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THE GENETIC CONTROL OF MICROSPOROGENESIS IN HIGHER PLANTS*

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In general, four different groups of genes control the fertility of higher plants. Three of them are directly correlated with the meiotic behaviour. The *as*-genes cause asynapsis, that is, the failure of homologous chromosomes to pair during the early stages of the first meiotic prophase. The *ds*-genes control the chiasma frequency or prevent chiasma formation at all. Both these gene groups act principally in a similar way, both in micro- and megasporogenesis. The third group, the *ms*-genes, become only effective in microsporogenesis and do not influence the meiotic behaviour of the embryosac mother cells. Thus, they cause male sterility. The fourth group is not related to the meiotic system but induces a misdifferentiation of the sex organs due to abnormalities in the differentiation of the growing points destined for flower formation. The gene action of the first three groups can be well defined and leads to mutants showing a relatively high degree of uniformity in each of the three groups. The fourth group, however, consists of genes having divergent effects. The respective mutants cannot be combined to a single uniform group. The sterility can be due to a number of completely different causes such as the failure of ovule differentiation, formation of open carpels or strong reduction or diminution of the sex organs under the influence of the mutated gene.

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Male sterility in higher plants is likewise not a uniform phenomenon. In general, we can distinguish three different types:

— cytoplasmic male sterility,
— genetic male sterility, and
— interaction of both these types.

In the present paper, a review upon the last two groups of male sterility is given. Since many reviews on cytoplasmic male sterility have appeared in the recent past (Caspari 1958; Edwardson 1956, 1970; Laser and Lersten 1972) it is not considered in the present paper.

The genetically conditioned male sterility can be due to a failure of archespore differentiation. The flowers of these mutants appear completely normal but there is no tissue in which microsporogenesis can take place. Other possibilities for genetically conditioned male sterility are the non-dehiscence of anthers, the failure of stamen development or the transformation of male sex organs into female ones. Some examples for these gene actions are given in the discussion. On the other hand, a relatively large group of genes seems to be present in the genome of all the higher plants that lead to the breakdown of microsporogenesis in the mutant condition. Only the genes of this relatively uniform group is being considered in the present paper. In the first part, the action of those genes is discussed for which a co-operation with the cytoplasmic male sterility is not known. This interaction is discussed in the second part. Furthermore, the species exhibiting male sterility have been arranged accord-

ing to their taxonomic position in the present text.

Unfortunately, not only the genetic background of male sterility but essentially the cytological situation of the genotypes is not studied in detail in many cases. Therefore, the time of action of many genes of this group cannot exactly be given. One of the aims of this review paper is to point out this lack of information. In fact, the phenomenon of genetically conditioned male sterility as a whole would be better understood when these details are known. We were not able to consider the whole literature. We aimed to present a survey of the known facts about this field of cytogenetic research and have tried to arrange the numerous heterogenous findings into a classified and orderly system with a hope that this shall facilitate the future analysis of the problem in a relatively simple way. Furthermore, the present work represents the first paper reviewing systematically the phenomenon of genetically conditioned male sterility in relation to genetic background and gene action.

(1) GENES CONTROLLING MICROSPOROGENESIS

The common feature of all the genotypes belonging to this group lies in their having a typical meiotic behaviour. Microsporogenesis begins completely normally specific. But in a certain stage of meiosis, characteristic for each gene of the group, a rapid degeneration of the living contents of pollen mother cells, microspores or pollen grains occurs and only the empty cells remain. Very often, the anthers of these genotypes are smaller, shrunken and non-dehiscent. Furthermore, they usually possess a different colour than that of the fertile plants of the same families or strains. Hand pollination and natural cross pollination lead to a normal seed set in the male sterile plants indicating thereby that the female sex organs of the flowers are fully functionable. In rare instances, a very small amount of stainable pollen grains is formed while majority of the pollen gets degenerated. Behaviour of these few pollen grains is also not uniform. In some genotypes, a very small seed set is obtained after selfing indicating the functionability of the pollen grains and male gametes. In other cases, however, the stainability of the pollen is not equivalent with their functionability.

Since male sterile plants usually do not differ morphologically from the fertile ones, their identification prior to floral anthesis is not possible in most of the cases. In many species, they, however, are easily discerned during fruit and seed development because of their sterility. As a result, these plants remain green and continue ontogenetic development while the fertile plants of the same families are already in a state of drying. In certain families, the cytological investigation of the male sterile plants is difficult because all the pollen mother cells (PMCs) present have already undergone microsporogenesis when the male sterility of these plants can be discerned morphologically. This is valid for legumes and grasses. In these cases, decapitation of the plants is necessary. This results in the development of lateral branches bearing flowers from which anthers with appropriate meiotic stages can be fixed. In other species such as tomato such inflorescences in the upper regions of the shoot are still formed when male sterility becomes evident in the lower clusters.

Most of the genes belonging to the *ms*-group cause the breakdown of microsporogenesis or of postmeiotic stages when present in the recessive state. However, this cannot be generalized because some instances are known in which dominant genes are responsible for these anomalies. Moreover, the phenomenon of male sterility can be due to the joint action of several *ms*-genes or due to an interaction of *ms*-genes and the cytoplasm. The findings existing are classified according to the four possibilities as given above.

(*a*) The action of single recessive *ms*-genes on microsporogenesis

The phenomenon of male sterility has been

extensively studied in a very small number of species in which a high number of spontaneously arisen or experimentally induced mutations are available. This is valid for tomatoes, peas, maize and barley. In the first two mentioned species, a thorough cytogenetic analysis including meiotic behaviour of the mutants has been made. In most of the other species, only a very small number of male sterile genotypes have been isolated so far.

