 275.

Fig. 9. *C, occidentalis* (2169) — tetraploid aposporic —, degenerating megaspore tetrad (the lower megaspore has no nucleus) with an aposporic cell developing immediately below it; the two cells immediately below the aposporic cell are probably in the first stages of aposporic enlargement; × 550.

stage. Those forms in classes one and two are facultative apomicts while those in class three are obligate apomicts. In the facultative apomicts there was no morphological way of determining whether a

particular ovule would develop apomictically or sexually; that is, there was no regularity in the position of the apomictic and sexual ovules in a head.

Type 1. C. occidentalis (2169) deviates less from the normal sexual development than any of the other apomictic forms; only 28 per cent. of the ovules recorded in table 2 showed any signs of apospory. Of the twelve cases of apospory only four were initiated in the prophase (fig. 13); apospory most commonly occurs after the completion of the megaspore tetrad stage. The aposporic processes in this apomict are essentially similar to those in the other apomictic forms and will be discussed in more detail below.

Only one case was found in which there was any hint that more than one embryo sac may develop aposporically (fig. 9). Directly below the basal megaspore, which has no nucleus and is degenerated, there is a young aposporic embryo sac and just below the latter are two integumentary cells, which are quite distinct in appearance; in comparison with the surrounding integumentary cells they are somewhat larger and have a larger proportion of cytoplasm, which is also of a more watery consistency.

The latest initiation of apospory was found in *C. occidentalis* (2169) (fig. 15); where a small one-celled aposporic embryo sac occurs along with a mature one in the normal position. It is possible that the „normal" embryo sac in this particular case may have been of aposporic origin, but judging from the low frequency of apospory in this form, it is more likely a sexual one.

Type 2. This type was found in all of the apomicts investigated except *C. occidentalis* (2169), discussed above, and *C. occidentalis* subsp. *pumila* apm. *hamiltonensis*, which is the single representative of type 3. In all of the forms of type 2 aposporic development is much more common than normal development, and apospory usually sets in while the EMC is still in prophase. The cell which develops aposporically is situated directly adjacent to the chalazal end of the nucellus. The initiation of apospory may be recognized by the enlargement of this and sometimes other adjacent and contiguous cells, and by the appearance of vacuoles in the cytoplasm of the enlarging cells (Plate, 1, fig. 4). The aposporic nucleus then becomes greatly enlarged, while its contents appear finely granular and weakly staining. The vacuoles continue to increase in size until they are the

most conspicuous feature of the ovule. In many cases the aposporic

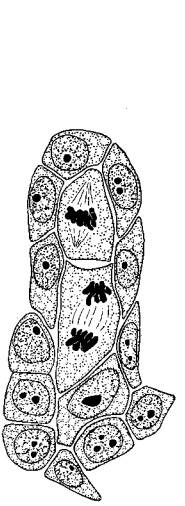


Fig. 10.

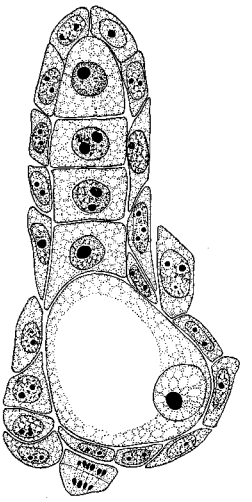


Fig. 11.

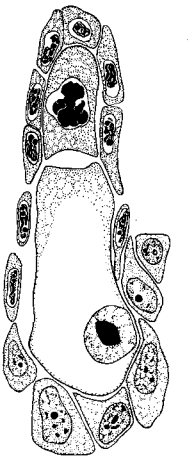


Fig. 12.

Fig. 10. *C. occidentalis* (2117) — triploid aposporic —, megaspore dyad with a cell immediately below showing signs of enlargement; × 550.

Fig. 11. *C. occidentalis* (2117), normal megaspore tetrad with an enlarged aposporic cell at its base; × 550.

Fig. 12. *C. occidentalis* (2117), the megaspore mother cell which has degenerated in prophase, being replaced by a one-celled aposporic embryo sac; × 550.

cell pushes its way into the nucellus, gradually crushing the EMC until finally the latter becomes merely a dark staining crescent of degenerated material (figs. 12, 13; Plate 1, figs. 5–7). At this latter stage the developing aposporic cell looks something like the enlarging chalazal megaspore of a normal tetrad. However, it can be readily recognized by: 1) the abnormally large vacuoles, 2) the presence of one rather than three masses of degenerated material at the micropylar end of the nucellus, and 3) the usually pronounced cavity at its chalazal end, which was formed by the aposporic cell during its first period of enlargement.

