THE NATURE OF THE DIECIOUS CONDITION IN MORUS ALBA AND SALIX AMYGDALOIDES.*

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In a recent article on "The Expression of Sexual Dimorphism in Heterosporous Sporophytes"† the writer referred to the nature of the sexual development in diecious plants, giving a number of examples of intermediate types of flowers and inflorescences as observed in various diecious species. It was maintained that sexuality as expressed in the sporophyte is a state which in most higher plants arises in the vegetative tissues. It has seemed to the writer that many geneticists have attempted to establish an arbitrary formula to explain sexual phenomena which cannot be applied to the great preponderance of known facts in regard to sex in plants and animals. The simplest sort of observations on a large number of species, especially when they are studied in phyletic series, will plainly indicate that sexuality is quantitative. The state of maleness or femaleness not only varies in degree in different individuals of the same species but also among many independent groups of species.

Morus alba L.

To discover something of the nature of dieciousness in a typical plant by mere observation, the writer chose for one study some trees of Morus alba L., the white mulberry, growing on his old home farm in Clay County, Kansas, where about forty years ago a small grove of this species was planted from nursery stock. These plants soon began to give rise to seedlings scattered along the ravines of the farm and there are now a considerable number of such trees, from ten to thirty years old, available for the study. The trees are all wild seedlings and have had no artificial treatment except that occasionally a limb has been torn down by the wind or removed by the ax and the tops of some have died off because of the dry climatic conditions of the prairie. It is important in such a study to know that the plants have not been grafted in any way.

Altogether 66 trees were studied while they were in full bloom in the months of May and June. The trees graded from apparently pure carpellate to pure staminate. Of course, the

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trees listed as pure may not have been absolutely monosporangiate, since catkins or flowers of the opposite nature may have been present and overlooked. When one has to go over a tree of considerable size, one may miss even what one is looking for with the greatest care, but the presumption is that they were really pure plants. But even if not, the general nature of the tree was typically carpellate or staminate and this is the important point. At the blooming period there is, besides the striking difference between the catkins, a marked difference in appearance between the staminate and carpellate trees. The staminate trees usually have few leaves at the time of blooming while the carpellate trees have abundant leaves and a more vigorous appearance in general.

Of the 66 trees examined, 28 were apparently pure carpellate individuals, 24 were apparently pure staminate, and 14 were intermediates—or 42+% carpellate, 36+% staminate, and 21+% intermediate. This is not far from 40% carpellate, 20% intermediate, and 40% staminate, which probably approximates the general condition. In other words the pure carpellate and pure staminate individuals are about equal to each other and the intermediate individuals number about one-half of either of the pure types, or about one out of every five trees studied.

The intermediate trees were of varying degrees of carpellateness or staminateness. It is simply stating a matter of fact then to say that in these individuals sexuality expressed as maleness or femaleness is entirely quantitative and may be developed in any degree.

Of the 14 intermediates, 5 were observed to be more carpellate or prevailingly so, 5 about half and half carpellate and staminate, 3 were prevailingly staminate, and 1 was a staminate tree with 3 of its main branches purely staminate but the fourth main branch was decidedly carpellate although still producing many staminate catkins. This tree is described in greater detail below.

The following descriptions show the characteristics of each of the 14 intermediate trees:

1. Carpellate tree occasionally having a carpellate catkin with a few stamens. The tree has only a very slight male tendency. No complete staminate catkin was discovered but some catkins were about half and half.

2. Carpellate tree with a few carpellate catkins having some stamens. This tree was about like the preceding in sexual expression.
3. Carpellate tree with here and there a carpellate catkin having a few staminate flowers.
4. Carpellate tree with a considerable number of staminate catkins, scattered here and there. In some cases the staminate catkins were in the same cluster as the carpellate. Also some carpellate catkins had a few stamens. This tree has female expression with a strong male tendency.
5. Carpellate tree with many staminate catkins and many mixed. There is a very strong tendency to male expression.
6. A tree with about half carpellate and half staminate catkins with some mixed. The staminate are apparently slightly more numerous.
7. Tree with about an equal number of staminate and carpellate catkins, with very many catkins having both stamens and carpels. A decidedly bisporangiate individual.
8. Tree with about an equal number of staminate and carpellate catkins and also with some mixed catkins.
9. A tree of about the same nature as number 8.
10. An individual having about equal numbers of staminate and carpellate flowers. Most of the catkins have both stamens and carpels in varying degrees.
11. Tree mostly staminate, with here and there a carpellate catkin or partially carpellate catkin on small branches in various parts of the tree.
12. Tree prevailingly staminate, with here and there a carpellate catkin; also with catkins having both kinds of flowers. Male sex is characteristic, with a considerable female tendency.
13. A tree prevailingly staminate, but with careful search showed a very few carpellate catkins. Male sex is expressed with a slight female tendency.
14. A wild seedling tree about 20 years old with a main central branch and three large side branches. One large branch had been cut off. This tree was a staminate tree covered with staminate catkins. Of the three large side branches two showed nothing but pure staminate catkins. The third had reversed the sexual state to a remarkable degree and was decidedly carpellate in all of its parts. It produced over 1000 fruits which came to maturity. However, the reversal was not complete for this branch produced some pure staminate catkins and also some catkins with both staminate and carpellate flowers. The main or central branch was also purely staminate except that near the top of the tree there was a small twig which produced four carpellate catkins which developed into fruits. In this twig evidently the same reversal of the sexual state had taken place as in the large branch farther down. The large reversed branch was 4 inches in diameter at the base and 12 feet long.