Brassica oleracea, B. campestris:

In *B. oleracea*, investigations on male sterile lines have been carried out in the varieties *capitata*, *botrytis*, *gemmifera* and *italica*. A case of genetically conditioned male sterility in green sprouting Broccoli (*B. oleracea* var. *italica*) was reported by Cole in 1957. Subsequently, the respective mutant was analysed cytogenetically. The plants are slow in growth, possess reduced vigour and brown, shrivelled, non-dehiscent anthers. The male sterility was found to be controlled by a spontaneously mutated recessive gene. In the homozygous condition, the *ms*-gene induces a breakdown in microsporogenesis immediately after the microspores are formed. The development of the PMCs up to this stage is normal. The separation of the four microspores in the microsporocyte does not take place; they remain fused together and get gradually destroyed (Cole 1959).

The linkage of another gene for male sterility designated as *ms*-1 with a gene controlling anthocyanine synthesis was studied by Sampson (1970). The anthers of this mutant are fleshy, pale green and nearly normal sized. In this material, a quadrivalent is present in all the PMCs besides 7 bivalents as a constant cytological feature. However, there is no relation between quadrivalent formation and male sterility.

A temperature sensitive male sterile mutant in Broccoli was investigated by Dickson (1970). Grown in the greenhouse, the plants form vestigial anthers. At a temperature of 24°C day and 17 C night, complete male sterility is observed. At 17 C day and 12°C night, only a slight degree of abnormal pollen development occurs. Finally, at 10°C, the plants are highly male fertile. The same mutant when subjected to fluctuating temperatures that average from 12° to 14°C produced small amounts of pollen having a fertility of 36-61 per cent. The average seed set ranged from 0-3·5 seeds per fruit with a mean of 1·2. In the fertile plants, the corresponding range was 2-14·5 and the mean 7·1 (Borchers 1971). The gene designated as *ms*-6 behaves as a recessive. Unfortunately, no cytological details are available. According to crossing experiments, the gene is not allelic to the *ms*- genes studied by Johnson (1958), Cole (1959), Nieuwhof (1961) and Borchers (1966).

In Brussels sprouts (*B. oleracea* var. *gemmifera*), a recessive gene becomes likewise effective after microspore formation. So far, no allelism tests of these two genes have been made. Nevertheless, it can be assumed, that the two mutants are not identical because of certain morphological differences. The anthers of this mutant are not shrivelled unlike most of the *ms*-mutants of other species. Moreover, the flowers are smaller and paler in colour. Male sterility is in this case likewise due to the action of a single recessive gene *ms*-2. The dominance of the allel *Ms*-2 is obviously not complete. Plants having the heterozygous constitution *Ms*-2/*ms*-2 possess about 1 per cent male sterile flowers. The expression of dominance is not influenced by temperature (Johnson 1958). Furthermore, plants of Brussels sprouts exhibiting either complete or partial male sterility have been found by Nieuwhof (1961). This type of sterility is controlled by recessive genes whose inheritance pattern seems to be complicated. Further cytogenetic details are not available. The same material was subjected to different temperatures and the expression of male sterility was studied. Out of 11 clones, three originated from plants having short stamens and shrunken non-dehiscent anthers. These were comple-

tely male sterile. In the field, many plants exhibiting partial male sterility were observed showing an irregular development of their androecium. Six clones derived from such plants were utilized for the experiments. At 17°C, the development of the stamens was normal but the amount of pollen grains was very low. At 10°C, however, more pollen was produced in both male sterile and partially male sterile plants. Their pollen fertility ranged from 45-58 and 55-77 per cent, respectively. The amount of pollen grains produced at 14°C was intermediate. Thus, high temperatures supress pollen formation in these genotypes. The female fertility of this material, in general, was good. At 14 and 17°C, seed set after hand pollinations was similar in all the clones but at 10°C, seed set of male sterile plants was a little lower than that of the male fertiles. Thus, the phenomenon of male sterility observed here does not have a direct correlation with the functionability of the female sex.

Gene *ms*-4 was found in an inbred line of purple cauliflower (*B. oleracea* var. *botrytis*) by Borchers (1966). The expression of this gene is stable and is not influenced by environmental factors and several chemical treatments. The anthers dehisc normally but the pollen grains are fused with each other forming a stiff pollen strip rather than loose amorphous pollen powder produced in normal anthers. Many of the pollen grains are small, non-staining and abortive; furthermore, their size is considerably variable. Details on the course of microsporogenesis are not given; therefore, the time and type of degeneration is not yet known. Gene *ms*-4 shows a monofactorial recessive inheritance.

Another male sterile mutant due to the action of a single recessive gene in cauliflower has been studied by Nieuwhof (1961). Plants homozygous for this gene have petals that unfold slowly and partially. Their stamens are small having poorly developed anthers that never dehisc. The course of microsporogenesis is not investigated.

In cabbage (*B. oleracea* var. *capitata*), two sterile plants were found which were not able to produce any seeds. They were maintained by means of cuttings. When pollinated with pollen of fertile cabbage and cauliflower, no seed set was obtained. Investigation of the embryo sacs indicated the presence of cellular growth that possibly leads also to female sterility (Nieuwhof 1961). Thus, neither the genetic situation nor the exact kind and causes of sterility are known in this material.

The findings just mentioned refer to different varieties of the species *B. oleracea*. Moreover, male sterile mutants were observed in two varieties of brown and yellow flowered *B. campestris*. One of them had a functional male sterility. In the other one, the anthers are reduced in size and contain non-viable pollen grains. The meiotic behaviour of this mutant is normal up to the formation of tetrads. Further development is inhibited. The microspores are present in the form of a crumpled mass of thin-walled cells containing very little cytoplasm that stains lightly. Tapetum develops normally until tetrad formation in the PMCs is completed. While the tapetal cells of fertile plant start degenerating and finally disappear, in male sterile plants the tapetum does not degenerate even after the disappearance of the pollen grains. The anomaly is due to a single recessive gene (Chowdhury and Das 1966, 1968).

