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THE EFFECT OF MUTATED GENES ON THE GERM CELL FORMATION IN PISUM

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The meiotic behaviour of an X-ray induced mutant of *Pisum sativum* was analysed. The recessive gene causes a strong reduction of chiasmata frequency resulting in a varying number of univalents in the pollen mother cells. The later meiotic stages are highly abnormal and lead to genomically unbalanced nonfunctionable germ cells. The mutant is sterile in both sexes.

In the light of the present finding the "gene action system" of the meiosis in *Pisum* has been discussed. A total of 39 genes have so far been controlling this fundamental biological process.

The fertility of an organism is dependent upon an undisturbed course of meiosis and a normal post-meiotic behaviour. An essential prerequisite is the formation of bivalents in the late stages of the first meiotic prophase and in metaphase I. It can only happen if two large groups of specific genes of the genome are present in the dominant condition. If they are recessive they would cause asynapsis, i.e. the lack of pairing of the homologous chromosomes (*as*-genes). The second group, the *ds*-genes, cause desynapsis, i.e. a reduction or a complete suppression of chiasmata formation. In both cases, a high proportion of univalents is expected, causing manifold irregularities in the later meiotic stages. Most of these mutants are sterile in both sexes. The cytogenetic investigation of this material shows that it is ideally suited for evaluation of the problem of the genetic control of meiosis and germ cell formation.

In the present paper, a highly desynaptic pea mutant was analysed which was obtained after seed irradiation.

Material and Methods

The meiotic behaviour of a completely sterile X-ray induced mutant of the variety *Dippes gelbe* Viktoria was studied. Suitable young buds were fixed in a solution of alcohol and acetic acid in a ratio of 3:1 and squashed in acetocarmine. The total segregation of the mutant in the M_3-M_5-generation was 128 normal: 55 mutant plants in segregating families showing that the meiotic anomalies are controlled by a single recessive gene.

Results

One of the characteristics of desynaptic mutants is that the degree of desynapsis, that means the degree of the reduction of the chiasmata frequency, varies considerably between adjacent pollen mother cells (PMCs) of the same anther. It is therefore necessary to study a large number of PMCs in order to clarify the range of action of these particular genes. It is relatively easy to obtain voluminous data on microsporogenesis because the number of PMCs per bud is very high. Corresponding investigations on megasporogenesis would be extremely difficult because of the very low number of embryosac mother cells in most of all plant species. However, it is possible to ascertain the proportion of functionable egg cells indirectly by studying the seed formation of such mutants after crossing them with the cytologically normal initial line. The existing findings show that the action of *ds*-genes is principally similar in both micro as well as megasporogenesis. Furthermore, it is not possible to study the action of these genes directly in those stages (pachytene and early diplotene) in which they actually act, because no methods is yet available by which chiasmata formation can directly be observed. Therefore, we are obliged to use the univalent frequency in metaphase I as a parameter to reconstruct the pachytene situation.

The meiotic behaviour of four different anthers of the same bud of mutant 82A is graphically represented in Fig. 1 a. The normal situation of 7 bivalents was never found in our material considering a total of 1824 analysable PMCs. Very rarely, 6 or 5 bivalents were noted indicating a low degree of dysynapsis resulting in a low univalent frequency. Mostly, the intensity of this gene action becomes discernible to a high extent. The configuration of 1 II+12 I for instance was observed in 28–36% of all PMCs studied. If we compare the four curves of Fig. 1a, there appears to be a

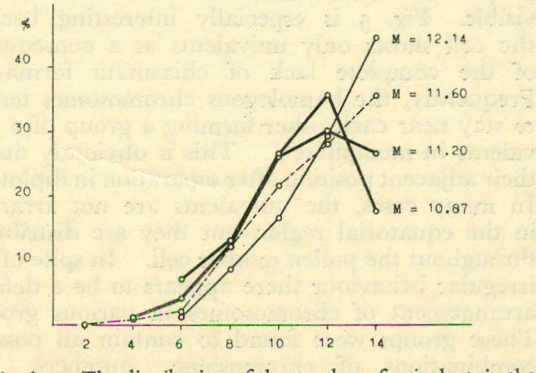

Fig. 1 a.—The distribution of the number of univalents in the pollen mother cells of four anthers of the desynaptic mutant 82A of *Pisum sativum*.

