Morphology of cannabis sativa L

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MORPHOLOGY OF CANNABIS SATIVA L.

by

JOYCE REED

THESIS
SUBMITTED IN PARTIAL FULFILLMENT OF REQUIREMENTS
FOR THE DEGREE OF MASTER OF SCIENCE.

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## INDEX

<table>
<thead>
<tr>
<th>Introduction</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Object of study</td>
<td>1</td>
</tr>
<tr>
<td>Methods</td>
<td>2</td>
</tr>
<tr>
<td>Obligations</td>
<td>2</td>
</tr>
<tr>
<td>The Archichlamydeae</td>
<td>3</td>
</tr>
<tr>
<td>Characters of Urticales</td>
<td>3</td>
</tr>
<tr>
<td>Characters of Cannabis sativa L</td>
<td>4</td>
</tr>
<tr>
<td>Historical resume</td>
<td>6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>The Inflorescences</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Flowering season</td>
<td>10</td>
</tr>
<tr>
<td>Staminate inflorescence</td>
<td>10</td>
</tr>
<tr>
<td>Pistillate inflorescence</td>
<td>11</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Floral Organs and their Development</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Staminate flower</td>
<td>13</td>
</tr>
<tr>
<td>Pistillate flower</td>
<td>13</td>
</tr>
<tr>
<td>Perianth of pistillate flower</td>
<td>15</td>
</tr>
<tr>
<td>Floral bract of pistillate flower</td>
<td>16</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nature of Ovary and Origin of Ovule</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovule</td>
<td>18</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Megasporangium and Female Gametophyte</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovule</td>
<td>22</td>
</tr>
<tr>
<td>Megasporangium</td>
<td>23</td>
</tr>
<tr>
<td>Female gametophyte</td>
<td>25</td>
</tr>
<tr>
<td>Fertilization</td>
<td>26</td>
</tr>
</tbody>
</table>
INTRODUCTION.

Object of Study.

Cannabis sativa L., the common hemp, has long been of interest because of its commercial, agricultural, chemical, and physiological importance and because of its dioecious character. The flower, fruit, and seed of Cannabis have been discussed by many writers. The dubiety regarding its floral structure, as to the correlation of the floral parts of the two sexes, and the nature of the organs that compose them, has been dealt with in an extensive literature. Since the plant is a strictly dioecious form the problem of its sexuality is of great importance and has been considered by numerous experimenters on diandinism. The nature, origin, ratio and significance of sex are questions which are being much investigated and discussed. Research on the subject of diandinism is going more and more into the inner morphology of plants in an attempt to solve the important question of sex determination and related problems of heredity. The critical morphology of the floral structures of Cannabis sativa L., especially that of the staminate flower, has been but briefly touched upon. Hence at the suggestion of Dr. R. B. Wylie this study was undertaken in the hope of contributing somewhat to the knowledge of the morphology of the plant, and perhaps aid in solving the great question of sexuality. Since the species is included in the Urticales, one of the primitive orders of the Archichlamydeae, a brief discussion of the characters of this great group will be entered upon.
Methods.

The material for the study was collected at different times in and around Iowa City, Iowa during the summers of 1912 and 1913. Most of the material was killed in one per cent chromo-acetic acid which proved very satisfactory for all stages of development. After washing, the material was run up to seventy per cent alcohol from which portions of each collection were embedded in paraffin. Microtome sections were cut 5-18 micrones in thickness. The stains used were Delafield's haematoxylin, Flemming's triple stain and Haidenhain's iron-alum-haematoxylin. Delafield's haematoxylin was used to advantage in the study of the younger stages. Flemming's triple stain and Haidenhain's iron-alum-haematoxylin proved to be better for the older stages and the latter was especially good in the study of the nucleus.

Obligations.

The work was carried on under the supervision of Dr. R. B. Wylie to whom I am greatly indebted for kindly encouragement, and helpful suggestions and criticisms. My thanks are also due to Professor B. Shimek for aid in securing reference books, and to Mr. C. H. Farr for his interest and numerous courtesies.
The Archichlamydeae.

The Archichlamydeae or Choripetalae, the more primitive of the two great divisions of Dicotyledones are distinguished in the main from the Sympetalae by apetaly or polypetaly. The group is vast and widespread including 61000 species and 180 families scattered in all regions. The most primitive of Dicotyledones belong here but the classification of the group is confusing and indefinite because of the fluctuating characters and the varying opinions concerning the details of classification. The general sequence of the divisions of the group is based upon the development of the perianth and floral axis and the arrangement of the floral members. However there can be no real sequence among the divisions of the Archichlamydeae, for they represent, mainly, parallel or divergent lines of development. The more primitive orders of the group are especially puzzling since they exhibit a rather heterogeneous assemblage of forms. This region has recently received much attention from morphologists but still greatly needs investigation in order that the various forms may be recombined into natural groups.

Characters of the Urticales.

The Urticales or Urticineae are among the more primitive of the Archichlaimdeae, being the ninth of the orders or alliances arranged by Engler (24) and given the same rank by Gray (30). The order contains about 1560 species and is largely tropical although also represented in our native flora. The Urticales include both herbaceous and woody plants with variously shaped always stipulate leaves and small, inconspicuous flowers closely aggregated into thick inflorescences. The flowers are monoecious, dioecious or polygamous and are cyclic in arrangement of
parts with a double perianth of similar members, or, seldom naked. The stamens are opposite the perianth segments and usually of the same number. The ovary is superior, composed of one or two carpels, usually unilocular and contains a single ovule with two integuments. The fruit is a nut or drupe and the seeds usually contain endosperm. The order Urticales has been variously divided into families and subfamilies by different authors. Goebel (28) divides the group into the families Urticaceae, Ulmaceae, Platanaceae and Cannabinae. Engler (24) and Britton (10) include in the order the families Ulmaceae, Moraceae and Urticaceae. According to Gray (30) the order comprises but one family, the Urticaceae which is subdivided into five tribes the Ulmeae, Celtideae, Cannabinae, Moreae and Urticiae.

Characters of Cannabis sativa L.

Cannabis sativa L., the species under consideration, is the sole representative of its genus in our flora. Gray (30) has included Cannabis and Humulus in the tribe Cannabineae of the family Urticaceae. Britton (10) has classified it in the family Moraceae separating it from the Urticaceae chiefly because of the character of its filaments which are straight in the bud instead of reflexed and of the ovule which is suspended instead of erect.

Cannabis sativa L. is a stout erect, annual, branching herb varying in size with the climate and soil in which it grows. In temperate regions it varies from 3 to 10 feet in height while in warmer climates it may attain the height of 15 to 20 feet. The leaves are digitate 5-11 palmately divided, alternate above and opposite-petioled below and beset with stiff hairs. Persistent subulate stipules are present. The
flowers are dioecious, small, greenish and axillary. The staminate flowers composed of a five segmented perianth and five drooping stamens are panicled. The pistillate flowers consist of a gamophyllous inconspicuous perianth closely embracing an ovoid ovary and are spike-clustered. The inner bark of the stem is fibrous furnishing the hemp fiber of the market. The plant is typically dioecious the pistillate plant being larger and more robust than the staminate and having a darker and more luxuriant foliage when mature. The two plants are scarcely differentiated before the flowering season. However when cultivated the staminate plants are recognizable at an early stage by certain minor characters which are useful to special experts in weeding them out. The staminate plants do not continue growth after flowering but turn yellow and soon die. The pistillate plants, however, continue their growth and become larger and stronger remaining green until killed by the frost.

This species is found widely distributed in all temperate regions of North America. It also inhabits cooler parts of India where it is native and whence it was transplanted to Europe and to America.
Historical Resume.

As before mentioned Cannabis Sativa L. has long been of interest to botanists and appears frequently in the literature. Most of the work centers around the structures of the pistillate flower and the nature of the dioecious character of the species. Since the recent papers on the subject are the more critical and also more available for study these only are taken into consideration. Frequent reference to the older works is made in the later publications thus extending the scope of the summary. The details of the literature bearing upon particular parts are taken up in connection with the several structures in the following pages and hence only a brief synopsis of the field covered will be made at this point.

The most ponderous and complete work on the subject of Cannabis was published by two Italian botanists, Briosi and Tognini (9), in 1894 and 1896. This work which appeared in two parts embraces a detailed study of the internal anatomy of the plant and is illustrated by numerous photographs and plates. Part I, published in 1894, deals with the flowers, the inflorescence, organography, organogeny, megasporangium, female gametophyte, microsporangium and male gametophyte. A discussion of the diclinism of the species is also included here. Part II, which appeared two years later, is a study of the internal anatomy of the vegetative organs, cotyledones, leaves, stipules, stem, and root.

In 1898 a contribution to the morphology of the pistillate flower and inflorescence of the Cannabineae was made by Zinger (69). Humulus japonicus and Cannabis sativa were studied comparatively as regards the nature and development of the inflorescence and floral
organs of the pistillate flower. The author constantly refers to the work of various writers on the subject and is inclined to agree with the views of the older morphologists rather than those of the more recent authors.

Montemartini (44) in 1902 published a work upon the morphology of the ovary and ovule of Cannabis. The vascular supply of these parts was studied with a view to interpreting the origin of the parts. The conclusions agree in the main with those of Briosi and Tognini (9) on this point.

A treatise on the morphology, teratology, and diclinism of the flowers of the Indian-grown Cannabis by Major D. Prain (50) was published in 1904. The description of the normal flowers is followed by accounts of abnormalities observed by the author. A full discussion of the nature of the ovary and origin of the ovule is taken up, the conclusions being supported by teratological phenomena observed. The subject of diclinism is discussed briefly with reference to the abnormalities and also to the work of older writers on the subject.

The work of Modilewsky (43) which appeared in 1908 is a study of the embry development of several genera of the Urticaceae. Elatostema sessile, Dorstenia drakeana, Urtica dioica, Dorstenia contrayerva, Urtica cannabina, Urtica pilulifera, Pilea grandis, Pilea nummulariaefolia, Boehmeria platyphylla, Celtis occidentalis, Cannabis sativa and Humulus japonicus are the species which were studied and compared. The female gametophyte, fertilization and development of the embryo are considered in the different species and general conclusions are drawn which apply to the group as a whole.
Since Cannabis sativa L. is dioecious, easily cultivated, and very wide in its distribution it has been the subject of much investigation in relation to questions of sex. Problems of sex differences, sex ratio, sex constancy, sex determination and sex inheritance have led to numerous experiments to which more detailed reference will be made in the discussion of dicylinism. Some of the more important of the investigations were carried on by Haberlandt (23), Saccardo (53), Heyer (34), Dusing (21), Hoffman (35), Fisch (25), Noll (46), Strasburger (62), and Sprecher (57).

A paper published by Noll (46) in 1907 dealing with the determination of sex in dioecious plants records many experiments chiefly upon hemp plants. Isolated pistillate individuals were pollinated in various ways in an effort to determine the time of sex differentiation and the cause of sex proportion. Although Noll's interpretations of his results are questioned by later authors on the subject, the experiments are carefully recorded and have contributed largely to the knowledge of the subject.

Strasburger (62) in 1910 made a contribution concerning the cause of sex determination. Refined experiments in an attempt to show the differentiation of sex in the pollen tetrad resulted negatively. However important results were obtained from investigations upon the sex proportion by cross-pollinating and cutting back plants of Mercurialis annua, Melandryum rubrum and other dioecious forms. A cytological study of the pollen mother cells of various dioecious plants including Cannabis sativa L. was made in the hopes of finding here an analogy to the heterochromosomes of the animal kingdom. The results
seem to prove conclusively that no such apparent differentiation is to be found in the chromosomes of these plants.