Viola orphanidis:

In *V. orphanidis*, plants having three different chromosome numbers are known (2n=20, 21, 22). No morphological variation is found to be associated with the variability in chromosome number. A Swiss collection of the species with 20 chromosomes was male sterile (Clausen 1930). Their PMCs were unable to complete the first meiotic division. From the degenerating PMCs, branched fibrillous attachments project out forming a network-like structure within the pollen sacs. The process of disintegration includes even the tissues present between the pollen sacs

Unfortunately, no meiotic data are given which would be necessary to judge the cytogenetic situation of the mutants.

Capsicum annuum and C. frutescens

A male sterile mutant of *C. annuum* designated as *ms*-3 was obtained by X-irradiation. The plants homozygous for the recessive gene do not differ from the fertile ones of the segregating families with regard to growth and morphology. Their considerably reduced anthers contained almost no pollen grains. Only 1-3 normally developed and stainable pollens were found per anther. The meiotic behaviour of the mutant has not been studied. The male sterility was neither influenced by environmental conditions nor by grafting on male fertile plants (Daskaloff 1968). Later, the author isolated five more male sterile mutants got after X- and gamma-irradiation (Daskaloff 1971, 1971/72, 1973a, b). In all these cases, sterility is governed by a single recessive gene; the genes being non-allelic. They are designated as *ms*-4 to *ms*-8. Mutant *ms*-4 is fully male sterile forming no pollen grains at all. Mutant *ms*-5, however, develops under greenhouse conditions a small amount of sterile and fertile pollen. In the field, it is completely male sterile. The functioning ability of these normally looking pollen grains has not been tested. The possibility of an interaction between gene *ms*-5 and the cytoplasm cannot be ruled out (Daskaloff 1971/72). In mutants *ms*-6, *ms*-7 and *ms*-8, a normal course of meiosis up to tetrad formation was observed. A complete degeneration of the mature microspores indicates that the disintegration of the cell contents takes place between microspore formation and their maturation (Daskaloff 1973a, b).

A similar meiotic situation is valid for a spontaneously arisen mutant in the Bell pepper variety "All Big". Disintegration of the microspores takes place immediately after tetrad formation. The expressivity of the recessive gene involved is stable in field and greenhouse (Shifriss and Frankel 1969). Moreover, a specific type of cytoplasmic male sterility has been found in the species which obviously is manifested as a result of interaction between cytoplasm and a nuclear gene (Peterson 1958).

Pochard (1970a) investigated material of *Capsicum annuum* having 8 per cent of haploid embryos. After treating the seeds with X-rays and EMS, he selected three male sterile haploid plants (*mr*-9, *mc* 509, *mc* 705). By application of colchicine diploid flowers were formed which were fertilized with pollen from fertile plants. The analysis of the progenies indicated that the male sterility in all the three genotypes is governed by a single recessive gene. According to Pochard, three different genes have mutated; however, allelism tests were not carried out. There are certain differences in the meiotic behaviour of the three mutants. While microsporogenesis in *mc* 509 is arrested at the tetrad stage, in mutants *mr* 9 and *mc* 705, the breakdown can take place at any stage from prophase I to microspore formation (Pochard 1970b).

In *Capsicum frutescens*, three different types of sterility were observed by Martin and Crawford (1951). The first type includes both male and female sterility; thus, it is not concerned with the problem being discussed in the present paper. The second type was male sterility manifested only under field conditions. The third type, finally, was male sterility only observable under green-house conditions. In the field, such plants are fully self-fertile and very productive. Unfortunately, also in these cases no cytogenetical studies have been made. Therefore, no detailed informations on gene action, meiotic behaviour and the direct causes of male sterility can be given.

Hebe parviflora and other species of the genus

In different species of the genus *Hebe*, naturally occurring male sterility in small, isolated populations in New Zealand was found to be obviously controlled by recessive genes (Frankel 1940). In *H. parviflora* and *H. subalpina*, degeneration occurs at pachytene. The bivalents coa-

cause this anomaly. One of the genotypes shows complete, other ones have partial sterility. Complete male sterility was observed in plants homozygous for gene *ms*-2 (Richmond and Kohel 1961). The anthers of the plants are indehiscent and the filaments are shortened. All the stamens are compactly placed along the staminal column. Pollen grains are formed but they are small and lack spines on their exine. Microsporogenesis appears to be nearly normal. The insignificant meiotic disturbances observed in these plants cannot be the cause of the complete male sterility of the mutant. Nevertheless, the pollen grains are non-functional while the female germ cells can be used for seed production when cross-pollinated.

Homozygosity for gene *ms*-1 causes complete or partial male sterility. Such a plant was derived from a monosomic plant; its meiotic behaviour is not known (Justus and Leinweber 1960).

Gene *ms*-3 of *G. hirsutum* causes not complete but partial male sterility (Justus *et al* 1963). The flowers of the plants homozygous for this gene contain either no pollen grains at all or a reduced amount of pollen depending on environmental factors which are not yet sudied in detail. Partially male sterile plants are more fertile under greenhouse conditions than in the field. This mutant is not comparable to the *ms*-mutants of other species discussed in this review because the little pollen produced in these plants is functional and can be used for self pollination or out-crossing. Thus, there must have been a normal course of microsporogenesis in some of the pollen mother cells and the degenerative processes characteristic for nearly all the male sterile mutants do not occur.

Another male sterile mutant of *G. hirsutum* having nectarless flowers and glandless leaves was studied by Weaver (1968). According to test crosses, the male sterility of this mutant is due to the combined action of two recessive genes designated as *ms*-5 and *ms*-6. This is the only report of male sterility in *G. hirsutum* that is conditioned by two genes. Details concerning the direct causes of sterility and the meiotic behaviour of the mutants are not known. Gene *Ms*-7 is responsible for male sterility when present in the dominant state. Its action will be described later.