Omitting the tree with the reversed branches, the remaining 65 individuals may be arranged to indicate roughly the degree of femaleness or maleness as follows:

Pure female expression.	Female with very slight male expression.	Female with considerable male expression.	Female with strong male expression.	Female and male nearly equally expressed.	Male with slight female expression.	Male with very slight female expression.	Pure male expression.
28	3	1	1	5	2	1	24

In the case of number 14, we have an example of the reversal of the sex condition in the vegetative tissues of a bud, which is more fundamental than the dimorphic hereditary expression which appears when two catkins of opposite nature are produced side by side, although the cause may be the same in either case. The main trunk with the male condition established, or at least with a strong tendency toward producing staminate catkins, suddenly gives off a bud with the opposite tendency which continues in scores of secondary buds and branches. The change from staminate to carpellate condition was, however, not complete. The reversal in sexual nature was not able to repress the male condition entirely, and so there are here and there catkins which have the staminate nature—an expression of maleness in the ultimate shoot, although the prevailing tendency of the entire branch is female. The seeds of the fruit are perfectly viable and little trees are being grown from it at the present time.

There could be no more striking example of the reversal of sex in a vegetative tissue and the prevailingly consistent behavior of its parent tissue than is shown in this tree. The process, however, can be no different, fundamentally, than what goes on in the vegetative tissues of monecious and bisporangiate flowers generally; but the case is interesting because of its bearing on diecism, and because of its persistence in the given branch. There is something in this derived vegetative tissue, derived apparently from male tissue, which compels female expression generally but permits a reversal to the male condition again occasionally. This "something" has nothing to do directly with the fundamental factors of male

and female organs or gametes, for all such factors are present in these cells; nor is there any relation to a reduction division, nor to any other possible shifting of hereditary sexual units. All of the sexual hereditary factors are present and functioning at certain points in cells derived by vegetative division from a previous, common, mother cell.

This interesting example shows that a sex reversal can and sometimes does take place in an old tissue whose cells are removed by thousands of vegetative divisions from the original zygote. It assures us that sex control is only a matter of finding out how to change the prevailing physiological state of the tissues in some way corresponding to the change of state which actually takes place in living bodies without any apparent external cause. It is reasonable to believe that a change in sexual state could be accomplished much more readily in the zygote, or the cells coming immediately from it, than in an older tissue where the particular condition presumably has been intensified by its longer continuance, whether the state is due to accumulated chemical bodies or to some other cause.

Salix amygdaloides Anders.

During the same period, the writer studied the diecious nature of the peachleaf willow, Salix amygaloides Anders., on the same farm. There is a grove of about 100 trees which has developed in a ravine that was formerly pure prairie, within the memory of the writer. One cannot be certain of the exact number of individuals, as this species sprouts abundantly from the base and forms clumps of shoots or trees which have a common vegetative origin. In older specimens it is not possible to tell absolutely whether a given clump represents a single individual or two or more.

Out of the 100 trees 9 individuals were found intermediate while the rest seemed to be normal staminate or carpellate. However, since the study was begun rather late in the blooming period there may have been some apparently carpellate individuals that had previously produced staminate catkins. The study was, therefore, confined to the nine intermediate individuals discovered. From a superficial knowledge of this species of willow, the writer believes that intermediates are quite rare and not commonly produced as in the white mulberry.

All of the nine intermediate individuals seemed to be primarily staminate, ranging from a few to many carpellate cat-

kins or bisporangiate catkins on a plant. One individual, however, had a considerable proportion of carpellate or fruiting catkins and produced considerable seed. Still it was prevailingly staminate.

In most cases, the carpellate catkins were staminate below and became carpellate at the outer end, usually at or somewhat above the middle. The axis of such catkins changed from the staminate state to the carpellate state. The staminate flowers below were normal for the species and the carpellate flowers near the tip appeared normal and finally discharged mature seed in the usual manner. But on the transition zone, between the staminate and carpellate parts, the axis seemed to be neutral in regard to sex and here bisporangiate flowers were frequently present, the same as is commonly observed in normal monecious inflorescences where one part is staminate and the other carpellate. In the neutral zone abnormal flowers were very frequent. In some cases structures developed which were partly staminate and partly carpellate. Or a stamen would have some carpellate characteristics or a gynecium take on some of the peculiarities of a stamen.