In some ovules the aposporic cell does not enter the nucellus, but forms a separate cavity at the chalazal end (fig. 11); under these conditions the EMC may proceed with meiosis undisturbed. In the cases illustrated the aposporic cell is not very large and may have

started development at a later stage than the EMC prophase, but the presence of several cases of meiotic figures and aposporic cells side by side in the same ovule is strong indication that meiosis is able to proceed unless the nucellar contents are actually injured by the developing aposporic embryo sacs. In still other ovules, more than one integumentary cell may enlarge and develop into aposporic

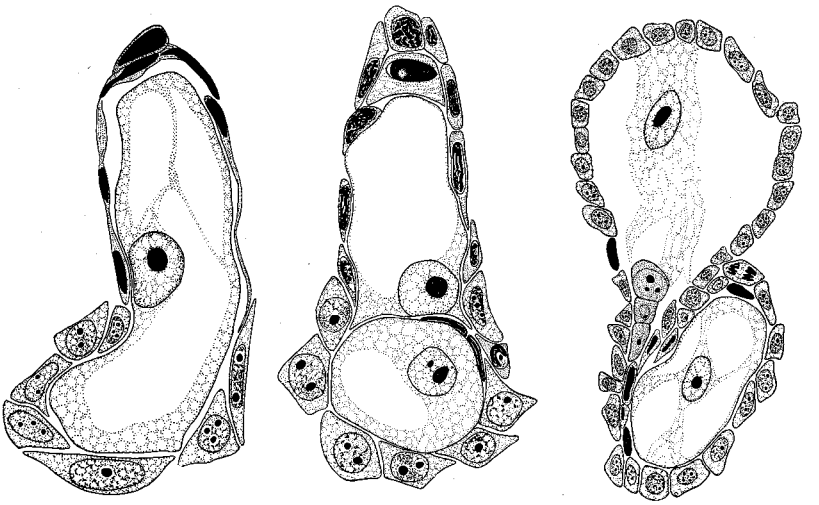


Fig. 13. Fig. 14. Fig. 15.

Aposporic embryo-sac development

Fig. 13. *C. occidentalis* (2169), a one-celled aposporic embryo sac which has so encroached upon the EMC — which probably degenerated in early prophase — that the EMC is reduced to a dark-staining crescent at the micropylar end; × 550.

Fig. 14. *C. occidentalis* (2117), the EMC has degenerated at prophase, immediately below it are two one-celled aposporic embryo sacs — multicellular apospory; × 550.

Fig. 15. *C. occidentalis* (2169), a reconstruction showing the chalazal end of a mature embryo sac in the normal position (with polar-fusion nucleus and antipodals showing); and a young one-celled aposporic embryo sac in the chalazal region; × 275.

embryo sacs. When this occurs the enlarging cells are usually immediately adjacent, but may be separated by a few cells. As a rule, one of them enters the nucellar cavity, and the others form separate smaller cavities in the chalazal region (fig. 14). Plate 1, figures 8 and 9, show two sections from the same ovule of *C. occidentalis* (2117) in

which four aposporic embryo sacs are developing. Plate 1, figure 10, from *C. occidentalis* apm. *hamiltonensis*, shows three young aposporic embryo sacs developing at once. The nucellus and EMC have alreday degenerated. This phenomenon will be referred to as multicellular apospory; that illustrated in Plate 1, figures 4–7, will be termed unicellular apospory. Both types occur together in different florets of the same head, and no relationship could be found between the position of the floret in the head and the type of apospory developed.