Male sterility in legumes

In Leguminosae, relatively large amount of information on the action of genes causing male sterility is available. The species investigated in this connection are *Glycine max*, *Lathyrus odoratus*, *Lupinus mutabilis*, *Phaseolus lunatus*, *Pisum sativum*, *Trifolium pratense*, *Vicia faba* and *Vigna sinensis*.

Pisum sativum

In the garden pea, 13 male sterile mutants have been obtained after X-irradiation. The course of microsporogenesis, the time of breakdown and the genetic behaviour of all these mutants have been fully explored (Gottschalk 1968, 1971, Gottschalk and Jahn 1964, Gottschalk and Baquar 1971, 1972, Klein 1968, 1969).

The action of the mutated recessive genes is concentrated either at early prophase stage or at the final stages of meiosis (figs 1, 2). In general the *ms*-genes of the genome cause—as in the other species mentioned above—breakdown of microsporogenesis at a specific stage followed by a disintegration of the living contents of the cells. However, only some genes of this group exhibit this type of behaviour. In some other mutants, meiosis continues but the plants are unable to form functional male germ cells. The following mutants belong to the first group:

— Mutants 69, 38B:
Degeneration in pachytene. These two mutants are obviously identical in showing a completely similar meiotic behaviour. Pairing of the homologous chromosomes takes place normally, but simultaneously, degeneration of all the chromosomes of the nuclei begins. Furthermore, the nu-

cleolus is considerably enlarged and highly vacuolated. At this time, the bivalents can still be observed as distinct entities of the nucleus. But later, all the bivalents appear to be clustered forming a structureless amorphous body (figs. 1, 2).

— Mutant 503:
In this, degeneration occurs between microspore and pollen formation. Furthermore, two more mutants have recently been isolated that show degeneration in leptotene and during diakinesis and prometaphase. All the other male sterile mutants of *P. sativum* show additional anomalies which are related to their meiotic behaviour.

In mutant 395, there is a completely normal course of microsporogenesis up to the second division. Quadripartition of the PMCs in order to form the microspores is initiated but not completed. In interphase II, broad cytoplasmic strands connecting the four microspores are observed. Degeneration of chromosomes and nuclei takes place during the end of microspore formation.

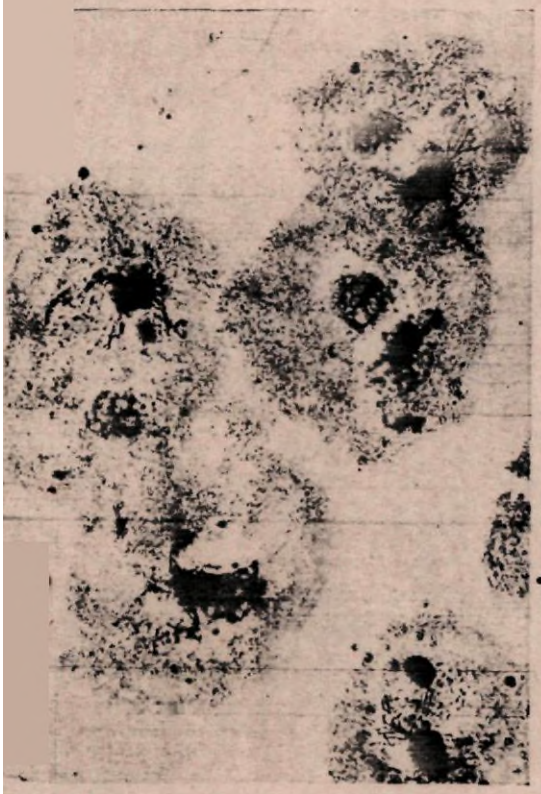

Fig. 1. Pollen mother cells of the male sterile mutant 38B of *Pisum sativum* during pachytene showing the beginning of the degeneration of the chromosomes.

Fig. 2. The same PMCs as illustrated in figure 1 in a somewhat later stage of nuclear degeneration. All the chromosomes are united forming an amorphous chromatin body.

Mutants 71A, 78 and 98A show a nearly similar meiotic behaviour. We are not yet able to state if these genotypes are identical and have originated from mutation of the same locus in three different irradiated embryos. It is conceivable that several genes of the *Pisum* genome cause identical meiotic anomalies when present in the homozygous state. This situation is realised in some *ds*-genes of *P. sativum* (Gottschalk 1968). Because of the sterility of the *ms*-mutants, it is very difficult and time-consuming to clarify the genetic situation existing between these mutants. They do not only show degeneration of their chromosomes and PMCs, but a broader spectrum of meiotic irregularities can be observed. All these anomalies are transferred enblock from generation to generation obviously due to a pleiotropic action of the genes involved. This pleiotropic spectrum comprises exclusively of premeiotic, meiotic and post-meiotic features which are as follows:

- — Considerable reduction of the number of PMCs per anther.
- — Premeiotic anomalies resulting in hypodiploid PMCs.
- — Poor stainability of the chromosomes.
- — Irregular chromosomal distribution in anaphase I.
- — Strong irregularities during the final stages of microsporogenesis. The chromosomal distribution in anaphase II is disturbed resulting in 4-16 microspores per PMC, the mean being 9·5.
- — Beginning of a third meiotic division at the end of microsporogenesis. This division continues up to metaphase.