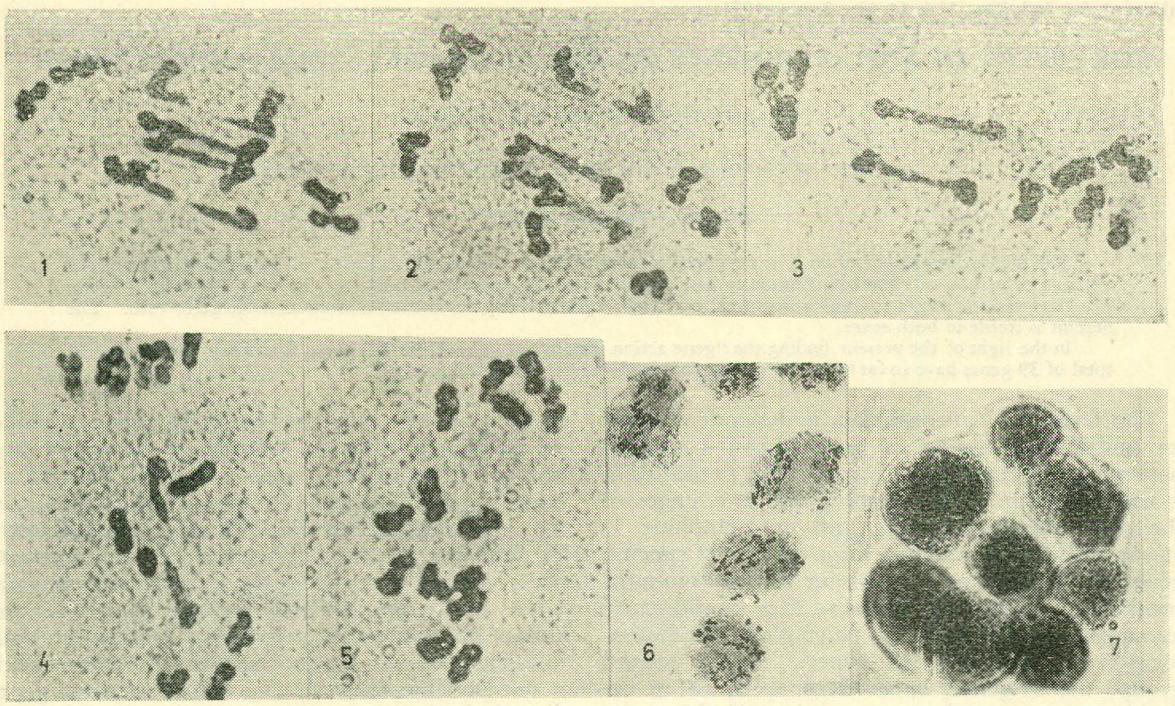

Fig. 1.—4 bivalents +6 univalents in early anaphase I of a PMC of univalent 82A. Fig. 2.—3 bivalents +8 univalents. Fig. 3.—2 bivalents +10 univalents. Fig. 4.—1 bivalents +12 univalents. Fig. 5.—14 univalents in metaphase I. Fig. 6.—Adjacent PMCs showing different anomalies in late stages of the second meiotic division. Fig. 7.—A pollen mother cell of mutant 82A containing 10 microspores of different sizes and chromosome numbers.

general agreement of the frequency of univalents besides the cells having 14 univalents. In spite of these minor differences the mean values of this character vary only slightly from 10.9 to 12.1. This means that reliable results on the action of this particular gene can be obtained by the analysis of a comparatively small number of PMCs derived from only a few anthers.

The range of the action of gene *ds*82A is evident from Figs. 1–5 showing the combinations of: 4 II+6 I; 3 II+8 I; 2 II+10 I; 1 II+12 I; 14 1. All these stages represent early anaphase I and the proportion of univalents and bivalents is clearly visible. Fig. 5 is especially interesting because the cell shows only univalents as a consequence of the complete lack of chiasmata formation. Frequently, the homologous chromosomes tended to stay near each other forming a group of 2 univalents in metaphase I. This is obviously due to their adjacent position after separation in diplotene. In many cases, the univalents are not arranged in the equatorial region but they are distributed throughout the pollen mother cell. In spite of this irregular behaviour there appears to be a definite arrangement of chromosomes in various groups. These groups were found to contain all possible combinations of chromosome numbers. The highest frequency of these ratios was noted to be 6+8 or 7+7 chromosomes in each group. It appears that there is a certain degree of polarity already in M. I.

Because of the presence of bivalents and univalents in metaphase I manifold meiotic irregularities can be expected in the following stages. In only one third of all PMCs studied two daughter nuclei were formed at the end of the first meiotic division. In nearly 40% of the cases, one to four micronuclei occurred besides two normal looking ones. In the remaining cells there was no regularity at all in the chromosomal distribution. The chromosome number in the interphase nuclei varied considerably. The "normal" distribution of 7+7 chromosomes in TI was found to be only in 12% of all analysable cells. The most frequent distribution was 8+6 chromosomes (50.8%); all distributions up to 12+2 were observed in our material. The union of 7 chromosomes, which is the haploid number in *Pisum*, in one or both the daughter nuclei at the end of the first meiotic division, is a matter of chance in these PMCs and is not equivalent to the formation of a balanced genome.