TABE 2. THE FREQUENCY OF NORMAL AND APOSPORIC EMBRYO SACS AT THE VARIOUS STAGES OF MEGASPORE-MOTHER-CELL DEVELOPMENT AND THE FREQUENCY OF UNICELLULAR AND MULTICELLULAR APOSPORY IN THE THREE TYPES OF *Crepis* APOMICTS

Species	Megaspore at late prophase		Megaspore at metaphase I to telophase II		Megaspore at tetrads and later		Total		Percent apospory	Type of apospory	
	Nor. [1]	Apo. [2]	Nor.	Apo.	Nor.	Apo.	Nor.	Apo.		uni-cellular	multi-cellular
TYPE 1.											
C. occidentalis typica (2169)	2	4	2	2	27	6	31	12	28	11	1
TYPE 2.											
C. occidentalis (2117)	5	23	1	2	2	3	8	28	78	11	11
C. occidentalis conjuncta (1589)	1	18	0	0	2	1	3	19	87	—	—
C. modocensis subacaulis (2159)	0	67	1	0	13	6	14	73	84	52	21
C. acuminata (2163)	1	21	1	0	4	7	6	28	82	15	13
C. intermedia (2173)	0	25	0	0	4	2	4	27	87	8	19
C. intermedia (2499)	3	34	0	4	7	7	10	45	82	35	10
TYPE 3.											
C. occidentalis pumila hamiltonensis	0	57	0	0	0	0	0	57	100	13	44

[1] Nor. — normal development; that is, absence of apospory.
[2] Apo. — apospory; one (unicellular apospory) or more (multicellular apospory) aposporic embryo sacs are present.

The various apomicts of type 2 differ from each other in two respects; first in the relative frequencies of unicellular and multicellular apospory, and second in the stage of EMC development when apospory begins (Table 2). In respect to the first point, the number of ovules was too small to establish significant differences between the various apomicts, except in the case of the two extremes: that is,

C. modocensis (2159) and *C. occidentalis* (2117), on the one hand, in which the majority of the ovules have unicellular apospory, and *C. intermedia* (2173), on the other, in which the majority of the ovules have multicellular apospory. *C. acuminata* (2163) is about intermediate between the two extremes.

In respect to the second point, *C. modocensis* (2159) and *C. occidentalis* (2117) show no sign of apospory until the meiotic prophase in the EMC is well advanced, and the PMCs are in the tetrad or early pollen-grain stage. In *C. intermedia* (2173), on the other hand, aposporic development frequently begins during the early prophase of the EMC, and while the PMCs are also in the prophase stage. In these forms there is a positive correlation between the early initiation of apospory and the occurrence of multicellular apospory.

C. acuminata (2163) is difficult to classify in regard to the onset of apospory. The EMCs degenerate some time during early prophase, and the PMCs also degenerate during mid prophase. However, the youngest ovules in which apospory can be found are 180–250 μ (average, 218 μ) long, while in*C. intermedia* (2173) the corresponding ovules are only 130–190 μ (average, 170 μ) long. Since the mature ovules and achenes are about the same size in the two apomicts, this indicated that apospory begins at a slightly later stage in *C. acuminata* (2163), and thus at nearly the same stage as in *C. modocensis* (2159) and *C. occidentalis* (2117). In other words *C. acuminata* (2163) is again intermediate between the two extremes. In both the greater frequency of multicellular apospory and the early stage at which the process begins, *C. intermedia* (2173) — and probably also (1700) — is in a class by itself, as compared with the other facultative apomicts.

In occasional ovules of all of the apomicts the contents of the nucellus are completely black and degenerate, but no aposporic cells have developed. In these ovules there is usually a small mass of degenerated tissue in the region where aposporic cells usually develop. This suggests that they represent cases of the degeneration of both the sexual and the aposporic cells. In the ovules which contain normal megaspore tetrads there are occasionally similar masses of degenerated tissue. Apparently the aposporic cells may occasionally start to develop, subsequently degenerate and wither, with or without the development of a normal gametophyte.

Type 3, obligate apospory. — This type of development was found

only in *C. occidentalis* subsp. *pumila* apm. *hamiltonensis*. The nature of the process is essentially as in *C. intermedia* (2173), except that multicellular apospory is even more frequent (table 2). Plate 1, figure 10 shows three aposporic embryo sacs developing at once in this apomict. Occasionally in apm. *hamiltonensis* the aposporic cell is separated from the nucellus by a row of normal integumentary cells, but the position of the former in the chalazal region is essentially as in the other apomicts. As in *C. intermedia* (2173), apospory begins when the PMCs are still in prophase. The main difference, therefore, between obligate apospory and the extreme expression of the facultative type found in *C. intermedia* (2173) is the complete failure of the EMC to develop beyond early prophase.

