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CONTROL OF THE SEXUAL STATE IN ARISAEMA TRIPHYLLUM AND ARISAEMA DRACONTIUM [1]

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Arisaema triphyllum (L.) Torr., being a dioecious plant with a considerable percentage of intermediate individuals, presented itself to the writer's mind as a very favorable plant for experiments on the control of sex, such experimental control seeming to be necessary to substantiate conclusions reached previously, mainly from taxonomic studies, as to the fundamental nature of the sexual state. Accordingly, work was begun on this plant in the spring of 1917. More recently work has also been done on *Arisaema Dracontium* (L.) Schott.

The first plantings were made in pots and were not very satisfactory, probably because there was no place in the greenhouse to keep them properly, and the writer was absent during the entire winter. However, one pure carpellate plant changed to a pure staminate plant, and some intermediate individuals also changed to the staminate condition. The writer was certain, therefore, that sex reversal took place, and the results so far as they went agreed with the reports of sex reversal in Arisaema by Atkinson (1), Gow (2), and Pickett (3). The study was continued by observations in the field and by experiments in garden plots.

FIELD OBSERVATIONS

The individuals in the field are pure staminate, pure carpellate, or intermediate in varying degrees from nearly pure staminate or carpellate to individuals having inflorescences with about an equal number of staminate and carpellate flowers. There are usually about 60 to 80 flowers on a fairly large inflorescence. The folding of the margins of the spathes appears to be an ordinary fluctuation. There are about equal numbers folded clockwise and contra-clockwise. The same conditions hold for the spathes of *Arisaema Dracontium*.

As stated, the intermediate plants show all degrees of sexual expression. The inflorescence may be carpellate except for one imperfect or perfect staminate flower, or it may be staminate with a single carpellate flower, or from such a condition up through all gradations to a monoecious inflorescence with about an equal number of both kinds of flowers. There is no definite position for the appearance of the opposite type of flowers. Pickett (3)

[1] Papers from the Department of Botany, The Ohio State University, no. 130.

has reported similar conditions. The staminate and carpellate areas are sometimes in definite spots, and when the transition line happens to pass through a flower one side is staminate and the other carpellate. The writer has published a detailed account of such cases elsewhere (6).

As a general rule the carpellate plants are the larger. There is an important difference in the longevity of the peduncle. The staminate peduncles die and dry up soon after blooming, while the fertilized carpellate peduncles, of course, remain in a vigorous condition until the fruit is matured. In the intermediate plants, there is every gradation of robustness and persistence, depending on the degree of carpellateness of the individual. Those that are largely staminate, though they may have several well-developed green fruits, soon wither, while those that have but a few staminate flowers remain green and erect. There is no essential difference in the vegetative characters of the staminate and carpellate individuals. Both one-leaved and two-leaved plants are either staminate or carpellate in about equal proportions.

A statistical study of the plants in the field was made with the results shown in table 1, all plants showing any evidence of monoeciousness being classed as intermediate.

TABLE 1. *Arisaema triphyllum in the Field*

Habitat	Carpellate Plants		Intermediate Plants		Staminate Plants	
	Number	Percentage	Number	Percentage	Number	Percentage
I. Rich beech forest.....	315	26+	167	13+	727	60+
II. Open pastured wood...	43	13+	40	12+	227	73+
III. Rich, moist mixed forest	9	23+	7	17+	23	59+
IV. Base of north-facing limestone cliff in ravine, rather dry....	16	10+	15	10	119	79+
V. Rich mixed forest containing black humus.	61	36+	38	22+	67	40+
Totals and averages for the 1874 plants.....	444	23+	267	14+	1163	62+

It will be seen by an inspection of the table that there is a large fluctuation in sex ratios for the different habitats. The staminate plants are always much in excess of either the carpellate or the intermediate individuals, and with the exception of the wet black humus habitat considerably over 50 percent of the total. The fourth habitat, representing a rather dry, poor soil, is in striking contrast to the fifth and shows how plants are affected by the ordinary field environments. The rich, wet soil has, in proportion, more than three times as many carpellate plants as the poor, dry soil, more than twice as many intermediates, and only half as many staminate individuals. As is shown below, these extreme fluctuations are due to the direct control of the sexual state by environmental factors and not to a difference in death rate between the two sexes.

Experimental Plots

In order to determine whether the sex could be reversed under definite control, a small number of plants was dug up on June 14, 1919, and transplanted into a special bed on the north side of the greenhouse at the west end. There were 25 pure carpellate plants, 5 intermediate plants, and 10 pure staminate plants. They were all treated alike, having most of their roots removed and all but a small fragment of the leaf blade cut away. The habitat was rather dry, and no artificial watering was done after the first few days. The date of transplanting was rather late, and it was feared that the incipient inflorescence buds might already have their sexual state determined. This fear was, however, mostly unfounded, as the next spring's crop of flowers proved. Under the treatment all the plants were expected either to stay in the pure male state or to change over to the male condition.

