

UNISEXUAL MAIZE PLANTS AND THEIR BEARING ON SEX DIFFERENTIATION IN OTHER PLANTS AND IN ANIMALS

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Differences in the size or shape of the chromosomes of the two sexes have been noted in many dioecious plants as well as in animals. A compilation by SINOTO (1929) gives 7 species of bryophytes and 51 species of angiosperms so characterized. In all but one case the female gametes are alike. The male gametes are of two kinds. They either differ in size or shape of one pair of chromosomes, or one class of gametes has one or more chromosomes that the other class does not have.

In 33 dioecious species of bryophytes and angiosperms examined, no allosomes have been found. Animals also have species in which no inequality in chromosome construction is evident. WINGE (1922) does not find any visible difference in the chromosomes of *Lebistes*, although there is definite evidence that one chromosome pair is associated with sex differentiation. Many other animal species have been examined and no heteromorphic chromosomes found.

Nevertheless, WINGE (1923) states: "I have no doubt that sex chromosomes are found altogether throughout the whole of the vegetable kingdom in dioecious species, though it will not perhaps always be possible to demonstrate the same. . . . Unisexual individuals of either sex can in their autosomes be equipped with male and probably female tendencies, but the sex chromosomes are the normal regulating mechanism which as a rule determines the sex."

According to this view one particular pair of chromosomes carries the main sex-determining agency whether or not the members are visibly differentiated. It is obvious that differences in the amount of chromatin can not be the primary genetic agency in sex determination.

Unisexual plants, produced in controlled pedigree cultures, from a monoecious species showing equal chromosome pairs, give an illustration of a working mechanism that differentiates the two sexes. Dioecious maize has been developed and propagated through four generations. Two classes of sex-determining gametes are produced by the male plants; the female plants are monogametic.

GENES AFFECTING THE FLORAL EXPRESSION OF MAIZE

Many genes have a specific effect on the floral expression of maize. Some of the more noticeable ones are tassel seed, tunicate ear, anther ear, and several of the genes for dwarf plants.

It is our purpose to show that some of these have a definite tendency to further the development of female flowers and suppress male tendencies while others have a directly opposite effect. In other words, there are specific sex genes in maize.

Several genetic factors producing pistillate-flowered maize plants have been described. EMERSON (1920) has given his results with tassel ear and tassel seed. These genes later were renamed tassel seed-1 and tassel seed-2. The inheritance of tassel seed-4 has been given by PHIPPS (1928). These three factors are all recessive and when homozygous the terminal inflorescences have their staminate florets replaced by pistillate ones. There is some difference in the formation of the inflorescence characteristic for each gene but in general the result is the same. The usual panicle of maize is replaced by a mass of silks closely enfolded by the terminal leaves. The ovules are more or less unprotected by the glumes and as the inflorescence grows out from the surrounding leaves the developing seeds are exposed. In this condition they are easily infected by the smut fungus, and by the time the seeds are ripe, the whole inflorescence presents an unsightly object having no utilitarian value and apparently having no advantage for survival under natural conditions.

In tassel seed-1 the seeds are arranged on small spike-like structures that have some resemblance to the lateral inflorescences. Many of these small ears are grouped together with a densely matted arrangement of pistils as shown in figure 1. In tassel seed-2 (figure 2) the seeds are arranged in pairs. The whole structure resembles the normal staminate inflorescence much more closely than the other types. The seeds at the base of each branch are only partially covered by the glumes but the glumes become longer as they are placed farther out on the branches, and at the tips the ovules are often completely covered. Aborted anthers may be found with these seeds in varying numbers and stages of development. At the base they may be entirely rudimentary. They become more frequent and better developed at the tips of the branches. On some plants no anthers are found. On others they may all be aborted. On some the anthers may produce pollen in varying amounts up to the normal amount for the individual spikelet. This pollen from tassel seed plants is capable of fertilization.

Tassel seed-4 as described by PHIPPS differs from the other types in having the seeds more crowded and irregular and in having more than one pistil associated with each spikelet. Many of these supernumerary pistils are modified glumes. Anthers are also present with the ovules in the terminal inflorescences.