Development of the embryo sac and embryo. When the aposporic cell has occupied the position of the nucellus its further development resembles, in general, that of a normal megaspore. The greater size of the vacuoles, however, often makes the young embryo sac longer than is the sexual one; and at the 2- or 4-celled stage the nuclei at the micropylar end may be thrust into the micropyle. The three successive divisions to form the eight-nucleated embryo sac are apparently very rapid, since only one or two of them were seen in all of the material examined; and they were entirely somatic in nature. After the embryo sac is formed the antipodal nuclei degenerate, while the polar nuclei may or may not fuse. Many of the mature embryo sacs completely resemble normal sexual ones, but various abnormalities are frequently present. One of these, the presence of synergids within the micropyle far below the egg nucleus (Plate 2, fig. 3), is apparently caused by the extreme elongation of the embryo sac at the 2- and 4-nucleate stages. Another is the failure of the nuclei at the micropylar end to become differentiated into egg and synergids (Plate 2, fig. 4).

In addition to the 8-nucleated embryo sac in the normal position, there are often smaller embryo sacs at its chalazal end (fig. 15, Plate 2, figs. 5 and 6). These generally have only one to four nuclei, even when fully mature, and are only partly differentiated. The two embryo sacs present in the same ovule may represent a normal and an aposporic one, or if multicellular apospory has taken place, two different aposporic ones. There is no way of telling at this late stage which is the case.

The endosperm and embryo of the aposporic embryo sacs start to develop before the buds have opened. Embryo development is preceded by a rapid series of successive divisions of the endosperm nucleus, so that embryo sacs containing many endosperm nuclei, and an undivided egg or 2–3-celled embryos are not uncommon (Plate 2, figs. 1–3 from *C. occidentalis* (2117), and figs. 7–11, from *C. occidentalis* (2169). These endosperm nuclei differ from ovule to ovule

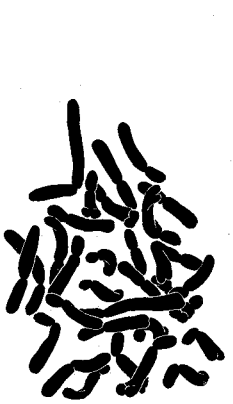


Fig. 16.

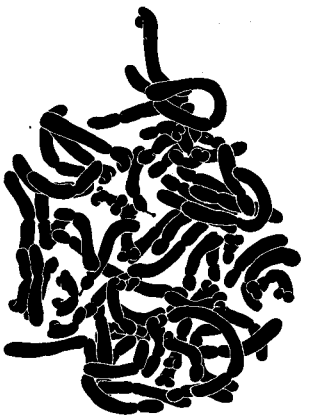


Fig. 17.

Fig. 16. *C. occidentalis* (2117), metaphase plate of a dividing endosperm nucleus with the somatic number of chromosomes; × 2500.

Fig. 17. *C. occidentalis* (2117), metaphase plate of a dividing endosperm nucleus with the 4n number of chromosomes. This plate is also illustrated in Plate 2, figure 5; × 2500.

in size and in the number of nucleoli that they contain. The smaller nuclei probably have the somatic (2n) number of chromosomes, and the larger ones twice that number (4n); that is, as mentioned above, polar fusion may or may not take place. Figure 16 shows an endosperm mitosis with about 33 (2n) chromosomes from *C. occidentalis* (2117) while that in figure 17 has about 66 (4n). This latter plate is the one shown in Plate 2, figure 5. Plate 2, figure 7 shows an embryo from an unopened bud of *C. occidentalis* (2169).

DISCUSSION

The mechanism of apospory. As has been emphasized in the descriptive section, the chief features of the early development of the

aposporic cell are: first, the vacuolisation of the cytoplasm; and second, the increase in size and decrease in staining capacity of the nucleus. Both of these processes appear to be associated with the intake of water. Apparently some internal change takes place in the cell destined to become an aposporic gametophyte, which enables its cytoplasm to take in a large amount of water (and perhaps nutrient material as well); and later permits the hydration of the nucleus. The anbormal vacuolisation of the cytoplasm is a phenomenon peculiar to somatic apospory; it does not occur in generative apospory (that is, in *Antennaria*). The hydration of the nucleus, however, is a conspicuous feature in three genera with apomeiosis, viz., *Hieracium* subg. *Archieracium*, *Antennaria*, and *Eupatorium* (GUSTAFSSON, 1935, p. 39); it is, in fact, more extreme in these genera than in *Crepis*. The first division of the aposporic nucleus in *Crepis*, though rare, has been seen among the few hundred nuclei examined, and hence is probably not carried through with as great rapidity as in *Hieracium* subg. *Archieracium* and *Antennaria*. This division in *Crepis* is, of course, purely somatic. Both generative and somatic apospory, therefore, are in many cases brought about by a physiological change in certain cells, which among other things produces a change in water relationships. In somatic apospory this change affects the cytoplasm before the nucleus, while in apomeiosis only the nucleus is affected.