A study of the variability of sex in Rumex acetosa L. and Cannabis sativa L. was published in 1913 by Andreas Sprecher (57). A splendid resume of the ground covered by earlier investigators is followed by a discussion of various phases of the question of sexuality. The author's contribution consists of experimental work upon the difference in variation which occurs in the staminate and pistillate plant of the same species when grown under different conditions. Variations in length, weight and osmotic pressure of the two sexes are recorded according to a statistical method.
THE INFLORESCENCES.

Flowering Season.

The flowering season of Cannabis sativa L. varies according to the climate and the season. In Iowa it is usually from June to September. The staminate and pistillate plants differ somewhat in respect to their time of flowering, the former blooming slightly earlier than the latter. After the staminate plants have finished blooming they turn yellow and die while the pistillate plants increase in size and vigor until late in September and remain green until killed by the frost.

Since Cannabis sativa L. is normally strictly dioecious, the flowers of the two sexes will be considered separately. As is usual in anemophilous forms, the flowers are small and inconspicuous displaying rather primitive characters in the single perianth and hypogyny. An essential difference in structure characterizes the two flowers - a condition uncommon among Angiosperms where hermaphroditism predominates.

Staminate Inflorescence.

The staminate inflorescence has been studied by Wylder (67), Eichler (23), Briosi and Tognini (9) and Frain (50) but the accounts of the authors do not differ materially.

The individual staminate flowers are short-stalked and drooping arranged in small determinate inflorescences or paniculate cymes. The cymes are located in pairs in the axils of stipules, usually on special floral branches but also found at the bases of these branches.
pairs of cymes both on and at the base of a floral branch show an inequality in size—one being larger than the other. Prain (50) mentions this fact in his description of the staminate inflorescence of the Indian form. The cymes on the floral branches and also the leaves in whose axils they are found become reduced and more crowded toward the apex of the branch. No leafy branch appears between the pair of flower clusters but Briosi and Tognini (9) have found a third panicle here which is usually smaller than the lateral ones, often being reduced to a single flower. Prain (50) in summing up the discussion of the staminate inflorescence states that "though simulating a panicle, the male inflorescence is not in reality a spiral of racemes but a spiral succession of cyme systems in which the axis is defined and gives out two branches, the lower smaller than the higher, which latter repeats the process".

Pistillate Inflorescence.

The pistillate inflorescence like that of the staminate plant is subtended by a leaf with two free stipules. In the axil of each stipule is a single, solitary pistillate flower enclosed in a leaf-like bract. The branch arising in the axil of the stipulate leaf between the two basal flowers forms a spiked inflorescence bearing an indefinite series of pairs of flowers which are located like the basal pair in the axils of the stipules. The inflorescence becomes very compact toward the apex, the leaves and stipules being much reduced. Toward the base of the stem the spikelets are often replaced by leafy branches.

The pistillate inflorescence has been studied by Wylder (67), B. Clarke (12), Eichler (23), Briosi and Tognini (9), Zinger (69), Montemartini (44) and others. The opinions of the various writers concerning
its true nature vary considerably. Wylder (67) considers the pistillate inflorescence as homologous with the lateral inflorescence of the staminate plant. Eichler (23) ranks the pistillate flower in its bract an equivalent of the staminate inflorescence which he terms a panicle. Zinger (69) opposing the view of a definite inflorescence described by Briosi and Tognini (9) states that the pistillate flowers form no inflorescence but are scattered in the axils of leaves on twigs of several orders. Montemartini (44) agrees with Zinger (69) and shows that the pistillate flowers are developed two by two in the axils of leaves representing the first small branchlets of the secondary axillary branch which develops between them.

The attempts of Wylder (67) to correlate the pistillate inflorescence with the staminate inflorescence and of Eichler (23) to correlate the pistillate flower with the staminate inflorescence seem useless since the two plants are very different. The view of a definite inflorescence set forth by Briosi and Tognini (9) appears to be offset by the presence of the flower pair at the base of the spike-like floral branch. The best explanation, then, of the arrangement of the pistillate flowers is as Zinger (69) and Montemartini (44) have shown that they are in pairs in the axils of the leaves of twigs of several orders.
Staminate Flower.

The individual staminate flower consists of five free perianth parts opposite each of which is a stamen (figs. 10, 11). The sepals are imbricated, oblong-lanceolate, pale-green, herbaceous segments. The stamens consist of a terminal, pendulous, oblong anther and a slender filament shorter than the anther (fig. 10). The normal staminate flower shows no trace of a rudimentary pistil (fig. 11).

The first indication of the staminate flower is a rounded elevation of meristematic tissue (fig. 1). The floral organs arise in acropetal succession — sepals and stamens. The primordia of the sepals appear first as little papillae on the periphery of the receptacle (fig. 2). These elongate curving inward and becoming quite distinct before the primordia of the stamens appear. The latter arise within and opposite the parts of the perianth appearing first as small elevations (figs. 3, 4, 5). The stamens elongate rapidly (fig. 6), the tip becoming enlarged to form the anther (fig. 8) and the filament elongating at the mother cell stage of the microsporangium. The stamens are straight in the bud and are roofed over by the imbricated sepals (fig. 10).

Pistillate Flower.

The pistillate flower proper, enclosed within the involving leaf-like bract, is composed of a thin, membranous, hyaline, perianth whose margin is slightly higher behind than in front, closely embracing but free from the ovoid unilocular ovary (fig. 24). Crowning the ovary are two filiform deciduous styles of which the posterior internal is slightly
larger than the external. The stigmas which occur around the periphery of the apex and along the opposing faces of the styles, are brush-like having their epidermal cells elongated into hair-like projections.

Within the ovary is a solitary uncinate ovule which is pendulous from the apex and attached by a short funiculus. In the normal pistillate flower there is no trace of an androecium within the perianth (fig. 24).

The pistillate flower appears in its earliest stage as a rounded mass of meristematic tissue in the axil of the young floral bract (figs. 12, 13). The floral members develop in acropetal succession - perianth and carpels. The perianth appears first as two small primordia which soon lose their identity and develop "en masse". The inner or posterior primordium opposite the involving bract is the first to appear (fig. 14) and is followed quickly by the outer anterior primordium (fig. 15). The axial growth of these two points is early checked and the whole zone at the periphery of the torus shares the growth resulting in an annular structure which is somewhat higher behind than in front. Within the perianth the carpels appear (fig. 16) one posterior and internal, the other external. These soon fuse laterally forming a cup-like structure. The ovule appears slightly lateral to the organic apex (fig. 18). It lies in the axil of the posterior carpel uniting with it (figs. 19, 20) so that as the posterior wall of the ovary grows upward the ovule is carried with it, reaching the top of the ovarian chamber before it is closed (figs. 21, 22, 23). The two styles appear at points representing the margins of the fused carpels (fig. 21). These become united at their bases thus closing the ovarian chamber (fig. 24).
The inconspicuous perianth of the pistillate flower was unknown to earlier writers who recognized the involving bract as the perianth. Payer (49) and at about the same time B. Clarke (12) first discovered the true perianth and it has since been recognized in descriptions of Cannabis. The opinions of different authors vary somewhat in regard to its nature and development. Bentham and Hooker (5) and others report that the perianth is weakly developed and is missing at times. However Prain (50), who examined many pistillate flowers of the Indian hemp asserts that it is always present in the normal flower except in solitary pistillate flowers at the apices of spikelets, where the involving bract is also absent. Payer (49) and Zinger (69) describe the development of the perianth from two leaves or leaflets which are formed independently of each other. Briosi and Tognini (9), however, show a different development for the flowers of Indian plants whose perianth they describe as arising from single ridge resulting in an almost perfectly horizontal perianth.

The present study of the floral envelope of the American Cannabis has shown a condition similar to that described by Payer (49) and Zinger (69) in which the perianth is developed from two primordia and is slightly higher behind than in front (figs. 14-24). Zinger (69) by using a clearing preparation was able to study the development of the floral organs from the exterior, and consequently could more readily observe the two beginnings of the perianth than Briosi and Tognini (9) who studied simply sections of the young flower. My observations of many serial sections of the young flower and also of the external appearance of the mature flower has led to the conclusion that the perianth agrees
with that described by Payer (49) and Zinger (69) rather than that shown by Briosi and Tognini (9).

Floral Bract of Pistillate Flower.

The nature of the involving leaf-like bract of the pistillate flower has been the subject of much discussion. It varies in shape being in the unclutivated Indian plants and the cultivated European plants usually acute or acuminate while in the cultivated Indian forms it is generally truncate. The earlier writers to whom the true perianth was unknown considered this bract as the perianth of the flower, but later authors have shown it to be a leaf-like floral bract. Schleiden (54) was the first to consider it as a bract and not the perianth. Irmisch and also Payer (49) pronounced it a bract in whose axil the flower arises. Briosi and Tognini (9) showing that the flower and bract are closely associated have termed the structure "brattea perigoniale". Zinger (69) disagreeing with the view of Briosi and Tognini (9) regarded the bract as a leaf of the secondary branch in whose axil the flower is formed. Montemartini (44) has termed it a leaf of a small floral branch not, however, in relation with the secondary branch as Zinger (69) believed.

The exact nature of the involving bract is difficult to determine since there are numerous gradations in the plant kingdom between foliage and floral leaves. Briosi and Tognini (9) have shown that a close relationship exists between the bract and the flower both in their origin and in their vascular supply. The bract and the floral organs develop from a common primordium the bract becoming differentiated first (figs. 12, 13). Also the vascular trace which in-
nerves the bract is traced from the pedicel of the flower. Hence, although the bract cannot rightfully be termed a part of the flower proper, it is as Briosi and Tognini (9) have stated, in very close relationship with it and perhaps their term "brattea perigoniale" is aptly applied.
NATURE OF OVARY AND ORIGIN OF OVULE.

The peculiar nature of the gynoecium of Cannabis has made it the subject of discussion by many writers. The nature of the ovary whether formed by one carpellary leaf or two and if two which leaf is fertile, has been speculated upon extensively. The origin and nature of the ovule has been equally disputed whether it is of epicarpellary, hypocarpellary or axial origin. Since this subject has been discussed so extensively in the literature an attempt is made here to bring together the principal theories of the various writers with the facts upon which the interpretations are based.

B. Clarke (12), probably the first writer on the nature of the ovary and ovule of Cannabis attempts to interpret the structure merely from dissections of the flowers. He states that the ovary is monocarpellary and that the posterior thickening represents the placenta formed by the uniting of the margins of the leaf-like carpel. The ovule is of foliar origin and located at the upper end of the placenta. No explanation is given for the presence of two styles.

The next attempt was made by Payer (49) who explains the nature of the ovary and ovule of Cannabis on organogenetic grounds. According to his theory the ovary begins as two carpels which are at first distinct but afterward unite at the base to form the styles. The anterior carpel becomes enlarged and forms the ovary while the posterior carpel remains rudimentary. The ovule is cauline arising from the posterior side of the stem apex, according to Payer (49) and is carried to the top of the ovary by intercalary growth. This theory
explains the origin of the posterior as well as the anterior style but does not offer any explanation for the larger size of the posterior style and the posterior thickening of the ovarian wall.

Doell (20) advances the monocarpellary theory which is also held by Clarke (12). However he further explains the second style as an excrescence on the ventral suture of the carpel.

Celakowsky (11) bases his theory on the views of Payer (49) describing the development of the ovary from two carpels of which the inner is abortive and the anterior is developed. The ovule is explained as foliar the growing point being displaced by the greater development of the anterior carpel.

