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CONCERNING ARTIFICIAL CROSSING IN *PISUM SATIVUM*[1]

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STIMULATED BY THE EXPERIMENTS OF DARWIN on the effects of cross- and self-fertilization in the plant kingdom, I began, in the year 1898 to make hybridization experiments with *Pisum sativum.* The group to which *Pisum sativum* belongs was especially interesting to me because in it are found exceptional results from the generally accepted principle of the advantageous effect of crossing different individuals and different varieties in contrast to self-fertilization. In most of the species with which Darwin worked (57 against 26 respectively 12), the seedlings from a cross between individuals of the same species almost always exceeded the competing individuals produced by self-fertilization in height, weight, vigor and frequently also in fertility, while in the case of the pea the amount of difference in height of plant produced by hybridization to those which were products of self-fertilization was as 100:115. Darwin believed that the reason for this behavior in peas was the result of many generations of repeated self-fertilization in the northern lands. Considering the limited material which Darwin observed (only four pea plant pairs were measured and observed) it appears to me, especially, since Darwin never emasculated

1 The detailed paper will be published in: *Zeitschrift für das landwirthschaftliche Versuchswesen in Oesterrei*ch, 3 (5), 1900.

the flowers, that these experiments should be repeated on a larger scale and with greater accuracy.

I also made artificial crosses between different varieties of *Pisum sativum*. The purpose was to study the immediate influence of the foreign pollen upon the constitution (form and color) of the seeds thus produced, and also to follow, in the next generation of hybrids, the inheritance of the constant, differentiating characters of the parental types used in the hybridization. In the second year of experiment, the behavior of the hybrids in respect to growth (especially height), seed production, and change in color and form of seeds and pods was compared with the corresponding characters of the descendents obtained from self-fertilization of the parents. Pollinations with two different pollen types (so called double pollinations) were performed on several flowers to determine whether there occurs a simultaneous action of both or whether there is predominance of one of them. Crossing the hybrids with their parental varieties — or pure varieties with hybrids — gave regular results. Finally, by making the necessary numerous weight determinations of individual peas, it was possible for me to draw conclusions regarding the position of the heaviest kernel in the pod.

METHODS

When the flowers were in the early bud stage, they were opened with a slender lancette, and the anthers were extracted by means of a hooked forceps. For pollination I employed ordinary writing pen-points. The advantage over the camel's hair brush usually employed is that the mass of pollen accumulated at the tip of the pen-point can be distributed over the stigma with much greater certainty. This has been proven to be true especially in my double fertilization experiments. In addition it is possible, after every pollination to cleanse the pen-point rapidly and completely with a rag. Covering the flowers to protect them against unwanted foreign pollination is, as the results of my experiments have shown, not at all necessary, since the blade vane and keel, even after emasculation has taken place, still fit together so closely that they form a natural protective covering against the entrance of large insects. Nevertheless, I have, as a precaution, protected many flowers, especially those which served for double fertilization experiments, by a small, close meshed bag of gauze whose edges were turned under and sewn together and whose opening could be drawn shut or opened again by means of a drawstring. In the year 1898, the experiments had primarily the aim to produce material by self-

fertilization as well as by crossing in order to repeat, next year, the competition experiments discussed by Darwin.

The plants grew in pots in a closed room under the most uniform conditions possible. In the year 1899, competing plants from seeds of equal weight were also grown in pots in a covered place. Concurrently, parallel experiments were also made by growing plants in the open. Crosses of nine different pea varieties were carried out between flowers of the same plant (geitonogamy), between flowers of the same variety but from different individuals (isomorphic xenogamy) and between flowers of different varieties. The seeds of the latter were distinct from one another either in regard to their form or by color or both characters (heteromorphic xenogamy).