The following was the result in the spring of 1920:

Of the 10 originally staminate plants, one died and 9 were still pure staminate.

All of the 5 originally intermediate or monoecious plants were pure staminate.

Of the 25 originally pure carpellate plants, 21 changed to pure staminate, 2 remained pure carpellate, and 2 had intermediate inflorescences. One of the originally carpellate plants gave rise to pure staminate "vegetative twins" which were counted as a single plant in the statistics. Of the two intermediate individuals, one had a carpellate inflorescence with two staminate flowers at the top and the other had a carpellate inflorescence with seven staminate flowers at the top.

Under the treatment, therefore, all the monoecious plants changed to the male condition, and out of the 25 carpellate plants 21 were reversed from a pure female condition to a pure male condition, 2 were changed from a pure female condition to a hermaphroditic condition, and 2 were either not influenced or else their buds had already developed the flower incepts beyond the stage at which reversal is possible.

The inflorescences were all removed in 1920 as soon as they were out of the ground, and all three plots were then treated with a thick layer of well rotted cow manure and kept very wet until the end of June. The plants made an extraordinary growth, and the intention was to reverse the sex in the opposite direction in all three plots, *i.e.*, from staminate to carpellate, the presumption being that high nutrition or abundant water supply or both combined would produce such a physiological condition in the corms that the incipient inflorescence buds would be thrown into the female state. The results in the spring of 1921 were as follows:

The 9 plants which were originally staminate and which had remained staminate the previous season were all reversed and carpellate, the female

condition being complete in all except one individual which had three staminate flowers at the tip of the inflorescence.

Of the 5 plants which were originally intermediate or monoecious, and which had been changed to the pure staminate condition the previous season, 4 were pure carpellate and one which had given off a large branch the first year and whose corm was thus reduced gave rise to 2 staminate shoots.

Of the 25 plants which were originally pure carpellate, and of which 23 had been caused to change their sexual state the previous season, one failed to bloom, one was pure staminate, one was intermediate with a carpellate inflorescence showing a single staminate flower at the top, and 22 were pure carpellate, having reversed their sexual state back to the condition they were in when originally transplanted.

The corms have given off a considerable number of small buds, so it is probable that future reversals will not be so nearly universal as in the first two seasons. The twin plant of the previous season had separated its corms completely, and both the twins that had been completely staminate the previous season were now completely carpellate.

The experiments show conclusively that it is possible to exercise almost complete control over the sexual state of *Arisaema triphyllum*. A slight disturbing factor is introduced because of the fact that the corms often produce buds of a sufficient size to rob the parent of much of its food supply. Sex reversal is complete in the individual in either direction from time to time, the male to the female or *vice versa*, or the reversal may be partial, from a pure male or female state to a hermaphroditic condition, or *vice versa* from a hermaphroditic condition to a pure male or female state. The exact factor which induces the reversal has not been ascertained. The writer was desirous to show first of all that sex reversal actually takes place in many plants. Apparently in *Arisaema triphyllum* the sexual state depends mainly on the water supply, or on the nutrition, or on both combined. According to Atkinson's (1) experiments the determining factor might be the varying amounts of the nutriment in the corm. Pickett (3) believes his field observations and experiments show that "the amount of water available at a certain period in development is directly or indirectly responsible" for the given sexual state. Apparently the sexual state may be determined in a feeble or in an intense manner. If the female state is of low degree, the inflorescence may change from carpellate below to staminate toward the top, or in some cases any patch of cells may change to the male state whatever their position in the spadix. If the sexual state is originally determined in the bud as male, the reversal usually takes place in patches at the sides of the spadix, involving one or more flowers, apparently without any relation to the age of the cells or to their position on the inflorescence axis.

Arisaema triphyllum is an unusually favorable perennial herb for experimental purposes in studies on sex control and reversal, and there should

be no special difficulty in determining the exact environmental factor or group of factors which determine maleness or femaleness in the incipient inflorescence bud.

ARISAEMA DRACONTIUM

Some work has been done on *Arisaema Dracontium* both experimentally and in the field, although it is much less common around Columbus than *A. triphyllum*. In this species, so far as the writer's knowledge goes, there are staminate and monoecious individuals. No pure carpellate plants have been discovered, although there are decided differences in the relative widths of the carpellate and staminate zone between different monoecious individuals. One plant was found that came very near to a pure carpellate inflorescence. The normal part of the flower-bearing spadix was purely carpellate, but on the sterile part above this there were six scattered staminate and imperfectly staminate flowers. One of these flowers was half carpellate. It is probable that in rare instances pure carpellate inflorescences are produced. One "staminate intermediate" plant was found having three carpellate flowers on one side at the base of the inflorescence, which was otherwise normally staminate; another staminate plant had two carpellate flowers near the middle of the spadix on opposite sides. Such intermediate staminate plants, even though they have the abnormal carpellate flowers fertilized, soon wither like ordinary staminate peduncles as in similar cases in *A. triphyllum*. Abnormal monoecious individuals were also found. One of these had two staminate flowers at the base, then a zone about one fourth inch wide of carpellate flowers, and above this a zone three fourths inch wide of staminate flowers; another had a single carpellate flower in the middle of the staminate zone; another was nearly completely staminate at the base, but in this there were three carpellate flowers, next there was a complete narrow zone of carpellate flowers, and above a wide zone of staminate flowers again. Three other monoecious inflorescences had each several staminate flowers at the base of the carpellate zone. One monoecious plant had the lower part in irregular staminate and carpellate patches. In those inflorescences which have staminate and carpellate flowers in patches, as well as in the ordinary monoecious type, there is often a general confusion of floral structures, as reported by the writer (6) for *A. triphyllum*. There are flowers partly staminate and partly carpellate, staminate flowers whose stamens have stigmas, and neutral or partly neutral structures of various abnormal shapes. These examples show that, as in *A. triphyllum*, there are intermediate individuals which do not have the ordinary types of sexual expression.