Briosi and Tognini (9) made a histological study of the ovary and ovule and describe the vascular traces of the base of the ovary. Four vascular traces are found, one anterior, one posterior, and two lateral. The anterior smallest trace enters the involving bract, the external lateral trace the anterior side of the ovary, the internal lateral trace branches to innervate a greater part of the carpellary wall and the posterior trace branches and passes into a small swelling opposite the base of the posterior carpellary wall. This latter trace innerves the ovule. These data overthrow the views of B. Clarke (12), Payer (49) and Celakowsky (11). However Briosi and Tognini (9) do not interpret these facts as solving the question as to whether the ovary is monocarpellary or bicarpellary or as to whether the ovule is cauline or foliar, but are inclined to regard the ovule as axial and the carpel as representing an intermediate form, referable neither to axis or leaf.
Zinger (69) regards the pistil of Cannabis as formed by two carpels of which the anterior only takes part in the formation of the ovary while the posterior constitutes only the back style. The ovule is formed by the apex of the flower axis and the lifting up of the ovule is accomplished by the elongation of the internode which separates the carpels.

Montemartini (44) describes the ovary as bicarpellary but contrary to the views of Zinger (69), Payer (49), and Celakowsky (27), shows that the posterior carpel is fertile and the anterior abortive and sterile. The ovule is described as independent - a cauline formation supported by the dorsal suture of the posterior carpel. The author also traces the course of the vascular traces which correspond in great part to that given by Briosi and Tognini (9).

Prain (50) who is the most recent author on the subject agrees with Montemartini (44) concerning the nature of the ovary and origin of the ovule of Cannabis, although apparently ignorant of his work. He explains the ovule as originating lateral to the apex and in the axil of the posterior carpel. The styles are developed on the margins of the fused carpels. He shows that the vascular traces described by Briosi and Tognini (9) seem to substantiate this theory - the two carpels being innerved by the two opposite lateral traces and the primary axial trace ending at the base of the ovarian chamber at a point corresponding to the position of the ovule. Prain's (50) description of teratological phenomena shows that the pistil is composed of two carpels of the leaf-tyle which are capable, under abnormal
influences, of becoming leaves and that the ovule is truly axillary to the posterior carpellary leaf.

In view of the fact that both an anatomical study of the vascular system of the pistillate flower, and also certain teratological phenomena are in harmony with this interpretation of the nature of the ovary and ovule, the more recent explanation advanced by Montemartini (44) and Prain (50) is the most acceptable.
MEGASPORANGIUM AND FEMALE GAMETOPHYTE.

The Ovule.

The ovule of Cannabis as described above, is cauline and lateral. It originates as a papilla of meristematic tissue (fig. 18) which soon becomes associated with the posterior carpellary wall (figs. 19, 20) and with its growth is carried upward to the top of the ovarian chamber (figs. 21, 22, 23). There is practically no funiculus, but a slight thickening corresponding to the path of the ovule along the posterior ovarian wall is distinguishable. The pendulous position, then, is due, not to the growth of the funiculus, but to the elevation of the hilum. In the mature flower the ovule fills the ovarian cavity and is attached above slightly to the posterior side of the cavity (fig. 24).

The direction of the growth of the ovule results in a form which is usually termed campylotropous. However, it has been variously described as anatropous because of its curved development, orthotropous because of its terminal position, and campylotropous owing to its uncinate form. Prain (50) maintains that the latter term is not accurate since the form of the ovule is due, not to its curving, but to the elevation of the hilum. He proposes the term "obcampylotropous". The orthotropous ovule is reported by Coulter and Chamberlain (17) as common for the Urticaceae and for the Archaechlamydeae in general. The campylotropous type is rare, being found among Dicotyledones in the Chenopodiaceae, Resedaceae, Cruciferae, Capparidaceae,
Caryophyllaceae, etc. - more or less specialized families of their alliances.

After the nucellus has become prominent and as a result of the growth of the carpels has reached the top of the ovarian chamber, the integuments appear. The inner originates first, appearing as an annular outgrowth around the base of the nucellus (fig. 22). Soon the primordium of the outer integument becomes visible (fig. 23). During their growth the integuments become very massive and when the embryo-sac is formed are completely coalesced over the apex of the nucellus (fig. 35). As Zinger (69) reports for the Cannabinaceae the integuments of the Cannabis grow together at the posterior side of the ovary and become fused over the tip of the nucellus so as to completely obliterate the micropyle.

According to Modilewsky (43) two integuments are common among the Urticaceae. Shattuck (56) also observed two and occasionally three for Ulmus americana. Coulter and Chamberlain (17) state that on the whole this condition is the rule among Monocotyledones and also prevails among the Archichlamydeae. Since a single integument is characteristic of the Sympetalae and higher forms the double integument can safely be regarded as a primitive structure.

The Megasporangium.

The material was not favorable for the study of the megasporangium and female gametophyte. However, certain peculiarities were very evident and at least deserve mention. Because of the campylotropous form of the ovule sections through the embryo-sac are difficult to obtain.
According to Briosi and Tognini (9) the archesporium of the megasporangium originates as a single hypodermal cell which as usual, divides periclinaly resulting in the formation of a primary parietal and primary sporogenous cell. The latter becoming the megaspore mother cell divides to form a row of three megaspores of which the inner one functions. They state that the embryo-sac is formed as is usual among Angiosperms but give no details in this connection.

The present investigation shows a variation from the mode of development described by the Italian botanists. A several celled archesporium was distinctly observed in a number of cases. The earlier development of the megasporangium, noted but rarely, showed only one primary archesporial cell (fig. 26). Whether or not this several-celled sporangium develops from a one-celled primary archesporium is a question. Treub (63) for Casuarina, Miss Benson (4) for Carpinus, Conrad for Quercus report many-celled archesporia, which according to Coulter and Chamberlain (17) are certainly not all hypodermal in origin. Treub (63) reports the archesporium of Casuarina as a group of hypodermal cells but his account and figures suggest that all of the sporogenous tissue may not be derived from the hypodermal layer.

The many-celled archesporium is the prevailing tendency among the Rosaceae and is also common in the Amentiferae. The Ranunculaceae show a great irregularity in the number of archesporial cells with a tendency to increase their number. Since the Rosaceae, Amentiferae and Ranunculaceae are recognized as primitive members of the Archichlamydeae the many-celled archesporium would seem to be
Characteristic of the more primitive Dicotyledones. However, Coulter and Chamberlain (17) state that this is true only in a general sense, for no large groups have this general tendency and the same feature has been reported for members of higher groups.

The divisions of the primary parietal cell result in the formation of 6-8 rows of cells placing the sporogenous cells deep within the sporangium (figs. 26-33). A conspicuous development of parietal tissue is noted by Coulter and Chamberlain (17) for certain of the Monocotyledones and Archichlamydeae. Since the suppression of the parietal tissue of the megasporangium is universal in the Sympetalae as far as they have been investigated a large development seems to indicate a primitive condition.

The Female Gametophyte.

With the growth of the megasporangium the sporogenous cells increase in size and take on the appearance of megaspore mother cells. The nucleus of each cell shows a distinct spirem on which prominent chromatin masses appear (fig. 29). This is evidently a presynaptic condition of the nucleus. A later stage shows one mother cell becoming dominant at the expense of the others. This one enlarges rapidly pushing the others aside (fig. 30, 31). The latter cells appear shrunken and deeply stained showing degeneration. A later stage of development shows only the dominant mother cell remaining, the surrounding cells having disappeared (fig. 32).

The further development of the female gametophyte was not observed. It was not determined whether the mother cell divides to
form megaspores or whether it functions directly as a megaspore. Four-celled (fig. 34) and mature embryo-sacs (fig. 35) seem to indicate that the development is as usual in Angiosperms. Modilewsky (43) reports that the antipodals are weakly developed and that certain large nourishing cells are present in the antipodal end of the sac. The embryo-sac is horse-shoe shaped narrowing toward the micropylar end. The nuellus also narrows at this extremity and becomes distinctly beaked (fig. 33). The integuments as noted before become coalesced over this beak closing up the micropyle.

Fertilization.

No traces of pollen tubes were found and hence it was not determined whether or not fertilization occurs here. Briosi and Tognini (9) failed to find pollen tubes but figure the development of the embryo. Zinger in 1898 observed the pollination of Cannabis and traced the course of the pollen tubes from the stigma to the embryo-sac. According to him the tube grows directly downward through the ovary and perpendicular to the integuments until it reaches the nuellus. The tube branches freely and forms blind sacs. However the phenomenon of fertilization was not observed. Winkler (65) records that earlier experimenters on hemp regarded it as parthenogenetic. Kirchner (41) states that seed formation in hemp is partly dependent upon parthenogenesis and suggests that some races of hemp may be parthenogenetic while others demand fertilization.
THE MICROSPORANGIUM.

Development of Microsporangium.

The androecium of Cannabis sativa L. consists of five, free, shortly stalked stamens arranged in a whorl opposite and inside the perianth parts (figs. 10, 11). As before noted the stamens arise lateral to the floral apex and hence may be regarded as foliar structures. Each stamen consists of an oblong anther somewhat longer than the slender filament at whose tip it is attached. The anther produces four microsporangia which later at the time of dehiscence form two loculi by breaking together of two sporangia. A notable feature connected with the development of pollen from the spore mother cells is the sterilization of potentially sporogenous tissue which occurs at several stages.

The development of the microsporangium agrees with that described by Coulter and Chamberlain (17) for most Angiosperms. The anther in cross-section is at first a round mass of meristematic cells surrounded by an epidermis (fig. 4). At an early stage the mass becomes four-lobed (fig. 7) and an archesporium is differentiated in each of the lobes (fig. 36). By periclinal divisions the archesporium gives rise to the parietal and sporogenous tissue (figs. 37, 38). The divisions of the parietal tissue result in four layers arranged concentrically around and completely investing the sporogenous mass (figs. 38, 39). Of these layers the inner next to the
sporogenous tissue becomes the tapetal or nourishing layer, the two middle layers disorganize during the divisions of the pollen mother cell, and the outer layer or endothecium becomes specialized for the dehiscence of the anther. During the divisions of the parietal layers the sporogenous tissue divides rapidly in all directions giving rise to a cylindrical mass of spore mother cells which is about 50 cells in length and 10-12 cells in diameter. The total number of sporogenous cells in one sporangium is, then, about 5655, and for one stamen 22620.

The Parietal Layers.

The "middle layers", the two layers of the wall between the endothecium and the tapetum, consist of tabular cells (figs.39,40) as Shattuck (56) observed in the species Umlus americana L. During the division of the pollen mother cells these layers become flattened and disorganize. The inner one breaks down first (fig.41) by a slow process becoming thinner and thinner and finally disappearing at the early tetrad stage of the mother cell. The outer parietal layer persists until later in the tetrad stage (fig.42) breaking down shortly before the tapetal cells disorganize.

The Tapetum.

The tapetum or "nourishing jacket" is a regular layer in contact with and completely investing the sporogenous tissue. The layer is apparently derived from the parietal tissue as is usual in the typical Angiosperm (fig.33).
At the mother cell stage of the sporogenous tissue the tapetum is well organized (fig. 39) - the cells appearing blocky and uninucleate with very granular contents (fig. 44). During the early presynaptic stage stages of the mother cells the nuclei of the tapetal cells divide mitotically and increase in size (fig. 45). The first nuclear division is regular and results in a uniform binucleate condition which persists for some time. The later divisions are more scattered. The nuclei present often peculiar lobed and irregular forms due to processes of nuclear fusion and subsequent divisions. Their form may be elongate two nuclei lying side by side until fused into a large elongated body, or, two or three spherical nuclei may unite resulting in an irregular form (fig. 49). Gates (26) working on Oenothera lata and Beer (2) on Oenothera longiflora and Oenothera biennis state that the later divisions of the tapetal cells are chiefly by amitosis. However, the recent work of Bonnet (31) on the tapetal cells shows that the irregular appearance of the nuclei is due entirely to nuclear fusions and not amitotic divisions. The author states "Les phenomenes d'amitose ne paraissent pas exister dans les cellules nourricieres. Toutes les apparences qu'on leur on attribuees s'expliquent par des irregularites mitotiques et des fusions nucleaires".

