

# CHANGE OF SEX IN HEMP

## Mutilation Makes Female Plants of *Cannabis Sativa* Produce Male Flowers— Change in Nutrition Probably Responsible for the Result

FREDERICK J. PRITCHARD

*Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.*

THE possibility of altering normal sex ratios in dioecious species of plants and animals is one of the most debatable topics of genetics. The experimental results thus far recorded are not only varied but occasionally contradictory. When viewed as a whole, however, they seem to indicate that maleness and femaleness are not always fixed characters, but frequently appear more like responses of the developing organism to external stimuli.

Of all the external factors that have been supposed to determine sex, food ranks first. Indeed, many biologists now believe that the determination of sex is in the last analysis a problem of nutrition. It is also remarkable that in nearly every instance in which food affects the sex ratio, favorable nutritive conditions tend to produce females and unfavorable conditions males.

Nevertheless the food theory of sex determination fails to account for the sex ratio of 1:1 commonly found among unisexual individuals.

A Mendelian theory of sex determination now popular not only explains normal sex ratios in dioecious species but receives considerable support from studies of sex-linked inheritance. The fundamental basis of this theory as expressed by a distinguished geneticist is that "sex has its beginning in gametic differentiation and is finally determined beyond recall in the fertilized egg by the nature of the uniting gametes." The hypothesis also carries the assumption that one of the sexes is heterozygous for a sex factor and the other homozygous.

Though subject to criticism, this theory is admirably adapted to the factorial method of analysis and appears to explain many facts of sex inheritance.

The difference of opinion regarding the effect of external stimuli upon sex ratios has led the writer to investigate the following questions: (1) Can sex ratios of dioecious plants be altered by modifying conditions external to their germ cells? (2) Is the alteration thus obtained limited to individuals of one sex? (3) How do the results harmonize with the Mendelian conception of sex determination?

### MATERIAL

As hemp is composed almost wholly of distinctly unisexual individuals it was chosen as a favorable species for the investigation of sex ratios. In addition to its separation of the sexes, it develops in its females a heavy and dense growth of foliage by which they may readily be distinguished from the males. In fact, at the time of flowering the plants may be recognized at a distance as male and female.

The proportion of males to females which normally appeared under the field conditions where the experiments were made was approximately 1:1. Monoecious individuals also appeared in relatively small numbers, as will be subsequently shown, but they were distinctly female in type and preponderatingly female in flower development.

### METHODS

Disturbances of the plant's physiological equilibrium were induced by the removal of flowers and vegetative parts and by the injection of chemical substances into the stem.

In addition to the removal of parts some plants were given further treatment by enclosing their tops in Manila bags to diminish the intensity of the light falling upon the newly developing flower buds.



#### MALE AND FEMALE HEMP PLANTS

At the left is the female or pistillate plant, at the right the staminate or male. In addition to the difference in flowers, the two sexes differ markedly in habit of growth, as is seen. By mutilation, each sex can be induced to take on the characters of the other; a change which may be due to interference with the plant's normal process of nutrition. Photograph by Lyster H. Dewey. (Fig. 19.)

The use of chemicals was limited to the year 1909. To facilitate their introduction small holes were cut into the pith cavities and afterwards closed with paraffin.

In 1914 no leaves or branches were detached but all flowers and flower buds were removed from branches and stem.

The counting of the flowers—a laborious process—was done by fives and tens but this nowise interfered with the observance of the staminate and pistillate character of the flowers.

#### EXPERIMENTS

Plants were grown for the investigations at Madison, Wis., in 1909, 1912, 1913 and 1914, but owing to a poor stand in 1912 and the writer's absence in 1913 when the treatments should have been given, the experiments for these two years were not completed. Hence, the results are limited to the two years' investigation in 1909 and 1914.

As no further opportunity has been found to continue the work it seems better to publish the results now obtained than to wait an uncertain period for the accumulation of further data.

In 1909, male and female hemp plants were used in approximately equal numbers. As each plant had at the time of operation already borne a large number of exclusively staminate or exclusively pistillate flowers, any degree of visible

monoeciousness could easily have been detected.

