SEX DETERMINATION IN HEMP (*CANNABIS SATIVA* L.)

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Until recently sex determination in hemp was thought to be epigamic, sex being primarily determined by some physiological stimulus at some stage in development after fertilisation. Schaffner (1921, 1923 *a*, *b*) especially maintains this opinion.

The writer, however, does not agree with him unreservedly, and suggests that sex in hemp is determined by a mechanism of the XY type. To demonstrate this, breeding experiments with the intersexes of the *Karafuto* variety have lately been carried out, and some of the results were published in August, 1925. At that time the writer particularly emphasised the fact that F_1 individuals from the selfing or mutual crossing of the female intersexes were females and female intersexes, with the exception of a few male plants.

Quite recently McPhee (1925) studied sex inheritance in hemp by breeding experiments similar to those conducted by the writer, and showed that sex determination in hemp can be explained on a genetic basis, the inheritance being of the XY type. It is satisfactory to find McPhee's results agreeing so closely with those of which an account is now given here.

EXPERIMENTS.

In the earlier experiments it was ascertained that both male and female plants of the *Karafuto* var. reversed their sexual expression after mutilation caused by the removal of certain parts of their branches and stems at an early stage of blooming, and also that the plants cultivated in a hothouse during the winter season showed a more marked sex reversion. The various breeding experiments were carried out on the following plan:

Female intersex selfed,
(Female intersex × Female intersex),
Female × Female intersex,
Female × Male intersex,
Female intersex × Male intersex,
Male intersex selfed,
(Male intersex × Male intersex).

The material used in these experiments was mostly that of the *Karafuto* var., though in a few cases the *Tochigi* var. was also used. The experiments were carried out in the Experimental Garden, as well as in the hothouse, of the Hokkaido Imperial University.

Exp. I. Female intersex selfed.

A. *Field Experiment.* After mutilation some branches of the female plants were enclosed in paraffin-paper bags, great care being taken in removing the flowers already in bloom. Self-pollination was carefully carried out when the anthers of the staminate or the hermaphrodite flowers on some female intersexes matured. Pollination was tried in the summers of 1923, 1924 and 1925. A similar process of mutilation was performed upon the F_1 plants in 1923 and 1924, but not in 1925.

TABLE I.

F_1 results from selfed female intersexes.

Seed No.	Seeds planted	Seeds germinated	Females	Female intersexes	Males
		(*In* 1924.)			
I–1–2–A	22	17	7	5	3
I–1–2–B	1	—	—	—	—
I–1–12	37	15	8	6	—
I–1–13	2	—	—	—	—
II–3–3	19	12	10	3	—
H–1–17	30	18	11	4	—
VI–1–17	44	17	7	6	—
VI–1–18	14	4	4	—	—
Total	169	83	47	24	3
		(*In* 1925.)*			
VI–1–17–A′	9	4	3	1	—
VI–1–17–B′	6	2	2	—	—
VI–1–17–C	13	9	5	4	—
VI–1–17–D	27	18	9	5	—
VI–1–17–E	8	5	3	3	—
Total	63	38	22	13	—
		(*In* 1926.)			
VI–1–17–H–3	6	3	1	1	—
VI–1–17–H–4	1	1	1	—	—
VI–1–17–H–6	17	7	3	4	—
VI–1–17–H–7	11	4	—	1	—
VI–1–17–H–9	6	2	1	1	—
VI–1–17–H–10	7	2	1	1	—
VI–1–17–H–11	4	2	1	—	—
VI–1–17–H–14	10	1	1	—	—
Total	62	22	9	8	—

* Plants grown from the seeds which had been obtained from the female intersexes grown in pots.

Female intersexes from the mating of "Female × Female-intersex" (I–1–2–N × VI–1–17–A) in 1925 were selfed, and six seeds obtained (cf. Exp. III B). These were sown in pots in the spring of 1926. Four of them bloomed in August, three being female intersexes and the fourth a female, although the process of mutilation was not followed with them. Self-pollination was tried upon these plants, but it failed.

