Theoretical and Applied Genetics 48, 23-34 (1976)
© by Springer-Verlag 1976

The Influence of Mutated Genes on Sporogenesis *

A Survey on the Genetic Control of Meiosis in *Pisum sativum*

W. Gottschalk and H.D. Klein

Institute of Genetics, University of Bonn (BRD)

Summary. The course of meiosis in higher plants is controlled by a large number of genes, the function of which can be discerned by means of mutants showing any kind of meiotic anomaly. In general, there are three main groups of genes belonging to this system. The *as*-genes control the pairing behaviour of the homologous chromosomes, causing asynapsis in the mutated condition. The *ds*-genes are responsible for chiasma formation and chiasma frequency, causing desynapsis in the mutated condition. *As*- and *ds*-genes influence micro- and macrosporogenesis in a similar way but the *ms*-genes become effective only in microsporogenesis, resulting in a complete breakdown of meiosis at a stage specific for each gene of the group.

In *Pisum sativum*, 58 mutants showing genetically conditioned meiotic anomalies have been cytogenetically analysed: 34 of them belong to the *ds*- and 7 to the *as*-group; one gene causes asynaptic as well as desynaptic effects; 13 genotypes are male sterile due to degeneration of the chromosomes; the remaining 3 genes cause less specific meiotic disturbances. The lethality of a mutant can be overcome by distinct environmental conditions but the mutant is sterile because of manifold meiotic anomalies.

One gene in the *Pisum* genome controls the transition from the vegetative to the reproductive stage of the plants. Other genes influence the differentiation of the growing points in such a way that the sporogenic tissues are not formed. In these mutants, no sporocytes are present which can undergo meiosis.

From the findings available for many species of the plant kingdom, it can be assumed that hundreds of genes controlling meiosis are present in the genome of each higher plant.

Introduction

The germ cell formation in an organism is a complicated procedure depending on a large number of partially synchronized processes. Therefore, a large number of genes may be responsible for the control of this process irrespective of the taxonomic position of the species within the plant or animal kingdom. This problem has been intensively studied in plants, particularly in species, from which voluminous collections of experimentally produced mutants are available.

The functioning of sexuality depends on the precision of the differentiation of those growing points determining flower formation. This process is controlled by many genes, nearly all of which must be present in the dominant condition to produce a fully functional flower containing operative male and female germ cells. Other genes of the genome become effective during the differentiation of anthers and ovules, preventing the development of those tissues which are responsible for germ cell formation. Even the transition from the vegetative to the reproductive status of the plant is genetically controlled.

During the past 10 years, in a great number of different plant species, an increasing number of mutated genes have been found which directly influence the process of meiosis in a nearly equal way. This is not surprising if we consider that meiosis is one of the few biological processes which operate in an identical way in all higher organisms. It can be assumed therefore, that this process is controlled by homologous genes. Consequently, mutations of these genes can be regarded as homologous ones, in an evolutionary sense, leading to similar irregularities of meiotic behaviour in different species.

It is not the purpose of the present paper to discuss the extraordinarily voluminous - and in many cases contradictory - findings existing in this field in the literature. This will be done in the frame of a review paper. We shall deal only with the meiotic behaviour of a large group of *Pisum* mutants studied for about 10 years at this institute. The garden pea is the spe-

* The investigations were supported by the Ministry of Research and Technology of the Federal Republic of Germany and by the European Atomic Community.

cies for which the most comprehensive findings on genetic control of meiosis are currently available. This material is well suited to estimating the proportion of genes in a given genome belonging to this control system. Cytogenetic details on the single *Pisum* mutants of this group have been published elsewhere (Gottschalk 1968, 1969, 1973, 1975, Gottschalk and Jahn 1964, Gottschalk and Villalobos-Pietrini 1965, Gottschalk and Baquar 1971a,b, 1972, Gottschalk and Konvička 1975, Gottschalk and Milutinović 1973, Gottschalk and Kaul 1974, Konvička and Gottschalk 1971, Klein 1968, 1969a,b,c, 1970, 1971, Klein and Baquar 1972, Klein and Milutinović 1971a,b,c, Klein and Quednau 1976).

Material and Methods

Fifty-six X-ray- and neutron-induced mutants of the variety 'Dippes gelbe Viktoria' of *Pisum sativum* (2n=14) were cytogenetically analysed. The microsporogenesis was studied in at least two generations. Nearly all the mutants of this group are sterile. So they can only be maintained by propagating plants heterozygous for the respective genes. In this way, the segregation of the genes has also been studied. All the genes discussed in the present paper behave as simple Mendelian recessives. In many cases, a more or less pronounced deficit of mutants in the segregating families was observed. This, however, is not a distinctive feature of this group of genes, being valid for many mutated genes of the *Pisum* genome. The action of the genes studied, in nearly all cases, is restricted either to micro- and macrosporogenesis or exclusively to microsporogenesis. Growing behaviour and vitality of the mutants are normal.

The sterile mutants of the segregating families, as well as the weakly fertile mutants of homozygous strains, were decapitated immediately after the start of flowering. The anthers of young buds were fixed in Carnoy and the chromosomes were stained in aceto-carmine or orceine.

The Empirical Findings

The method we use is comparable to the methods used by gene physiologists to clarify the genetic control of a biosynthesis. However, we are using not biochemical, but cytogenetical procedures. The meiosis, in particular the microsporogenesis, is divided into 18 stages and we select mutants showing cytological anomalies at different meiotic stages. These mutants are analysed cytologically and genetically. In this way it is possible to clarify the dependence of the whole process upon the action of specific genes step by step. So far we have isolated nearly 60 different genes of the *Pisum* genome belonging to this control system. We know when they become effective and what functions they have in the framework of the total process.

Essentially, there are three gene groups which show a distinctive action on meiosis: the *as*-genes, the *ds*-genes and the *ms*-genes. The first two groups act during the early stages of the first meiotic division. In the mutated, i.e. in the recessive condition, they cause similar meiotic anomalies, although they induce completely different mechanisms of disturbance. The present paper also includes some mutant genes which influence earlier processes of ontogenetic development, preventing either flowering or the differentiation of tissues relevant for spore formation. The action of the *ms*-genes is restricted to microsporogenesis, macrosporogenesis being undisturbed in the mutants homozygous for them.

a) Genes influencing earlier ontogenetic stages

The evaluation of our comprehensive collection of *Pisum* mutants has shown that already the transition from the vegetative to the reproductive state of the plant is controlled by specific genes of the genome. Mutant 172 is homozygous for such a gene (Gottschalk 1969). The plants are very vigorous and do not differ from the initial line in their gross morphology but the growing points destined for flower formation never differentiate. Instead, they remain active, continuing the formation of somatic organs. In place of the inflorescences, strongly shortened lateral branches are produced in these plants. Thus, they occupy an intermediate position between normally flowering and non-flowering plants. If we consider the flower to represent an extremely shortened shoot, it is evident that certain tendencies in this direction are still discernible in the mutant, but no termination of mitotic activity in these organs was observed over 11 successive generations.