All of these tassel seed plants produce lateral inflorescences that are similar to the normal carpellate floral structure of maize. They differ in

having the seeds crowded into an irregular position due to the development of the second of the paired flowers that is usually aborted. Tassel seed plants in nearly every case can be identified from normal plants in this way even if the terminal inflorescence does not develop.

Three other tassel seed types have been named but their descriptions have not yet been published. They are dominant in their inheritance but otherwise are similar to the others just described. The dominant gene responsible for the tunicate ear of maize, when homozygous, also forces the development of ovules in the terminal inflorescences as described by COLLINS (1917) and shown here in figure 3.



FIGURE 1.—A terminal inflorescence of tassel seed-1 shortly after pollination.

Another group of genetic factors allows stamens to develop among the seeds of the lateral ear. Anther ear and several factors for dwarf plants fall in this class. Such anthers are usually poorly developed and are not permitted to shed pollen under natural conditions since they are enclosed by the husks. For a more complete discussion of genetic characters of this kind, see EMERSON (1924).

ENVIRONMENTAL INFLUENCES AFFECTING THE FLORAL EXPRESSION OF MAIZE

When grown in a shortened day, normal maize regularly produces seeds in the tassel as shown in figure 4. In such cases, often, no lateral inflores-

cences are formed. Both pistillate and staminate flowers are produced in varying amounts. The seeds produced in the tassels in this way give normal offspring when grown in a normal environment. The sporadic occurrence of seeds in the tassels on the main stalk and on the side branches of plants grown under natural field conditions, and the occurrence of tassel-like structures on the ears, are more difficult to account for. They are not readily associated with any external conditions since the usual fluctuations



FIGURE 2.—A terminal inflorescence of tassel seed-2 at the time of pollination, upper leaves removed.

of fertility and moisture do not consistently bring about these changes. They are not due to any simple combination of genetic factors since they do not reappear in later generations in the descendants of deviating plants much more than from normal plants. On the other hand, some strains of maize have a noticeable tendency to produce these abnormal flowers while other strains never do. This fact shows that some germinal combinations are more easily changed in this respect than are others. STRASBURGER, CORRENS, SCHAFFNER, YAMPOLSKY and others have given much evidence

showing the effect of external conditions upon the sexual expression of plants. RIDDLE, CREW, BANTA and others have done the same with animals. For the literature and an excellent discussion of this subject, the reader is referred to SHARP (1934). Since all other characters are modified in their expression by environmental influences, it is certainly to be expected that those complex characters associated with sex will also be modified by external conditions. But this variation does not preclude some

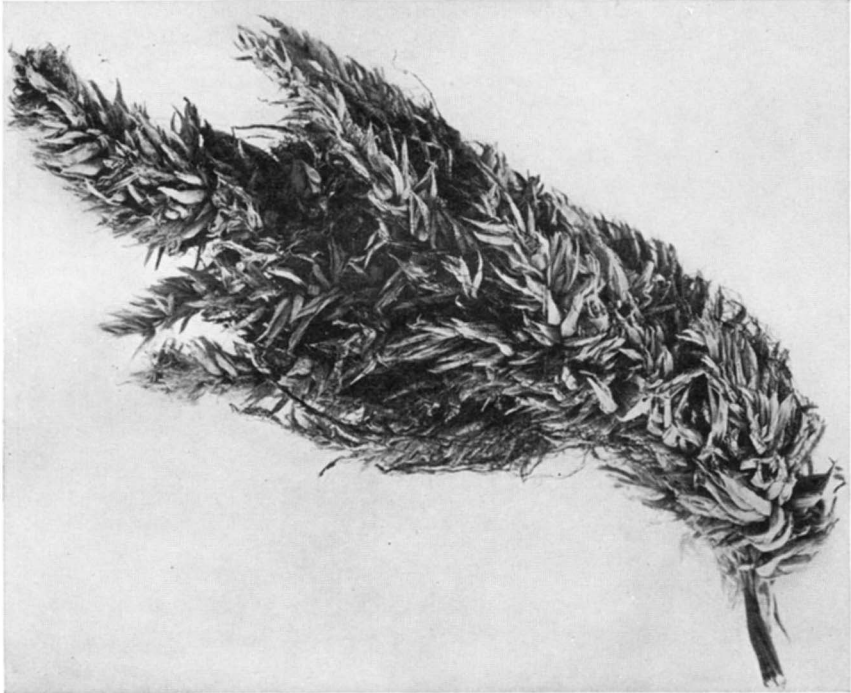


FIGURE 3.—Panicle of homozygous tunicate maize with mature seeds.

transmissible mechanism by which the numbers of two sexes are roughly apportioned.