Apomixis and Polyploidy. Crepis is no exception to the general rule in apomictic groups that the diploid species are sexual, and the apomictic forms are polyploid. It furthermore supports the opinion expressed by GUSTAFSSON (1935, p. 61) that there is no direct correlation between the degree of polyploidy and the development of apomixis. The tetraploid *C. occidentalis* (2169) is less strongly apomictic than the triploid of the same species (2117). On the other hand, the only obligate apomict known in this genus, *C. occidentalis* apm. *hamiltonensis*, has the highest chromosome number of any of the forms here investigated. There is little doubt that the mechanisms which produce apomixis all function better in polyploids than in diploids. Several authors have shown that polyploidy produces various physiological changes in the cells, affecting among other things their osmotic relationships and their water and food content (cf. MÜNTZING, 1936, pp. 295–298). These changes may be responsible for the greater development of apomixis in polyploids.

The probable genetic mechanism of apospory. The entire process of somatic apospory as it occurs in *Crepis* shows a striking resemblance to that described by ROSENBERG (1907) for *Hieracium* subg. *Pilosella*. The position of the aposporic embryo sac and its relationship to the normal one are identical in the two genera; and in both of them the species are, for the most part, facultative apomicts. There is every reason to believe, therefore, that both the mechanism of apospory and the genetic behavior of the species as a result of this process are similar in the two groups. OSTENFELD (1910) has shown that in crosses between the facultatively aposporic *Hieracia* subg. *Pilosella*, the tendency for apospory may or may not be carried into the F_1; that is, it behaves as if it were controlled genetically, either by single genes or by systems of complementary genes. The same has been shown by ANDERSSON-KOTTÖ (1932) for the fern *Scolopendrium*, and by BERGMAN (1935) for *Leontodon hispidus*. The situation in *Crepis*, therefore, is probably similar. Evidence for this hypothesis is the fact that closely related forms may have different types of apospory, for example, *C. occidentalis* (2169) and (2117).

There is no correlation between the type of apospory and the systematic relationships of the various apomicts, as is evident from Table 2. There is, furthermore, no correlation between the type of development that occurs in the PMCs and that in the EMCs (see above, p. 204). The different types of abnormalities found behave as if they were governed by independent genetic mechanisms.

Hybridization and apomixis. As has been demonstrated elsewhere (BABCOCK and STEBBINS, 1938) these species of *Crepis* constitute another group in which hybridization and polyploidy are closely associated with apomixis. They differ from most apomicts, however, in that the meiotic abnormalities usually associated with hybridization are not strongly developed. In the case of some apomicts, such as *C. occidentalis* (2169), there is every reason to believe that in the absence of apomixis, the form could maintain itself perfectly well by means of sexual reproduction, without sterility more than is found in many sexually reproducing polyploids. In other words, apomixis in *Crepis* does not appear to be caused by hybrid sterility, although in many cases this sterility makes apomixis the only means by which a particular form can survive. The opinion of HOLMGREN (1919), ROSENBERG (1930), and GUSTAFSSON (1935), that apomixis is an ac-

companying but not a resultant phenomenon of hybridization, is well supported by the evidence from *Crepis*. Hybridization may, in many cases, bring together complementary genes which produce apomixis; but in the absence of such genes, neither hybridization alone or hybridization plus polyploidy are able to bring about apomixis.