A second group of genes becomes effective during the differentiation of anthers and ovules. A characteristic representative of this group is the neutron-induced mutant 2228. As revealed by paraffin sections, there is no differentiation into specific tissues in their anthers. The total content of the two thecae is filled with a homogeneous, unspecific tissue surrounded by the endothecium; neither pollen sacs nor the tapetum and adjacent layers are present. The function of the archespore tissue can not be taken over by other cell groups, so that no cells are available in these plants

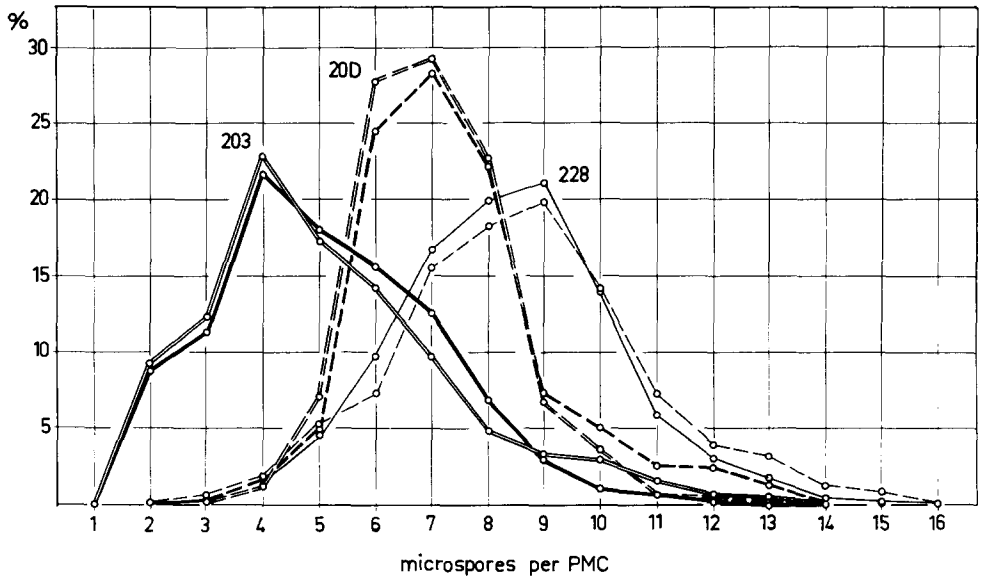

Fig. 1. Frequency distribution of the number of microspores per PMC in the asynaptic mutants 203, 20D and 228 evaluated in two subsequent generations

to undergo microsporogenesis. The recessive gene influences the corresponding tissues of the female sex organs in a similar way: the cells of the nucellus show degenerative alterations and functional embryo sacs are not formed. Thus, the mutant is sterile in both sexes and no germ cells are produced (Klein and Milutinović 1971c). Mutants 178 and 2254A show the same situation in principle, at least with regard to the male sex organs.

b) The *as*-genes

One of the fundamental meiotic processes - the pairing of the homologous chromosomes - is controlled by a whole group of different genes of each genome. They are designated *as*-genes, because they cause a s y n a p s i s, the lack of pairing when present in the homozygous recessive condition. Thus, one of the most essential prerequisites for regular meiosis is missing. They are known in many plant species. In our radiation genetic experiments in *Pisum*, eight *as*-genes of the genome have been isolated so far. They do not act in an additive manner, each of the eight genes alone causing lack of pairing. In principle, complete and partial asynapsis are possible. In the case of complete lack of pairing, only univalents between pachytene and metaphase I are present in the PMCs. This holds for mutants 228 and 211 of our collection. The other six mutants of this group (nos. 20D, 203C, 231, 2241, 2805, 2984) show partial asynapsis, i.e. a varying degree of pairing of homologous chromosomes. In the pachytene of these genotypes, pairing in some segments of the chromosomes is observed, while other segments remain unpaired. Unfortunately, the pachytene chromosomes of *Pisum sativum* can not be identified in spite of the low chromosome number of the species (n = 7). Therefore, it is not possible to determine the unpaired regions and to state reliably whether the phenomenon of asynapsis refers in all cases to the same segments of the same chromosomes. In these mutants, normal pairing in all chromosomes of the genome has never been observed, although a great number of PMCs have been analysed and found undergoing this stage of microsporogenesis. The frequency of univalents in metaphase I can be used as a parameter for evaluating the degree of asynapsis of these genotypes. The higher the number of univalents per PMC, the lower the degree of pairing and the higher the degree of asynapsis (Fig. 1). Because of the high proportion of univalents, manifold irregularities arise in the subsequent meiotic stages, resulting in a highly varying number of microspores per PMC. The variation ranges between 1 and 16 (Fig. 2). Thus, balanced germ cells containing a full genome are not produced in these mutants, so they are completely sterile. In principle, the *as*-genes act in a similar way on micro- and macrosporogenesis, resulting in sterility in both sexes.

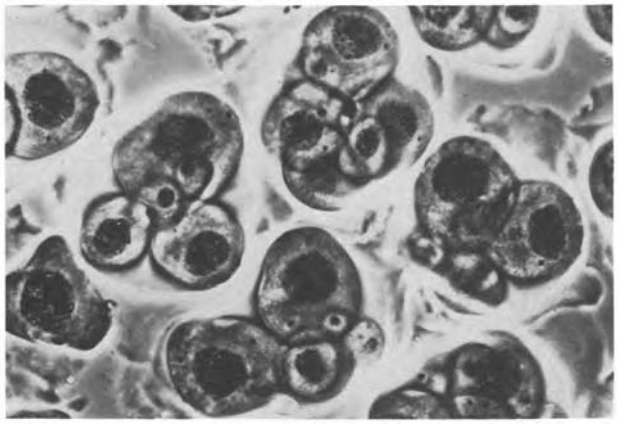

Fig.2. Microspore formation in PMCs of the asynaptic mutant 228. The number of microspores per PMC is increased and varying from cell to cell. The size and the chromosome number of different microspores of the same PMC are likewise varying. These anomalies are due to the high frequency of univalents in the early and middle stages of the first meiotic division

One of our *Pisum* mutants - no. 2984 - can not be classified definitely as to the mode of its meiotic disturbances. There is pairing of the homologous chromosomes, but not in all chromosome segments. In the paired segments, chiasmata can be formed but their frequency is very low (Gottschalk and Konvička 1975). Considering the length of the paired segments, a higher chiasmata frequency would be expected. Thus, asynaptic as well as desynaptic tendencies are discernible in this genotype. It occupies an intermediate position between desynaptic and asynaptic mutants. The gene responsible for these anomalies seems to belong to the *as*-group, so it is designated as^{2984}.

c) The *ds*-genes

The second group - the *ds*-genes - act temporarily a little later in the course of meiosis. They can only become effective if the homologous chromosomes pair normally. This happens if all the *as*-genes of the genome are present in the non-mutated condition. Recessive *ds*-genes cause the phenomenon of d e s y n a p s i s, i.e. the reduction of chiasma frequency or complete lack of chiasma formation following normal pairing of the homologous chromosomes.

