THE FLUCTUATION CURVE OF SEX REVERSAL IN STAMINATE HEMP PLANTS INDUCED BY PHOTOPERIODICITY[1]

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In past experiments on sex reversal in the hemp, *Cannabis sativa* L., the writer did not succeed in producing 100 percent reversal of the individuals of any given population grown in the greenhouse during the short day period of winter (Schaffner 9, 10). The highest percentage of reversal obtained was 88+ percent for carpellate plants and 93+ percent for staminate plants. When these results were interpreted by some as indicating a multiple factor constitution of sex determination, with females, female intersexes, male intersexes, males, and supermales as the possible Mendelian combinations, further experiments became necessary to obtain definite information on the subject (see Hirata 3, 4). An experiment was devised through which "pure females" were rejuvenated after the usual blooming period and then changed to male expression in the second or rejuvenated ontogenetic cycle (Schaffner 11).

It is evidently desirable to learn how to set the ecological stage so that 100 percent of sex reversal would appear in the staminate plants in the first or usual ontogenetic cycle. A record of sex reversal in relation to photoperiodicity throughout the changing daily light period of the different seasons also seemed desirable. The aim, then, in the present experiments was to obtain, if possible, 100 percent of sex reversal of the staminate plants, including the "supermales," of a given plot; to find out how perfect the curve of reversal might be, and also to discover how definitely the percentage of reversal for any seasonal planting might be predicted.

The staminate plants only were studied because of a lack of proper greenhouse space and because the carpellate plants take a much longer period to reach maturity. The carpellate plants were mostly removed as soon as their sex reaction was definitely developed. The plants were grown on greenhouse benches with about 8 in. (20 cm.) of good, rich soil and were well watered. The temperature was kept at about 55–65 degrees Fahrenheit (13–18° C.) but the fluctuation was often considerably beyond these points. The plants were not disturbed in any way by removing flowers, branches, or leaves so whatever unusual effects were obtained were due entirely to difference of daily illumination.

Yampolsky (13) was able to cause sex reversal in staminate plants of *Mercurialis annua* by subjecting them to severe pruning, thus disturbing

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the sex balance present in the individual which under the given environment was expressing purely male characters. New methods of sex control and disturbance are continually coming to light. Recently Allan and Zotov (1) reported reversal of sex in both directions in the flowers of *Clematis foetida* caused by the presence of a rust (*Aecidium otagense*) in the inflorescence tissues and Holdaway (6) was able apparently to alter the sex ratio of the "flour beetle," *Tribolium confusum*, by a method of starvation, which was not due to differential mortality of the sexes.

The plantings were begun on July 15 and were made semi-monthly for ten months, or up to May 15. There were, therefore, 21 successive plantings all told. The general results are tabulated in table 1. Any plant that

TABLE 1. *Sex Reversal in Staminate Hemp. Greenhouse Plantings, 1929–1930*

Date of Planting	Total No. of Staminate Plants	Pure Staminate	Plants Showing Stigmas	% of Pure Staminate	% Showing Stigmas	Staminate Flowers on Reversed Plants	Flowers Showing Stigmas on Reversed Plants	% of Flowers with Stigmas	Average No. of Flowers with Stigmas on Reversed Plants
July 15.....	78	78	0	100	0	0	0	0	0
Aug. 1.....	95	90	5	94¾	5¼	365	17	4⅖	3⅖
" 15.....	69	50	19	72½	27½	898	33	3½	1¾
Sept. 1.....	114	69	45	60½	39½	1659	295	15	6½
" 15.....	38	15	23	40	60	753	140	15⅗	6
Oct. 1.....	51	12	39	23½	76½	662	165	20	4
" 15*...	10	1	9	10	90	96	69	42	7⅔
Nov. 1**...	5	0	5	0	100	20	54	73	11
" 15.....	20	0	20	0	100	75	192	72	9½
Dec. 1**...	11	1	10	9	91	55	53	49	5⅓
" 15†....	58	18	40	31	69	176	159	47½	4
Jan. 1.....	26	5	21	19	81	107	107	50	5
" 15.....	28	8	20	28½	71½	481	149	22	7½
Feb. 1.....	63	20	43	32	68	950	277	22½	6½
" 15.....	34	14	20	41	59	497	127	20⅓	6⅓
Mar. 1.....	84	48	36	57	43	1706	280	14	6
" 15.....	63	46	17	73	27	912	115	11⅕	6¾
Apr. 1.....	35	28	7	80	20	529	54	9⅕	8
" 15.....	16	14	2	87½	12½	143	15	9½	7½
May 1.....	19	19	0	100	0	0	0	0	0
" 15.....	38	38	0	100	0	0	0	0	0

* Cutworm and mice destroyed about two-thirds of the plants.