Seeds were sown in the hothouse in June, 1925. Before flowering all the male plants were picked out. Among the female plants four individuals reversed their sexual expression. Each of them was bagged after removing the branches of those already in bloom. Both selfing and crossing were carried out. The results of the crossing are given later under Exp. II. Forty seeds were obtained by selfing. Part were sown in the hothouse in January, 1926, the results of which are given in the following experiment. The remaining seeds were sown in pots in May, 1926, and the plants were not subjected to mutilation. The result was as follows:

Seed No.	Seeds planted	Seeds germinated	Females	Female intersexes	Males
No. 1 (0–5)	25	19	3	11	—

Five of these plants were very dwarf, and two of them withered before blooming.

As will be shown later, it seems worthy of notice that even under normal conditions many female intersexes appear among the offspring of selfed female intersexes. It is possible, even under normal conditions, that all the offspring may express intersexuality by the selection and the selfing of female intersexes.

B. *Hothouse Experiment.* As mentioned above, fifteen seeds obtained by the selfing of the female intersexes in the hothouse were again sown there in January, 1926. The six individuals obtained all showed various grades of intersexuality. These plants were submitted to selfing or crossing for further study (cf. Exp. II B). The most striking feature about them was their long life and their tendency to ever-flowering. The detailed description of the habit of each individual need not be given here. Generally speaking, however, the male tendency increased gradually until June. Then, after a short period of weakness, a rejuvenative tendency appeared and they continued blooming until September or even later. But at that time the sexual transition, from female to male, could not be seen definitely, and they expressed various intersexual forms.

The same process seems to occur in other winter-plants, as will be shown later, and is doubtless due to photoperiodism, the relative length of day and night, as has been shown by Garner and Allard (1923).

It is remarkable that individuals of the same pedigree (No. 1), all of which show female intersexuality in the hothouse, do not always show it in the field. This fact, therefore, proves that environment plays an important rôle in the expression of sex in hemp.

From these results it is clear that every individual from the selfing of female intersexes is female or female intersex, except a very few males (see Tables I and II). It is probable that the occurrence of such males in the 1924 experiment was due to an accidental pollen-contamination from some of the male plants, for no male plants were seen in the experiments performed more critically.

Exp. II. Female intersex × Female intersex.

A. *Field Experiment.* The method of experiment was the same as that of the former field experiment, but the plants were crossed mutually with each other. But since a plant's own pollen grain may also have been mixed with that of the other individual used, the term, "mutual crossing," is not used in its strictest sense. The results of the 1924 experiment are shown in Table II.

TABLE II.

F_1 results from mutual crossing of female intersexes.

Seed No.	Seeds planted	Seeds germinated	Females	Female intersexes	Males
I–1–12 × I–1–2–A	20	12	4	3	1
I–1–12 × I–1–2–B	18	9	5	1	—
I–1–12 × I–1–2–C	1	—	—	—	—
I–1–12 × II–3–1	9	3	3	—	—
Total	48	24	12	4	1

These results are practically the same as those of the earlier field experiments.

B. *Hothouse Experiment.* As mentioned above (p. 67), the seeds obtained by the crossing of the hothouse-plant in 1925 were sown there in the winter of 1925–1926. The results are shown in Table III.

Most of the plants sown in the beginning of December withered before the end of May, but those sown at the end of December, or in January, were long-lived, even if they did not rejuvenate. The sex of the rejuvenated plants was at first the re-reversed form, that is, the female tendency appeared again, but they seemed again to go toward

TABLE III.

F_1 results from mutual crossing of female intersexes.