The 263 plants treated were mutilated by removing their flowers and flower buds, their leaves, and varying proportions of their stems; the tops of twenty were also bagged; and the stems of sixty others injected with 1 to 2 ounces of one or more of the following chemical solutions: calcium nitrate 1/10%; zinc sulphate 1/10%; dextrose 5%; maltose 5%; peptone 1%; asparagin 1/2%; potassium iodide 3%; pyridin  $\frac{n}{25}$ ; formic acid  $\frac{n}{16000}$ ; acetic acid  $\frac{n}{60}$ ,  $\frac{n}{30}$ ; sodium hydrate  $\frac{n}{300}$ ,  $\frac{n}{100}$ ,  $\frac{n}{60}$ .

Alteration of sex occurred under several different treatments. Either covering the top with a Manila bag or injecting into the stem a solution of dextrose, maltose, glucose, asparagin or pyridin was accompanied by a modification of sex. In each instance, however, the removal of parts constituted a part of the treatment. In fact the removal of parts was the only factor common to all the sex-developing responses. Hence it was probably the chief cause of sex alteration.

Of the 163 plants which reproduced flowers after treatment twenty-nine or 17.8% developed some flowers of the opposite sex. Four of these plants were males, the other twenty-five females.

TABLE I.—*Proportion of Monoecious to Dioecious Hemp Plants Found on Successive Dates at Madison, Wis., in 1909*

Date of examination	Number of dioecious plants		Number of monoecious plants	Percentage of monoecious plants	Field
	Male	Female			
September 23.....	49	43	8	8.0	A
September 27.....	96	99	5	2.5	B
September 30.....	0 <sup>1</sup>	174	26	6.5(13.0)	A
October 1.....	0	187	13	3.25(6.5)	B
October 1.....	0	84	16	8.0(16.0) <sup>2</sup>	B
October 18.....	0	47	3	3.0(6.0)	B

<sup>1</sup> As no male plants under these conditions formed perfect flowers, the omission of male plants in the counting records from September 30 to October 18 gives the percentage of monoeciousness for only the female type. The true percentages of monoeciousness are one-half the values inclosed in parentheses as represented by the figures at their left.

<sup>2</sup> Late maturing plants.

It may be argued that temperature or some other factor due to an advancing season was the effective stimulus rather than the removal of parts but this makes little difference as it would still be an external stimulus. The same statement may be made with regard to the possible effects of chemicals and diminished light intensity. However, the percentage of monoeciousness was determined on several successive dates in two neighboring fields designated respectively as A and B. These results are presented in Table I.

The percentage of monoecious plants in field A was much larger than in field B. This may have been due to wider spacing, as the plants in field A stood farther apart than in field B and consequently were larger and better fed; or it may have been caused by some inherent difference in the seed.

If we exclude the records of late maturing plants, made on October 1, the table shows no evidence of an increased percentage of monoeciousness as the season advanced.

The results for 1914 are presented in Table II. As they show unmistakable evidence of sex alteration from the use of external stimuli they are published in detail. Both the number and character of flowers removed as well as those that subsequently developed are included.

As shown by the table, sex was not altered by bagging the tops but was very decidedly altered by the removal of flowers. Of the fourteen male plants that formed flowers after the operation only three developed pistils but every female plant produced both stamens and pistils in abundance. In fact the proportion of stamen-bearing flowers formed on female plants greatly exceeds that ordinarily formed on monoecious plants.

As a check on the experiments recorded in Table II, twenty-eight female plants were tagged at the time of the foregoing operations and carefully examined at the end of the season for the appearance of male sex organs but not a single stamen had developed.

It is evident from the experiments and their checks that changes in sex

were induced by the removal of flowers and flower buds, probably through alteration of the food supply. The production of pollen and ovules is an exhausting process. As soon as male hemp plants shed their pollen they turn yellow and die. The removal of flowers and flower buds from the female plants when their reserve food is at a minimum probably makes the nutritive conditions less favorable for the development of the new buds and in accordance with the food theory of sex determination, causes an excess of male development. The appearance of pistillate flowers upon a few treated male plants, however, is difficult to explain upon this basis unless we assume that these particular flowers received more than their share of the food supply.