So far, we have isolated 34 desynaptic mutants in our collection and cytological data for at least two generations are available. About 10 other genotypes are under study, most of which also belong to this group. Thus, about 40 desynaptic *Pisum* mutants are known at

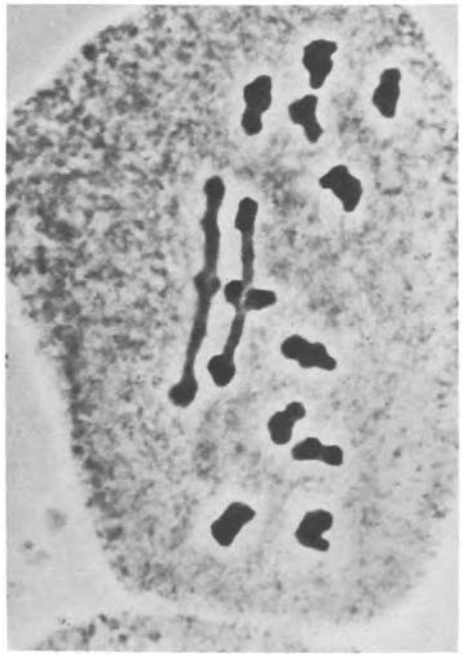

Fig.3. Metaphase I in a PMC of the desynaptic mutant 2989 having 2 bivalents and 10 univalents

present. It can be concluded that the chiasma behaviour of a higher plant is controlled by a strikingly high number of different genes of the genome, in *Pisum* certainly by more than 100 genes. Each single gene must be present in the dominant condition in order to accomplish normal chiasma formation. If a single gene of this group mutates, a reduction of the number of chiasmata or complete lack of chiasma formation results. The different genes of the group generally show a similar action. They are distinguished from one another only in the intensity of desynapsis, i.e. the extent of the reduction in chiasmata frequency.

The common feature of all the desynaptic mutants is that there is a specific situation in each PMC of the same anther with regard to the degree of desynapsis. Unfortunately, it is not possible to analyse chiasma behaviour directly because the middle stages of the first meiotic prophase are not analysable in most plant species, but the effect of the *ds*-genes is apparent - as in the *as*-mutants - in the presence of univalents. The homologous chromosomes can not remain united in the form of bivalents because of the lack of chiasmata. It is easy to evaluate the number of univalents and bivalents in metaphase I (Fig.3). From these data, reliable conclusions can be drawn on the situation existing during the earlier meiotic stages:

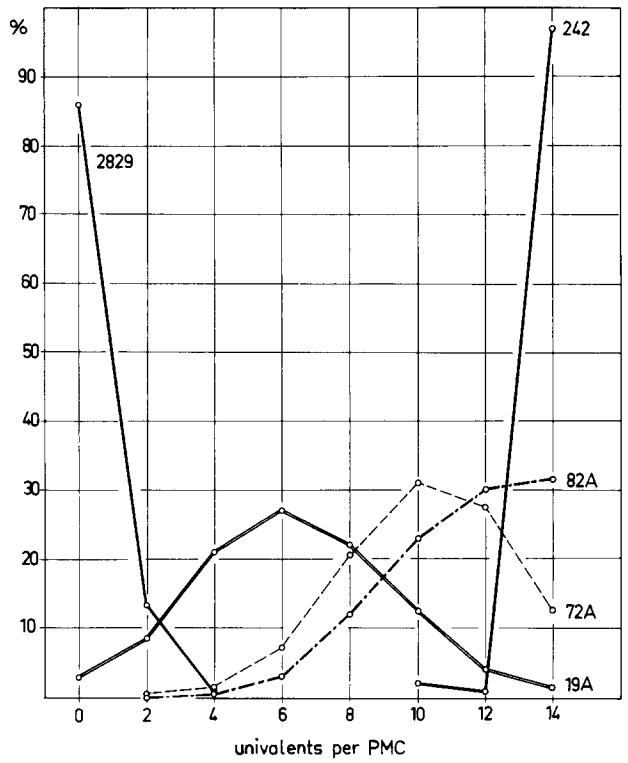

Fig. 4. Comparison of the degree of desynapsis in mutants 2829, 19A, 72A, 82A and 242 of *Pisum sativum* demonstrated by means of the number of univalents per PMC

- the lower the number of chiasmata,
- the higher the number of univalents per PMC;
- the higher the degree of meiotic anomalies,
- the lower the fertility of the mutant.

There is a broad variation in the number of univalents in different PMCs of the anthers of such a mutant. Thus, the action of each *ds*-gene becomes discernible in the form of a characteristic curve for the criterion "number of univalents per PMC". The curves for the univalent frequency of some desynaptic *Pisum* mutants are presented in Fig. 4. The graph shows that the whole range, from almost normal chiasma behaviour to the complete lack of chiasma formation, is already covered by the action of a small number of *ds*-genes controlling this process. It can be expected that there is a relatively high number of weakly desynaptic mutants in our *Pisum* collection which, however, have not yet been detected. We select our asynaptic and desynaptic mutants in segregating M_2- to M_x-families by their sterility or their strongly reduced fertility. Weakly desynaptic mutants have a relatively high proportion of bivalents, so that their fertility is relatively good, in many cases comparable to that of other mutation types and even the initial line. In those cases in which the lack of chiasma formation refers only to one arm of the homologous chromosomes, rod bivalents occur which do not negatively influence the course of meiosis. These mutants can be fully fertile although they belong to the group of desynaptic genotypes. For these reasons, weakly desynaptic mutants will only be found by chance. For instance, this holds for mutant 2829 of our collection. In about 90 % of its PMCs, seven bivalents are found. The remaining cells contain 2 or 4 univalents at most, while PMCs with a higher proportion of univalents are not present (Fig. 4). This is the weakest degree of desynapsis hitherto known in *Pisum sativum*. Some weakly desynaptic mutants are known for *Brassica oleracea* (Gottschalk and Konvička 1971, 1972, Konvička and Gottschalk 1971) and *Drosophila melanogaster* (Sandler *et al.* 1968), among others. An extraordinarily strongly desynaptic genotype of our collection is mutant 242. In 97-100 % of all PMCs analysed, only univalents were observed. All the other desynaptic mutants studied occupy an intermediate position between these two extremes.