Seed No.	Seeds planted (date)	Seeds germinated	Observation up to June		Individuals rejuvenated (observed on Sept. 7)
			Females	Female intersexes	
No. 3×5–1 (0–1)	60 (Dec. 7)	37	—	35	—
,, 2×No. 1 (0–2)	38 (,, 7)	33	13	20	2
,, 3× ,, 4 (0–3)	2 (,, 14)	—	—	—	—
,, 2–NC–1*	29 (,, 7)	23	12	11	1
,, 2–NC–2*	21 (,, 14)	18	5	18	—
,, 3–NC*	44 (,, 14)	38	6	24	5
,, 4–NC–1*	45 (,, 28)	26	2	23	10
,, 4–NC–2*	27 (Jan. 4)	13	2	9	3
,, 4–NC–3*	44 (,, 11)	32	6	26	8
,, 4–NC–4*	35 (,, 18)	22	2	20	3
Total	345	242	48	186	32

* These seeds were from female intersexes which were not bagged, no male plants being present.

the male direction and thus expressed intersexuality. According to observations made on September 7, the sexes of the rejuvenated plants were as follows:

Seed No.	Individuals, intersex again	Individuals, intersex turned female	Individuals, female turned intersex
No. 2×No. 1 (0–2)	2	—	—
,, 2–NC–1	—	1 (1)	—
,, 3–NC	4	1	—
,, 4–NC–1	3	7 (1)	—
,, 4–NC–2	1	1	1
,, 4–NC–3	4	3	1
,, 4–NC–4	1	2	—
Total	15	15	2

The number in parentheses indicates plants which expressed intersexuality in October.

Some of the plants expressing intersex again were selfed or crossed, and many seeds matured.

Many individuals from No. 3 × 5–1 (0–1) showed a much higher reversion, and at the end of their blooming period appeared as males. They were entirely sterile on the female side. Certain of them were crossed with the plants of No. 1 (0–5), viz. female intersexes from the selfing of female intersexes, and the seeds produced were sown in pots in the hothouse in May 8, 1926. They grew almost to normal stature, their height being from 0·8 to 2·4 metres. The result was as follows:

Seed No.	Seeds planted	Seeds germinated	Females	Female intersex	Males
0–5–4 × 0–1–1	20	10	1	9	—

These intersexual plants were selfed or crossed and many seeds matured.

From the results of the rejuvenescence we can see that the same individual shows different sexual expressions according to the period of flowering, a fundamental piece of evidence that sexual expression in hemp is comparatively easily affected by different physiological conditions.

Exp. III. Female × Female intersex.

A. *Hothouse Experiment.* Some female plants raised in pots were isolated from the garden about two kilometres to avoid any stray pollen from male plants. This experiment was begun in the summer of 1924, and part of the seeds were sown in the hothouse in October and December of the same year. The following table shows the results:

TABLE IV.

F_1 *results from female × female intersex.*

Seed No.	Seeds planted	Seeds germinated	Females	Female intersexes	Males
I–1–2–N × VI–1–17–A	30*	25	16	5	1
I–1–2–N × VI–1–17–A	20†	16	11	3	—
Total	50	41	27	8	1

* These seeds were sown on Oct. 11. † These seeds were sown on Dec. 1.

Two of the plants grown from the seeds sown in December expressed themselves in February, one as a female and the other as a female intersex. They rejuvenated in the spring and bloomed again in August. The former remained unchanged in sex but the latter now appeared as a normal female.

Five seeds from the cross made in the hothouse in 1925 were sown there in December 14 of the same year. From them four plants grew and all of them were female intersexes.

B. *Field Experiment.* The remaining 55 seeds of the above experiment were sown in the spring of 1925 as usual. The mutilating process was not followed with these plants. The result is shown in Table V.

TABLE V.

F_1 *result from female × female intersex.*

Seed No.	Seeds planted	Seeds germinated	Females	Female intersexes	Males
I–1–2–N × VI–1–17–A	55	48	37	8	—

Eight plants represented female intersexes under normal conditions. These plants were selfed, the result of which has been shown already (cf. Exp. I A).

Twelve seeds descended from the crossing between a female plant and No. 1 (female intersex) in the hothouse in 1925 were sown in pots on May 25, 1926. Seven of them germinated and two of them were female intersexes, the rest being females.

Exp. IV. Female × Male intersex.

