SEX PROBLEMS IN HEMP

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ALTHOUGH sex has been recognized in animals since before the time of recorded history the recognition of sex in plants is, with few exceptions, less than 250 years old. In Mesopotamia and in Egypt, where the date palm has been cultivated for ages, the distinction of male and female palms has been made for at least 4000 years as shown by inscriptions describing and illustrating the manner of pollinating the female flower clusters by racemes of male blossoms, in practically the same way as that still used by the Arabs and Egyptians. The earlier herbalists ascribed sex to various plants. Thus one kind of fern was called Male Fern and another Female or Lady Fern, and one kind of Dogwood was named *Cornus mas* and another C. *foemina*. These differences, however, were specific or even generic and had nothing to do with the sex of the plants.

The failure to distinguish sex in plants is due to the fact that in the popular mind sex distinction is associated with distinction of *individuals* of different sex, as in the domestic animals and in man. In Europe hemp and the hop were the only commonly cultivated plants in which the plants are dioecious. Here the pollination is accomplished by air currents and there is nothing similar to the sexual processes in animals. The remainder of the commonly cultivated plants possessed perfect flowers and in some of them, wheat, barley, oats, pea and bean, are usually pollenized ***before*** the flower had opened. Thus the necessity for sex was not recognized.

Nehemiah Grew in 1682 indicated the stamens as male organs and the pistils as female organs, from morphological reasons only. Camerarius in 1694 was the first investigator to report the results of actual experiments by which he proved the correctness of Grew's assumptions. He excluded pollen from the pistils of *Mercurialis*, ***Moms,*** *Zea mays* and other plants and obtained no seeds except when he applied pollen from the male flower of the same species. The publication of the results of these experiments was followed by numerous experiments both in Europe and in America which confirmed the sexual nature of stamens and pistils and led soon to successful attempts at hybridizing various kinds of plants.

Hemp, *Cannabis sativa*, is a native of Asia and has been cultivated both for its fibre and for the drug "hashish" which is in great demand in Central Asia, Persia, Afghanistan and Northwestern India. Its culture as a fibre plant has spread all over the temperate regions of the world and it has escaped and become a weed of low lands in various places. Thus it is very abundant along the Missouri River and tributary streams in Eastern Nebraska and Western Iowa.

The plant as grown for fibre is 8 to 12 feet or more tall while the varieties grown for the drug are usually only about 3 to 4 feet tall. The male plants are usually more slender than the female plants, sometimes slightly paler green, and have but few and small leaves at the top. The clusters of male flowers are large and conspicuous. As soon as the pollen has all been

shed the plants die, long before the female plants. The latter are usually stouter and leafy to the apex, the flower clusters being smaller and less conspicuous (before the seeds begin to form). The female plants have a strong, somewhat pungent odor, especially after the seeds are beginning to form.

Normally, when sown in the summer time, about 45 to 47 per cent of the plants that grow to maturity will be males, but the ratio may vary considerably in adjacent plats from the same lot of seed. Sometimes up to 55 per cent will be males. Usually the male plants will have no female flowers but a small percentage of the female plants will develop, as they grow older, a few male flowers. In 1926 or 1927 the writer found a plant, resembling a male plant in habit, with perfect flowers, the pistil and stamens apparently being functional. The flowers did not develop until just before an early frost which destroyed the plant.

THE EFFECT OF AGE OF POLLEN

Perhaps the first systematic experiments to determine the factors involved in the determination of sex in any plant were those begun by Dr. Theophilus Ciesielski in 1871 in Poland. These experiments covered a series of eight or ten years or possibly longer, but were not reported until 1911. The earlier experiments had to do with the effect of close or distant planting, with only negative results. Planting at different phases of the moon also was without effect upon the sex ratio. Growing the plants in light and in shade, in moist and in dry soil, and in rich and in poor soil were also equally ineffective. The ratio remained about the same; 40 to 50 per cent of the plants were males, the remainder females. Similar result were obtained whether the pollen was taken from the top, middle or lower portion of the inflorescence. The only definite results were those where the pollen used was of different ages.