RESULTS

The several pollination types generally produced no decisive difference in relation to the absolute number of the mature seeds, or in relation to the proportion of the mature seeds of the number of the potentially seed setting ovules. Neither could a definitive influence of hybridization as compared to self-fertilization, with respect to the weight of the peas, be detected. The proportional heights in the case of the descendents from self-fertilization and from geitonogamy was on the average 94:100, in the case of the competing ones from self-fertilization and from isomorphic xenogamy 95:100.

This result thus agrees with the former experiments of Darwin. In the competition experiments between descendents from self-fertilization and those from heteromorphic xenogamy (hybrids), only certain hybrid forms showed increased growth over the self-fertilized plants. Other combinations, however, lacked such an “advantage” as a result of crossing against self-fertilization. The relative strength of the hybrid as compared to the self-fertilized maternal variety, for example, a hybrid from a cross of a relatively short variety with a relatively tall one, should usually be interpreted simply as an inheritance from the father rather than an “advantage” resulting from the cross, as such, in comparison to the self-fertilization. An interpretation in the later sense is justifiable only in such cases in which a hybrid exceeds, in height, the descendents of self-fertilization not only of the maternal variety but also of the paternal variety. The taller type always has the greater influence, regardless of whether it characterized the maternal or paternal variety. The descendants of a relatively short type after pollination with the pollen of a relatively tall one, appear relatively

greatly increased in height, as Andrew Knight has already observed; in the reverse case the hybrids are only slightly dwarfed, if at all.

A direct influence of the foreign pollen on the seeds could be determined in certain cases of artificial crossing of different varieties of peas. Certain specific combinations yielded this effect with regularity. The characters which were taken into consideration to recognize such an influence, related to the form of the seeds and the color of the storage tissue. The peas of the varieties used were either round and smooth or only slightly wrinkled, or they were more or less cubical (*Pisum quadratum*) and at the same time deeply wrinkled. The color of the storage tissue was either many shades of yellow or green.[2] My experiments showed the fact that the above mentioned differences of the same entity, *i.e.*, the characteristic "trait" of the individual varieties were found not to be equivalent in regard to their inheritance. Quite regularly one of the characters (in question) from either the paternal or the maternal plant (the dominating character according to Mendel) is developed exclusively while the recessive character of the other parental plant is not developed. The latter one, nevertheless, will reappear at least in part in the seeds of the hybrid plant. In agreement with Mendel's data[3] the round smooth form shows dominance over the cubical deeply wrinkled one, and the yellow color of the storage tissue over the green color. This is equally true whether the seed or pollen (of the parental plants) carried the character (in agreement with Mendel). The appearance of the dominating or the recessive character is not an all or none phenomenon. In individual cases I was sometimes able to establish, with certainty, a simultaneous appearance of both, *i.e.*, transition stages. The principle, established by the investigator above, of the regular non-equivalence of characters in inheritance, is confirmed by my experiments on *Pisum sativum*. Likewise the observations of Körnicke, Correns and de Vries on *Zea Mays* as well as those made by de Vries in his species crosses,[4] completely corroborate it. It proves to be of the highest significance for the study of inheritance in general. A possible modification of the seed coat in the direction of the pollen plant as a result of crossing needs to be interpreted in a very different way from that in the storage tissue, respectively the endosperm. The seed coat is a purely maternal product, but the endosperm (according to the investigations of Nawaschin and

2 "Die Farbe des Speichergewebes war entweder gelb oder grün in mannigfachen Nuancen." [Original German text.]