The tapetal cells reach their maximum development during the formation of the tetrads at the time of the disorganization of the inner of the middle layers. At this time the cells are very large (figs. 47, 48, 49) and may have 2, 3, or 4 nuclei which contain an abundance of darkly staining material, presumably chromatin.
In the later stages the nuclei fuse and the cells, shortly before disorganization, contain one or two nuclei (figs. 50, 51). After the microspores have separated from their tetrad arrangement the nourishing cells break apart, become vacuolated, take peculiar irregular forms and stain darkly. Their nuclei become indistinguishable and the chromatin masses appear scattered about in the cytoplasm of the cell (figs. 52, 53). Their breaking down is coincident with the formation of the wall of the pollen grain.

At times the tapetal cells present the appearance of disorganizing at earlier stages than usual. The cells appear as they do in the later stages being separated, irregular and darkly staining. This condition is found at almost any stage in the development of the pollen mother cell. Gates (26) observed a similar appearance in the tapetal cells of Oenothera Lamarckiana, and attributed the appearance to the degeneration of the cells. However, in Cannabis no microsporangia were observed in which the tapetal cells had entirely disappeared prematurely as Gates (26) reports in Lamarckiana. Since the sterility in the sporogenous tissue of Cannabis sativa L. is similar to that observed by Gates (26) in Oenothera Lamarckiana, it is significant to note the apparent similarity in the sterility of the tapetum.
DEVELOPMENT OF MICROSPOROE.

The general development of the microsporangium was followed in the preceding chapter with especial emphasis upon the formation and ultimate fate of the wall tissue, including the tapetum. The present chapter deals particularly with the divisions of the pollen mother cell and the formation of pollen.

Since Strasburger (59) in 1894 placed the alternation of generations in plants on a chromosome basis and explained the significance of the reduction divisions, much study has been given to this subject in both the animal and plant kingdoms. The nature of the chromosomes and the part they play in heredity have been discussed in an extensive literature. As a result of the many investigations the later stages of the reduction division from the end of prophase onward are generally agreed upon by most cytologists, especially those who have studied plants. The recent papers have been in regard to the earlier stages from the resting condition of the mother cell to synopsis.

Although extensive work has been done upon the structure and morphology of Cannabis sativa L., the study of the reduction division of the pollen mother cells has been taken up but briefly. Briosi and Tognini (9) mention that the division of the pollen mother cell is, as in all Dicotyledones, "a simultaneous division into four parts". Strasburger (62), in a recent paper dealing with the sex-determining factors of dioecious plants, reports the results of a cytological study of several species including Cannabis sativa L.
The observations were made in an attempt to find a difference in the chromosomes which might correspond to the sex determining "heterochromosomes" or "idiochromosomes" of the animal kingdom. Both the pollen mother cells and the somatic cells of the root of Cannabis were investigated and several stages in the reduction division figured.

The small size of the pollen mother cells which are only 13 microns in diameter renders the study of the divisions somewhat difficult to follow in detail. The present investigation includes the chief events of the divisions of the pollen mother cell with especial reference to the prophase of the heterotypic division.

Heterotypic Division.

The sporogenous tissue of the sporangium at the mother cell stage consists of a cylinder 10-12 cells in diameter and about 50 cells long. A wide variation in the stage of development of the sporogenous cells is noticeable especially during the homoeotypic division which is more rapid than the heterotypic. In the different sporangia of an anther and the different anthers of a flower the variation is much greater and all stages of both divisions were traced within a single flower. No uniformity exists as to which end of the loculus contains the younger stages, for progression may be made from either the upper or lower end of the sac to the opposite extremity. In the lower part of one sporangium the mother cells are in the resting condition at the end of the heterotypic division while in the upper end they are in telophase of the homoeotypic. An adjacent loculus shows the four cells resulting from the hom-
oetypic division below and the telophase stage of the second division above. Some sporangia show zones of development, as for example one in which the dyad stage of sporogenous cells appears in both ends, with later stages of metaphase and anaphase in the central region.

Because of the large mass of mother cells in one sporangium some variation is not unusual as was found by Allen (1) in Lilium canadense. However, the lack of uniformity between the anthers of a single flower seems greater than has been observed in the normal development of pollen mother cells. Gates (26) who found such an irregularity in Oenothera lata, a sterile hybrid, connects this condition with the failure of pollen development. Since sterility appears in the later stages of the pollen development of Cannabis similar to that observed by Gates (26) in Oenothera, the coincidence is worthy of note.

In early prophase the pollen mother cells pass through the usual growth period resulting in a marked increase in the size of both the cells and their nuclei (figs. 54-56). The cytoplasm at this stage is exceedingly dense (fig. 54) and granular, showing few vacuoles and staining darkly. The cells are angular exhibiting no evidence of rounding off as yet. The nuclei are about 7 micromes in diameter and contain each a relatively large globular nucleolus. An extremely delicate reticulum composed of thread-like strands upon which small chromatin granules are distinguished forms a network around the nucleolus (fig. 54).

As the nucleus grows the reticulum becomes more pronounced
and the chromatin granules larger (figs. 55, 56). The network can now be seen more distinctly and the chromatin granules become spread out along its length thus making the linin thread indistinguishable (57). This appearance gives ground for the theory of Gregoire (41) who maintains that the reticulum is at times composed of one general material which varies in density so as to give the appearance of granules. The cytoplasm surrounding the nucleus becomes less dense (figs. 56, 57) and somewhat vacuolated.

In a very few instances the mother cells at this stage appear to be disorganizing as evidenced by their shrunken condition and dark stain (fig. 58). Gregory (32) in a sweet pea hybrid and Gates (26) in Genothera lata report sterilization at this early stage.

As the nucleus approaches synapsis the chromatin becomes more conspicuous and more closely aggregated. The nuclear framework which now consists of a continuous spirem (fig. 58) shows no evidence of being double. At this time the nucleus has reached its growth limit and measures 11 microns in diameter being surrounded by a very delicate nuclear membrane. The cytoplasm of the cell begins to round off slightly (fig. 59).

Synapsis which as usual consists of the contracting of the nuclear framework into a knot, was found as a very common condition in the material studied (fig. 59). Although this stage was long regarded as an artifact due to faulty fixation of material it is now generally recognized as a constant character of the mother cell. Recent careful investigations on both the animal and plant sides show that the peculiar phenomena connected with this stage are
uniform. Special significance has been attached to this stage by some who maintain that an exchange of material between the maternal and paternal chromosomes takes place here. Other investigators among whom is Gates (27) refute this theory. The latter author in a paper on chromosome reduction states that synapsis can have no special significance since the chromosomes are known to be paired in the somatic tissue of the sporophyte, and because there is no satisfactory evidence of the existence of smaller units of structure than the chromosomes. The theories in regard to the significance of this period can perhaps never be substantiated satisfactorily by observations alone, but will remain to be solved by experimentation.

The contracting of the spirem into synapsis goes on rapidly resulting in a compact mass usually at one side of the nuclear chamber (fig. 59). The nucleolus remains distinct and may be either within the mass of chromatin or at one side of it. This condition is doubtless a prolonged period since it is so commonly seen.

The loosening of the synaptic knot results in a much coiled, delicate, uniform spirem (fig. 60). The chromatin granules can no longer be distinguished in this homogenous thread, nor was any evidence found of either a longitudinal split or a pairing of threads. The duration of the spirem stage is very short and segmentation soon takes place.

The cutting up of the spirem results in rather long straight, curved, or bent, segments (fig. 61). The mode of reduction here seems to be undoubtedly telosynaptic. X, V, U figures are formed by the
segments and the end to end arrangement of a pair of chromosomes is often very evident (fig. 61). Soon the segments contract and blocky bivalent chromosomes appear distinctly (fig. 62), taking up their position at the periphery of the nucleus.

The phylogenetic significance of the mode of reduction has been somewhat questioned by Gates (27). He concludes that either mode of reduction may take place and often both modes occur in the same species. From a study of various species he states that in forms which have long thread-like chromosomes the pairing may be expected to take place side by side while in forms with short, stout chromosomes the pairing is likely to be end to end. The short blocky nature of the chromosomes of Cannabis and their telosynaptic origin seem to substantiate this generalization of Gates (27).

At the dyad stage of the chromosomes a count showed the reduced number to be 10 (fig. 62), a number which was later found to agree with that of Strasburger (62) who made a count from a plate view of the spindle in metaphase. He also determined the diploid number of chromosomes, 20, in a somatic cell of the root.

Previous to the formation of the spindle the chromosomes aggregate in the center of the cell. The nuclear membrane and nucleolus disappear and spindle fibers are seen extending toward the chromosomes (fig. 63). The usual multipolar spindle is distinguished which, however, soon takes a bipolar form. The chromosomes, which are at first scattered along the spindle, later come together in the equatorial region (fig. 64) and are closely packed together. The pulling apart of the chromosomes (fig. 65) results in a compact
chromatic mass at each of the spindle poles (fig. 66)

During the reconstruction of the daughter nucleus a nuclear membrane is formed around each mass (fig. 67) and the nucleus begins to grow in size. The chromosomes become separated (fig. 66) and, as the nucleus becomes larger, seem to stretch out becoming scattered along the reticulum. The chromatin masses, however, remain distinct (fig. 69) and a count of these shows in no case more than ten present in one nucleus.

Homoecotypic Division.

Although the process involved in the division of the pollen mother cell nucleus extend over a long period, the division of the daughter nuclei is rapid. Practically all the stages of this division may be traced within a single sporangium.

The daughter nucleus increases in size preparatory to beginning the second division. The spirem spreads out, the segments being distinct and located at the periphery of the nuclear chamber. The nucleus becomes more rounded (fig. 69) and at the time of the disappearance of the nuclear membrane is nearly spherical.

With the breaking down of the nuclear membrane cytoplasmic fibers appear extending toward the chromosomes (fig. 70) which are massed in the nuclear chamber as in the preceding division. The small spindle figures are formed rapidly and lie either parallel or at right angles to each other (fig. 71). The chromosomes separate as in the heterotypic division (fig. 72) and appear later massed at the poles (fig. 73). The reconstruction of the daughter
nucleus resembles the process described for the first division and results in four nuclei (74) arranged tetrahedrally in the cell.

A noticeable sterilization occurs at the tetrad stage of development as is shown by the shrinking and dark stain of whole tetrads or parts of tetrads. A more detailed account of this peculiarity follows in the chapter on sterilization.

Much evidence has been accumulated recently in support of the individuality and permanence of the chromosomes. The resting stage of the spore mother cells, their daughter and grand-daughter nuclei show the chromatin in definite masses which apparently correspond to the "chromocentren" of Rosenberg (52) and the "prochromosomes" of Overton (47).

Special Wall of Young Tetrad.

Inside of the mother cell wall another wall is developed around the protoplast which becomes distinct early in the prophase of the heterotypic division, being first observed during the formation of the dyad chromosomes (fig. 61). A similar wall was observed by Mottier (45) in Acer negundo L. in the spirem stage of the mother cell and described as a "soft thick wall". During the succeeding stages of division this wall becomes thicker and at the tetrad stage of the mother cell is very conspicuous in the iron-alum-haematoxylin preparations where it stains brown (figs. 75, 76). Beer (3) who observed similar walls in the tetrads of Ipomoea describes them as "massive mucilaginous walls" which give reactions to callose and pectose. Soon after the formation of the nuclei of the tetrad certain thickenings appear on the inner side of this wall (fig. 66) correspond-
ing in position to the cell plates between the four nuclei. These thickenings increase in size becoming ridge-like projections (figs. 65, 66) growing toward the center of the cell. Partition walls between the cells are formed apparently independent of the investing wall and connecting with the ridge-like thickenings (fig. 76). Each cell of the tetrad then invests itself with a delicate cell wall (fig. 68) which later becomes differentiated into the intine and exine of the pollen grain. While the wall of the microspores is being formed the heavy wall of the tetrad disorganizes (fig. 77) and finally disappears (fig. 78).