In the male hemp plant the stamens usually begin to dehisce a little while after sunrise when the atmosphere begins to become drier. These flowers were collected early in the morning shortly before the stamens opened and the pollen from some of these was scattered on the flowers of female plants placed indoors or at a considerable distance to prevent chance pollination. Some of the male flowers collected at sunrise were kept until sunset and were then used to pollenize other female flowers. With slight variations as to details these experiments were repeated several years. The seeds were saved and planted out-of-doors next summer. From the flowers pollenized with freshly produced pollen the seeds gave 85 to yo per cent male plants while the seeds from flowers pollinated with 12 hour old pollen gave yo to 100 per cent female plants. Ciesielski emphasizes the fact that he repeated these experiments several successive years, always with the same results. He concludes, therefore, that there is a tendency toward maleness in fresh pollen and that as the pollen becomes old this tendency changes from maleness to femaleness.

In 1921 Lilienfeld reported the results of experiments performed by him to test the correctness of Ciesielski's conclusions. He could not find any differences in the sex ratio whether the pollen used was fresh or 12, 30 or 36 hours old.

Ignorant of Lilienfeld's work the writer began experiments along the same line a short time later. This work was carried out in the greenhouse at first and then out of doors. The number of seeds obtained in the greenhouse experiments was too small to enable any definite conclusions to be drawn. Out of doors the female inflor-

escences were bagged and allowed to remain covered one or two weeks. Then the bags were temporarily removed and all flowers whose ovaries had started to enlarge (indicating pollination before bagging) were removed. Then the flowers were pollenized with pollen of the desired age and again covered until the male plants were all removed a few days later. Pollination was accomplished in various ways. For fresh pollen in most cases anthers were selected that were just opening and the pollen dusted from these on the female flowers. Sometimes unopened anthers were torn open and the contents applied to the stigma. The flowers were then rebagged. For older pollen, clusters of male flowers on the point of opening were bagged early in the morning after removal of all previously opened flowers. Since nearly all of the male flowers open at about the same time the pollen in the bag will be approximately of equal age when the bag is removed from the inflorescence. Such bags of pollen were held various lengths of time. When it was desired to use pollen of any given age the corresponding bag of pollen was placed on a female influorescence that had been bagged as described above, and shaken and beaten until all the flowers were pollinated. This bag was allowed to remain or a fresh bag was placed over the inflorescence until all bags were removed after the destruction of all male plants. Pollen used in these series of experiments was fresh, 8-g hour, 14-16 hour, 24 hour, 42 hour and 64 hour pollen.

The seeds resulting from these pollinations were planted next year and the ratios of male and female plants determined when they began flowering. The plants grown in 1926 from pollinations made in 1925 gave for "fresh" pollen 44.2 per cent male plants, the control from open pollinated seeds from other branches of the same parent plants giving 49.3 per cent males. The seeds produced from "old" pollen gave 43.4 per cent male plants and their controls 48.7 per cent males. In 1927 the figures for seeds resulting from 1926 pollinations were 47.2 per cent males for "fresh" pollen, 53.5 per cent males for 10 to 12 hour old pollen, and 47.1 per cent males from pollen 17-27 hours old. The controls were respectively 53.5 per cent, 49.9 per cent and 50 per cent males. Although the figures showed considerable variations it is clear that they agree rather closely with those obtained by Lilienfeld and do not give any support to Ciesielski's reports. Either the latter failed to make accurate experiments or the variety of hemp used by him responded in an entirely different manner from the varieties used by Lilienfeld or the writer.

THE EFFECT OF MUTILATION

The sex question in hemp had already been attacked from another angle by Pritchard in the years 1909–1914. This investigator removed the flowers and flower buds and even leaves and portions of the tops from various hemp plants of both sexes. Omitting the earlier experiments in which the numbers involved were few the results of his 1913 mutilations may be considered. Fourteen male plants which bore no female flowers had all their flowers and flower buds removed. Eleven of these produced only male flowers on their new growth but on two of the plants a few sex-intergrade flowers and female flowers appeared among the male flowers (four female flowers to 7390 male flowers) and one produced all (461) female or intersex flowers. Of the 31 female plants that had their flowers removed four had at the time a few male blossoms, a total of 1016 male to 29,950 female flowers, or a little over 3 per cent. After new growth took place these four plants gave 17,137 male

blossoms and 18,861 female blossoms, or almost 48 per cent male plants. The twenty-seven female plants that had no male blossoms at first had on their new growth 160,862 flowers of which 24,242 were male (or intersex) flowers, approximately 15 per cent.