The evidence from *Crepis*, furthermore, supports the opinion that the degeneration phenomena in the PMC meiosis of many apomicts are the result not of chromosomal incompatibility due to hybridization, but of the influence of specific genes or gene combinations. GENTSCHEFF (1937) is of the opinion that the abnormal behavior of the chromosomes in apomicts of *Hieracium* subg. *Archieracium* is due to a gradual degeneration of the tapetum as well as of the surrounding tissue. The most conspicuous abnormality in the PMCs of *Crepis* — the degeneration of the pollen in *C. acuminata* (2163) — is most certainly produced in this fashion. Furthermore, in both *Crepis* and *Hieracium* the degeneration phenomena although most frequent in allopolyploids, also occur in polyploids which are apparently autopolyploid. *C. acuminata* (2163) is, on the basis of external morphology, an autopolyploid. *Hieracium canadense*, in which both Alton Gustafson (1933) and GENTSCHEFF (1937) have demonstrated the semi-heterotypic division, is so close morphologically to *H. umbellatum* that some systematists have treated it as a mere synonym, and not even as a variety or a subspecies; therefore, it may be an autopolyploid of the sexual *H. umbellatum*. Since in *Hieracium excellens* × *aurantiacum* Ostenfeld (1910) has shown that there is segregation for pollen degeneration in the F_1 generation, there is good reason to believe that all of the types of pollen degeneration and extreme meiotic abnormalities characteristic of apomicts are the result, not directly of hybrid incompatibility, but of genetic mechanisms. Some of these may find their expression through the coming together of complementary genes in the hybrids, but this does not require the hypothesis that the hybridization is the cause either of apomixis or of the abnormalities in the PMCs.

Facultative vs. obligate apomixis and variability. If the apomicts of *Hieracium* subg. *Pilosella* (ROSENBERG, 1907) are compared in respect to the frequency of apospory to those of *Crepis*, *H. excellens* probably corresponds most closely to *C. occidentalis* (2169). In the

former, apospory occurs „in many ovules, where the EMC had been divided into tetrads" (ROSENBERG, *op. cit.*, p. 156); and this is about as has been described above for *Crepis occidentalis* (2169), representing type 1 of the aposporic series. OSTENFELD (1910) found that in a cross with *H. aurantiacum* as pollen parent, *H. excellens* produced 20 apomictic progeny and 6 hybrids, 3 of which were vegetatively weak, and 3 vigorous, apparently sufficiently so to have survived under normal conditions. In other words, this particular cross resulted in considerably more apomictic than sexual reproduction. Since *H. excellens* and *H. aurantiacum* are only fairly close to each other systematically, it is possible that if *H. excellens* were pollinated with some more closely related form, a greater proportion of vigorous offspring would be produced sexually. Nevertheless, there is reason to believe that in nature both *H. excellens* and *C. occidentalis* (2169) reproduce at least as frequently and perhaps more frequently by the apomictic than by the sexual method.

The type of development in *Hieracium flagellare* and *H. aurantiacum*, in which an aposporic embryo sac develops in nearly every ovule (ROSENBERG, 1907, pp. 157–158), is more nearly like type 2 as described for *Crepis*. The only evidence as to the actual frequency of apomixis in these species is derived from the classic experiments of MENDEL (CORRENS, 1905). He was unable to obtain hybrid offspring from *H. aurantiacum* in spite of repeated efforts. Neither *H. flagellare* or *H. aurantiacum* were used by Ostenfeld as ovulate parents for his hybrids. The results suggest that the actual frequency of sexual reproduction in facultative apomicts of this type is much less than that indicated by the frequency of reduced embryo sacs. There is reason to believe, moreover, that sexual reproduction is much less common in the *Crepis* apomicts of type 2 than is indicated by the presence in them of 13 to 22 per cent of one-celled embryo sacs derived from reduced megaspores. In the first place, examination of mature ovules indicates that, as in *Hieracium*, some of the embryo sacs degenerate; many of these are undoubtedly derived from megaspores with an abnormal chromosome complement. This is particularly likely in the 33 and 55 chromosome types, but even in those with 44 chromosomes there is occasional elimination of univalents and formation of microcytes, so that some of the megaspores have less than the normal haploid number. Furthermore, there are

probably some embryo sacs which develop to maturity in spite of an abnormal chromosome complement, but of which the egg cell may form a lethal zygote in combination with some gametes from the pollen parent. In addition, the phenomenon of self-incompatibility, although it has not been demonstrated in these particular species, is so widespread in *Crepis* that it very likely prevails here. If this is true, then a reduced egg cell could not be fertilized by a plant of the same apomictic clone, and the functioning of even the normal reduced eggs would be expected only in localities where two or more clones of the species grow near together. Finally, the degeneration of pollen in whole series of related clones of *C. acuminata* and other species would make wide crossing necessary for sexual reproduction of these species in many localities. When all of these facts are taken into consideration, the most likely situation is that in the apomicts of type 2 not more than about 5 per cent. of the individuals are produced sexually, and in clones that are isolated geographically the proportion of sexual offspring may be only a fraction of one per cent.