3 G. Mendel in *Verhandl. des naturf. Ver. Brünn*, Vol. 4:1, 1865. [Publ. 1866. Tr.]

4 Vol. 18 (3), 1900 this publication.

Guignard) a derivative of the embryo sac, which was fertilized by the (heteromorphic) pollen, in other words a product of the fusion of the second pollen tube nucleus with the so called embryo sac nucleus or the endosperm double nucleus. The storage tissue consists, on the other hand, apparently of products of the ovule. While in both of the latter cases we are dealing with hybrid- or crossing-effect, in the first case, a change in a product or part of the maternal organism would be involved in consequence of an action of the hybrid egg, *i.e.*, we would have here an indirect effect of the heteromorphic pollen. Only in this way would possible effects on the seed coat as well as effects on the pod and the mother organism at large be at all understandable on the basis of contemporary knowledge. I denote such cases as "xeniodochien." The hypothesis of direct action on the somatic cells of the mother plant by the pollen cells without the intermediary of the hybrid egg cell (or respectively an embryo sac) appears not to be tenable (the hybrid egg cell, could die very early).[5] On the other hand the possibility of a direct effect by the pollen cells on the somatic cells, especially of the stigma, in the sense of a release of further development of the ovary in the typical direction may well be possible.

In certain cases of form- (and also in part color-) differences in the parental varieties where there was an indication of intermediacy in the hybrids, each of the parental varieties showed relatively more influence on the character (especially the form) of the hybridization-products when it furnished the ovule than when it furnished the pollen.

In the seeds obtained by self-fertilization of the first generation hybrids, the characters yellow and smooth appeared to have a higher value or hereditary potency than the characters green and wrinkled exactly as in the cross pollinated seeds of the mother plant. However, while in the artificial production of products of heteromorphic xenogamy, the first named characters are almost without exception dominant, and the latter "recessives" appear in pure form or as admixtures only in a very few cases, in the seeds of the first hybrid generation, the former characters are expressed in pure form in a majority of cases only, and the recessive character appears in pure form in a minority. In the first case an almost absolute dominance exists, in the second a mere prepotency (in a fixed proportion). Combinations of both character groups are also here rare, but perhaps less rare than in the former case. The ratio of seeds carrying the dominant, prevailing character to those carrying the recessive is about 3:1. A comparison of the progeny obtained from reciprocal crosses of different varieties

5 Contrary to Darwin, "*The Variations of Animals and Plants under Domestication*," Vol. 1, Chap. 11.

showed, in agreement with the results analogous to those reported above for the products of reciprocal pollination, that in certain experimental cases the egg cell appears to be a more effective transmitter of the dominating color character than the pollen cell. Further experiments, however, are required to make a definite statement. The combination of two dominant or recessive characters in one parental type results in the seed production of the hybrid having a behavior similar to that of the respective isolated characters. An alteration in the potency as, for instance, an increase in the dominance thus is not produced.

Interesting results which are of unexceptional regularity were obtained by pollination of emasculated hybrids with parental pollen. Pollination of the hybrid by the parental type with the dominating character, regardless of whether it is the paternal or maternal variety, produces exclusively seeds with the dominating character. When fertilized by the parental type with the recessive character, the number of bearers of the recessive character are increased over that of self-fertilization of the hybrid. The influence of the character "yellow" in the seeds in the hybrid was in this case reduced by 57 per cent, while that of the character "green" was reduced by 43.5 percent.

In addition flowers of the pure varieties were pollinated with hybrid pollen. If the former had a recessive seed character, a mixture of seed types always appeared, however if they had the so-called dominant seed character then pollination with hybrid pollen did not change the typical uniform seed type showing the dominant character. Thus the reproduction products of the hybrid pollen with pure varieties bearing the recessive character are just as polymorphic as those of the pollen of pure recessively marked parental types with a hybrid. This is not surprising since we are simply dealing with products of recriprocal crosses.