The young microspores in many cases appear shrunken and degenerating. A definite period of degeneration, which will be discussed later, occurs at this stage similar to that observed in the young tetrad.

Male Gametophyte.

The germination of the microspore beginning with the division of the nucleus takes place after the spores have separated from their tetrad arrangement. The microspore nucleus is relatively large, central in position and exhibits definite chromatin masses in the reticulum (fig. 85). The division of the nucleus was not observed. The daughter nuclei resulting from the division (fig. 88) are different in size; the tube nucleus being larger than the generative. The generative cell with its cytoplasm about it lies in the pollen grain and as far as observed maintains its spherical form.

Experiments were tried in germinating the fresh pollen
during the summers of 1912 and 1913. Successful results obtained by Dr. R. B. Wylie with the pollen of Sambucus canadensis L. and other species placed in sugar solution, led to these trials. The pollen of Cannabis sativa L. was taken from unopened flowers collected in good condition and placed in sugar solutions varying in strength from 1/2 to 10 per cent. Although the experiment was repeated frequently no pollen tubes could be grown. Briosi and Tognini (9) report that the pollen of Cannabis placed in a damp chamber germinated in 4 or 5 hours sending out tubes with heavy walls.

Although the greater number of the pollen grains used for experimentation appeared shrunken and sterile, a few presented the normal, plump appearance of functional pollen. These latter grains would be expected to germinate under favorable conditions. The negative results obtained from the experiments in germinating the pollen lead to one of two conclusions. Either the medium in which the grains were placed was not a suitable one for its germination, or, none of the pollen was functional. Briosi and Tognini (9) succeeded admirably in germinating the pollen of the Italian hemp in a damp chamber. The pollen of the American Cannabis placed in a nutritive solution in which the pollen of other species had been successfully germinated, failed to develop tubes. Whether or not this indicates sterility in all the pollen of the species investigated is a question.

Wall of Pollen Grain.

The young microspore is invested with a thin wall (fig. 81)
which becomes differentiated into the inner intine and the outer exine (fig. 85). The mature pollen grain (fig. 88) is spherical when normal, 20 microns in diameter and has a wall which is very thin in comparison with the spore costs of most land plants. The outer cutinized layer is delicately aculeolate when observed in the dry condition but appears smooth when immersed in a liquid.

At three points equidistant from each other a small papilla-like protuberance appears on the pollen grain marking a thin spot in the exine for the exit of the pollen tube (figs. 85, 88). A similar number of such points is noted by Coulter and Chamberlain (17) for the Cupuliferae, Proteaceae, Geraniaceae, Chagraceae, Boraginaceae and Compositae. Each point in section shows a small aperture opening into a narrow canal that crosses the exine and enters a small chamber between the intine and exine (fig. 91). The outer layer is thin at this point and enlarges around the opening to form an annular lip. Corresponding with each aperture the intine forms a hemispherical body (fig. 91) which Briosi and Tognini (9) state is stored with cellulose to furnish material for the walls of the pollen tube.

Dehiscence and Pollination.

The mature anther wall consists of the epidermis and outer-most parietal layer or "endothecium", a name given by Furkinje (51) to the dehiscing anther wall within the epidermis or exothecium and since become restricted to the outer parietal layer. Briosi and Tognini (9) have described the reticulate system of lignified thickene-
ings and also the method of the dehiscence of the wall. A non-lig-
nified and contractible membrane connects the thickenings, and the dry-
ing of the membrane produces a consequent constipation of cells. The
epidermal layer in drying also contracts more strongly than the endo-
theecium and consequently exerts a force which tends to make the wall
curve outwards and break at its weakest point. The endothecium and
the epidermis become smaller and weaker toward the connective tissue
and as a result of the tension of the anther wall tend to rupture
at these places in a longitudinal line.

Pollination is affected by the wind as is usual in the prim-
itive unisexual flowers of Angiosperms and is the rule in the Urti-
caceae. The flowers of Cannabis display those negative characters
common to anemophilous plants - the absence of light-colored floral
envelopes, perfume and honey. The loose staminate inflorescences,
stalked and drooping staminate flower and pendulous stamens lend
themselves admirably to the shaking of the wind. On the other hand
the large, brush-like character of the stigma, the location of the pis-
tillate flowers in the axils of leaves are adaptations which fit the
flower to catch and retain the wind-blown pollen. The character of
the pollen itself fits it for wind distribution. Its abundance, in-
coherency, dryness, smoothness and lightness render it easily carried
and distributed in the air. Although the sexes are separated, pollina-
tion is well cared for because of the great abundance of spores. An
estimation of the number of grains produced by one flower based upon
a count of the mother cells gave about 452,400. Strasburger (60) for
one staminate hemp plant computed 12,500,000 grains. This number is
perhaps conservative since it must include only a count of the flowers which were on the plant at a given time without regard to those which had gone or to those which would appear later. Considering that the plants in the uncultivated state grow thickly and with the sexes intermixed pollination seems to be well assured.
STERILIZATION OF POLLEN.

Sterilization in the microsporangium of Cannabis sativa L. is a very conspicuous feature. As was noted in the preceding chapter the degeneration of potentially sporogenous cells occurs at several stages in the development of the pollen. An attempt is made here to describe the breaking down of the spores and also to obtain definite counts showing the ratio of the sterile to the apparently functional spores.

The development of the mother cells of the microsporangium proceeds in a normal manner to the tetrad stage with the exception of a scattered few which degenerate at various early stages (fig. 58). However, at this stage a marked difference in the cells is noted, some appearing plump and normal (fig. 77), others showing evidences of breaking down or being almost completely degenerated (figs. 79, 80). The latter take a deep stain and are shrunken. Gates (26) in a study of the microsporangium of Oenothera lata and Oenothera Lamarckiana, found many irregularities in the tetrads and also evidences of their breaking down.

The sterilization does not occur in a regular manner but varies in different sporangia and different anthers. One sac may contain only a few degenerating cells while another may be almost completely sterile. Irregularity is also noted in the degree of sterilization within the tetrad, 1, 2, 3 or all the spores breaking down. The abortion of spores often occurs in regions, one
end of a sporangium showing practically all the cells shrunken while the remainder contains normal cells. Again the functional and abortive microspores may be interspersed. At times a whole anther sac and even a whole flower shows sterility, while on the other hand some exhibit no evidences of degeneration.

Owing to the irregularity of the sterilization much difficulty is experienced in attempting to determine the exact ratio of sterile microspores to those which appear to be functional. Numerous counts made from prepared slides to determine the percent of abortion that occurs at the tetrad stage showed that approximately 40 per cent of the number degenerates (Plate XXIII).

The young microspores after they have separated from the tetrad arrangement resulting from the division of the mother cell, show various degrees of degeneration. Some of the spores which began to break down earlier appear now to be almost completely disorganized (fig.84). Others are evidently just beginning to degenerate (figs.82,85) and appear slightly shrunken.

As the microspores enlarge and become rounded those which formerly appeared to be entering upon a period of degeneration are now darkly staining and small (fig.87). These, however, do not constitute a large per cent of the spores being only about 10 per cent of the number (Plate XXIII). The remaining microspores appear almost normal at this stage but often show slight evidences of shrinkage (fig.86) which is doubtless the beginning of the sterilization so evident in the mature pollen grains.

The mature pollen in the fresh condition was examined during the summers of 1912 and 1913 and presented a peculiar appear-
ance. The greater number of grains was badly shrunken (figs. 89, 90) their form being angular and irregular. Even pollen found in unopened flowers showed this appearance of degeneration and only a few were round and plump appearing as normal functioning grains. The ratio of shrunken to normal grains was determined to be about 50 to 1 or 98 per cent of the number (Plate XXIII). Counts both of the fresh material and of prepared slides gave a great preponderance of badly shrunken grains. The fact that the fresh pollen showed evidences of degeneration precludes the idea of apparent abortion due to faulty fixation of material.

It has been shown that at several stages in the development of the microspore and also in the mature pollen, sterilization of potentially sporogenous tissue is evident. The proportion of the sterility that occurs at the various stages is of interest in this connection. Considering that each mother cell would give rise to four spores, the abortion of spores appearing at scattered stages of the mother cell before its division, is approximately 1 per cent of the whole. At the tetrad stage 40 per cent of the remainder becomes sterile. Another period of degeneration occurs at the early microspore stage when approximately 10 per cent if the remainder, or, 5 per cent of the whole break down. The mature pollen shows the greatest amount of sterilization. 98 per cent of the pollen grains or about 44 per cent of the total number of spores that would have been formed if no sterilization had occurred, become aborted. The sum total of these various losses through sterilization between the mother cell and the mature spore, approximates 99 per cent of
the total potential number of spores and leaves but 1 per cent of apparently functional pollen. (See Plate XXIII).

The significance of this sterilization is taken up in the chapter on the discussion.

**Sterilization of Pollen.**

<table>
<thead>
<tr>
<th>Stage</th>
<th>Per cent becoming sterile</th>
<th>Per cent of total potential pollen</th>
<th>Per cent remaining</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early sporogenous cells</td>
<td>1%</td>
<td>1%</td>
<td>99%</td>
</tr>
<tr>
<td>Tetrad</td>
<td>40%</td>
<td>39.6%</td>
<td>50.4%</td>
</tr>
<tr>
<td>Microspore</td>
<td>10%</td>
<td>5%</td>
<td>45%</td>
</tr>
<tr>
<td>Mature pollen</td>
<td>98%</td>
<td>44.1%</td>
<td>1%</td>
</tr>
</tbody>
</table>
EMBRYO AND FRUIT.

A study of the embryo development was not attempted since the material proved unfavorable. Prain (50) reports the presence of albumen in the seed and that the posterior cotyledon of the embryo is noticeably larger than the anterior one.

The fruit is a nut enclosed in a leathery husk and completely filled by a fleshy kernel. In terming the fruit a nut meaning a one-seeded fruit, enveloped by a husk, with a hard shell composed of more than one carpel, we accept the theory of a bicarpellary ovary. The fruit is smooth, ovoid, and of a greenish gray mottled appearance. The husk is the remains of the involving bract and the mottling is due to the remains of the perianth.

A layer of irregular columnar cells in the endocarp is responsible for the crustaceous character of the shell.
DISCUSSION.

Relative Rank of Species.

As previously noted, Cannabis sativa L. has been classified in the Urticales one of the primitive orders of the Archichlamydeae. Accepting the evidence that the simpler flowers are primitive rather than derived, this classification is warranted by certain features which may be regarded as primitive as far as our present knowledge of the lines of descent can determine.

The small inconspicuous flowers are characteristic of primitive forms which are wind-pollinated. The dioecious habit also prevails among the lower seed-bearing plants and is nearly always associated with wind pollination. The tendency to dioecism is general for the Urticaceae and the more primitive groups and hence may be regarded as a primitive character.

The staminate and pistillate flowers differ from each other in many respects. On the whole the staminate flower displays more primitive characters than the pistillate. The parts of both flowers are definite and cyclic in arrangement, features which entitle them to a comparatively high rank. However, the single inconspicuous perianth marks them as less specialized than the higher forms which have a double showy perianth. The staminate flower has separate floral parts which are developed independent of each other. The pistillate, however, has both perianth parts and carpels united or
coalesced, which tendency is more advanced than that of separate development displayed by the staminate flower. Hypogyny as is regarded by Coulter and Chamberlain (17) is undoubtedly a primitive feature.

The ovule is campylotropous, which is a rare type and especially peculiar to the Urticaceae where the orthotropous ovule is common. Its cauline origin and double integument mark it as primitive. Lower forms in both Monocotyledones and Dicotyledones have ovules of cauline origin and hence the character is to be regarded unspecialized. The two integuments found in Cannabis prevail also among the Urticaceae and Archichlamydeae in general. Since a single integument characterizes the Sympetalae and higher groups the double integument is doubtless a primitive feature.