Furthermore, there are some other specific pecularities which are not observed in other male sterile *Pisum* mutants. The main effect of the genes in question—the degeneration of the living contents of PMCs—does not occur in a distinct meiotic stage. On the contrary, there are three different stages during which the genes are operative. Disintegration can occur during interphase II preventing microspore formation. In other cells of these mutants, degeneration takes place when the microspores are formed. In this case, the formation of intine and exine takes place not around the individual microspores but around the whole PMC. This is one of the most characteristic anomalies in the three mutants and also in this behaviour they resemble each other fully. If degeneration at this stage would not occur, giant pollen grains would be formed having the tetraploid instead of haploid chromosomes number. In a relatively small proportion of PMCs, degeneration occurs in the completely developed pollen grains.

It is pertinent to mention here that some groups of neighbouring PMCs in the anthers of these male sterile mutants exhibit a nearly undisturbed course of microsporogenesis. In spite of this normal behaviour, the mutants never set seed. Therefore, it can be concluded that these "normal" PMCs are unable to produce functional male gametes. This differential behaviour could be due to differences in the degree of expressivity of the mutant gene. It would be worth while to study the causes of these differences.

Mutant 67 of our collection shows in general the same meiotic and premeiotic behaviour as mentioned above. Also in this case, the earliest anomalies are observed in interphase II and the whole PMCs are surrounded by a thick cell wall. After degeneration, only pycnotic and clumped portions of protoplasm remain (Klein 1969). This mutant does not show the complete pleiotropic spectrum found in mutants 71A, 78 and 98A. Therefore, it is obviously not identical with the other three genotypes.

Mutant 195B of *P. sativum* belongs likewise to the group of male sterile genotypes but it shows a completely divergent behaviour that is not comparable with the action of the other *ms*-genes. Hence, it occupies a special position within the whole group. In this mutant, there is an undisturbed normal course of meiosis up to diakinesis. Degeneration begins during prometaphase

esulting in an amorphous chromatin body ike that in mutant 38B. But the meiotic tage of this agglutination is not firmly ixed. It can also occur during later stages ıp to the end of second meiotic division. The specific pecularity of this mutant, ıowever, is that there is no breakdown of nicrosporogenesis. On the contrary, meio- is continues in spite of the anomalies nentioned above and microspores are pro- luced. But the principal effect of the gene s also in this case the complete degenera- ion and disintegration of the cell contents. t takes place between microspore and ollen grain formation. Thus, mutant 195B hows some specific characteristics of the ypical male sterile mutants, while it deviat- s from them with regard to other features. Since degeneration of the precursors of the nale gametes finally takes place, it seems o be feasible to place it in the male sterile group.

All the male sterile *Pisum* mutants show a nonohybrid segregation. In some geno- ypes, a statistically significant deficit of ecessive plants was observed in the segre- gating families. A similar deficit is known or many *Pisum* mutants irrespective of the pecific action of the concerned mutated genes (Gottschalk 1964). It is probably lue to weak competitive ability of the ollen tubes containing the mutated genes.

Vicia faba

In the broad bean, a male sterile mutant s known forming a very low amount of ollen grains which could not be stained in ceto carmine. On selfing such plants, no eed set was observed. However, cross ollination led to the formation of seeds. Hence, the female sex organs are normally unctioning (Bond *et al* 1964). This is obvi- usly a true case of genetically conditioned nale sterility, but there are no details vailable on the course of microsporogene- is. Therefore, it is not yet clear at what neiotic stage the gene acts and in which way the degeneration of the PMCs, micros- pores or pollen grains occurs.

Lathyrus odoratus

In the sweet pea, a recessive gene leading to male sterility is known since the beginning of this century (Bateson *et al* 1903, 1908). According to Gregory (1905), certain anomalies occur already during the premeiotic mitoses resulting in a reduced number of PMCs in the anthers. However, the gene becomes mainly effective during microsporogenesis. A special meiotic feature of the *ms*-plants consists in the delayed disappearance of the nuclear membrane. Moreover, the bivalents in most of the PMCs are randomly distributed and not orientated to the equatorial plate. After the end of the second division, certain anomalies in cell wall formation are observed. Obviously, no independent microspores are formed but there is only a chamber formation within the microsporocytes. The final arrest of the meiosis is not firmly fixed; it can take place any time between diakinesis and anaphase II. These differences in the expression of the gene seem to be environmentally controlled (Fabergé 1937). The female sex of the plants is fully functional (Punnett 1923, 1925, 1927, 1932).

Trifolium pratense

A recessive *ms*-gene in *Trifolium pratense* was studied by Smith (1971). The pollen sacs of the plants homozygous for this gene were shrivelled and the anthers were orange-coloured instead of yellow. They contained only 3·6% stainable pollen grains. Considerable differences in size were observed within the unstained, non-functional pollen grains. A 3:1-segregation of gene *ms*-1 was found; meiotic study of the plants is not yet carried out.

Glycine max

The species *Glycine max* represents one of the examples where genes for male sterility are acting but where no cytological details are available. Plants homozygous for gene *ms*-1 are indistinguishable from fertile plants till to pollen formation and pod development (Brim and Young 1971). They produce only a few one-seeded fleshy pods. Thus, they can be identified either by their pollen sterility or by their extremely reduced seed

set. In a natural crossing block, 99% of the seeds produced in the male sterile plants were the result of cross pollination.

Vigna sinensis

In the cowpea (*V. sinensis* ssp. *cotjung*), a male sterile mutant was studied by Sen and Bhowal (1962). The plants excelled the fertile plants in vegetative growth, but their floral organs were reduced and the anthers were small and not fully developed. Archespore cells were present but obviously in a reduced number. Only a few PMCs were formed and these showed breakdown of microsporogenesis in early prophases. The latest meiotic stage observed in the meiocytes was the diakinesis. These cells degenerated very rapidly without any further growth. Crossing experiments indicated that male sterility is controlled by one recessive gene.