All of the evidence shows that there is a clear-cut physiological gradient in maize from femaleness at the base of the plant towards maleness at the top. In both types of inflorescences this gradient is also operating from the base to the tip. In mixed inflorescences the female flowers are nearly always at the base and the male flowers at the tip. In related species of grasses, a similar situation exists. The genetic factors just described operate in some way to determine the points on this gradient where the development of the flowers begins. In such a complex of interacting forces their action is seldom complete but in general they determine the *balance*

of control such that the effect of one or the other allele directs development in one way or the other.

In studying the effect of genetic factors upon the floral expression of plants it is the usual practice to hold the environmental conditions as uniform as possible. In making such a study there is a natural tendency to overlook or minimize the importance of the external influences that may bring about similar results. For the same reason the physiologist uses ma-



FIGURE 4.—Seeds produced in the terminal inflorescences of normal maize when grown in a shortened day, compared with a normal lateral ear (center) grown on the same kind of a plant in its usual environment.

terial that is germinally stable and varies the environmental conditions in many ways. He, likewise, has a tendency to overlook or minimize the importance of the germinal control of floral expression.

The normal monoecious maize plant is fundamentally a perfect-flowered organism, as shown by WEATHERWAX (1923). In the flowers of the terminal panicles the stamens are fully developed while the carpels are present only in a rudimentary condition. In the lateral spikes the reverse is the case. The stamens, ordinarily, do not develop beyond a primordial stage. The change from functionally carpellate to functionally staminate flowers is merely a suppression or stimulation of one or the other type. This change

may be brought about both by transmissible genes or by environmental influences. But under the usual conditions in which the plants are grown the flowers are relatively constant in their development. Otherwise maize would not be such an important crop plant.

Many unisexual plants and animals give evidence that they are basically bisexual and carry the potentiality to develop some or all of the opposite sex characters. Sex reversal is simply a process of stimulation of certain potentialities that are usually dormant and of suppression of other characters normally functioning.

ORIGIN OF DIOECIOUS MAIZE

The pistillate-flowered maize plants described above are wholly or largely female in function. Several other genes are known that sterilize entirely the pistillate flowers. One of these is silkless, a gene that causes the ovaries to abort. The lateral inflorescences are otherwise normal in development, but the spike, enclosed in its usual protective husks, is entirely barren. Stalks wholly without lateral flowers are associated with at least two other genes. Both of these types lacking pistillate flowers have well-developed stamens in the terminal inflorescences and are entirely male in function.

In 1925 the production of dioecious plants in a species that had previously never shown this condition was attempted by crossing a functionally pistillate individual with a functionally staminate one. For this purpose a silkless plant was used as the male and tassel seed-2 as the female. The behavior of the first and later generations of this cross have been described (JONES 1931, 1932). Briefly, the first generation was entirely normal with both types of flowers in their usual position. In later generations double recessives of tassel seed-2 and silkless were found to be indistinguishable from tassel seed-2 by itself. Apparently this tassel seed gene has the ability to nullify the action of the silkless gene. Plants having the composition $sk\ sk\ ts_2\ ts_2$ produce seeds both in the lateral as well as the terminal flowers and are female in function. When such plants are crossed with silkless individuals heterozygous for tassel seed, $sk\ sk\ Ts_2\ ts_2$, and male in function, the result is a progeny made up of the same two classes as the parents.

In this way the dioecious condition propagates itself. Fourteen families in all have been grown. Eight of these were carried two generations and six were continued to the fourth generation. The results with the fourteen families are given in table 1. In a total of 967 plants, 390 are male and 515 female, with 61 monoecious hermaphrodites.