This hypothesis agrees with the observation of these *Crepis* apomicts made in the field. In regions where *Crepis* is abundant and which are close to or within the ranges of the diploid forms, almost every colony contains a large number of apomicts, some of which are represented by many individuals and some by only a few (Babcock and Stebbins, 1938). On the other hand, the isolated localities near the periphery of the range of the groups are usually occupied by only one or two apomictic forms, and these have relatively wide ranges. One of these isolated apomicts, *C. intermedia* apm. *lacustrensis* (2499), is known to produce sexual embryo sacs (table 2), yet it has been collected in three different localities within a radius of twenty miles (32.2 kilometers) and at each station the numerous individuals were all identical with each other and with those of the other stations. In this apomict sexual reproduction, though possible, must take place very rarely or not at all.

It is now evident that the entire pattern of variation in these species of *Crepis* — extreme polymorphism near the center of the range of the group, but relative constancy in any particular colony, and comparative constancy near the periphery of its range — can be explained on the basis of predominantly apomictic reproduction,

with a varying proportion of the sexual type. New hybrids and segregating types can constantly be produced by this latter method. If these new forms are vegetatively vigorous and adapted to their environment, they will maintain themselves by apomixis. Their constancy will depend first on the percentage of aposporic embryo sacs produced, second on the degree of gametic sterility present, and third on the degree of geographic or ecological isolation from other apomicts. In connection with the second point, it is probably no mere accident that many of the most widespread and constant of the apomicts are „unbalanced" chromosomal types — that is, those with 33, 55, or 77 chromosomes. These types undoubtedly have a relatively low frequency of sexual reproduction, due to the rarity with which the gametes contain an euploid number of chromosomes.

In view of the variability which can be produced by even very occasional sexual reproduction in these apomicts, there is no reason to postulate any other mechanism for the production of new variants. The process of „apogamic mutation", for which the only clear evidence is that of Ostenfeld (1921) in the obligate apomicts of *Hieracium* subg. *Archieracium*, probably does not occur in *Crepis*. DARLINGTON (1932, pp. 472–474; 1937, p. 475) has suggested that this phenomenon is explained by means of crossing over and segregation, followed by the formation of diploid „restitution nuclei". This process is obviously impossible in *Crepis*, since no restitution nuclei are ever formed in the megasporocytes. In *Hieracium* subg. *Pilosella*, ROSENBERG (1907), p. 156) has described rare instances of dyads in *H. excellens*, but his illustrations (Plate 1, figs. 19 and 20) resemble, both in the character and position of the nuclei, the situation produced by the encroachment of an aposporic cell into the nucellus while the megaspore is still in prophase (cf. his fig. 16; Plate 1, figs. 5 and 6 of this paper). The only evidence for „apogamic mutation" in subgenus *Pilosella* is a single „mutant" in an F_2 population derived from *H. excellens* × *aurantiacum* (OSTENFELD, 1910 p. 263). Since the different F_1 individuals of this particular cross have different chromosome numbers (Rosenberg, 1917), and the particular plant (No. 463) which was the parent of the mutant was an aneuploid (Rosenberg, *op. cit.* p. 167), this „mutant" may have been the result of the elimination of chromosomes during the somatic divisions in the formation of the embryo sac or the embryo. Such elimination occurs in the first

division of the generative aposporic embryo sac of *Antennaria* as well as in the purely somatic divisions in the antipodal region of the embryo sacs (STEBBINS, 1932). There is, therefore, no good evidence for „apogamic mutation" of the type found in *Archieracium* in any group with somatic apospory.

C. occidentalis subsp. *pumila* apm. *hamiltonensis* is as yet the only obligate apomict known among the angiosperms with somatic apospory. Its chromosome number (2n = 77) indicates that it must be a secondary derivative from other polyploid types, and in view of what is known about the other polyploids of *Crepis*, apm. *hamiltonensis* was almost certainly derived from facultative apomicts. In other words, obligate apomixis in *Crepis* has been derived from the facultative type. The suggestion has been made elsewhere (BABCOCK and STEBBINS, 1938) that this is also the case in *Antennaria* in spite of the opinion of Gustafsson (1935, p. 60) to the contrary. A careful cytological study of apomicts of *Antennaria*, as well as of *Taraxacum* and *Hieracium* subg. *Archieracium*, in regions in which closely related sexual and apomictic forms grow side by side, will be necessary before the question can be settled as to whether obligate apomixis always arises suddenly in these groups, or whether it is preceded by the facultative type.