The genetic control of the process as a whole becomes clear if we consider all the data available from the 34 desynaptic *Pisum* mutants. Two or three of them may be identical, but all the others are different genotypes as concluded from their differing meiotic behaviour. Because of the voluminous amount of data available, it is not appropriate to use the curves for the trait "number of univalents per PMC" to characterize the differences in degree of desynapsis for the whole group. Therefore, we have used the mean values for this character. They are graphically presented in Fig. 5. For many genotypes, the values for several generations are considered in order to judge the reproducibility of the findings. The degree of similarity of the values for the same mutant in successive generations is surprisingly high considering that chiasma frequency is influenced by many environmental factors. This shows that the expression of the genes in question is relatively consistent. Figure 5 shows a series of *ds*-genes covering practically all the values between nearly normal chiasma formation and complete lack of chiasmata which are theoretically conceivable. In this graph, the broad palette of the influence of the *ds*-genes on chiasma behaviour is clearly visible.

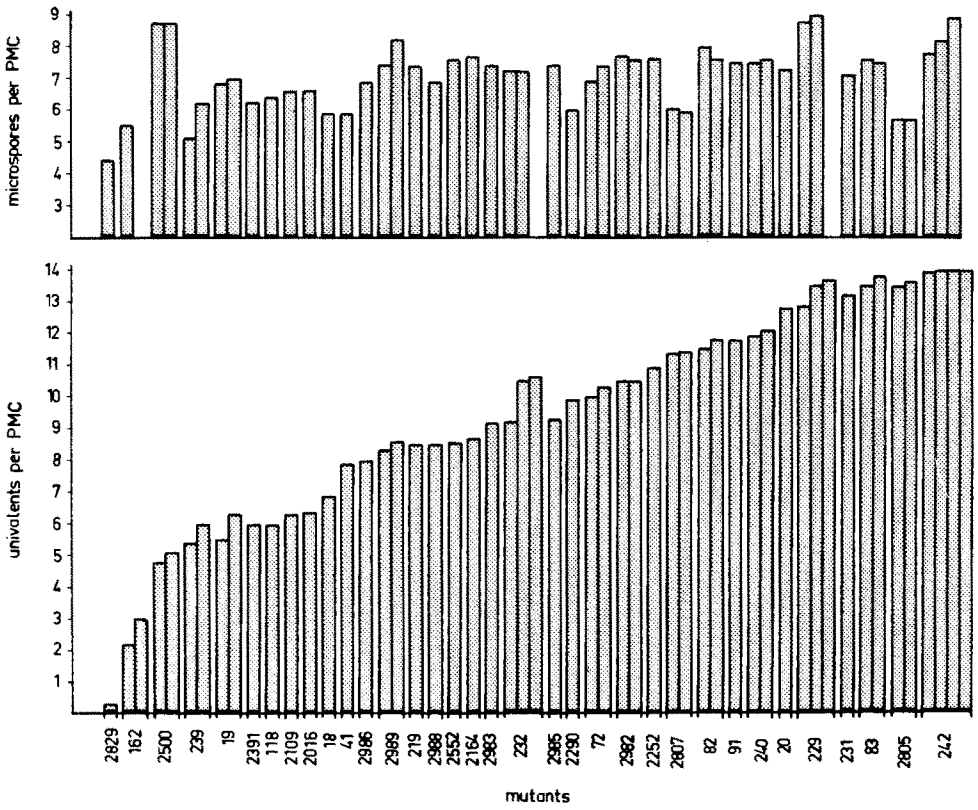

Fig.5. Bottom: The mean values for the character "number of univalents per PMC" of 34 desynaptic *Pisum* mutants. Upper part: Means for the character "number of microspores per PMC" of the same genotypes. Each column represents the mean value for one generation

Although the mechanisms resulting in desynapsis and asynapsis are supposed to be completely different from each other, their consequences for the course of meiosis are generally similar. This is due to the presence of univalents which are the common feature of both groups. Only the chromosomes of the bivalents are regularly distributed at anaphase I. The univalents are either randomly incorporated into the daughter nuclei of the first meiotic telophase, or they form micronuclei. This depends on the degree of desynapsis of the mutant. In a weakly desynaptic mutant, a certain polarity is apparent in the PMCs at the end of the first meiotic division. Most of the chromosomes are united in two daughter nuclei, while the others remain outside them. This polarity, however, can not be expected in the strongly desynaptic or in the asynaptic mutants. In these cases, a certain degree of polarity is also produced but the causes are not yet understood (Klein and Quednau 1976). An example of this situation is given in Fig.6. As a consequence of the anomalies just mentioned, the later meiotic stages of these mutants are strongly disturbed (Fig.7).

One should expect that an increasing extent of meiotic anomaly will occur with an increasing degree of

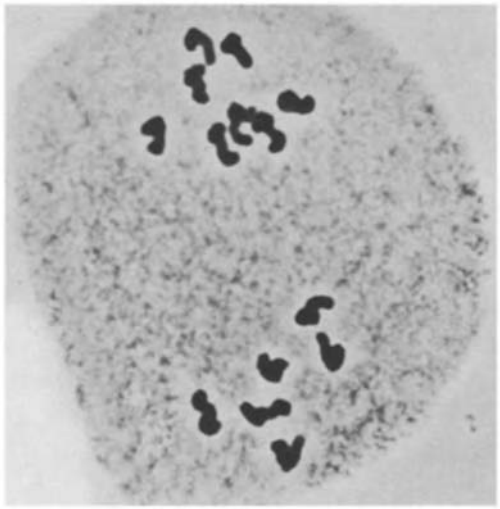

Fig.6. Metaphase I of a PMC of the highly desynaptic mutant 242. The photomicrograph reminds of a telophase, but it shows the situation in MI. All the chromosomes (2n = 14) are present in the form of univalents but a polarity within the cell is evident: there are two groups of 6 and 8 chromosomes