GENETIC COMPOSITION OF THE DIOECIOUS PLANTS

The evidence that the female plants are homozygous for both tassel seed and silkless is derived from several sources. In the first place, plants

heterozygous for both factors when selfed give normal, silkless and tassel seed plants, in a ratio of 9:3:4. This is as expected on the assumption that the recessive silkless gene has no effect when associated with homozygous recessive tassel seed. The actual count is 36 normal, 17 silkless and 30 tassel seed with 14 individuals having normal tassels but no lateral inflorescences. Such plants can not be classified either as silkless or normal but may be either. Dividing them in the same ratio as the same two types found and adding to the others, a ratio of 46:21:30 is obtained where expectation is 54:18:24.

If the female plants are homozygous for seed sterility, even though producing good seeds, when crossed by recessive silkless with normal tassels, they should give all silkless plants. Five progenies have been grown totaling over 100 individuals and all but one have been silkless. This one could easily be an out-cross.

Some tassel seed plants produce a small amount of pollen. Such pollen used on female plants gave a progeny entirely made up of tassel seed plants.

With the evidence from these three sources it is proved that the female plants are $sk\ sk\ ts_2\ ts_2$ in composition and are monogametic. The male plants are $sk\ sk\ Ts_2\ ts_2$ in composition and are digametic. Male and female plants in the third generation of dioecious propagation are shown in figure 5. Representative terminal and lateral inflorescences of a female plant and lateral sterile inflorescences only of a male plant are shown in figure 6.

The growth and arrangement of the terminal flowers of tassel seed are such that it is difficult to guard against undesired pollen in controlled pollinations. For this reason the lateral spikes are generally used for pollination. In many cases the female plants make no lateral inflorescences necessitating the use of terminal flowers. Even the lateral flowers can not be completely protected. In every case in which pollen from other kinds of corn gains access, the result is a normal monoecious hermaphrodite. Moreover, outside pollen also makes possible female-appearing plants heterozygous for silkless ($ts_2\ ts_2\ Sk\ sk$) instead of the double homozygotes. These plants can not be detected and when used as females give hermaphrodites, females and males in the ratio of 1:2:1. If we take all progenies in table 1 with two or more hermaphrodites, we have a count of 34 hermaphrodites: 87 females: 45 males—a fair approximation of a 1:2:1 ratio.

Twenty-one progenies produced only male and female plants. Twenty-one more showed only one hermaphrodite. In many cases, following generations gave no hermaphrodites, showing that these unexpected individuals could have been out-crosses. In the remaining 12 progenies out of a total of 54, there were two or more hermaphrodites. It seems unreasonable to

attribute all of these to accidental out-crossing and to parents heterozygous for silkless, especially in view of the fluctuating variability of the female plants themselves, although these two possibilities undoubtedly account for many of the off-type plants.

DEVIATION IN SEX RATIO

The 21 progenies with no hermaphrodites produced 170 males and 210 females, a ratio of 1 to 1.24 or 44.7 percent of males. A deficiency of males



FIGURE 5.—Dioecious maize: female (left) with mature seeds in both terminal and lateral inflorescences; male (right) with pollen-bearing panicle and sterile lateral inflorescence.

has been noted in many dioecious plants, particularly by CORRENS (1928) in *Melandrium* and *Rumex*, and WINGE (1923) in *Cannabis* and *Humulus*. In these plants WINGE attributes the differences to unequal fertilizing ability of the male-determining pollen grains with their smaller chromatin content. He cites NOHARA'S (1923) results with *Spinacia* showing equality in the number of both sexes in a species where no chromosome differences have been found. A difference in chromosome number or size is apparently not the deviating factor in this material. There is no reason evident why

the female plants with two homozygous recessive factors and their associated chromosomes should have a higher survival value than male plants with only one such pair.