In connection with the foregoing effects of mutilation upon the sex ratio in hemp may be noted the report of J. E. Higgins upon *Carica papaya*, in which sex changes have been brought about in a similar manner. This herbaceous tree, if it may so be called, is normally dioecious although trees with hermaphroditic flowers are not rare. Upon cutting back a male tree it frequently happens that the new shoots produce female flowers or hermaphroditic flowers, but Higgins could not induce female trees to change their sex by cutting them back.

SEX CHROMOSOMES

The discovery of chromosome differences in the two sexes of certain insects thirty years ago, followed by the realization that this is a very general condition throughout most of the animal groups, led to investigations to determine whether similar conditions held in those plants in which the sexes are separate. The earlier studies by Strasburger gave negative results but in 1917 C. E. Allen reported sex chromosomes in *Sphaerocarpus*, one of the Hepaticae. Six years later they were reported in a number of Dicotyledons and in one Monocotyledon. Among the former was the hop (*Humulus lupulus*), a near relative of hemp. In the next year, 1924, Hirata, a Japanese investigator, found both X and Y chromosomes in one variety of hemp. The male plants were heterozygous with 18 autosomes + X + Y and the female plants homozygous, 18 + X + X. The male gametes were of two kinds, with either X or Y chromosomes + g autosomes. The female gametes all had g autosomes + X. In another variety the two could not be distinguished by size and shape. McPhee in the same year reported his investigations in which the variety of hemp studied did not show distinguishable differences between X and Y chromosomes.

THE EFFECT OF LIGHT

In the meantime Dr. J. H. Schaffner had been carrying on studies on the sexual expression in hemp and other dioecious and monoecious plants. Planting hemp seeds in December he obtained approximately equal numbers of plants that had the aspects respectively of male and female plants. However, 88 per cent of the female plants produced some intersex flowers or some purely male flowers and 84 per cent of the male plants produced some intersex flowers or female flowers. These plants were the descendents from two generations of plants that when grown in the summer were pure male or female with no intersexes. Only a few of the flowers showed complete sex reversal, most of them being intermediate, with malformed and nonfunctional stamens and pistils or organs showing the characters of both, e.g. stamens bearing stigmas, or pistils with microsporangia. The few complete reversals consisted of normal male flowers producing functional pollen on the female plants and, much more rarely, normal female flowers on male plants. This author is very emphatic in his conclusions that the sexual differentiation is not explicable by a "Mendelian hypothesis of sex" but is the result of environmental factors. The particular factor involved in the experiments just described was the "abnormal environment, mainly a lack of light." The author carried out further experiments which led him to conclude that not merely shortened daylight but also reduced fertility

of the soil increased the tendency toward sex-intermediacy or sex reversal.

McPhee repeated some of these experiments with several different strains of hemp, using chambers which could be closed at different times of day so that the plants could all be grown during the summer and yet have different daylight periods. Although he did not obtain such striking percentages of change as Dr. Schaffner did he found that with the shortening of the daylight the percentage of intersex flowers was increased, although the plants retained their general aspect of male or female plants. He was successful in obtaining a few viable seeds from female flowers produced on male plants and pollenized by pollen from the same plants. Only four such seeds produced plants that grew to flowering. Of these three were male plants and one female. On the other hand many more seeds were obtained from flowers on female plants pollenized by pollen from male flowers on the same plant. Out of **161** such plants grown to flowering, 158 were typical female plants. The three male plants are believed by McPhee to have been due to accidental pollination from male plants. Flowers on these female plants were pollinated from male flowers produced on the same plants under the influence of shortened daylight and from these a few second generation plants were obtained, like their parents all female. Apparently the condition of femaleness is a matter of heredity.