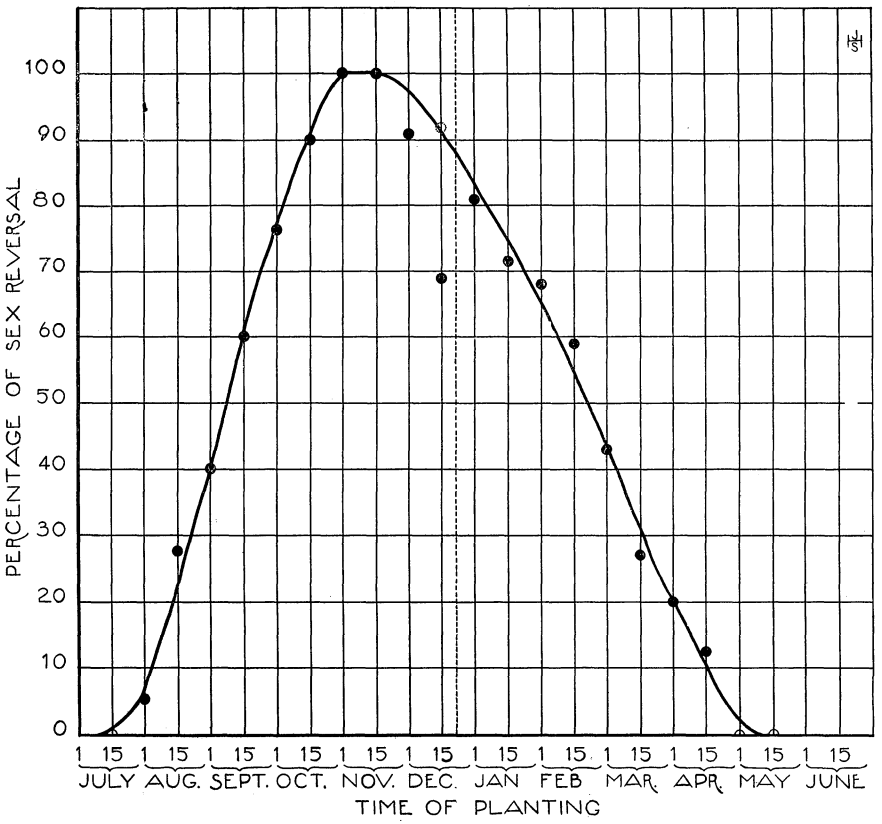
** Failed to sprout properly and nearly all died.

† Abnormal condition because of very bright weather at one period and accidental low temperature in the greenhouse.

showed flowers with fairly well-developed stigmas either on imperfect or normal ovularies or at the ends of stamens was counted as showing reversal to femaleness, since the stigma is a typical female organ. Many of the plants would never have produced normal seeds. The real problem was to find the existence of the female sex potentiality in the male plants and the ecological conditions which would swing the sex balance in the opposite

direction during the growing period after sprouting. The degree of perfection attained in the reversed sexual expression is of no special importance in these plants since it is itself dependent on the degree of action of the ecological conditions.

It will be noted that the percentage of reversed individuals rose gradually with decreasing daily illumination from zero to one hundred and then fell down again to zero with increasing daily illumination. There is one record that is decidedly below expectation, that of Dec. 15. This was plainly due to an accident which happened in the period of vegetative growth. There was an unusual amount of very bright weather at the critical period and then a very low temperature in the greenhouse room caused by the clogging up of the steam pipes for several days. A record of a previous year's planting for the same date is given as a dotted circle in the graph showing the



TEXT FIG. 1.

fluctuation curve (text fig. 1), which fits very well with the 1929–1930 curve. This record was of a planting of Dec. 15, 1920, having 42 staminate plants with 39 individuals or 93 percent showing reversal to femaleness.

Unfortunately three of the plantings show very low numbers, namely those for Oct. 15, Nov. 1, and Dec. 1. However, the expected ratio of reversal was maintained. The first reduction of the individuals was caused by an invasion of mice which injured the plants. The second and third plots failed to sprout properly for some unknown reason, so that many of the young seedlings died.