It has already been mentioned, that most of the univalents are not arranged in the equatorial plate. Those ones, which are placed in this region, start dividing already in anaphase I. This suggests that chromosomes as well as chromatids are present in the telophase and interphase unclei

of these cells. Such a behaviour results into an additional irregularity of chromosome numbers at the end of the first meiotic division. Similar irregularities were noted in the second division also. In Fig. 6, adjacent PMCs in late anaphase or telophase II are seen which show varying effects of the acting *ds*-genes.

It is expected that strong disturbances of microspore formation occur due to the additive effects of various irregularities in previous meiotic stages. A broad variation of the number of microspores, ranging from 3–12 per PMC, could be observed in our material (Figs. 7 and 8). The highest frequency was found to be 8 (27.3% considering a total of 1199 PMCs). Their chromosomal situation is unbalanced in number as well as in genomic constitution; therefore, their development into functionable germ cells is not possible.

The "Gene Action System" of the Meiosis.—The mutant described above belongs to the group of desynaptic genotypes. Our collection of *Pisum* mutants contains nearly 40 genes which are responsible for the control of the meiosis and it was possible for us to establish a "gene action system" by the help of this material. The method used consists in the selection and cytogenetical analysis

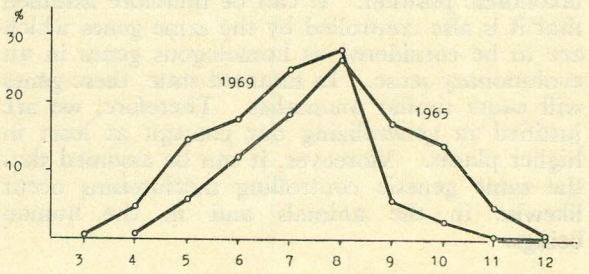

Fig. 8.—The distribution of the number of microspores per PMC in two subsequent generations of mutant 82A.

of a large number of mutants showing genetically conditioned disturbances of meiosis. In this way it will be possible to clarify the phenomenon of the genetic control of meiosis step by step. Fig. 9 elucidates the action of 39 genes belonging to this system and the meiotic stages in which they become effective. The total course of meiosis is divided into 18 subsequent stages, in which three groups of different genes occupy a special position with regard to the control of specific meiotic processes. Firstly, the *as*-genes which become effective in zygotene where they hinder pairing of the homologous chromosomes and cause univalent instead of bivalent formation. Three such genes have been isolated so far during our mutagenic treatments. *as*-genes are likewise known in *Zea*,[1] *Avena*,[2] *Rumex*,[3] *Hevea*,[4] *Sorghum*[5] among others. The second group—the *ds*-genes—can only become effective if the homologous chromosomes pair normally, i.e. if all the *as*-genes of the genome are present in the dominant condition. When recessive, *ds*-genes cause desynapsis as described in detail in the present paper. These genes are responsible for the chiasmata formation and their frequency. It is very surprising that a high number of such genes is present in the *Pisum* genom. So far, 19 desynaptic mutants have been selected in our experiments which differ in the intensity of desynapsis, i.e. in the frequency of univalents. Most of these mutants are completely sterile excepting a few which are weakly fertile. These fertile mutants were crossed with one another in order to study their genetic relationships. The F_1-hybrids were cytogenetically normal and completely fertile and they displayed dihybrid ratios in the F_2-generation. This is also true for some genotypes which show a more or less similar meiotic behaviour. It can be concluded from these findings that the genes in question are