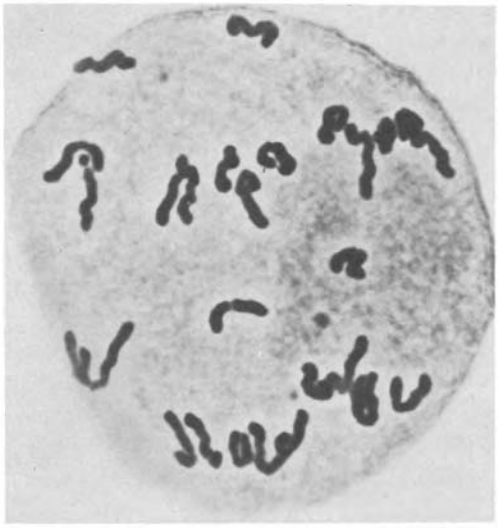

Fig.7. Late anaphase II of the desynaptic mutant 232. Chromatids are distributed in 3 spindles; moreover, 4 laggards are present. An increased number of microspores will be formed in this PMC

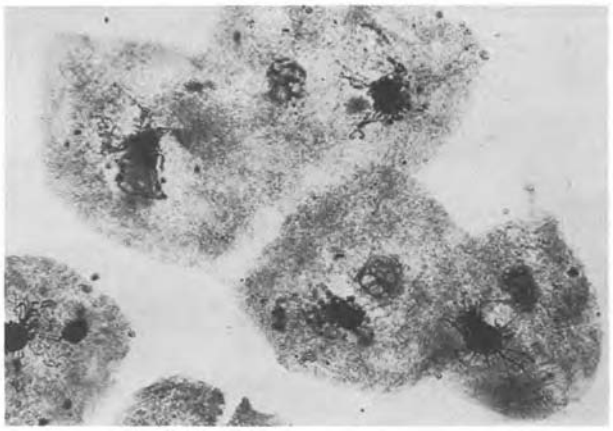

Fig.8. Pollen mother cells of the male-sterile mutant 38B in pachytene showing the beginning of the degeneration of the chromosomes. The nucleoli are highly vacuolated

desynapsis, but this is not the case in the present material. If we compare the number of univalents and the number of microspores per PMC of the same desynaptic mutant, there is no strong correlation (Fig.5). Since even a relatively low degree of desynapsis causes strong meiotic disturbances, there are practically no marked differences with regard to the number of microspores per PMC between weakly and strongly desynaptic mutants. However, there are two exceptional cases which should be discussed in more detail. The relatively weakly desynaptic mutant 2500 (mean number of univalents per PMC about 5) shows an extraordinarily high number of microspores per PMC. This would not be expected if only the frequency of univalents were responsible for the degree of meiotic anomaly. This genotype shows additional meiotic anomalies, particularly extensive chromosome or chromatid breakages resulting in a high number of fragments (Klein 1971). The reverse situation is exhibited in mutant 2805. It forms almost exclusively univalents in its PMCs, so a very high number of microspores should be expected. This is, however, not the case when the results of two successive generations are considered. The mean values for the number of microspores per PMC are equivalent to those in some weakly desynaptic mutants. The reason for this situation is a pronounced stickiness of chromosomes or chromosome groups found during metaphase I. In many cells, nearly all the chromosomes are clumped together in the region of the equatorial plate and a restitution nucleus is formed beside some micronuclei. In the second meiotic division, this effect does not appear to the same extent, so that a relatively low number of microspores is formed in many PMCs. Mutant 2807 also has a relatively low number of microspores, in spite of a high univalent frequency. In this case, the causes of the negative correlation are not yet clear. A similar case is known for a desynaptic mutant of *Brassica oleracea* having a regulating mechanism, which partly reduced the anomalies in later meiotic stages (Gottschalk and Konvička 1971).

d) The *ms*-genes

In general, *as*- and *ds*-genes act in a similar way on micro- as well as on macrosporogenesis. In both cases, meiosis proceeds up to the final stages and microspores are formed but, due to their unbalanced genomic constitution, no functional germ cells are produced in any of the asynaptic or in most of the desynaptic mutants.

The third group we have to discuss is the *ms*-group. Each gene of this group causes complete breakdown of microsporogenesis but macrosporogenesis remains uninfluenced by these genes. Thus, a genetically conditioned male sterility occurs. Microsporogenesis begins completely normally in these genotypes. At a specific meiotic stage characteristic for each *ms*-gene, degeneration of the chromosomes, the nuclei and finally of the whole PMCs occurs (Fig.8,9). In contrast to the action of the *as*- and *ds*-genes, there

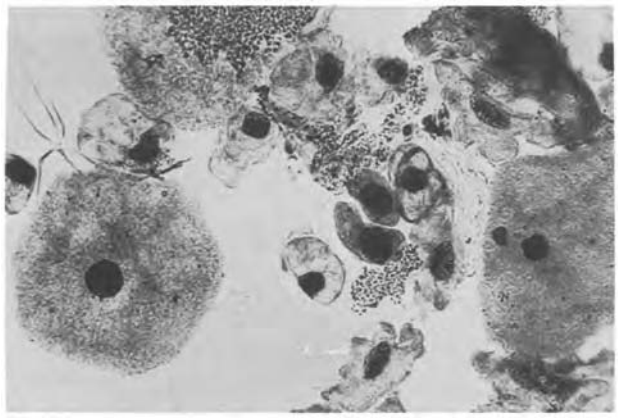

Fig.9. The same PMCs as illustrated in Fig.8 in a somewhat later stage of microsporogenesis. The nuclei are present in the form of structureless spherical chromatin bodies as a result of degenerative alterations

is no continuation of microsporogenesis and male germ cells are not produced at all. The various genes of the *ms*-group differ from one another in the meiotic stage at which they become effective.

Despite the uniform meiotic behaviour of the male sterile mutants known in higher plants, genetically conditioned male sterility is not a uniform phenomenon. So far, at least four different groups of male sterile genotypes are known, caused by:

- the action of single recessive genes;
- the action of single dominant genes;
- the joint action of several recessive genes;
- the interaction of *ms*-genes with a specific type of cytoplasm. The *ms*-mutants hitherto studied in *Pisum sativum* belong to the first group.

It is obvious that most of the *ms*-genes become effective during the final meiotic stages between interphase II and pollen formation. A second, smaller group acts during the early and middle stages of the first meiotic prophase, preventing further meiotic development and pollen formation. Only a very small number of *ms*-genes becomes effective between diakinesis and metaphase II (Fig.11). This regularity is not only observed in *Pisum*, but also seems to be valid for all higher plants. The causes of this unusual distribution are not yet known. A review paper on this problem containing many details from the literature has recently been published (Gottschalk and Kaul 1974).