In addition to the remarkable correspondence between the daily photoperiodicity and the percentage of individuals showing sex reversal, a consistent fluctuation is also in evidence in the percentage of flowers with stigmas on the reversed plants. This rose from $4\frac{2}{5}\%$ for the Aug. 1 plants to 73% and 72% for the Nov. 1 and Nov. 15 plants and then fell down gradually to $9\frac{1}{2}\%$ for the April 15 plants. The movement of reversal shows much more irregularity in the increasing light of spring then in the autumn. This was probably due to exceedingly variable weather conditions in the winter and spring as compared with the autumn weather. A considerable difference is also noticeable between the fall and spring curves. If one could employ a proper artificial illumination without variation in intensity, an ideal fluctuation curve would no doubt be developed; or any place on the fortieth parallel where there is uniform sunlight for the proper seasons might also give ideal results.

The percentage of sex reversal under a given set of ecological conditions can be predicted with much accuracy. Any plantings between May 15 and July 15 will produce 100 percent of the staminate plants with pure male expression. Plantings from Nov. 1 to Nov. 15 will give 100 percent of the staminate plants showing a greater or less degree of femaleness. Plantings made about Sept. 5–10 or about Feb. 20–25 will give about 50 percent of the staminate plants pure and 50 percent with some degree of femaleness expressed.

As reported in previous papers on sex reversal, all sorts of abnormalities in floral structure are developed, which is to be expected because the tissues from which the flowers and floral parts are produced may be made up of mosaics of cells in various sexual states in various degrees of development. Among the abnormalities observed were the following:

1. Flowers with helicoid stigmas.
2. Structures with the texture of stigmas but representing neither ovulary nor stamen.
3. Pollen-bearing stamens ending in a single stigma at the tip.
4. Imperfect ovulary on one side with two stigmas and two nearly normal microsporangia on the other side.
5. One vestigial ovulary with a perfect stigma.
6. An imperfect ovulary with two stigmas.
7. A nearly perfect ovulary with three stigmas, one smaller than the other two.

8. A flower with one imperfect ovulary with two stigmas, one imperfect ovulary with one stigma and a normal microsporangium at the side, and with one normal stamen which, however, had a stigma at the tip.

9. A flower with a fairly well-developed ovulary with two stigmas considerably below the apex and with the apex occupied by a large ovoid stigmatic mass.

10. Ovularies with three stigmas.

11. A flower having a stamen with an imperfect stigma appendage at the base on the inner side.

12. A stamen with an imperfect ovulary and single stigma on the inner side near the base.

One odd reversed staminate plant was studied in detail. It had 15 pure staminate flowers, 16 flowers with stigmas, and 5 flowers with normal ovularies and stigmas without a trace of stamen vestiges. It first had normal staminate flowers below; then it began to reverse, having staminate flowers with stigmas; and at the very last, at the tip of the inflorescence, several normal carpellate flowers developed with the normal carpellate floral sheath. This is the reverse of what usually occurs. In the carpellate plants reversal from femaleness to maleness usually comes late in the ontogeny, while in the staminate plants normally the reversal to femaleness comes early and in the later stage of the ontogeny the tissues produce only staminate flowers. This individual was, therefore, just the opposite in reversal behavior from the usual condition. The reversal was from normal male expression to normal female expression as the ontogenetic gradient developed. In past experiments with carpellate plants, cases have developed in which the individual progressed to complete staminate expression. The female state was changed completely to the male state in the advanced stage of maturity (Schaffner 8).

According to recent cytological evidence presented by Hirata (5) hemp has the common type of allosome distribution, namely a homomorphic set (AA) for the carpellate plant and a heteromorphic set (AB) for the staminate plant. But such difference in chromosome constitution does not imply any direct relation to sex determination. To say that it does, according to our present evidence from both plants and animals, is a mere assumption based on a very imperfect correspondence. Even if the correspondence were complete we would still have to have some sort of positive proof before making such an hypothesis. Such proof has not been produced, but practically all of our experimental and taxonomic evidence indicates exactly the opposite. After sex reversal has taken place there is no correspondence, for then the female tissues show an AB set of allosomes and the male tissues an AA set of allosomes. The sexual state therefore is dependent on the determination of physiological balance independently of the allosomes.

Whenever there is a sufficient change in the ecological condition, the physiological reactions change and with them the sexual state and expression. The allosomes seem to have nothing to do with the matter. They are entirely passive. Since this is the experimental evidence and is in harmony with the passivity of the allosomes in relation to secondary and primary sex determination of the gametophyte we are fully justified in assuming that they are also passive in the egg when the sex of a dioecious sporophyte is first determined.