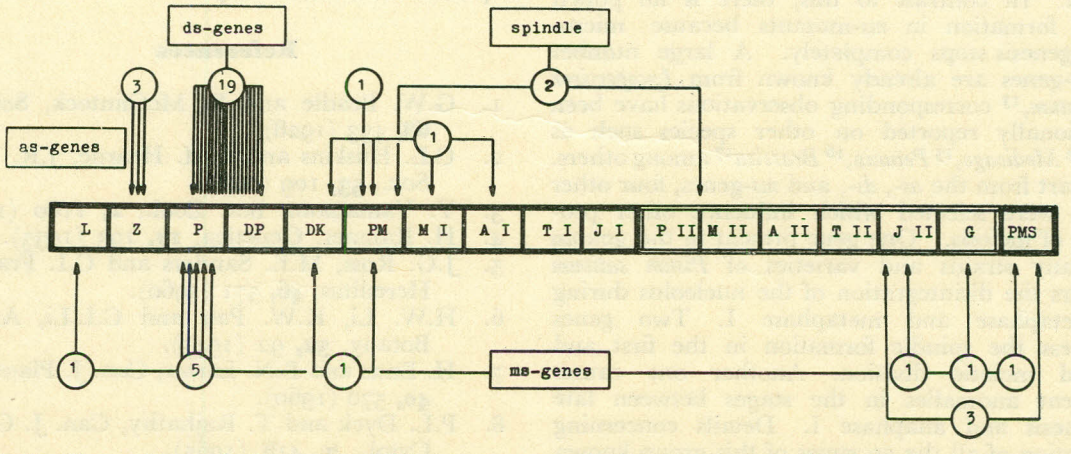

Fig. 9.—The position of the action of 39 mutant genes of the *Pisum* genom belonging to the gene action system of the meiosis. L = leptotene; Z = zygotene; P = pachytene; DP = diplotene; DK = diakinesis; PM = prometaphase; MI, II= metaphase I, II; AI, II = anaphase I, II; TI, II = telophase I, II; JI, II = interphase I, II; P II = prophase II; G = microspore formation; PMS = postmeiotic stages.

neither identical nor multiple alleles; on the contrary, they belong to a polymeric, nonlinked system. As this question has not yet been examined in the sterile mutants of this group it cannot be reliably stated whether multiple alleism besides polymery plays any role in the control of chiasmata formation. Desynaptic mutants are sparsely known also in other species such as *Triticum*[6] *barley*[7] *Avena*,[8] *ryegrass*,[9] *Oryza*,[10] *Lycopersicon*,[11] and *Brassica*.[12] Asynaptic and highly desynaptic mutants show principally similar behaviour in later meiotic stages although they are due to completely different primary actions of the *as*- and *ds*-genes.

The third group with a controlling function in meiosis represents the *ms*-groups. In recessive condition, *ms*-genes cause a complete breakdown of microsporogenesis in a specific meiotic stage. In contrast to the *as*- and *ds*-genes, the *ms*-genes influence exclusively the microsporogenesis whereas the megasporogenesis remains uneffected. Therefore, mutants homozygous for *ms*-genes are male-sterile. So far, 13 *ms*-mutants of the *Pisum* genome have been selected which show a very uniform meiotic behaviour. The microsporogenesis runs completely undisturbed until a specific stage is reached. In this stage a chromosome degeneration occurs followed by a complete disintegration of the protoplast leaving only the membrans of the PMCs intact. The different genes of this group differentiate only with regard to the timing of their activity. It can be seen from the figure. that the *ms*-genes of *Pisum*, yet known, act either during different stages of the first meiotic prophase or during the end of microsporogenesis or in postmeiotic stages. The asynaptic and desynaptic mutants continue their meiosis in spite of strong irregularities. Pollen grains are formed although they are not functionable because of their unbalanced genomic constitution. In contrast to this, there is no pollen grain formation in *ms*-mutants because microsporogenesis stops completely. A large number of *ms*-genes are already known from *Lycopersicon esculentum*,[13] corresponding observations have been occasionally reported on other species such as *Zea*,[14] *Medicago*,[15] *Petunia*,[16] *Brassica*[17] among others.

Apart from the *as*-, *ds*-, and *ms*-genes, four other genes were selected which influence other processes of meiosis. One gene present in the genom of many strains and varieties of *Pisum sativum* hinders the disintegration of the nucleolus during prometaphase and metaphase I. Two genes suppress the spindle formation in the first and second meiotic division. Another one causes different anomalies in the stages between late diakinesis and anaphase I. Details concerning the action of all the 39 genes of this group known so far can be found in previous publications.[18]

There are still some gaps present in this "gene action system" especially in the range between telophase I and telophase II. The work on a large number of other mutants of our collection showing low fertility or sterility, is under way, and it is expected that some of these gaps will be filled in near future.

Conclusions

Our collection of 800 *Pisum* mutants consists of 720 to 750 different genes about 50 of which have some or the other controlling action during meiosis. As only a relatively small proportion of the whole genome is known so far, it is certain in the light of our results that hundreds of different genes are responsible for the control of meiosis. For an undisturbed meiosis it is necessary that all these genes are present in the dominant state. The transition of one single dominant gene of this giant group to the recessive condition results in a specific meiotic disturbance. Other groups of genes become effective during the pre-and post-meiotic stages and must be included in the "gene action system" controlling germ cell formation.

The meiosis takes place in all the species which are capable of sexual reproduction principally in the same way without consideration of their taxonomic position. It can be therefore assumed that it is also controlled by the same genes which are to be considered as homologous genes in an evolutionary sense. In mutated state, these genes will cause similar anomalies. Therefore, we are justified in generalizing our concept at least in higher plants. Moreover, it can be assumed that the same genetic controlling mechanisms occur likewise in the animals and in the human beings.

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