VARIATION IN PISTILLATE PLANTS

It is a common observation that inbred strains of maize vary markedly in the relative development of their pistillate and staminate flowers. Some strains are good pollen producers and are less well-developed in seed formation. Others give good grain yields when properly pollinated by other

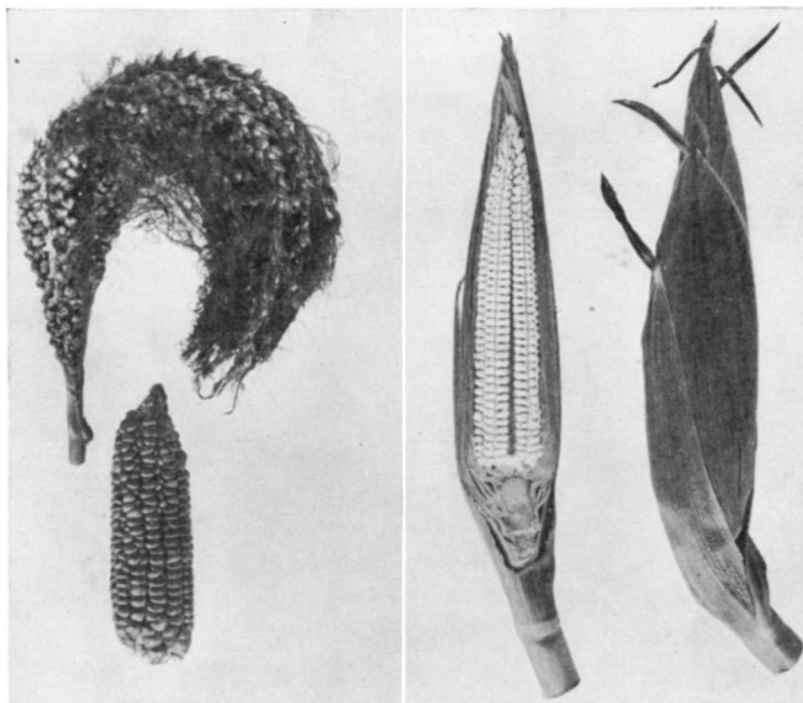


FIGURE 6.—Dioecious maize: mature seeds in the terminal and lateral inflorescences of a female plant (left) and sterile lateral inflorescences of a male plant (right).

plants but shed very little pollen themselves. Other strains are about equally well-developed.

Tassel seed types also vary in the amount of seed borne in the terminal panicle and in the amount of pollen produced. In the original tassel seed-2 plants obtained from Dr. R. A. EMERSON, no anthers were formed. A representative terminal inflorescence is shown in figure 2. After crossing with normally tasseled plants for several generations during the process of establishing the dioecious condition and then for four generations afterwards a tendency to form anthers has been strongly developed in all fami-

TABLE 1
The progeny of dioecious maize families in successive generations.

FAMILY NUMBER	YEAR GROWN	CLASSIFICATION OF PROGENY		
		♂	♀	♂
1	1930	11	7	0
	1931	9	14	0
	1932	11	6	1
	1932	12	9	0
	1933	1	15	0
Total		44	51	1
2	1930	5	6	0
	1931	7	4	1
Total		12	10	1
3	1930	5	12	0
	1931	5	4	0
	1932	8	12	0
	1932	10	8	1
	1933	8	3	1
	1933	7	11	0
	1933	9	6	0
	1933	5	8	1
Total		57	64	3
4	1930	6	14	0
	1931	6	11	1
	1932	12	9	0
	1933	4	12	0
Total		28	46	1
5	1930	10	10	0
	1931	11	10	1
	1932	10	8	2
	1932	10	12	0
	1933	11	5	0
	1933	5	9	3
Total		57	54	6
6	1930	12	8	1
	1931	6	13	2
	1932	8	13	0
	1932	0	9	5
	1932	9	7	1
	1932	18	5	0
	1933	7	8	1
	1933	5	12	2
	1933	2	8	6
	1933	7	12	0
	1933	5	8	0
Total		79	103	18
7	1930	9	6	1
	1931	8	9	1
Total		17	15	2

TABLE 1 (Continued)