Phaseolus lunatus

In lima beans, one male sterile plant occurs in a population of about 20,000 plants due to spontaneous mutation. The respective gene shows a pleiotropic spectrum affecting the gross morphology of the plant besides the functioning ability of the male sex organs (Allard 1953). The action of the gene is discernible even at the seedling stage. The shoot apex of the primary axis stops its function in a very early stage of ontogenetic development and the whole shoot system is developed from lateral axes. As a consequence of this anomaly, the vegetative development of the plant is initially delayed. Later, the plants exceed the fertile plants of the same family in stature. In addition, the mutated gene influences leaf morphology and organisation as well as the chlorophyll content. The anthers are shrunken and fail to dehisc. In principle, pollen grain formation takes place, but majority of them are empty. A maximum of 7% stainability was obtained. Fruits were never formed in these plants. Since a detailed analysis of the microsporogenesis has not been made in this mutant, no exact information on the causes leading to the production of the empty pollen grains is available. Genetic analysis revealed the sterility to be conditioned by one recessive gene.

Lupinus mutabilis

From the material got from Peru, about 35 per cent of the plants examined by Pakendorf (1970) were found to be male sterile. In these, microsporogenesis proceeds normally up to tetrad formation but the tetrads degenerate before separating from each other. This degeneration is coupled with a progressive increase of the endothecial layer whose cells are without supporting bands. While the tapetal cells in the fertile plants disintegrate, in the sterile they remain intact and nonvacuolated. The nature and type of sterility is not known; moreover, the female fertility was not determined.

Helianthus annuus

Male sterility in sunflower was reported in a Russian material by Kuptsov (1935). The specific plants were characterised by non-emergence of stamens. When bagged, no seed set was obtained. After cross pollination, there was a normal seed setting. The anomaly was obviously conditioned by a recessive gene. Moreover, genetically controlled male sterility in Russian varieties was observed in France that was likewise due to the action of a single recessive gene (cited after Putt and Heiser 1966).

Putt and Heiser (1966) found two types of male sterility in sunflower: complete and partial sterility. Complete sterility was exhibited in an ornamental red-rayed variety grown in Bloomington/Indiana and in another stock grown in Morden/Manitoba. In both the cases, the plants possessed anthers that split apart before exsertion. The male steriles of the Bloomington stock had either no pollen or pollen grains of uneven size that remain clumped and unstained in aceto carmine. The early stages of microsporogenesis were completely normal. Further details indicating the degeneration of microsporocytes, microspores or pollen grains are lacking. The genetic control of this anomaly is not yet fully clear. While in three crosses the

sterility appeared to be controlled by a single recessive gene, is two others the control appeared to be due to duplicate genes.

In the male sterile plants of Morden stock, the pollen grains were uniformly smaller than those of the fertile plants and were nonclumping. In two thirds of the sterile plants, no pollen stained. In the remainder, only 1 per cent partially or normally stained pollen grains were found. The anomaly is likewise due to the influence of a single recessive gene. Any meiotic irregularities could not be observed in this material; therefore, it is not yet clear at what postmeiotic stage the gene causes pollen abortion."

The authors made reciprocal intercrosses between the two male sterile stock and got fertile F_1-hybrids. Therefore, the male sterility cannot be due to identical genes or alleles.

Besides plants exhibiting complete male sterility, partially male sterile plants were isolated in two inbred lines of the species. The genetic basis of this type of sterility within the two lines is not fully clear. However, according to crossing experiments, it appears that the genes controlling sterility in the two lines are not identical although they originated from the same variety.

Daucus carota

The first cases of male sterility in carrots are documented in Knuth's book published in 1898. The author mentions about certain "physiologically female" plants occurring randomly in wild populations of *D. carota*. They developed either leafy stamens or stamens with unrolled filaments and non-dehiscent anthers. Moreover, partially male sterile wild carrots have been found by Beiyerinck (1885) in the Netherlands.

A single male sterile plant was detected in a commercial stock of the variety "Tendersweet" by Welch and Grimball (1947). The cytological behaviour of the plant and the genetic control of this anomaly were not studied. The female sex was normal; therefore, it appears to be a case of true genetically conditioned male sterility. A similar, but obviously not identical case was observed in some other breeding lines of the species. In two out of four cases, pollen grains were produced whose viability remains unknown while two others did not produce any pollen. In all the four plants, seed set occurred as a result of open pollination.

One more recessive gene *ms*-5 and one dominant gene *Ms*-4 controlling male sterility in cultivated carrots were reported by Hansche and Gabelman (1963). In the male sterile plants, the collapse and desiccation of the stamens took place just before floral anthesis. Also in this material, no meiotic investigations have been made. This is likewise valid for the material investigated by Braak and Kho (1958). In these male sterile carrots, the flowers remained unrolled and anthers were nondehiscent. Thus, the umbels possessed female and sexless flowers due to staminal abortion.

A complete microspore abortion in sterile plants of *D. carota* was observed by Zenkteler (1962). This anomaly was associated with heterozygosity for a reciprocal translocation leading to the formation of chromosome bridges and fragments. Microspore formation was coupled with a periplasmodium formation of the tapetal tissue and with anther wall deterioration.

The interaction of male sterility genes with cytoplasm in *D. carota* is described in section 2 of the present paper.

Lycopersicon esculentum

In Solanaceae, an intensive study of male sterility has been carried out mainly by Rick. Moreover, genetically conditioned male sterility is known in egg plant and tobacco.

From the tomato genome, 24 *ms-genes* are

known (Rick 1944, 1945, 1948, 1966, Rick and Butler 1956, Rick and Boynton 1967, Rick and Khush 1965). The time of action of these genes is distinct and specific; a survey is given in figure 3. The earliest action is observed in mutants homozygous for *ms*-3. This gene becomes effective during the latest premeiotic stages. Only degenerated PMCs are observed in mature anthers. As for many genes of the group, the time of action is not strongly precise. In some of the PMCs, microsporogenesis proceeds up to metaphase and telophase I.