The development of organs in such transition zones is very interesting, since it indicates that the differential sexual state is not sufficiently strong in one direction or the other to make the factors which control the expression of one or the other set of organs entirely latent or entirely active. In consequence of the lack of such control, there is an attempt, so to speak, to develop both male and female characters in an organ which phylogenetically, and normally in its ontogeny, is purely male or purely female in expression. The reversal of the sexual state in the middle of the catkins is not abrupt but there is a gradual change in the tissues from one condition to the other. It is a quantitative change; and thus it necessarily follows that the characters developed through the activity of the hereditary factors are also quantitative in respect to maleness and femaleness. In these catkins the reversal of sex is from male to female as in the inflorescence of Zizania aquatica L. In other plants like Tripsacum dactyloides L. it is just the opposite.

The conclusion from the evidence presented above seems to be inevitable, that sex expressed as maleness or femaleness is not an irreversible, Mendelian, hereditary character, dependent on the presence of a single hereditary factor or group of factors, but is a physiological state or condition which influences the

activity and latency of the factors that control the development of sexual gametes or organs, or other sexual peculiarities possessed by the organism.

An examination of the life cycle of one of the higher plants, like Selaginella kraussiana (Kunze), shows that sexuality as expressed in the male and female gametophytes, or as slightly indicated in the microsporophylls and megasporophylls of the sporophyte, is not Mendelian and cannot have any relation whatever, primarily, to Mendelian factors, or Mendelian combinations and segregations; for the simple reason that the beginning of sexual differentiation is initiated in the tissues of the mature sporophyte. Furthermore, when the reduction division occurs in which the synaptic chrosomes are segregated and with them the possible Mendelian factors the four megaspores or the four microspores resulting are not half of one sex tendency and half of the other, but all of the four spores resulting from a reduction division are of the same sexual state and all four give rise to females or all four to males, depending on which sexual state was established in the tissue from which the sporocyte originated. Again, after the gametophytes have developed and matured their gametes, the resulting zygote is not determined as male or female, or more properly speaking as microsporangiate or megasporangiate, but the resulting sporophyte is neutral until it begins to form its strobili when both male and female expression originate side by side in its vegetative tissues as stated above. It is simply impossible to think of a Mendelian formula for the sexual expression of such plants when the ontogenetic processes do not permit combinations and segregations of sex-determining factors to take place in the chromosomes. In consequence of the above facts it comes about that in this species of Selaginella the proportion between the sexes of the gametophyte generation is about 1 female to 5,000 males instead of about 1 to 1, as would follow if sexuality were controlled by factors segregated in the reduction division.

This conclusion has been evident to the writer ever since he began to plot the life cycles of plants as an aid to teaching in general botany. In the first edition of his "Laboratory Outlines for the Elementary Study of Plant Structures and Functions from the Standpoint of Evolution"* the following statement is made: "Male or female sex is not an inherited

* Journal of Applied Microscopy and Laboratory Methods, 5 : 2056. 1902.

quality, but depends on the environment present during the germination of the spore and the development of the embryo, and it can be directly controlled in many cases by artificial means." A statement of similar import has been included in the several editions of the "Outlines" published independently from time to time. In the fourth edition entitled "Laboratory Outlines for General Botany," 1915, the more carefully worded statement appears on page 23 as follows: "Maleness or femaleness is not an hereditary character or factor, but a condition, and often depends on the environment present during the germination of the spore or the development of the embryo." In the meantime, after the knowledge of mutations and the Medelian discoveries had become generally known, the problem of sex has received renewed attention from numerous investigators many of whom attempted to develop a general Mendelian formula for sex, assuming the one sex to be homozygous and the other heterozygous. There have, however, been many whose experimental work has been in substantial agreement with the undeniable conditions as presented by the Selaginella type of plants. The remarkable work of O. Riddle on pigeons has opened up a new line of attack on the problem connected with the higher animals which may well be carefully considered by experimental botanists.

In conclusion the writer will present a number of paragraphs from an article published in 1910, on "The Nature and Development of Sex in Plants,"† in which the problem is discussed from various angles. Among other conclusions expressed the following seem appropriate here:

"Sexuality expressed as maleness or femaleness, whether in gametes, sexual organs, or individuals, is a condition and not a character" (factor).

"Sex may be determined sometime before reduction and thus independently of any process going on during either a vegetative or reduction karyokinesis; it may be determined during the reduction division; it may be determined during the fertilization stage; or finally, it may be determined after vegetative growth has begun."

"In some cases, when the sex is once determined it cannot be changed in the vegetative body nor any vegetative spore or propagative bud; in other cases, it may be changed in the vegetative body after being developed as male or female."

† Proc. Ohio Acad. of Sci. 5 : 321-350.