8	1930	9	10	1
	1931	7	14	0
Total		<u>16</u>	<u>24</u>	<u>1</u>
9	1930	8	4	1
	1931	9	8	1
Total		<u>17</u>	<u>12</u>	<u>2</u>
10	1930	4	14	2
	1931	6	15	1
	1932	7	14	1
	1932	6	7	6
	1933	2	14	1
	1933	7	7	6
	1933	4	10	1
Total		<u>36</u>	<u>81</u>	<u>18</u>
11	1930	3	17	1
12	1930	8	10	2
13	1930	5	13	1
14	1930	6	10	2
	1931	5	5	2
Total		<u>11</u>	<u>15</u>	<u>4</u>
Total of all families		390	515	61

lies, indicating an accumulation of germinal modifying factors, in spite of the fact that good female plants have been selected as progenitors in each generation. Figure 7 shows eight stages of variation from nearly complete female to nearly complete male, represented by plants in the fourth generation of dioecious propagation.

In the first stage all of the flowers have well-developed ovaries and pistils. There are a few rudimentary anthers in the terminal flowers of the main spike and at the tips of some of the branches. The whole inflorescence is curved and drooping due to the weight of the developing seed. In the intermediate stages there are no pistils in the flowers at the tips of the main spikes. The flowers with functioning ovaries become fewer in number and those with anthers more and more prominent. The panicles become more upright and spreading. Finally stages are found in which very few seeds are produced and in some the pistillate flowers form no seeds. Their presence is noted by the few scattering pistils.

Some of the hermaphrodites found in the dioecious progenies may be females modified to a point where no silks are visible in the tassels. Such plants would still have lateral ears with irregular rows. No such plants have been noted although not all of the hermaphrodites were examined with this point in mind.

Many of these stages of variation are found within the same progeny or in the same family in different generations. There are, however, persistent differences in different families with respect to the stage of sex integradation indicating genetic control in some way. These differences can not be easily shown statistically.

VARIATION IN STAMINATE PLANTS

The silkless gene was first found in an inbred strain of yellow flint (JONES 1925). A similar gene, found by Dr. E. G. ANDERSON in other material, proved to be the same when tested. Silkless has usually been quite clear cut in its expression. A few seeds have been found in the lateral inflorescences

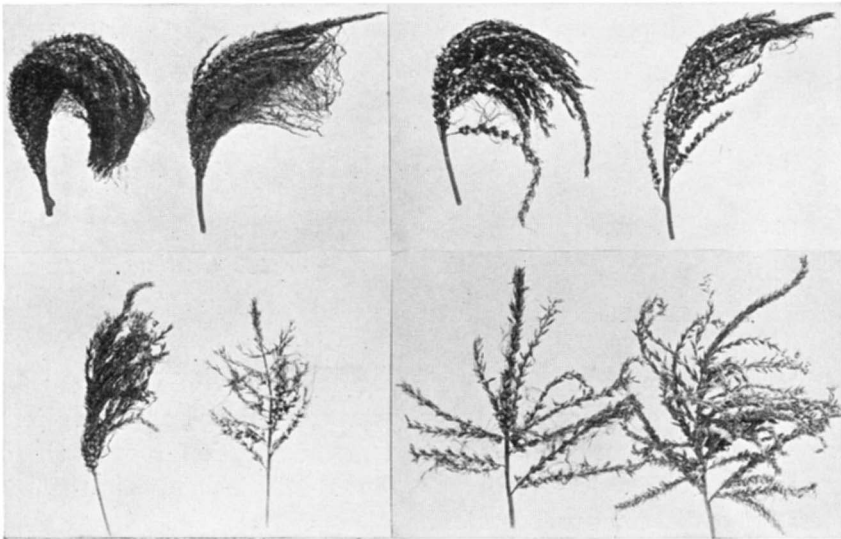


FIGURE 7.—Mature panicles from female plants of dioecious maize in the fourth generation showing variation in the number of pistillate and staminate flowers.

of several male plants. Some male plants in the fourth generation in one dioecious family produced a few short pistils on two ears that were otherwise barren. Pollen was applied to these but no seeds developed. A sterile structure of this kind, with one short pistil, is shown in figure 8 together with other cobs showing variation in the number of anthers produced. In many silkless families anthers are formed at the tips of nearly all of the barren spikes. These anthers are poorly developed and rarely contain pollen.