Three examples representative of this group may demonstrate the action of *ms*-genes. Mutant 38B of our collection shows normal meiotic behaviour up to early pachytene. Pairing of the homologous chromosomes takes place but, simultaneously, a continuously increasing agglutination of all the bivalents occurs resulting in a structureless, amorphous chromatin mass (Fig.8,9). A few hours later, no chromatin is observed in the PMCs. After another few hours, even the cytoplasm has disappeared. Obviously, the whole living cell content is reabsorbed by the adjacent tissues of the anther. Thus, early pachytene is the final stage of microsporogenesis in this male sterile mutant.

Mutant 33C is characterized not only by the degeneration of the archespore tissue but also by some additional meiotic anomalies demonstrating a certain degree of pleiotropic action of the mutated gene. Seven bivalents are formed, but the chromosomes are unusually strongly spiralized even in the later stages of the first meiotic prophase. Therefore, a high degree of optical definition is apparent in diplotene and diakinesis, in striking contrast to the corresponding stages of the initial line. The nucleolus is abnormally big and highly vacuolated. Moreover, it does not completely disintegrate in prometaphase I. Droplets of nucleolar substance are distributed over the cytoplasm during all the later stages of microsporogenesis. In anaphase I and II, no spindles are differentiated. Microspores are formed and the postmeiotic stages are initiated. The main action of gene ms^{33C} becomes discernible prior to pollen mitosis. At this stage, degeneration of the cells begins, resulting in vacuolation and deformation of the old microspores.

Gene ms^{71A} causes the earliest meiotic anomalies during anaphase II, consisting of irregularities in chromatid distribution and resulting in an increased number of nuclei per PMC at the end of the second meiotic division. Microspore formation begins, but is not completed. The cell walls characteristic for the pollen grains (intine and exine) are not formed around the microspores but around the PMCs, so that each PMC of this mutant becomes a giant pollen grain containing the tetraploid instead of the haploid chromosome number (Fig.10). A third division, which follows immediately after completion of meiosis, is initiated in a part of the PMCs of this genotype. It represents a mitosis and only runs up to metaphase. At this stage, the degenerative action of gene ms^{71A} becomes effective. The initiation of a third meiotic division is not

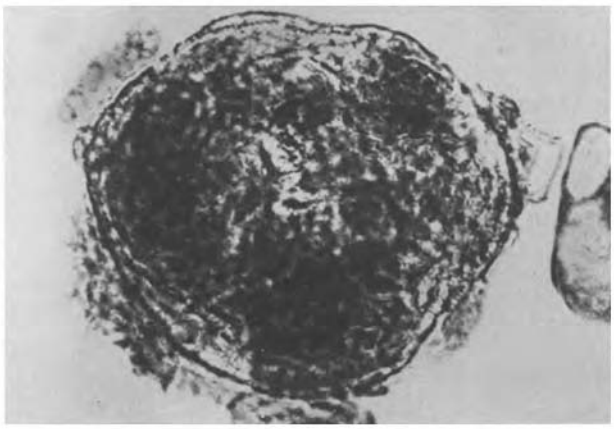

Fig. 10. Abnormal pollen formation in the male-sterile *Pisum* mutant 71A. Intine and exine are not formed around the microspores inside the PMC, but around the PMC as a whole. Thus, the PMC becomes a giant tetraploid pollen grain

limited to mutant 71A. It is also observed under the influence of genes as^{211} and as^{228}.

e) Other *Pisum* genes influencing meiosis

As well as the *as*-, *ds*- and *ms*-genes, a small number of other genes in *Pisum* is known to influence distinct processes of meiosis, but can not be included in one of the three groups mentioned. One of our mutants shows relatively unspecific anomalies between diakinesis and anaphase I. In another mutant, certain restrictions on the functioning of the spindles in both the meiotic divisions become discernible, which are obviously genetically controlled. Another recessive gene, which seems to be widespread in *Pisum sativum*, hinders the disintegration of the nucleolus at the end of the first meiotic prophase. Similar, but weaker, effects are shown as part of the action of gene ms^{33C}. As the anomalies just described are consistently observed in all the plants belonging to the respective genotypes, they are certainly genetically conditioned.

A very interesting genotype is the X-ray induced mutant 227A of our collection. It is primarily a chlorophyll-deficient lethal mutant of the variegate type, dying before flowering. Its lethality, however, can be overcome by cultivating the plants under specific environmental conditions, i.e. by avoiding low temperatures and high light intensities. Under these conditions the plants form a normal amount of chlorophyll. They remain somewhat smaller than the initial line, but are able to undergo normal ontogenetic development and flower abundantly. They are, however, completely sterile. This sterility is due to a whole range of different meiotic anomalies, such as

- formation of univalents beside the bivalents,
- chromosome maldistribution in anaphase I,
- fragmentations, laggards, bridges, cytomixis.

As a consequence of these disturbances, the number of microspores per PMC is abnormally high, ranging between 3 and 12. Bi- and multinucleate microspores, such as dyads and monads, are observed in the anthers because of spindle inactivations. In addition, hypo- and hyperdiploid PMCs are produced through maldistributions and non-disjunctions during the later premeiotic mitoses. The frequency and extent of these anomalies vary considerably from anther to anther. Finally, all the microspores formed degenerate and no pollen grains are produced (Gottschalk 1969).

This gene action is of interest because it comprises effects characteristic for *as*- or *ds*- as well as for *ms*-genes. Unfortunately, whether the gene also acts in the way described on macrosporogenesis was not studied. It was not possible to separate the chlorophyll deficiency from the lethality and the sterility. Therefore, the whole complex showing monohybrid segregation may be due to the action of a single pleiotropic gene. This genotype is a particularly interesting example of the negative selection value of a mutant. Under normal conditions, the pleiotropic gene of the simultaneously mutated group of closely linkes genes may cause lethality. Under specific environmental conditions, the lethality does not become effective but the plants are sterile. Thus, the mutant contains two negative features which hinder its propagation in any case.