A noteworthy character brought out by this study is the many-celled archesporium of the megasporangium. Briosi and Tognini (9) report a one-celled archesporium for this species which would indicate a more advanced condition. The many-celled archesporium noted in this investigation is doubtless a primitive character since as Coulter and Chamberlain (17) state it occurs quite commonly in the Amentiferae, Ranunculaceae, Rosaceae and other lower forms. The occurrence of both the one-celled and many-celled archesporium in the same species may indicate that the plant is shifting its habits.

A conspicuous development of parietal tissue appears in the megasporangium. This is probably correlated with the many-celled archesporium and is certainly a primitive character since higher forms have a tendency toward its suppression.
The branching of the pollen tube of Cannabis has been reported by Zinger (69). Since this feature is common among Gymnosperms and the Amentiferae of Angiosperms it is doubtless characteristic of lower forms.

Cannabis sativa L. although displaying mostly primitive characters, has certain tendencies which may be regarded as more advanced. The primitive characters as noted above are the small, inconspicuous, dioecious flowers, the single, bracteate perianth, hypogamy, the cauline ovule, the double integument, the many-celled archesporium, the great development of parietal tissue of megasporangium and the branched pollen tube. On the other hand the definite number of floral parts, the cyclic arrangement of parts, the zonal development of the floral organs of the pistillate flower and its zygomorphism are more advanced characters.

Diclinism.

The problem of sex in plants and animals is very old and has long been of interest among biologists because of its relation to the important question of heredity. Sexuality is almost universal in the organic kingdom being lacking in the lowest forms only.

It is not always present in the higher plants, but, as Schaffner (55) states, their relationships and morphology clearly point to a sexual ancestry. Sexual difference in plants extends not only to the sexual generation but to the sporangium and entire sporophyte as well. The staminate and pistillate flowers differ in number, position and structure, the former being specialized for the pro-
duction and distribution of pollen, the latter for the protection and ripening of the ovules and the distribution of seed.

As before noted the strictly dioecious character of Cannabis, its easy cultivation and world-wide distribution have caused it to be the subject of much experimental work and discussion in relation to this question. Problems of the character of the sexual dimorphism, the difference between the staminate and pistillate individuals, sex ratio, sex constancy and the time of the determination of sex in the individual have been studied through investigations upon Cannabis.

Differences between the staminate and pistillate individuals of Cannabis are not easily discernible until the time of flowering. After blooming a marked difference is noted. The staminate plants turn yellow and soon die, while the pistillate become more luxuriant and persist until after the ripening of the seed. The staminate plant with its drooping stalked flowers aggregated in loose inflorescences is specialized for the production and distribution of pollen. On the other hand the pistillate plant with its sessile flowers arranged in compact inflorescences is specialized for catching the wind-blown pollen. The persistence of the latter plant also allows time for the ripening of the seed.

Goebel (29) in an article on the sexual dimorphism of plants states that the staminate and pistillate individuals are rarely unlike in secondary sexual characters before blooming and cites hemp as an example. He explains that in Dicotyledones a post-fertilization development of the vascular system in the pistillate
plants is responsible for its longer life, while the weak vascular supply of the staminate plant causes its early disappearance.

Sprecher (57) made a study of the sexual differences in Cannabis sativa and Rumex acetosa. He measured the variability in length, weight and osmotic pressure of the staminate and pistillate plants when grown under different conditions and concludes that the variation is much more extended in the pistillate individuals than in the staminate.

The ratio of sexes in the lower plants is far from being equal. In dioecious Angiosperms the ratio is more nearly equal but usually shows a preponderance of the pistillate individuals. Hemp has been considered by many experimenters in relation to this question. Heyer (34) examined 40,000 plants of Cannabis and determined the proportion of sex to be 100 staminate to 114.93 pistillate; Haberlandt (33) in Austria found the ratio of the same species to be 100 staminate to 120.4 pistillate plants; Fisch (25) counting 66,000 plants at Erlangen found the ratio of 100 staminate to 154.24 pistillate individuals. As Schaffner (55) states the case of hemp shows a ratio which is exceedingly variable. However, the variation in proportion seems constant for the various races observed when large counts are taken.

The question then arises as to what conditions cause this constancy of sex relation and why is it recognizable in such large counts? Until recently it was generally believed that sex in plants is largely determined by conditions of environment, such as temperature, light, nourishment, conditions of growth, etc. This belief was
founded upon certain successful experiments in Cryptogams where the sex organs are developed upon small, free-living, few-celled prothallia. Here, by the changing of external conditions, the thalli could be induced to produce either male or female sex organs - unfavorable conditions causing the male organs to appear and favorable the female. From experiments performed upon various groups of plants it seems proven that environmental factors do influence either directly or indirectly the development of sexual organs in plants where both tendencies exist. However recent and more careful experiments show that in strictly dioecious forms such factors do not determine, at least in the life history of the individual, which sex shall develop.

Perhaps no dioecious Angiosperm has been investigated as much as Cannabis in an attempt to solve the problem of the determination of sex. Haberlandt (35), Saccardo (53), Heyer (34), Hoffmann (35) and many other experimenters worked with this species in attempting to determine the influence of external conditions upon sex. Their conclusions are as follows:

1. Sex is not influenced by changes in external conditions such as temperature, light, soil, etc.

2. Sex cannot be determined by the position of the seed upon the mother plant or by any general characters of the seed.

3. Sex of dioecious Angiosperms is already determined in the seed.

Noll (46) experimented with dioecious Angiosperms including Cannabis sativa L. in an effort to determine the cause of the def-
inite ratio of staminate and pistillate individuals and the time of sex determination. His experiments lead to the conclusion that sex must be determined at the time of fertilization by the paternal sex cells. His theory concerning the differentiation in the pollen grains is that two spores from a tetrad have a dominant and two a recessive staminate tendency. Hence the union of a sperm from a pollen grain having a dominant staminate tendency with the egg gives rise to staminate offspring. Also the union of a sperm from a pollen grain having a recessive staminate tendency with the egg gives rise to pistillate descendants.

Correns (16) experimenting with monoecious and dioecious Angiosperms agrees with Noll (46) that the paternal sex cells determine the sex of the descendants, that the tendency of the egg is always pistillate and that the differentiation of paternal sex cells takes place in the reduction division of the pollen mother cell. However, the theory of Correns (16) differs from that of Noll (46) for he argues that two spores resulting from the tetrad have a staminate and two a pistillate sexual tendency.

Strasburger (62) in a recent publication records the results of experiments and observations concerning the cause of sex determination in dioecious Angiosperms. He attempts to prove the theory of Noll (46) and Correns (16) concerning the differentiation of sex in paternal sex cells by pollination experiments, but his results are negative. The division processes of the sporogenous cells of several dioecious forms were investigated in an effort to determine the cytological basis of sex separation. The work of Stevens (58),
Wilson (68) and others has shown that certain sexual differences of chromosome groups exist in the spermatozoa of insects. Darling (18) reports the presence of certain "accessory" or "idiochromosomes" in Acer negundo which he states are analogous to those found in the animal kingdom. However, Mottier (45) in a recent investigation of the pollen formation of the same species denies the existence of such chromosomes here. The observations of Strasburger (62) indicate that there are apparently no differences in the size or number of chromosomes in the pollen produced from a given tetrads of microspores.

The present study which follows the divisions of the pollen mother cell in greater detail than was done by Strasburger (62) confirms his theory that in the pollen of Cannabis sativa L. there is no significant difference in the division products resulting from one pollen mother cell. Neither in the size nor number of chromosomes is there any indication of a differentiation which might be correlated with the observed condition in insects.

The inheritance of sex in plants as well as animals is a debated question. Correns (16) as a result of cross-pollination experiments concludes that the staminate plants of dioecious forms are heterozygotic and the female homozygotic and that sex is inherited according to Mendelian rule. Strasburger (62) concludes that the staminate tendency is subjected to a weakening in forms which have resulted from self pollination. The ratio of staminate to pistillate individuals is somewhat dependent upon the chance pollination of the species and the preponderance of pistillate individuals would be due to the recessiveness of the staminate
tendency in more than half of the pollen grains.

Strasburger (62) would agree that both sexual tendencies exist in the individual one being dominant and one recessive. The original separation of the sexes is completed sometime in the haploid generation and is not necessarily bound to the reduction division as Correns (16) and Noll (46) believe. Primary sex separation then precedes fertilization and its separation in the reduction division is only secondary.

The strictly dioecious character of Cannabis sativa L. has led to much discussion concerning the nature of its diclinism. Some regard the unisexuality of this species as inherent and primitive. Others believe that the flowers have become unisexual by the atrophy of the androecium in the pistillate and of the gynoecium in the staminate flowers.

Briosi and Tognini (9) regard the diclinism as a primitive condition since there are no rudimentary sex organs in either the staminate or pistillate flower. Furthermore a certain meristematic activity noted in the flowers seems to indicate a disposition to produce new organs. They observed certain meristematic structures between the perianth and ovary of the pistillate flower and consider these as beginnings of stamens rather than rudiments. The rare occurrence of hermaphrodite flowers is taken to be indicative of a condition which is beginning rather than one which is inherent and primitive.

Praia (50) agrees with Briosi and Tognini (9) that the diclinism is primitive supporting his theory by observations of ter-
atological phenomena. In the hermaphrodite flowers of Maria hemp a single stamen is found closely applied to an open monocarpellary ovary. In the Sheria hemp various combinations between staminate and pistillate floral organs exist. Staminody of the pistil resulting in the substitution of a stamen for a style is the commonest phenomenon. The author states that since the alternation of the sexual organs precludes any comparison between the parts of the staminate and pistillate flower the diclinism is in the highest degree inherent.

Dicliny is generally considered as a derived condition since many families show transition stages from the monoecious to the dioecious forms. The experimental studies of Noll, Cowrens and Strasburger indicate that both a staminate and pistillate tendency is present in dioecious Angiosperms. In their phylogenetic development the paternal sex cells have become differentiated and a separation of sexes results.

In this investigation of the American Cannabis no hermaphrodite, monoecious or other intergrading conditions have been noted. The plants observed were growing in waste fields. The accounts of teratological phenomena reported by various authors indicate that abnormal conditions appear more commonly in cultivated places. Since the plants are given more favorable growth conditions in cultivated fields the tendency toward monoecism would seem to be a reversion to an ancient type brought about by unusual conditions. If the dicliny here is derived rather than primitive the differentiation of sex has gone far. The flowers themselves have lost
all suggestions of the suppressed parts and differ completely in form, structure, and function. The differentiation has also extended to the staminate and pistillate plants which have become specialized for pollen and seed bearing.

Sterilization of Pollen.

The phenomena of sterilization so evident in the microsporangium of Cannabis sativa L. lead to the question of its cause and significance. Sterility in pollen formation has recently received much attention from cytologists because of its common occurrence in hybrid forms, and because of its significance in the determination of the hybrid origin of species. Sterilization has never been reported for Cannabis sativa L. Hence its appearance here leads to a comparison of this species with other plants in which sterility has been observed.

That hybrids are frequently sterile has long been known and commented upon. According to Webber and Swingle (64) hybrids arising from the union of widely different parents are commonly sterile often producing little or no pollen and more rarely having defective ovules. All students of hybrid forms agree that the pollen is more likely to be imperfect than the ovules. Hence the commonest consequence of hybridization is the imperfect formation of pollen grains in the offspring. Often the anthers are completely empty or they are small and do not open.
Juel (39) who made a study of the pollen development in the sterile Syringa Rothomagensis, a cross between S. vulgaris and S. persica, found many irregularities in the reduction division. Degeneration sometimes begins as early as synapsis, but usually the tetrad divisions are completed.