SPECIFIC SEX-DETERMINING GENES

In this dioecious maize there are two specific sex-influencing genes whose locations are known. One of these is kept in the heterozygous con-

dition in one sex, the other homozygous in both sexes. The interaction of both is necessary to maintain the unisexual condition. A clear presentation of a similar case has been made by ALLEN (1932).

So far the chromosome formation of this material is entirely normal. No visible differences in the male and female-determining chromosomes can be seen. The enforced heterozygosity maintained in one sex makes it possible for chromosome irregularities such as deficiencies or inversions to persist if they should occur and are viable. There is also the probability that recessive genes will accumulate in the protected chromosome in the male and this in time may have an effect on the sex expression.

In this material the male is the heterozygous sex. By using tassel seed

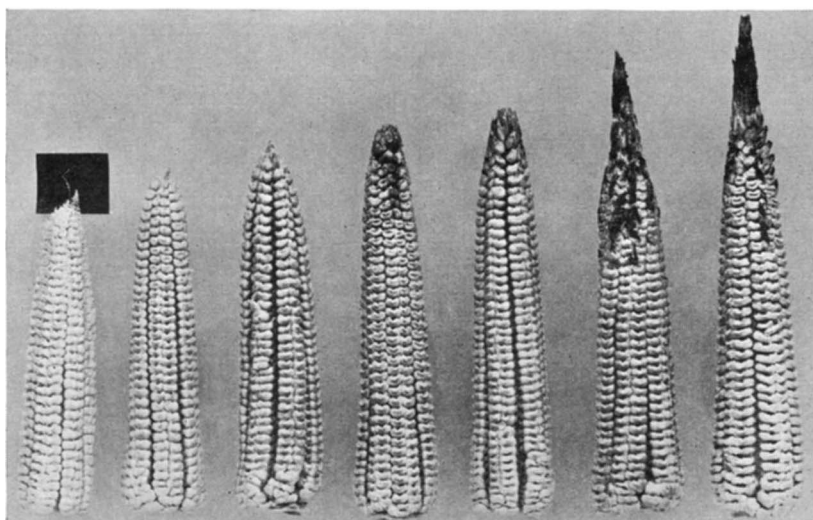


FIGURE 8.—Sterile lateral inflorescences from male plants in the fourth generation showing variation in the number of pistils and anthers.

genes with the gene for barren stalk, EMERSON (1932) has developed two types of dioecious maize. In one of these the male is the heterozygous sex, in the other the female is the heterozygous sex. The latter has been made by using one of the dominant tassel seed genes instead of a recessive.

No male plants have been found with ovules in the tassels or on the terminal flowers of side branches where they occur infrequently on normal plants. On the other hand there is a marked tendency for these individuals to produce anthers in the lateral spikes even though heterozygous for the tassel seed gene. The terminal flowers are well-developed and produce an abundance of pollen. This is due in part to their nutritional advantage in being carried on plants that do not have the burden of producing seeds. Nevertheless the silkless gene seems to be a specifically male-promoting

gene, suppressing female tendencies. The tassel seed gene is distinctly a female-promoting gene. The staminate flowers in the panicle are suppressed and the carpellate flowers that have long been dormant are forced into development. The secondary flowers in the spikelet of the lateral inflorescences are also forced into development as shown by the irregular arrangement of the kernels and their small and crowded condition. And in addition, the sterilizing effect of the silkless gene is entirely suppressed when both are acting together.

RELATION OF DIOECIOUS MAIZE TO SEX
IN OTHER ORGANISMS

Although we should not expect to find any close agreement between this sex mechanism in maize, that has come into operation almost overnight, with processes that have been developing in other species through geological epochs, there are certain features in common. In its important characteristics this situation in maize is closely in line with the theories of sex-determination advanced by CORRENS and GOLDSCHMIDT and supported by MULLER and PAINTER. One specific gene holds the *balance of control* over the sex of the offspring. In its action this gene is wholly dependent upon the support of at least one other gene located in a different chromosome. Other genes working with physiological modifiers, both internal and external, influence the development of the sex organs and bring about varying degrees of intergradation between the two sexes. None of these factors has been specifically located but definite evidence for their existence is at hand. They are complex in their action and widely distributed in the chromosomes.

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