McPhee found that there was great difference in the ability of various strains of hemp to be affected in the sex ratio by different lengths of daylight. Some' strains appeared to be entirely unaffected by such treatment and produced pure males or pure females, regardless of the shortening of the daylight period. Other strains were very readily modified by the same treatment,

DISCUSSION

When we bring together the various observations with reference to the sexual conditions in hemp we find a number of apparently contradictory conditions. In at least one variety of hemp Hirata has demonstrated the presence of distinguishable X and Y chromosomes, the male plant being heterozygous and the female homozygous. In other varieties these chromosomes are not distinguishable but it can hardly be doubted that they are there even though alike in appearance. The plants grown from seed whether in summer daylight length or in shortened light periods are in about equal numbers, male and female, so far as the vegetative characters are concerned. Yet by cutting back the plants and growing them in shortened light periods Schaffner caused such plants to show partial or in a few cases complete sex reversal. From these series of observations Schaffner is very positive in his conclusions that the heterozygous and homozygous chromosome condition has nothing to do with the determination of sex in hemp. From his studies in other dioecious plants he is even more firmly convinced of this fact. McPhee, noting that the male or female aspect of the plant is fixed from the beginning regardless of the environment, believes that the sex is determined in a Mendelian manner, but that the sexual expression may be modified by environment. The fact that seeds from female plants pollenized by pollen produced, under the influence of the environment, on the same plants give rise only to female plants would seem to substantiate the idea that the chromosome condition is the determiner for sex.

Hirata suggests that the Y chromosome has a tendency toward maleness, the X chromosome a tendency toward femaleness, The male tendency in Y over-

balances the female tendency in X so that a plant heterozygous for X and Y is normally male and an XX plant is normally female. In the autosomes the sexual tendencies are about equally balanced. The sexual determination is brought about by the excess respectively of the enzyme "andrase" for the male or of the enzyme "gynase" for the female. Under abnormal environment or under the influence of mutilation the dominant enzyme formation may be partially suppressed, allowing the opposite sex to express itself partially (intersexuality) or completely (sex reversal).

The writer believes that both views may be reconciled in the following manner. The sexual expressions as male or female are the result of different metabolic rates in the individual, as Riddle has suggested for the pigeon. A marked change in this metabolic rate from a higher to a lower rate or the reverse will change the expression of sex. If the change is to an intermediate metabolic rate the result will be the production of intersex forms. Under normal environmental conditions the determiners for the higher or lower metabolic rate are the sex chromosomes, hence the usual approximately equal numbers of males and females. Since this chromosome condition exists from the fertilization of the egg the embryo in the seed is already predetermined for a certain metabolic rate, i.e. for a definite sex. Hence we find that the plant will have the vegetative characters of a male plant or of a female plant from the beginning. If the external environment (light, moisture, etc.) is normal the plants will produce male or female flowers, respectively. Extensive mutilation will affect the metabolic rate, as will shortened daylight, infertile soil, etc. Depending upon the degree of this alteration there will be produced a few or many intersex flowers or even a complete sex reversal.

Sex reversal has been brought about by Schaffner in *Arisaema triphyllum* by simply transplanting the corms of male plants from poor, dry soil to rich, moist soil, or of female plants from rich, moist soil to poor, dry soil. An intermediate type of soil may produce monoecious plants with both male and female flowers in the same spathe. Hemp, *Arisaema* and *Carica* and other plants in which sex changes have been reported as a result of changes in environment or of mutilations are species which are as it were only sporadically dioecious in families or orders in which the normal condition is hermaphroditism or monoecism. Most of the Urticaceae (in the wide sense) are either monoecious or hermaphroditic. Even in *Carica papaya* perfect flowers are not rare and the nearly related plants (e.g. Passifloraceae) are hermaphroditic. Clearly sexual differentiation of the plants into male and female plants is not an ancient or very deep seated character. This is quite in contrast to the sexual condition in animals which is very ancient and deep seated. Even in the family Salicaceae we find intersexuality. Probably there are no families of flowering plants where the distribution of sexes in different individuals is deep enough to prevent partial reversal by environmental changes.

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