The production of 50 percent pure male plants and 50 percent male plants with a partial reversal to femaleness at the planting periods of September 5–10 and Feb. 20–25 is just as definite as the production of approximately equal numbers of males and females in the decidedly uniform environment and determinate physiological gradients in the seeds. In the first case there was no shifting of allosomes or hereditary potentiality associated with the reversed determination; in the second case there was a definite association corresponding with the sexual state ascertained at a later period. The problem is to find out in what way and by what means this correspondence is brought about. Since the sex determination in the 50 percent of reversed individuals was accomplished in spite of the fact that supposed allosome determiners for a contrary result were present and were not changed nor shifted, there is no evidence that the allosomes acted as determiners previously. The whole evolutionary sequence of the time of sex determination would bring us to the same conclusion. Our knowledge of plant evolution indicates that allosomes are but one result of the evolution of unisexuality and have nothing to do primarily with the determination of differential sexual states and structures, because these were completely established long before unisexuality appeared. Furthermore, if the allosomes or any supposed sex-determining units which they might contain really determined sex, then sex reversal would be impossible and the carpellate hemp, which possesses a homomorphic set of allosomes and is thus claimed to be homozygous for sex, could not show maleness unless this were introduced into it from some outside source. When gametes are developed from haploid gametophytes or directly through reduction from diploid male and female normal or reversed individuals, their primary sexual states and structures do not follow their allosome content but are determined by the previous sexual states of the tissues from which the gametes are derived. There is no correspondence between specific allosome condition and the primary sexual states and peculiar sexual morphological differentiations characteristic of the egg and sperm; yet these are certainly due to differential sexual states.

Summary

Taking advantage of the difference in length of daylight from July 15 to May 15, a series of bimonthly plantings, in the greenhouse, of *Cannabis sativa* was carried on in order to find out the fluctuation in sex reversal of

staminate plants as induced by photoperiodicity. A very perfect fluctuation curve was developed, ranging from zero reversal for the July 15, May 1, and May 15 plantings to 100 percent reversal for the Nov. 1 and Nov. 15 plantings. There are no super-males in respect to reversal, since every staminate plant can be caused to express femaleness. The degrees of readiness or difficulty of reversal shown by different individuals are to be ascribed to degrees of differentiation, both physiological and morphological, which the individuals have attained.

The determination of sex in the hemp is not caused by an allosome differential, but these chromosomes must be assumed to play a passive rôle. Determination is brought about through differential physiological states developed in gradients in both the egg and the vegetative body.

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SPECIAL LITERATURE

1. **Allan, H. H., and V. D. Zotov.** A case of malformation in *Clematis foetida*. New Zealand Jour. Sci. Tech. **9**: 362–365. 1930.
2. **Heitz, E.** Geschlechtschromosomen bei *Pellia fabbroniana* (diöcisch) und *P. epiphylla* (monöcisch). Ber. Deutsch. Bot. Ges. **45**: 607–610. 1928.
3. **Hirata, K.** Sex reversal in hemp. Jour. Soc. Agr. For. Sapporo **16**: 145–168. 1924. (English abst. in Exp. Sta. Rec. **54**: 527. 1926.)
4. ——. On the sex determination in hemp, *Cannabis sativa* L. Jour. Soc. Agr. For. Sapporo **19**: 39–54. 1927.
5. ——. Cytological basis of the sex determination in *Cannabis sativa*. Japanese Jour. Genetics **4**: 198–201. 1929.
6. **Holdaway, F. G.** Nutritional status and sex determination. Nature, July 26, 1930.
7. **Lindsay, Ruth H.** The chromosomes of some dioecious Angiosperms. Amer. Jour. Bot. **17**: 152–174. 1930.
8. **Schaffner, J. H.** Complete reversal of sex in hemp. Science **50**: 311–312. 1919.
9. ——. Influence of environment on sexual expression in hemp. Bot. Gaz. **71**: 197–219. 1921.
10. ——. The influence of relative length of daylight on the reversal of sex in hemp. Ecology **4**: 323–334. 1923.
11. ——. Further experiments in repeated rejuvenations in hemp and their bearing on the general problem of sex. Amer. Jour. Bot. **15**: 77–85. 1928.
12. **Sinoto, Y.** On the chromosome number and the unequal pair of chromosomes in some dioecious plants. Proc. Imp. Acad. Tokyo **4**: 175–177. 1928.
13. **Yampolsky, C.** Induced alteration of sex in the male plant of *Mercurialis annua*. Bull. Torrey Bot. Club **57**: 51–58. 1930.