A. *Field Experiment.* The results were obtained in the same manner as those from the former field experiments, with the exception that the pollen from a male intersex was used. This cross was made in the summer of 1923, but not since then. Table VI shows the results.

TABLE VI.

F_1 *results from female × male intersex.*

Seed No.	Seeds planted	Seeds germinated	Females	Males	Male intersexes
I–1–21 × T–1	9	3	2	1	—
III–3–10 × III–3–6	22	14	9	2	3
III–3–24 × III–3–6	58	42	17	3	19
Total	89	59	28	6	22

T–1 = Male intersex of *Tochigi* var.

B. *Hothouse Experiment.* In the *Tochigi* var. sown in November, 1924, two male intersexes appeared. Crosses were made with these plants, and the seeds obtained were sown in November, 1925. They bloomed in January, 1926. The result is shown in Table VII.

TABLE VII.

F_1 *result from female × male intersex.*

(*Tochigi* var.)

Seed No.	Seeds planted	Seeds germinated	Females	Males	Male intersexes
I × T–*a*	16	13	4	3	3

In general the result is not different from those obtained from the *Karafuto* var. Only two individuals of the female plants rejuvenated and in April they expressed female intersexuality.

Exp. V. Female intersex × Male intersex.

Field Experiment. This cross was made in 1924. The offspring were not subjected to any process of mutilation. The result is shown in Table VIII.

TABLE VIII.

Seed No.	Seeds planted	Seeds germinated	Females	Males	Male intersexes
III–3–12 × III–3–6	38	25	13	9	—

In general the results are similar to those obtained in the former experiments.

Exp. VII. Male intersex selfed.

(Male intersex × Male intersex.)

As shown in a previous paper, there are generally two types of male intersexes; in one of them fairly perfect carpellate flowers grow on the upper part of the inflorescence, and in the other, very incomplete hermaphrodite flowers appear on the nodes, the female organs being entirely abortive. The former type, however, is always conspicuously protogynous, so that self-pollination of the male intersex here becomes very difficult. We had therefore to content ourselves with mutual crossing instead of self-pollination.

In order to make the cross, the plots were divided into two, and seeds were sown at different times for each plot. Male plants were removed from the plots before blooming. As mentioned above, the first type, the seed-producing male intersexes, are conspicuously protogynous so that it is very easy to distinguish them from male plants. Pollen from male intersexual plants, grown from the seeds sown earlier in the plot, was brought to the stigma of the male intersexes which had been sown later in the other plot.

By these means, eighteen mature seeds were obtained on three individuals. Ten of them germinated, eight of them bloomed; six were female and two male.

Hence it is evident that the offspring from the various crossings of the male intersexes can show various sex forms.

DISCUSSION.

Since environment evidently plays an important rôle in the sexual expression of hemp, it might be thought that sex is determined only by external conditions, and not by any Mendelian factor. Judging, however, from the fact that under any conditions the sex ratio in hemp is not decisively changed from the normal 1 : 1 ratio, the writer (1924) suggested that essentially the mechanism of sex determination must be of the XY type. After this suggestion was made, the theory was elaborated in detail by McPhee (1925) and the writer himself with similar breeding experiments. A part of the writer's results was reported also in 1925. It was shown that though the offspring from the various crossings of the male intersexes express various sex-forms, all the offspring of selfing, of the mutual crossing of female intersexes, and of the hybridisation of the female with the pollen of female intersexes are females or female intersexes. It is to be regretted that McPhee did not touch upon the subject of intersexualism, because this phenomenon must be of great significance in pointing to the direction in which we may look for the complete solution of the problem of sex determination.

From the fact that in hemp, although the species is prevailingly dioecious, various intersexes may occur under abnormal conditions such as a short duration of daylight, or the mutilation of certain parts of the plant early in the blooming season, and also from the fact that certain plants show intersexuality even under normal conditions, it is probable that the factor (or factors) for the opposite sex exist in any given individual, just as traces of the opposite Mendelian character are found in extracted dominants or recessives in certain cases. For we are not justified in assuming that a hermaphrodite factor (or intersex factor) is responsible for the occurrence of intersexuality. The factor which induces the opposite sex, seems to function only under special conditions. Granting so much we may now ask what these factors are, and in what manner they are concerned in the physiology of sex determination.