A group of four genes causes breakdown of microsporogenesis during different stages of the first meiotic prophase: *ms*-10 in early prophase, *ms*-1 in mid prophase, *ms*-5 obviously during several subsequent stages of prophase and *ms*-18 in diplotene.

So far, no *ms*-genes of the tomato genome are known that specifically influence the stages metaphase I to anaphase II. But there is a large group of *ms*-genes which effect the final meiotic stages; these being *ms*-6 in telophase II and tetrad stage, *ms*-2, *ms*-17 in tetrad stage, *ms*-4, *ms*-9 in early microspore stage and *ms*-13, *ms*-14 in late microspore stage.

The action of the *ms*-genes mentioned above are more or less limited to specific stages of microsporogenesis. Besides, three other genes can become effective during any meiotic stage: like, *ms*-8, *ms*-12 from prophase through tetrad stage; *ms*-7 from prophase to mid microspore stage. Moreover, a case of variable male sterility exhibited in a spontaneous mutant of the variety "San Marzano" of *Lycopersicon esculentum* was found by Rick and Boynton (1967). This trait is controlled by a recessive gene designated as *vms* mapped on chromosome 8. Microsporogenesis in these plants is normal and degeneration occurs after microspore formation. Most of the anthers do not contain stainable pollen grains. The few stained ones observed are obviously non-functional as all efforts to self these plants failed to produce seeds. The female sex organs are fully functioning as could be evidenced by hand cross pollination.

The mutant shows a high degree of temperature sensitivity with regard to the degree of expression of male sterility. Under Californian field conditions between June and October, the plants develop a defective corolla. The stamens are highly reduced, malformed and discoloured and show some additional morphological abnormalities. Even when the opened thecae are teased pollen grains are never shed. Before June and under greenhouse conditions throughout the year, flowers are nearly normal. Under greenhouse conditions, the plants show good fertility. When the same clones were again grown in the field in the following summer season, they exhibited male sterility. Such behaviour was consistantly obtained from rooted cuttings or in seed progenies. This varied gene expression is due to the differences in temperature. A minimum temperature of 30°C in the field and 32°C in the greenhouse are required to evoke the male sterility of the mutant. The sensitive stage in which the high temperature acts as stimulus seems to be flower primordia of 0·075 mm or smaller diameter. Buds of this size are still highly mitotic; meiosis takes place in them after 10 days.

In Russian material, two male sterile lines due to spontaneous mutations have been investigated by Solovjeva (1970). In both the cases, sterility was inherited as a recessive character. Among the sterile plants, a different expression of the genes was observed such as complete lack of stamens, underdeveloped or feminized stamens containing ovule like organs at their base. In some flowers, there are undeveloped stamens lacking pollen sacs. Male sterility was obviously not complete because in single flowers 1-3 normal stamens containing viable pollen grains were formed. When crosspollinated, the male sterile plants are able to form seeds.

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ORCEIN-BANDING IN PLANT CHROMOSOMES

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Very rarely, few pollen grains are formed. In the field, a normal seed set of these plants was observed indicating the full functionability of the female organs. Although no genetic details are available, it appears to be clear that this type of male sterility is genetic.

Cucurbita maxima:
The earliest observations of genetically controlled sterility in *C. maxima* were made by Hutchins (1944). This material was cytologically analysed by Singh and Rhodes (1961). The archespore cells do not enter into meiosis; on the contrary, they enlarge and develop vacuoles. Their nucleus becomes hypertrophied and the cells degenerate. This is followed by tapetal disintegration. In some cases, the pollen mother cells neither degenerate nor pass through microsprogenesis. Instead, they develop a thickened wall comparable to that of the pollen grains. This represents one of the very rare cases in which the action of a *ms*-gene becomes discernible already before the initiation of meiosis. In the same species, another male sterile mutant was analysed by Francis and Bemis (1970). Meiosis proceeds normally up to quartet stage after which breakdown occurs sometime during the maturation of the microspores. No pollen is produced in the shrivelled and necrotic anthers. This anomaly is correlated with a specific tapetal abnormality. During microsporogenesis, the tapetal cells undergo an additional cell division resulting in a two layered tissue which is not found in fertile plants of the species. At the onset of microspore formation, the tapetal cells increase to double size and expand into anther locules; simultaneously, the microspores start deteriorating. According to crossing experiments, the two mutated *ms*-genes mentioned above seem to be alleles.

In another male sterile genotype of *C. maxima*, the sterility in due to the abortion of the androecium is bud stage (Scott and Riner 1946). This is a case of "functional male sterility" which is not discussed in the present paper.

Cucumis melo:
Bohn and Whitaker (1949) found male sterility in a powdery resistant line of *C. melo* governed by the recessive gene *ms*-1. This gene expresses its action at the end of the second meiotic division by arresting pollen development. A second gene of this group—*ms*-2—was detected by Bohn and Principe (1964) in another powdery resistant line of the species. Meiosis of plants homozygous for *ms*-2 is normal up to telophase II. Cell wall formation during the development of microspores is highly irregular leading to cells that vary in size, chromosome number and spatial arrangement. No nuclear membranes are observed after the cell wall formation is initiated and chromatin fragments become dispersed in the cytoplasm. The final degeneration takes place during microspore formation. Genes *ms*-1 and *ms*-2 are not identical and probably not linked. A material of *C. melo* supplied by Whitaker was studied by Chauhan and Singh (1968). They found, that the microspores fail to separate and remain cemented together. They possess only little cytoplasm and a degenerated nucleus. The authors analysed the activity of acid phosphatase in the tapetum and found clear differences between male fertile and sterile plants, the latter lacking the enzyme.