In order to determine the effect of double-pollination with its own pollen as well as with that of another variety upon the seeds themselves, it was necessary to select as the seed plant a variety with a so called recessive character and to take the pollen, for the heteromorphic xenogamy, from a variety with the dominant character. Several pods with different colored seeds, yellow and green, were gathered, as well as those with all seeds exhibiting either the recessive or dominant character. The seeds with the recessive character must, with great probability, be due to self-fertilization. Those with the dominant character must with certainty, be due to cross-fertilization. Possible exceptions within the first group, in which in spite of cross-fertilization by a variety with a dominant character the recessive character of the seed plant still manifests itself, could of course be

recognized in plants which had been grown from those seeds and been left to self-fertilization. A modification of growth in comparison to the mother form (in case of divergent types of growth of the father) as well as the occurrence of differently colored or shaped seeds, especially in one and the same pod, would in such cases disclose the hybrid character and the origin of the seeds from self-fertilization would be excluded. On the contrary if the product obtained resulted from self-fertilization, then naturally, the adult plants grown from these seeds would precisely copy the mother variety in the type of growth and in the type of seeds produced by self-pollination. In the case of all seeds exhibiting the recessive characters, it is therefore highly probably that only self-fertilization operated. In the case of differences (dominant and recessive characters) of seeds in the same pod — a case which occurred extremely rarely in heteromorphic xenogamy of emasculated flowers – a partial effectiveness of the foreign pollen is certain, and that of [additional] self-fertilization is very likely. In any case my experiments, in agreement with the results of Fritz Müller[6] on *Ruellia silvico*[7] and *formosa*, makes it impossible to support the statement made by Darwin and later by Sachs[8] — which has been pronounced generally and has been adopted in many text books that, upon simultaneous application of two different kinds of pollen on the same stigma, only one of them will effect fertilization. At least for pollination between varieties of one species this statement is not valid.

Likewise in double fertilization of the hybrids with their own pollen, or with pollen of the same kind, and with pollen from one of the parental varieties both can act; by no means is one kind of pollen excluded by the other in the fertilization or prevails over it regularly. The same is true for double fertilization of a parental kind with its own and with hybrid pollen.

An orderly relationship between the absolute weight of the specific pea and their arrangement in the pod has been denied by some observers.[9] Both authors gave no consideration to the aborted seed anlage, and this accounts probably for their results of an “irregular

6 Abhandlungen des naturf. Ver zu Bremen, Vol. 12, page 379.

7 The Effects of Cross and Self Fertilization in the Vegetable Kingdom: “It is a much more remarkable fact that pollen from another individual of the same variety surpasses the pollen of the same plant.”

8 *Pflanzenphysiologie*, 1882, page 957.

9 Fruwirth, Über den Sitz des schwersten Kornes in den Fruchtständen bei Getreide und in den Früchten der Hülsenfrüchte, in Wollny’s Forschungen, Vol. 15:49, and Feldmann, Beiträge zur Kenntniss der Individualität des Saatkornes. 1897.

change in position of the heaviest seed." Numerous weighings made by me gave the result that in incompletely filled pods the heaviest seed lies on the average approximately in the center. If, in my tables, one adds the number of funiculi and divides the result by the sum of the respective number (counted as funiculi) of the heaviest kernels, one finds that the proportional number is 1.7 (159:94) not 2 which would be the midpoint of funiculi number. As, with complete wholly intact pods, this calculation results in the same proportional number, I believe that the conclusion is justified that the position of the heaviest kernel is not essentially dependent upon the number and arrangement of the fully formed or aborted seed, but rather is determined, primarily, already before further development of the anlagen, and lies generally somewhat above the middle of the sequence.

POSTSCRIPT

Correns[10] has just published experiments which also deal with artificial hybridization of different varieties of *Pisum sativum* and observations of the hybrids left to self-fertilization through several generations. They confirm, just as my own, Mendel's teachings. The simultaneous "discovery" of Mendel by Correns, de Vries,[11] and myself appears to me especially gratifying. Even in the second year of experimentation, I too still believed that I had found something new.

10 G. Mendel's Regel über das Verhalten der Nachkommenschaft der Rassenbastarde. *Ber. der Deutschen Bot. Gesellsch*., 18 (4): 158-168, 1900.

11 Das Spaltungsgesetz der Bastarde. *Ber. der Deutschen Bot. Gesellsch*. 18 (3): 83, 1900.