And here we may consider briefly the investigations of Goldschmidt on *Lymantria* (1920 *a*, *b*, 1921) and of Bridges on *Drosophila* (1922, 1925), cases which have afforded important evidence in connection with the nature of sex determination. Goldschmidt's investigations deal with the production of intersexes in crosses between the different races of gypsy moth, *Lymantria dispar*. He was able to produce intersexes of practically all degrees, not as sporadic occurrences, but as regular phenomena of the crosses. Studying the various races and the intersexes from different crosses he has formulated an explanation for their occurrence. He recognises the chromosome theory of sex determination, and applies it to the present case on the basis of the ZW type. He believes that a male determining factor (M) is located in the Z-chromosome. As the female tendency, however, does not segregate, but is strictly maternally inherited, he suggests that a female factor may be located in the W-chromosome and that it exerts its influence on the cytoplasm of the egg during its development. Hence every gamete which is produced in the female individual is invested with a female tendency as well as a male tendency. The cytoplasmic female tendency is denoted as $\boxed{F}$. Goldschmidt proves from his results that the sex of a given individual is determined by the balance between the activity of these two sets, and also that these sex factors differ amongst themselves in their valency (= quantity) according to the different races.

A similar indication that sex is determined by the balance between male-determining and female-determining agencies has been found also by Bridges' most elaborate study. In *Drosophila melanogaster*, he has found a strain which continually produces intersexes. Genetic tests and cytological examination show that these individuals contain two *X*-chromosomes and three members of each set of autosomes. He considers that the *X*-chromosome has a net female tendency, and that in the autosomes are male determiners giving rise to a net male tendency, sex being decided by the relative strengths of the tendencies of the two opposed sets of factors. Thus intersexes are produced when the excesses in opposite directions cancel each other, since the two sets of factors are of almost exactly the same weight.

Comparing the two cases, we must suppose that in *Lymantria* the change of the balance between the valency of the factors is caused by the crossing of the different races, while in *Drosophila* it is brought about by the non-disjunction of chromosomes. Nevertheless there is agreement upon the fundamental point that the sex of a given individual is determined by the balance between the activity of two opposed sets of factors, one set tending to produce the female characters and the other to produce the male characters. Now, hemp is a case in which the activity of the sex factors is easily changed by the effect of the environment. Can we apply the case of *Lymantria* or *Drosophila* to the sex mechanism of hemp?

Since in hemp the male is heterogametic, the *Lymantria* type cannot hold good. Nor does the *Drosophila* mechanism seem adequate, because in hemp irregularities in the maturation divisions (non-disjunction) do not seem to occur. Therefore the following compromise between them is suggested as an explanation of sex determination in hemp. It is evident that in hemp the chromosomes, especially the sex chromosomes, the *X* and the *Y*-chromosome[1], seem to determine the sex. It is here suggested that both the female producing and the male producing factors are contained in the respective sex chromosomes, but in the *X*-chromosome the valency of the female factor is higher than that of

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[1] The terms *X* and *Y*-chromosome are not here used with a strict morpho-cytological meaning, for the cytological identification is not completed.

the male factor, while in the Y-chromosome that of the male factor is higher than that of the female; and hence, the X-chromosome has a net female tendency and the Y-chromosome a net male tendency. Moreover, the female tendency produced by the X-chromosome must be weaker than the male tendency of the Y-chromosome. Individuals with two X-chromosomes, therefore, express themselves as female; those with one X-chromosome and one Y-chromosome as male. On these lines we may suppose that the mechanism of sex determination in hemp is not beyond the range of "genic balance" suggested for the cases of *Lymantria* and *Drosophila*. How, then, can this view of "genic balance" be applied to the occurrence of intersexes in hemp?