Discussion

The total number of mutants in higher plants now known to have genetically conditioned meiotic anomalies is certainly higher than a thousand. In some species, whole groups of genes are known belonging to the control system of meiosis. This is true for 24 *ms*-genes of the genome of *Lycopersicon esculentum* (Rick 1944, 1945, 1948, 1953, 1966, Rick and Butler 1956, Rick and Khush 1965, Rick and Boynton 1967) and for 44 *ms*-genes of the genome of *Zea mays*, 15 of which have been studied in detail (Singleton and Jones 1930, Beadle

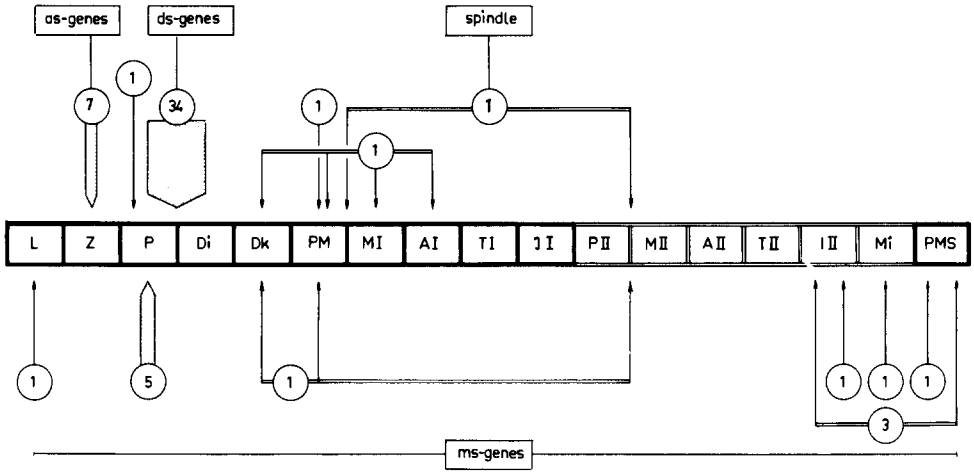

Fig.11. The gene action system of the meiosis of *Pisum sativum* including 58 genes of the genome. Middle: The subsequent stages of the course of meiosis (leptotene, zygotene,... microspore formation). Solid lined: first meiotic division. Double lined: second division. PMS: postmeiotic stages. The arrows show the time of action of the respective gene or genes. The number of genes belonging to the different groups of the system are given in the circles

1932, Emerson 1932, Weijer 1952, Madjolelo *et al.* 1966). Most findings, however, have been obtained in single mutants of a great number of different species and genera. Numerous findings concerning male sterile mutants are restricted to the observation that a genetically conditioned male sterility occurs in these genotypes, demonstrated by the presence of nonfunctioning pollen grains and functioning female sex organs. However, no detailed meiotic investigations have been made on this material. Only the action of about 100 *ms*-genes on specific stages of microsporogenesis in 48 species of 12 different families has been described (Gottschalk and Kaul 1974). There is much confusion in the literature especially about the delimitation of asynapsis and desynapsis because these two phenomena can only be distinguished from each other by analysing the zygotene and pachytene. This is only possible in a few species, while clear cytological findings in most species are obtained in the later stages of the first meiotic prophase, and in metaphase I at the earliest. In most cases, only the proportion of univalents and bivalents in metaphase I has been evaluated. From these findings, conclusions have been drawn concerning the pairing behaviour of the homologous chromosomes in earlier meiotic stages. However, it is not possible to classify mutants of these two groups reliably by this kind of evaluation. A clear decision can only be made in pachytene. If complete pairing occurs, the univalents in metaphase I are caused by desynapsis. If there is no pairing, the univalents are attributed to asynapsis. This decision is often possible even in those species where a pachytene analysis can not be made. This has often not been considered and many mutants designated as asynaptic in the literature will certainly belong to the group of desynaptic mutants. In the case of partial asynapsis, it is almost impossible to exclude an additional desynaptic effect. It we consider the high number of *As-* and *Ds*-genes present in the genomes of higher plants, it is possible that mutants arise in mutation experiments which are homozygous for closely linked *as-* and *ds*-genes. The cytogenetic clarification of such a situation is extraordinarily difficult. Such a case is possibly *Pisum* mutant 2984, already described in the empirical section.

If we want to estimate the proportion of those genes of a given genome which influence meiosis in any way, we have to look for a species in which a great number of genes of the *as-*, *ds-*, *ms-*, and possibly of some other groups, is known. This is the case in *Pisum sativum*. A total of 56 genes of our collection have been found to have distinct control functions during meiosis. Moreover, a clearly asynaptic *Pisum* mutant has been studied by Koller (1938). The "meiotic" genes of our collection are presented in Fig.11, showing their time of action and the meiotic processes which they control. About 30 sterile or weakly fertile mutants more are under study. At least some of the genes will belong to the

gene action system of meiosis. The total number of mutants in our *Pisum* collection is about 800, 200 of which are lethal and can not be tested for meiotic behaviour. Of the remaining 600 mutants, only a relatively small number have been cytologically investigated so far. Since about 60 mutants of our collection show specific meiotic anomalies, it can be assumed that numerous genes of the genome will be responsible for the control of this process. It is, however, practically impossible to calculate the number of genes belonging to this system reliably even if we consider the findings already available. Because of the meiotic differences which are consistently observed in different mutants of the *as*- or *ds*-group in successive generations, we can assume that the respective mutants are not identical. For practical reasons, however, it is extraordinarily time-consuming to prove whether some of them are due to the action of multiple alleles. Their sterility, means that this test could only be performed by crossing plants heterozygous for the respective *as*- or *ds*- genes with each other. These plants can not be produced systematically because of the sterility of the mutants. They could only be taken from segregating families of the mutation types which ought to be tested, but they can not be discerned because of the recessiveness of the genes. However, some of our weakly desynaptic mutants, which produce small amounts of seeds, were developed into pure lines. They show closely similar meiotic anomalies. Hybrids between them have completely normal meiotic behaviour and are fully fertile. In F_2, 9:7-segregations were observed in this material. This indicates that the desynaptic effects of the parental mutants are due neither to identical genes nor to multiple alleles. On the contrary, they are caused by different loci located on different chromosomes. A similar situation can be assumed for most of the other genes of the system which have not been tested. This is in accordance with the corresponding results obtained in *Drosophila* (Sandler *et al.* 1968).

Considering not only our results, but also corresponding findings from the literature, it is obvious that chromosome pairing and chiasma behaviour are controlled by highly polygenic systems. The genes involved do not act in the form of additive polymery. On the contrary, the mutation of a single gene of these big gene groups into its recessive allele causes complete sterility in the majority of cases. Because of the great number of genes responsible for the control of meiosis, this genetic system as a whole is very susceptible to mutations.

The course of meiosis is very uniform in all sexually propagating species, so it may be assumed that this fundamental biological process is controlled by homologous genes in all species, irrespective of their taxonomic position. We can expect that the genes isolated in *Pisum* will cause similar meiotic irregularities in other species, if they are changing from the non-mutated into the mutated condition. Consequently, we should be entitled to generalize our findings at least in higher plants. Homologous genes of the groups discussed will probably also be present in the genomes of higher animals and human beings, but their presence has not yet been demonstrated because of the difficulties of cytogenetic analysis in these organisms.