Rosenberg (52a, 52b) in a study of the pollen development of Drosera longifolia obovata, a hybrid of D. rotundifolia and D. longifolia, observed that many pollen grains lose their contents and also that the embryo-sac development usually stops at the binucleate stage.

Gregory (32) noted an early degeneration in prophase of the pollen development of hybrid sweet peas.

Tischler (62a) ascribes sterility in the hybrids of Ribes and Bryonia and also hybrids of Lathyrus odoratus to the influence of long culture.

Gates (26) made a critical study of the pollen development in hybrids of Oenothera lata x O. Lamarckiana. Sterility appears here rarely in the early stages of the mother cell but is more evident in the tetrad stage. Oenothera Lamarckiana also shows irregularly shaped pollen grains and occasionally complete sterility.

Sterilization in the pollen of a number of species of Potentilla was observed by Wullff (66). The author explains the cause of the sterility as a series of influxes of the outer life conditions of the plant and suggests that perhaps parthenogenesis may be found in these forms.

Holden (36) records certain peculiarities in Betula pumila
which suggest its hybrid origin. Large areas of abortive spore-
mother cells were observed in the staminate cones and later the
degeneration of three spores of each tetrad was noted. The author
cites other peculiarities of the plant and states that spontaneous
hybrids can be diagnosed as hybrids by the investigation of their
internal anatomy.

Jeffrey (38) in a recent article in which he attempts to
prove that the genus Oenothera is subject to spontaneous hybrid-
ization, states that sterility is a noticeable characteristic of
species crosses or true hybrids. He observed the spores and pollen
of liverworts, mosses, ferns, lycopsids, selaginella, quillworts, lepi-
dodendrons, equiseta, cycads, ginkgo, conifers, gnetales, monocotyledon-
cous and dicotyledonous Angiosperms and always found known hybrids
to be characterized by abortive spores which have little or no
protoplasmic contents. He considers the sterilization of spores
as an indication of the hybrid origin of species. Jeffrey (38)
finds sterility in many species of Oenothera and concludes that
these forms are subject to spontaneous hybridization. Then the
mutation theory of De Vries based upon observations of various
species of this genus, according to this author appears to be only
a useless hypothesis. However, more investigation is needed in order
to establish a cytological and morphological basis for the phenomena
of mutation.

The occurrence of sterilization in the microsporangium of
Cannabis arouses the question as to whether or not the species is
a hybrid or is giving rise to mutants. According to Jeffrey (38),
Holden (36), and others whose works are quoted above, the sterility of pollen in varying degrees is a peculiarity of hybrids. The obscure and ancient origin of this species makes it impossible to determine this point; but the marked sterility of potential pollen as proven for the first time by this study casts suspicion upon its ancestry. Because of the similarity of the pollen condition to that of hybrids the study of the plant from the standpoint of mutation would doubtless prove profitable.

In spite of the recent morphological and cytological studies on the sterilization of hybrids no satisfactory explanation of its cause has been made. According to Gates (26) some of the suggested causes are: (1) lack of nutrition in the parts affected causing failure in the development of the tapetum and sporogenous cells; (2) the influence of long culture; (3) irregularities of development in the germ cells due to some lack of harmony between idioplasts; (4) some more deep-seated phenomena affecting the whole plant. These categories are more or less general having no definite limitations. However, they serve to designate the widely different points of view with which this subject is regarded.

Lack of nutrition due to the early degeneration of the tapetal cells is not a possible explanation for the sterility in Cannabis. The irregularity of the degeneration of the tapetal cells does not permit of any correlation with the degeneration of the sporogenous cells, since the tapetum often appears to be breaking down after the sporogenous tissue has begun to degenerate.

Sterility as a result of long culture appears in many cul-
tivated plants whose origin is unknown. De Vries (19) names many common garden plants which have long been propagated by vegetative means and are sterile, sometimes even destitute of flowers. Although Cannabis sativa L. is widely cultivated, it is always propagated by the seed. If the formation of the seed is dependent upon the act of fertilization then the pollen must be normal and functional. Whether or not fertilization takes place in Cannabis is discussed later.

Irregularities in the pollen development due to a lack of harmony between idioplasts is proposed by Gates (26) as a possible explanation of sterility in Oenothera lata. He states that the intimate union of paternal idioplasts in synapsis might lead to the development of incompatibilities between the plasms causing irregularities in the reduction divisions. This explanation is more or less theoretical based upon the unproven fact of the exchange of material between maternal and paternal plasms in synapsis. The lack of harmony between plasms of a hybrid derived from widely different parents is conceivable. However, since Cannabis is not a known hybrid this explanation of the sterility of the pollen would hardly suffice. Furthermore irregularities during the early stages of the mother cells are very few, the greater part appearing at the end of the reduction divisions.

Gregory (32) suggests that sterility of the pollen is the expression of more deep lying phenomena affecting the whole plant. A similar view is held by Tischler (62a). The American Cannabis differs from the European and Indian forms in many particulars.
evidently due to a difference in climate and soil. It is conceivable that sterility in the pollen formation might also be caused by the different climatic and soil conditions.

In many apogamous Angiosperms the pollen is defective or reduced. Strasburger (61) observed apogamy in Elatostema sessile, the pollen development of which is irregular. Miss Pace (48) also observed apogamy in Atamosco and substantiates the theory of Strasburger (61) that a diploid egg is incapable of fertilization. Wullff (66) as was previously noted also suggests that this condition may exist in Potentilla where the pollen is sterile. Cannabis, according to Winkler (65), is perhaps parthenogenetic or apogamous. If this is the case, then a certain correlation may exist between the parthenogenetic development of the egg and the defective condition of the pollen. Hence, as was previously shown by germination experiments the pollen may be entirely sterile if it is not necessary for fertilization and seed formation.

Investigation of the embryo-sac of Cannabis is needed to throw light upon this problem. The peculiar shape of the ovule renders a study of the female gametophyte difficult, and it is doubtless due to this fact that not much is known concerning its development.
SUMMARY.

1. The floral organs of the staminate flower appear in acropetal succession - sepals and stamens.
2. The floral parts of the pistillate flower arise in acropetal succession - perianth and carpels.
3. The perianth of the pistillate flower is developed from two primordia and is slightly higher on the adaxial side.
4. The floral bract of the pistillate flower is closely associated with the flower proper.
5. The ovary is bicarpellary in origin.
6. The ovule arises lateral to the apex of the stem, becomes associated with the posterior carpellary wall and is carried upward to the top of the ovarian chamber where it assumes a pendulous position.
7. The ovule is campylotropous and has two integuments.
8. The styles are developed on the margins of the fused carpels.
9. The species is dioecious and the flowers show no indication of suppressed organs.
10. The archesporium of the megasporangium is many-celled, but one cell functioning.
11. The wall of the microsporangium consists of an endothecium, two middle layers and a tapetum.
12. The tapetal cells divide mitotically in the early stages. Later fusions of the nuclei result in irregularities in their number and form.
13. The disorganization of the tapetal cells occurs normally at the early microspore stage of the pollen, but frequently at various earlier stages.

14. A large mass of sporogenous tissue is developed in the microsporangium, a single stamen having about 22,620 spore mother cells.

15. The reduction divisions of the pollen mother cells reveal no peculiarities in the form and behavior of the chromosomes.

16. The chromosomes remain distinct in the resting phase between the heterotypic and homotypic divisions.

17. A special wall is developed around the pollen mother cell which persists and becomes conspicuous at the early tetrad stage, later disappearing.

18. The pollen grain at maturity is invested by a comparatively thin wall in which are three exit points for the pollen tube.

19. The mature pollen contains a tube nucleus and a spherical generative cell.

20. All attempts at germination of the pollen resulted negatively, no pollen tubes being formed.

21. The dehiscence of the anther is in a longitudinal line, but there are no special lines of cleavage.

22. Pollination is affected by the wind.

23. Sterilization of potentially sporogenous tissue in the microsporangium occurs at all stages of pollen formation.

   a. Early sporogenous cells --- 1 per cent.
   b. Tetrad ------------------- 39.6 per cent.
   c. microspore --------------- 10 per cent.
d. Mature pollen ------- 44.1 per cent.

24. The pollen remaining unshrunken and apparently functional constitutes 1 per cent of the total potential pollen.
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69. Zinger, N.
EXPLANATION OF PLATES.

All figures were drawn with Spencer camera lucida. Figures 24 and 25 were made with Bausch and Lomb 1 1/2 objective and Zeiss ocular 4; figures 10 and 11 with Bausch and Lomb 3/4 objective and Zeiss ocular 4; figures 1 - 9, 12 - 23 with Spencer 2/3 objective and Spencer ocular 8; figures 26 - 33, 36 - 43, 92 with Spencer 1/6 objective and Spencer ocular 8; figures 34, 35, 44 - 54, 90, 93 - 101 with Bausch and Lomb 1 1/2 objective and Spencer ocular 8. The approximate magnification in diameters is as follows: figures 24 and 25, x 80; figure 10 and 11, x 120; figures 1 - 9, 12 - 23, x 168; figures 26 - 33, 36 - 43, 92, x 825; figures 34, 35, 44 - 54, 90, 93 - 101, x 1725.
ABBREVIATIONS USED.

a. sp. c. --------- active sporogenous cell.
an. -------------- anther.
ar. -------------- archesporium.
at. -------------- antipodals.
c. -------------- carpel.
cyst. -------------- cystolith.
d. sp. c. --------- degenerating sporogenous cell.
e. ---------------- egg.
emb. s. ------------ embryo sac.
end. -------------- endothecium.
ep. -------------- epidermis.
fil. ------------- filament.
fl. b. ------------- floral bract.
i. int. ------------ inner integument.
i. mid. l. -------- inner middle layer.
mid. l. ----------- middle layer.
n. --------------- nucellus.
o. int. ------------ outer integument.
o. mid. l. -------- outer middle layer.
ov. --------------- ovule.
p. ---------------- perianth.
par. --------------- parietal tissue.
p. ar. c. ------- primary archesporial cell.

pol. gr. ------- pollen grain.

p. sp. c. ------- primary sporogenous cell.

s. ------------ sepal.

sng. ------------ synergid.

sp. c. --------- sporogenous cell.

sp. m. c. ------- spore mother cell.

st. ------------ stoma.

stg. ------------ stigma.

stm. ------------ stamen.

tet. ---------- tetrad.

tro. --------- trichome.
Staminate Flower.

Figure 1. Longitudinal section through beginning of flower.
Figure 2. Appearance of sepals.
Figure 3. Appearance of stamens.
Figures 4, 5. Transverse and longitudinal sections of later stage.
Figures 6, 7. Longitudinal and transverse sections of still later stage.
PLATE II.

Staminate Flower.

Figure 8. Longitudinal section of advanced stage showing enlargement of anther.

Figure 9. Transverse section of same stage.
PLATE III.

Staminate Flower.

Figure 10. Longitudinal section of mature flower bud showing erect stamens roofed over by the imbricated sepals.
Figure 11. Transverse section of mature flower bud.


**Fig. 11**

The image shows a diagram labeled "Fig. 11". It appears to be a scientific illustration, possibly related to cellular structures, given the labels "s" and "stm". The diagram is labeled "PLATE IV" at the top, indicating it is part of a series or collection of illustrations. The content suggests it might be from a biological or chemical context, possibly showing cross-sections or models of cellular or molecular arrangements.
Figure 12. Longitudinal section through papilla which is to give rise to the flower and bract.
Figure 13. Appearance of floral bract.
Figure 14. Appearance of posterior perianth papilla.
Figure 15. Appearance of anterior perianth papilla.
Figure 16. Later stage showing appearance of carpels.
Figure 17. Transverse section of flower and bract at stage shown in figure 15.
Figure 18. Beginning of ovule.
Figure 19. Later stage showing association of ovule with posterior ovarian wall.
Figure 20. Transverse section of stage shown in figure 19.
Figure 21. Longitudinal section through later stage of flower showing the closing of the ovarian cavity and the beginning of the stigmas.