The writer believes that in hemp the valency of the factors in the sex chromosomes is more or less different in practically all individuals, so that by crossing there must occur various changes in the relative balance between the action of the two opposed sets of factors. This is in agreement with the fact that intersexes occur when the two opposing valencies tend to cancel each other, and the difference between them becomes relatively small. The nearer the opposing valencies are to equilibrium the more easily is intersexuality produced. This relation is exactly the same as that pointed out by Bridges. Thus even under ordinary conditions, we are able to obtain intersexual individuals, as has been shown in the foregoing experiments. So too by the selection and self-pollination of an individual showing a higher grade of intersexuality we find all of the offspring expressing intersexuality even under normal environment.

Now let us look into the function of factors upon their substrata, the physiological interpretation of factorial action. Before discussing this problem, however, the distinction between the valency and the activity of a factor must be emphasised. To the writer it seems reasonable that "the valency of a factor" should mean the value which is given to the nature of the factor. Therefore the valency of a factor is determined either by the quantity of the factors, or the ability of the enzyme-production of the factor, or by both; so that the enzyme-productivity of the factors being the same, the valency would, of course, be determined proportionally only by the quantity of the factors. On the other hand, "the activity of a factor" would mean the reaction induced by the factor according to its valency. It is conceivable that the action induced by the factor may be nothing but the enzyme-reaction upon the substratum, so that the enzyme-theory could be applied for it. Quite recently Hedin (1926) has shown that at the state of equilibrium between enzyme (E), substratum (S), and the compound of enzyme-substratum (ϕ), the equation

$$[E-\phi]\cdot[S-\phi]=K\cdot[\phi]$$

is admissible. In it K represents the dissociation constant. From this equation, we are able to suggest that the action of a factor may be related to the quantity of the enzymes as well as to the amounts of the substratum. Further, it is probable that the enzyme represents an autocatalytic reaction inhibiting the action of the other enzyme produced by the opposing factor, and also that in general the velocity of the reaction is determined by the law of mass-action, especially in the definite amount of substratum. Hedin, however, has shown that with a small amount of enzyme the velocity of the reaction is proportional to the amount, but with a large amount it is not proportional. In the latter case the products of the reaction per unit quantity of the substratum are proportionately diminished because they inhibit the reaction more markedly than in the former case. Attention must also be paid to the concentration of the substratum. We can, however, recognise that though the reaction conforms to the law of mass-action until the optimum concentration of the substratum is reached, once this point is passed the reaction is gradually inhibited.

Reverting to sex in hemp, it is clear that the enzymes influencing the sexuality are relatively sensitive to the conditions in a substratum which is definitely affected by the environment. In considering intersexuality, therefore, we must have regard not only to the slight differences in the valencies of the opposing factors, but also to the state of the substrata. It is probable that the optimum concentration of substratum may differ somewhat for different enzymes. Let us suppose, for example, that the andrase (the enzyme produced by the male factor) is present in slightly larger quantity than the gynase (the enzyme produced by the female factor)—normally this individual is male. If the quantity and the concentration of the substratum are adapted to the function of the andrase during development, the individual will become a male, even if the genetical constitution is the same as that of an intersex. In this way we can explain the infrequent occurrence of intersexuality under ordinary conditions. When, however, the self-inhibiting action of the andrase comes into play, the state of the substratum eventually becomes capable, during the development of the individual, of initiating the activity of the other factor, the gynase, with the appearance of female characters; and so we arrive at intersexuality. If, however, the substratum is from the beginning suited to the action of gynase, the matter becomes more complicated. Here we must assume two sorts of reactions. In the one the enzyme present in greater quantity (andrase) may be active at the beginning of the reaction, while in the other on the contrary the state of the substratum induces earlier activity of the gynase, so that even if this is present in smaller quantity than the andrase, it may yet, owing to the favourable nature of the substratum, produce an effect in the earlier part of the reaction. In both reactions, however, the self-inhibiting action is stronger than that of the former case, so that intersexuality must occur more readily, and at the same time be of higher grade. The same argument is of course applicable to the case of a slight excess of gynase. These assumptions would be sufficient to explain the experimental results. How, then, is the substratum influenced by the physiological stimuli?