SUMMARY

1. According to the classification of apomictic phenomena which has been adopted for this work, reproduction in the polyploid forms of the North American species of *Crepis* is by means of somatic apospory followed by diploid parthenogenesis.

2. Meiosis in the pollen mother cells of the sexual, diploid forms of *Crepis acuminata* and *C. occidentalis* is normal. In tetraploid *C. occidentalis* and *C. intermedia* PMC meiosis is characterized by the presence of 2 to 7 multivalents and 1 to 4 univalents. In both of these forms 30 to 40 per cent. of the sporocytes show chromatin bridges and fragments at first anaphase, indicating chiasma formation in inverted segments. Aside from the irregularities produced by the above mentioned causes, meiosis is completed normally.

3. In one apomict of *C. occidentalis* with 2n = 77 chromosomes, chromatin bridges are very common, and fusion of chromosome

groups belonging to different spindles frequently occurs at second metaphase or anaphase. This results in the production of triads or dyads, and of microspores with the unreduced number of chromosomes.

4. The frequent presence of chiasma formation in inverted segments in the polyploids, along with its absence in the diploids, is cytological evidence for the allopolyploid nature of the former. This agrees with the evidence from the morphological appearance of the forms investigated. Evidence is presented to show that the presence of multivalents in these forms is not incompatible with the assumption of their allopolyploid origin.

5. Degeneration of the pollen mother cells takes place in *C. acuminata* during the mid-prophase of meiosis. This is preceded by the degeneration of the tapetal cells.

6. Megasporogenesis and the formation of the embryo sacs in the diploid sexual forms is normal in every respect.

7. The polyploid apomicts may be grouped into three classes in respect to the frequency of apospory and the time at which it begins. In a tetraploid form of *C. occidentalis* apospory is found in 28 per cent. of the ovules and usually begins after the megaspore tetrads have formed. In the majority of the apomicts, including polyploid forms of *C. occidentalis*, *C. acuminata*, *C. intermedia*, *C. modocensis*, *C. monticola* and *C. pleurocarpa*, apospory occurs in 78–87 per cent. of the ovules, and usually begins while the megaspore mother cell is still in prophase. In one form of *C. intermedia* aposporic development begins when the PMCs are still in meiotic prophase. In this form, the aposporic development of two or more cells occurs in most of the ovules. This phenomenon is termed multicellular apospory. It occurs in the other apomicts, but the development of a single cell, or unicellular apospory, is more common in them. The apomicts of these first two classes are facultative apomicts.

8. In one apomict of *C. occidentalis* with $2n = 77$ chromosomes, apospory occurs in every ovule. It begins while the PMCs are in meiotic prophase, and is most often of the multicellular type.

9. Occasional abnormalities are found in the development of the aposporic embryo sac. Embryo formation in the apomicts begins before the buds open, and is preceded by the rapid division of the endosperm nuclei. These may have either 2n or 4n chromosomes, depending on whether or not the polar nuclei fuse.

10. The great hydration of the aposporic cell, which affects first the cytoplasm and then the nucleus, suggests that the mechanism of somatic apospory involves a change in water relationships at the chalazal end of the nucellus.

11. The close parallel between these species of *Crepis* and those of *Hieracium* subg. *Pilosella* suggests that the genetic mechanism of apospory is the same in the two groups. In *Crepis* the majority of the apomicts are of hybrid origin (allopolyploids), but hybridization is probably an accompanying phenomenon rather than the cause of apomixis.

12. Various lines of evidence indicate that the actual proportion of sexual to aposporic development in the facultative apomicts is much less than that which would be expected if all of the embryo sacs formed from megaspores should function. The presence of predominantly apomictic reproduction, together with the occasional production of segregating and hybrid types by means of the sexual process, accounts very well for the pattern of variability found in these species.

13. The chromosome number of the only obligate apomict found in *Crepis* indicates that it has been derived secondarily from facultative apomicts. In *Crepis*, obligate apomicts apparently do not arise directly from sexual forms.

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