Figure 22. Later stage showing beginning of inner integument of ovule.

Figure 23. Beginning of outer integument of ovule.
PLATE VII.

Pistillate Flower in Bract.

Figure 24. Longitudinal section of nearly mature flower showing brush-like stigma and final pendulous position of ovule.
PLATE VIII.

Pistillate Flower.

Figure 25. Longitudinal section through ovary after fertilization.
PLATE VIII

Fig. 25
PLATE IX.

Megasporangium.

Figure 26. Longitudinal section through young ovule showing primary archesporial cell.

Figure 27. Slightly later stage showing primary sporogenous cell divided.

Figure 28. Later development of megasporangium showing several-celled archesporium.
PLATE X.

Megasporangium.

Figure 29. Section of ovule showing group of sporogenous cells.
Figure 30. Section through ovule showing one sporogenous cell becoming dominant.
PLATE XII.

Megasporangium.

Figure 31. Section through ovule showing one sporogenous cell greatly enlarged and others disappearing.
PLATE XIII.

Megasporangium.

Figure 32. Section through ovule showing embryo-sac initial.
PLATE XIV.

Megasporangium.

Figure 33. Section through tip of ovule at mature stage of embryo-sac showing integuments fused over beaked nucellus at micropylar extremity.
PLATE XV.

Female Gametophyte.

Figure 34. Four celled embryo-sac.

Figure 35. Mature embryo-sac.
PLATE XVI.

Microsporangium.

Figure 36. Transverse section through portion of stamen showing young archesporium and parietal tissue.

Figure 37. Later stage of same.

Figure 38. Section of microsporangium showing differentiation of tapetum.

Figure 39. Later stage of same.

Figure 40. Section of sporangium at synaptic stage of mother cell.
PLATE XVII.

Microsporangium.

Figure 41. Section through sporangium showing beginning of degeneration of inner "middle layer".

Figure 42. Degeneration of outer "middle layer".

Figure 43. Mature anther wall showing endothecium with thickening bands.
PLATE XVIII.

Tapetal Cells of Microsporangium.

Figure 44. Early uninucleate stage of tapetum.
Figure 45. Nuclei dividing at synapsis stage of spore mother cell.
Figure 46. Uninucleate cell at dyad stage of spore mother cell.
Figure 47. Three nucleate cell at same stage.
Figures 48, 49. Tapetal cells at tetrad stage of mother cells showing peculiarly shaped fusion nuclei.
Figures 50, 51. Tapetal cells at microspore stage showing beginning of degeneration.
Figures 52, 53. Tapetal cells almost completely degenerated.
PLATE XIX.

Pollen Mother Cell.

Figure 54. Nuclei in a resting condition.

Figures 55, 56, 57. Nuclei showing reticulum and chromatin becoming more distinct.

Figure 58. Nuclei with definite spirem before synapsis.

Figure 59. Nuclei in synapsis.
Pollen Mother Cells.

Figure 60. Nucleus at spirem stage.
Figure 61. Segmentation of spirem showing telosynaptic formation of chromosomes.
Figure 62. Nucleus with dyad chromosomes.
Figure 63. Formation of multipolar spindle.
Figure 64. Bipolar spindle with chromosomes grouped in equatorial region.
Figure 65. Pulling apart of chromosomes.
Figure 66. Massing of chromosomes at spindle poles.
Figures 67, 68, 69. Reconstruction of daughter nuclei.
Figure 70. Daughter nuclei entering into homoeotypic division.

Figure 71. Appearance of bipolar spindles.

Figure 72. Pulling apart of chromosomes.

Figure 73. Chromosomes massed at poles of spindles.

Figure 74. Reconstruction of grand-daughter nuclei.

Figure 75. Young tetrad showing prominent special wall.

Figure 76. Nuclei of tetrad walled apart. Development of ridge-like thickenings on inner side of wall.

Figure 77. Later stage of tetrad showing outer wall beginning to disorganize.

Figure 78. Tetrad whose wall has disappeared. Microspores breaking apart.

Figure 79. Same stage showing one cell of tetrad degenerating.

Figure 80. Whole tetrad degenerating.
PLATE XXII.

Pollen.

Figure 81. Young microspore after separation from tetrad.
Figure 82. Microspore at same stage slightly shrunken.
Figure 83. Microspore at same stage smaller and shrunken.
Figure 84. Microspore nearly completely broken down.
Figure 85. Later stage of microspore.
Figures 86, 87. Microspores at same stage showing different degrees of sterilization.
Figures 88. Normal mature pollen grains.
Figures 89, 90. Pollen grains, shrunken and sterile.
Figure 91. Section through point of pollen tube exit (after Briosi and Tognini).
Diagram showing the amount of sterilization that occurs in the sporogenous tissue of the microsporangium.
Sterilization of Pollen

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Percent at spore mother cell stage.

Percent at Tetrads stage.

Percent at early microspore stage.

Percent at mature pollen stage.

Percent of functional pollen remaining.
PLATE XXIV.

Figure 92. Section of mature leaf.
Figures 93, 94. Beginning of development of glandular hair.
Figures 95, 96. Transverse and longitudinal sections of later stage of development.
Figures 97, 98. Sections through 4-celled glandular hair.
Figure 99. Transverse section of multicellular hair.
Figure 100. Longitudinal section of multicellular glandular hair.
Figure 101. Unicellular hair.
Economic Uses and Vegetative Structures.

A conspicuous development of bast is noted in the stem of Cannabis. The fibers are very long often reaching a length of 22 millimeters and are multinucleate. It is for the bast of the stem that the plant is cultivated so widely.

Cannabis sativa L. is the commercial hemp which is cultivated in many parts of the world for its products. Although a native of Asia it spread westward throughout Europe and southward through the Indian peninsula. The chief continental hemp producing countries are Italy, Russia and France and it is also grown in several parts of Canada and the United States and India. The plant is cultivated in different countries for three products - the bast fiber of the stem, the resinous secretion developed in hot countries upon its leaves and flowering heads, and its oily seeds.

The fiber of hemp has long been used extensively in the rope industry and is also used in the production of yarns, the manufacture of sail cloth, sheeting, sacking, etc. The coarser quality is made into ropes and similar material and the finer into cloth. The plant is cultivated for its fiber in almost all European countries and most extensively in European Russia which is the chief hemp-exporting district. The finest hemp fiber is grown in the province of Piedmont, Italy where the climate and soil are such as to permit
a rapid growth of the plant at first, thus resulting in the formation of long fibers.

The preparation of the fiber for the market involves several processes. The plants are pulled or cut, then bound into sheaths, dried, and stacked. A process of combing removes the seeds and the stalks are tied into bundles. The retting or rotting of the stems is accomplished by immersing the bundles in water where they remain from 10 days to 2 weeks, until the fiber is separated from the woody core. The stalks are then dried and the fibers are separated and cleaned by hand or machinery, after which the material is ready for the market. The average yield of hemp fiber per acre is about 1000 pounds.

The ordinary unicellular superficial hairs which are common to Dicotyledones are very conspicuous in Cannabis where they often reach a great length (fig. 101). These are common in all parts of the plant especially upon the leaves, bracts, and stem. The hairs are simply prolongations of epidermal cells and appear dead and devoid of cell contents at maturity. Boodle and Fritsch (7) report that they are common in the Urticaceae.

Glandular hairs which are multicellular, epidermal outgrowths with a head and more or less definite stalk, are noted more particularly on the floral parts. When mature the cells of the head vary from one, two or four cells (figs. 97, 98) to many (figs. 99, 100). The resinous secretion for which the species is cultivated in the East is secreted from these glands especially from those of the pistillate inflorescence.
Cystoliths which are aggregates of calcium carbonate are common on the upper side of the leaf. They are contained in trichomes the basal portion of which is strongly swollen (fig. 92). Longer hairs on the lower side of the leaf also contain smaller cystoliths. Of these the stalk of the cystolith may be said to be formed by the body of the hair which has become solid owing to calcification and silification.

The medicinal and intoxicating properties of hemp have been known in the oriental countries since an early time. In Persia, Northern India, Arabia, and parts of Africa and Brazil it is grown partly and often mainly for the resin which is produced by the glands upon the leaves and the flowering heads. The plant as a drug or intoxicant for smoking and chewing occurs in three forms—"bhang" which is the dried leaves and small stalks,"ganja" or the flowering heads of the pistillate individual and "charas", the resin collected as it exudes from the plant. The composition of the drug is obscure but it acts typically as an intoxicant resembling alcohol in many features of its action but varying widely in its effects upon different individuals and races. The early symptoms of its use are highly pleasurable and for these it is largely consumed in the East. The drug is employed extensively for medicinal use in all parts of the world.

The seeds of hemp are combed from the pistillate plants when the fibers are prepared for the market and sold for various uses. The finest ones are kept for sowing, a large quantity is sold
Cannabis indica, a special variety of Cannabis sativa L., differs from the American grown Cannabis in several particulars owing to the difference of climate and soil. The plant grows to a much larger size and it is the resin of the Indian form that has the medicinal and narcotic properties before mentioned. Much comment on the subject of the American Cannabis has been recently caused by the increased cost of the Indian drug and the question as to whether or not an active variety can be successfully cultivated in this country. A study of the American grown Cannabis in comparison with samples from various other sources was carried on by C. R. Eckler and F. A. Miller (22). Hemp seeds from different localities and of different varieties were grown and their medicinal effect tested upon animals. From these experiments it was shown that the soil, climate and geographical location have a decided influence upon the activity of the American and the Indian Cannabis, since repeated plantings from selected seeds of both forms did not yield a product testing over 65 per cent as active as the Indian grown drug. Consequently if the American Cannabis is made officinal much difficulty will be experienced in obtaining active lots which will compare favorably with the Indian drug.
Abnormalities in Cannabis have been observed to occur commonly in certain localities and their presence is taken to bear upon the origin of the dioecious character of the species. The most striking and common of these have been reported by various writers — the most recent accounts being made by Briosi and Tognini (9) and later by Prain (50).

In the cultivated hemp fields of Bengal where all the staminate shoots are pruned away from the pistillate plants there is always present a considerable number of pistillate plants which are termed "Khasia". These individuals are marked by a total absence of narcotic resin for which the crop is grown and although they flower as copiously as the normal pistillate plants they never set seed. The flowers are similar in appearance to the normal flowers except that the glandular hairs are not discernable from the non-glandular owing to the absence of resinous material. Although these plants are recognizable at a fairly early stage by their robust growth and darker color they are left standing in the field because their differentiation is not effected sufficiently early to admit of their being replaced. This condition is reported by Prain (50) and Kerr (40) from the locality of Bengal but is not known in other regions. Prain (50) examined carefully hundreds of these plants and found the ovule enclosed within a perfect ovary but without any trace of fertilization. The author suggests that this may be due to impregnation by imperfect pollen or the result of an over stimulation.
of the vegetative functions since the condition is found exclusively in cultivated fields.

Monoecious conditions of hemp have been reported as quite common in certain regions. Prain (501 states that this condition is not unusual in the Indian Cannabis and describes several distinct monoecious flowers. Plants of staminate habit and appearance have staminate flowers on the lower portion of each branch and only pistillate on the upper part. The converse condition is also found where plants of the staminate type have the lower one-third to one-half of each floral branch bearing nothing but pistillate flowers and the upper portion only staminate. The last condition is so common in India that it has received the special name of "Sheoria" hemp. Pistillate plants are often observed with one or more branches bearing only staminate cymes which in cultivation are carefully removed. The "Moria" hemp of India is a common monoecious condition in which the axillary spikelet occurring between the basal pair of pistillate flowers develops stamens.