Here attention should be called to the experiments of Garner and his colleagues (1923, 1924). Investigating the regulatory action of the relative length of day and night upon flowering and fruiting, they have shown that prompt initiation of sexual reproduction may be associated with partial loss of turgor, lower concentration of hydrogen-ion, and so forth, when certain species—so-called short-day plants—are exposed to a reduced daily illumination period. Since hemp is a short-day plant, it follows that in the winter-plants the same conditions would be brought forth by a relatively short daily illumination. Hence with a cell state favouring the development of the reproductive organs, the reaction of the dormant sex factor would be concurrently accelerated. The same state may be induced not only by a short duration of day-light, but also by a process of mutilation on certain parts of the plants. In brief, when the vegetative growth of the plants is more or less inhibited, the activity of the dormant factors would be more readily induced. The writer intends to start some experiments on these lines with hemp in the near future.

Since it is possible that external conditions affecting the cell state may play an important part in the expression of genetic factors, we ought in considering Mendelian characters, to give more attention to environmental influences. The writer believes that these are of two kinds. In the one the environment affects only the activity of the enzymes, but in the other it affects also the quantity of the enzyme itself. The former corresponds to the so-called modifications, which are not heritable; in the latter the stimulation is so great that the enzyme-productivity of factors is inhibited or accelerated so as to affect the manifestation of the ordinary valency of the factors. It seems very probable, therefore, that in the latter case the constitution of the factor may frequently be changed. Thus, once the reconstruction of the factor occurs, and the valency of the factor is consequently changed, we can understand that the change might be handed on to the offspring. The occurrence of intersexuality in hemp is induced by an abnormal state of the protoplasm stimulating the activity of the enzyme produced by the opposite sex factor, though in many cases it is suppressed by the functioning of the other sex enzyme under a normal state. In short, the sexual expression in hemp depends on two agencies, *i.e.* the combination of sex factors (the quantitative difference of andrase and gynase), and the different states of substratum caused by the different environments.

The writer considers that the suggestion regarding sex determination proposed in the report of 1924 may be regarded as on the whole satisfactory, and the formulae there given may be repeated here:

	Diploid	Haploid
Super male	$18 + Y + Y^*$	$9 + Y$, $9 + Y$
	$18 + y + Y^*$	$9 + y$, $9 + Y$
	$18 + x + Y$	$9 + x$, $9 + Y$
Male	$18 + X + Y$	$9 + X$, $9 + Y$
	$18 + x + y$	$9 + x$, $9 + y$
Male intersex	$18 + y + y^*$ (?)	$9 + y$, $9 + y$
	$18 + X + y$	$9 + X$, $9 + y$
Female intersex	$18 + x + x$	$9 + x$, $9 + x$
	$18 + x + X$	$9 + x$, $9 + X$
Female	$18 + X + X$	$9 + X$, $9 + X$

* These forms may have difficulty in existing.

In the above formulae x and y correspond to XY respectively, but x has a more marked male tendency than X, and y has a more marked female tendency than Y, *i.e.* the difference in valency between x and y is smaller than between X and Y.

It seems true that in the primitive form of sex—the hermaphrodite—the difference between the two opposite valencies is so slight as to result in almost complete balance. We agree that dioecious forms have been evolved from hermaphrodite ones. How, then, have the differences in the valency of a factor originated? This is a fundamental question in genetics generally as well as in sex determination. The idea that a factor may undergo evolution according to definite rules is involved in the answer. Until, however, the mechanism of factorial evolution is cleared up, the question cannot be solved. At present, although it is very unsatisfactory, there is no other alternative but to apply the mutation theory in explanation.

For a complete solution of the mechanism of sex determination further experiments are necessary, and these the writer hopes to carry out.

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