A statistical study of two habitats for two years gave the results shown in table 2.

The plants in the second habitat (III and IV) are apparently dying out rapidly because of changing ecological conditions and the ravages of

the Arisaema rust. In the first habitat the plants are thriving. In both habitats and in both years there is an enormously greater number of staminate individuals than of monoecious individuals.

TABLE 2. *Arisaema Dracontium in the Field*

Habitat	Monoecious Plants		Staminate Plants	
	Number	Percentage	Number	Percentage
I. Rich, moist, open mixed wood, 1920	9	8+	97	91+
II. The same in 1921	125	18+	568	81+
III. Open pastured wood, 1920	2	4+	43	95+
IV. The same in 1921	1	3+	27	96+

On May 28, 1920, a plot on the north side of the greenhouse was planted with several monoecious individuals and a considerable number of staminate individuals, the leaf surface and the roots being reduced. The intention was to give the plants an environment that would keep the staminate plants staminate and change the monoecious individuals to staminate individuals. In the spring of 1921, three of the originally monoecious plants bloomed; two were monoecious and one was pure staminate. Seventeen of the originally staminate plants bore inflorescences, of which sixteen were pure staminate and one typically monoecious. This monoecious plant had changed from the staminate condition in spite of the fact that the nutritive supply was decreased. Presumably, its bud for the following year's shoot was already sufficiently developed to have its sex determined, or else it had a sufficient amount of the proper food supply stored in the corm to cause a reversal of sex notwithstanding the unfavorable treatment given.

But the important result is that in *A. Dracontium* staminate individuals do reverse to monoecious individuals, and that monoecious individuals do change to staminate individuals. The conditions of sexuality are in general the same as in Jack-in-the-pulpit. Sex in the green-dragon is a condition and not dependent on homozygous and heterozygous hereditary factors. The general hereditary constitution is apparently such that when the female state is established in the incept of the monoecious inflorescence, the condition is not strong enough to continue through the entire developmental cycle; but after the bud has developed a zone of carpellate flowers, the sexual state is reversed, perhaps through senile changes of the cells, specific differentiations, or exhaustion of available food supply, and only staminate flowers are produced above. *Arisaema Dracontium* is, therefore, a species with decidedly male characteristics and can be compared with a similar condition in the gametophyte generation of *Selaginella Kraussiana* and other like species where, because of the small percentage of megasporophylls and megaspores produced, the female gametophytes are outnumbered to an extreme extent by the males; or it may be compared with such animals as the European cuckoo bird, which is said to produce about four males or even more to one female.

CONCLUSIONS

The foregoing study shows that sex in Arisaema is dependent on a functional state and not on hereditary factors; that the sexual state is readily controllable and is reversible in either direction, the male to the female or the female to the male, and then back again; and that the dimorphism which appears in the inflorescences of these diploid organisms cannot be due to homozygous and heterozygous factors or chromosome constitutions.

The condition of affairs found in Arisaema is in perfect agreement with that discovered by the writer in *Cannabis sativa* (5) and by Yampolsky in *Mercurialis annua* (7), which are very different types of plants and belong to different subclasses of a different class. The factorial hypothesis of sex cannot be entertained by botanists, and it is the writer's conviction that sex in the higher animals is no more determined by Mendelian factors than in plants and that chromosome differences where they exist are merely indicators of sex and not determiners. Chromosome differences may influence the metabolism of the cell and thus influence the determination of sex, but it is possible to overcome this influence in the cell and to cause a reversal of the sexual state even in the presence of an allosome difference. In fact, the sexual state is commonly reversed in the somatic cells of animals through various external causes, as by the injection of proper hormones, removal of sex glands, etc.

In nature, we see quite generally the existence of positive, negative, and neutral conditions, and the physicist is inclined to interpret these conditions in terms of positive and negative electricity. Whatever the fundamental cause of the positive and negative states of matter will be found to be, it will probably also give the clue to the nature and cause of the remarkable duality and dimorphism which we call sexuality and which is a characteristic of all plants and animals except the very lowest.

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