Literature

Beadle, G.W.: Genes in maize for pollen sterility. Genetics 17, 413431 (1932)

Emerson, R.A.: A recessive zygotic lethal resulting in a 2:1 ratio for normal vs male sterile and coloured vs colourless pericarp in F_2 of certain maize hybrids. Science 75, 566 (1932)

Gottschalk, W.: Die Wirkung mutierter Gene auf die Morphologie und Funktion pflanzlicher Organe. Bot. Studien 14, 1-359 (1964)

Gottschalk, W.: Investigations on the genetic control of meiosis. The Nucleus, Seminar Volume, 345-361 (1968)

Gottschalk, W.: A mutant gene in *Pisum* causing lethality and sterility. J. Cytol. Genet. 4, 25-31 (1969)

Gottschalk, W.: The genetic control of meiosis. Genetics 74, Suppl.; 99 (1973)

Gottschalk, W.: The genetic control of meiosis. Proc. XII. Intern. Bot. Congress Leningrad 1, 215 (1975)

Gottschalk, W.; Baquar, S.R.: The effect of mutated genes on the germ cell formation in *Pisum*. Pakistan J. Sci. Ind. Res. 14, 223-227 (1971a)

Gottschalk, W.; Baquar, S.R.: Desynapsis in *Pisum sativum* induced through gene mutation. Canad. J. Genet. Cytol. 13, 138-143 (1971b)

Gottschalk, W.; Baquar, S.R.: Breakdown of meiosis in a mutant of *Pisum sativum*. Cytobiologie 5, 42-50 (1972)

Gottschalk, W.; Jahn, A.: Cytogenetische Untersuchungen an desynaptischen und männlich-sterilen Mutanten von *Pisum*. Z. Vererbungslehre 95, 150-166 (1964)

Gottschalk, W.; Kaul, M.L.H.: The genetic control of microsporogenesis in higher plants. The Nucleus 17, 133-166 (1974)

Gottschalk, W.; Konvicka, O.: Die Meiosis einer partiell sterilen Mutante von *Brassica oleracea* var. *capitata*. Cytologia 36, 269-280 (1971)

Gottschalk, W.; Konvicka, O.: Desynapsis bei *Brassica oleracea*. Biologia 27, 179-186 (1972)

Gottschalk, W.; Konvička, O.: Die Meiosis einer partiell asynaptischen *Pisum*-Mutante. Cytobiologie 10, 458-467 (1975)
Gottschalk, W.; Milutinović, M.: Trisomics from desynaptic mutants. Nucleus 16, 1-10 (1973)
Gottschalk, W.; Villalobos-Pietrini, R.: The influence of mutant genes on chiasma formation in *Pisum sativum*. Cytologia 30, 88-97 (1965)
Klein, H.D.: Die Beeinflussung der Meiosis durch mutierte Gene. Diss. Math. Nat. Fak., Univ. Bonn, 1-97 (1968)
Klein, H.D.: Desynapsis und Chromosomenbrüche in einer Mutante von *Pisum sativum*. Mutation Res. 8, 277-284 (1969a)
Klein, H.D.: Desynaptische Mutanten mit Unterschieden im Univalentenverhalten. Genetica 40, 566-576 (1969b)
Klein, H.D.: Male sterility in *Pisum*. The Nucleus 12, 167-172 (1969c)
Klein, H.D.: Asynapsis and extensive chromosome breakage in *Pisum*. Caryologia 23, 251-257 (1970)
Klein, H.D.: Eine *Pisum*-Mutante mit zahlreichen meiotischen Störungen. Cytologia 36, 15-25 (1971)
Klein, H.D.: Baquar, S.R.: Genetically controlled chromosome breakage and reunions in the meiosis. Chromosoma 37, 223-231 (1972)
Klein, H.D.; Milutinović, M.: Expressivitätsschwankungen mutierter Gene in der Meiosis. Genetica 3, 285-294 (1971a)
Klein, H.D.; Milutinović, M.: Genetische Kontrolle des Konjugationsprozesses der Meiosis in röntgen- und neutronen-induzierten Mutanten. Genetica 3, 1-12 (1971b)
Klein, H.D.; Milutinović, M.: Genbedingte Störungen der Infloreszenz- und Blütenbildung. Theor. Appl. Genetics 41, 255-258 (1971c)
Klein, H.D.; Quednau, H.D.: Die biometrische Bewertung des Univalenten-Verhaltens in konjugationsgestörten Mutanten von *Pisum sativum*. Theor. Appl. Genetics (in press; 1976)
Koller, P.C.: Asynapsis in *Pisum sativum*. J. Genet. 36, 275-306 (1938)
Konvička, O.; Gottschalk, W.: Cytologische Untersuchungen an strahleninduzierten Mutanten von *Brassica oleracea* var. *capitata*. Biol. Plant. 13, 325-332 (1971)
Madjolelo, S.D.P.; Grogan, C.O.; Sarvella, P.A.: Morphological expression of genetic male sterility in maize (*Zea mays* L.). Crop Sci. 6, 379-380 (1966)
Rick, C.M.: A new male-sterile mutant in the tomato. Science 99, 543 (1944)
Rick, C.M.: A survey of cytogenetic causes of unfruitfulness in the tomato. Genetics 30, 347-362 (1945)
Rick, C.M.: Genetics and development of nine male-sterile tomato mutants. Hilgardia 18, 599-633 (1948)
Rick, C.M.: New male-sterile mutants. Rep. Tomato Genet. Coop. 3, 19-20 (1953)
Rick, C.M.: Abortion of male and female gametes in the tomato determined by allelic interaction. Genetics 53, 85-96 (1966)
Rick, C.M.; Boynton, J.E.: A temperature-sensitive, male-sterile mutant of the tomato. Amer. J. Bot. 54, 601-611 (1967)
Rick, C.M.; Butler, L.: Cytogenetics of the tomato. Adv. Genet. 7, 267-382 (1956)
Rick, C.M.; Khush, G.S.: Chromosome engineering in *Lycopersicon*. In: Chromosome Manipulation and Plant Genetics. Suppl. Heredity 20, 8-20 (1965)
Sandler, L.; Lindsley, D.L.; Nicolette, B.; Trippa, G.: Mutants affecting meiosis in natural populations of *Drosophila melanogaster*. Genetics 60, 525-558 (1968)
Singleton, W.R.; Jones, D.F.: Heritable characters in maize. XXXV. Male sterile. J. Hered. 21, 266-268 (1930)
Weijer, J.: A catalogue of genetic maize types together with a maize bibliography. Bibliographia Genetica 14, 189-425 (1952)

Received January 23, 1976
Communicated by H.F. Linskens

Professor Dr. W. Gottschalk
Dr. H.D. Klein
Institut für Genetik
der Universität Bonn
Kirschallee 1
D-5300 Bonn (Germany/BRD)