If the effect of flower removal on the food supply has been properly interpreted, it is evident that the proper method of inducing pistillate development in male plants is through high feeding, especially about the time of flower formation. This should be done through the soil in such a way as not to interfere with the plants' normal physiological activities.

#### CONCLUSIONS

The foregoing experiments show that sex of hemp is alterable by the removal of flowers. While only a few male plants produced pistils, they constituted 14 to 21% of the total number of males reproducing flowers after the operation. It is quite probable that if the proper stimulus were used pistil formation could be induced in all the males. The females were very responsive to the stimulating effect of flower removal. In fact in the second year's experiments every female operated upon produced an abundance of stamens.

The results do not seem to support the theory that sex is wholly a matter of zygotic constitution—one dose or amount of an inherited sex factor producing one sex and two the other, but indicate that both males and females are potential hermaphrodites as believed by Darwin and Strasburger.

TABLE II.—*Alteration of Sex Ratios in 1914*

Plant number	Number of flowers removed <sup>1</sup>		Additional treatment	Number of flowers appearing after operation			
				Under sack		On remainder of plant	
	Male	Female		Male or perfect	Female	Male or perfect	Female
1	16,030	0	Top bagged	640	0	1,040	0
2 <sup>1</sup>	55	9,780	Top bagged	101	432	8,420	621
3 <sup>1</sup>	560	5,500	Top bagged	875	97	5,085	5,910
4	0	7,355	Top bagged	43	980	722	8,100
5	0	7,370	Top bagged	11	65	655	3,280
6	18,875	0	Top bagged				
7	0	9,090	Top bagged	250	320	210	1,300
12	10,280	0	Top bagged	630	0	4,860	0
13	0	9,690	Top bagged	56	415	225	4,360
14	0	4,640	Top bagged	0	0	950	4,295
15	0	10,640	Top bagged	42	385	463	2,510
16	25,185	0	Top bagged	870	0	10,150	0
17	0	3,690	Top bagged	32	450	505	2,840
18	20,275	0	Top bagged	575	0	4,535	0
19	0	7,450	Top bagged	120	1,265	605	5,580
22	11,520	0	Top bagged				
23	0	11,680	Top bagged	890	780	4,750	9,340
27 <sup>1</sup>	341	8,990	Top bagged	720	750	3,250	3,870
37	23,910	0	Top bagged	480	0	2,590	0
38	28,370	0	Top bagged	540	0	5,140	3
39	0	7,820	Top bagged	15	420	52	2,530
40	0	10,650	Top bagged	184	470	269	3,410
41	0	9,380	Top bagged	5	880	3	2,340
42	0	8,920	Top bagged	258	470	275	3,490
43	0	8,220	Top bagged	75	380	125	7,850
46	0	9,370	Top bagged	125	350	336	3,160
8	17,450	0	None			840	0
9	0	9,625	None			550	4,575
10	8,140	0	None			0	461
11	0	10,211	None			375	6,735
20	0	10,360	None			2,005	2,270
21	23,845	0	None				
24	0	8,240	None			3,210	9,480
25	0	10,770	None			2,705	8,390
26	0	9,110	None			267	4,560
28 <sup>1</sup>	60	5,680	None			382	8,460
29	23,500	0	None			7,205	0
30	0	10,290	None			2,280	10,220
31	17,320	0	None			9,110	0
32	9,750	0	None			2,250	1
33	0	8,460	None			215	5,280
34	0	10,530	None			850	8,410
35	0	9,760	None			45	6,770
36	23,440	0	None			1,060	0
44	10,440	0	None			830	0
45	15,360	0	None			1,560	0
47	0	6,890	None			39	370
48	0	4,140	None			400	1,120

<sup>1</sup> Monoecious plants.<sup>2</sup> These were all the flowers on the plants at the time of operation.