In *C. sativus*, two male sterile lines were developed after gamma irradiation. The anthers of the plants are rudimentary but microsporogenesis proceeds normally till to microspore formation. The degeneration occurs after the course of pollen mitosis. Both the lines are identical with respect to the gene *ms*-2 (Whelan 1972). Another type of male sterility was observed by Barnes (1961) to be due to the abortion of the staminate buds. This seems to be a case of "functional" male sterility which is not directly related to the problems being discussed in the present paper.

Gossypium hirsutum:
In cotton, male sterility is known so far only in *G. hirsutum* where six recessive and one dominant genes have been found to

gulate into an amorphous mass that disintegrates quickly. Simulaneously, the tapetum tissue degenerates. An apparently similar situation is valid for male sterile plants of *H. transversii*. In all the three cases, the respective gene acts rapidly and regularly like that described for mutants 38B and 69 of *Pisum sativum*.

In *Hebe salicifolia* var. *communis*, degeneration takes place at the end of the second meiotic division. Tetrads are formed but the PMCs collapse rapidly. Hence, no pollen grains are produced. An even later degeneration was observed in a plant which probably was a natural hybrid between *H. leiophylla* and *H. salicifolia*. Pollen grains were formed but they shrivelled and collapsed rapidly and regularly. In *H. traversii*, the normally appearing pollen degenerated along with the tapetal cells.

A completely different effect of a *ms*-gene was observed in *Hebe townsonii* influencing the timing of microsporogenesis resulting in a chimerical pattern of different meiotic stages in the pollen sacs. Degeneration occurs after pollen grain formation.

In *Gramineae*, the phenomenon of genetically controlled male sterility has been studied in *Avena*, *Dactylis*, *Hordeum*, *Sorghum*, *Triticum* and *Zea*. Comprehensive findings are available in *Hordeum vulgare* and *Zea mays* while in the other genera just mentioned only a few genes of the group are known so far.

Zea mays

According to the catalogue of genetic maize types, 44 *ms*-genes of the genome were known in the beginning of the fifties, most of them are already mapped (Weijer 1952). Many of the respective mutants have not yet been analysed cytologically; therefore, we can only give a very rough view of their action. 17 *ms*-mutants are described by Beadle (1932); all the genes responsible for this anomaly are recessive. In all these cases, female fertility is not influenced. In general, the anthers of these plants are not exerted. Thirteen genotypes of this group are not able to produce functionable pollen grains and hence are completely male sterile. Mutants *ms*-6, *ms*-12 and *ms*-17 however, are only partially pollen sterile. In *ms*-6, three different kinds of anthers are developed. Most of them are not exserted. A few, when exserted, do not dehisc while some others are able to shed functionable pollen grains. The degeneration takes place in the majority of the PMCs just before, during or after meiotic phase I while in the remaining few degeneration is delayed till to pollen formation. A similar situation is valid for *ms*-17 (Emerson 1932). *Ms*-12 is likewise a gene causing partial pollen sterility. It has not been studied in detail therefore, no precise features on the type and time of its action are known. Mutant *ms*-15 was available only in a heterozygous state and was not analysed further.

Genes *ms*-8, *ms*-9 and *ms*-17 induce degeneration of microsporocytes in the early and middle stages of the first meiotic prophase. Gene *ms*-6 acts during diakinesis and anaphase I. All the other genes studied induce breakdown of meiosis after microspore formation. Mutant *ms*-1 was for the first time reported by Eyster (1921) and cytologically analysed by Beadle and McClintock (1928). The gene involved was designated as *ms*-1 by Singleton and Jones (1930).

The morphology of mutants *ms*-2, *ms*-7 and *ms*-11 was studied by Madjolelo *et al.* (1966). According to their findings, degeneration of the pollen grains occurs between 5 and 10 days after the completion of meiosis. In general, the plants of these three genotypes were found to be shorter than their male fertile counterparts. This difference becomes more obvious after the breakdown of pollen grains.

Besides the male sterile mutants mentioned above, some more genes influencing microsporogenesis are known in corn

Mutant "*variable sterile*-1" studied by Eyster (1934) shows a lack of cytokinesis. The essential difference of this mutant from the other ones is that not all but only a relatively small number of PMCs is affected by the recessive gene.

Sorghum vulgare and S. bicolor

So far, 7 genes causing male sterility in *S. vulgare* are known and all of them are recessive. Gene *ms*-1 suppresses obviously pollen formation completely while the female sex organs are normally functioning. Monogenic inheritance for this gene was observed by Ayyangar and Ponnaiya (1937). In plants homozygous for *ms*-2, the anther size is half the normal size or less. No PMCs could be found indicating that the gene action becomes effective during early stages of anther differentiation. However, gene *ms*-2 shows certain variations in its expressivity. In a few scattered populations, plants of this genotype with apparently functioning anthers were observed. These plants can be discerned from their pointed shape of the panicle due to longer rachis and shorter rachis branches. Moreover, they possess an increased number of lateral branches. The gene is linked with genes *A* (awn formation) and V_{10} (chlorophyll formation). Furthermore, it could be shown, that genes *ms*-1 and *ms*-2 are non-allelic (Stephens 1937, Stephens and Quinby 1945). Another two male sterile *Sorghum* genotypes have been studied by Ayyangar (1942).

All the male sterile mutants of the species mentioned so far originated spontaneously. Some more mutants of this type were selected in radiation genetic experiments. Mutant *ms*-1645 obtained by Barabas (1962) after having used X-rays in combination with colchicine shows a specific reaction to photoperiod. The action of the recessive gene becomes only discernible under short day conditions. Under these presuppositions, only 1-2 per cent obtainable pollen grains are produced and there is no seed set.

In *S. bicolor*, a male sterile mutant was obtained after gamma irradiation; the gene was designated as *ms*-7 by Andrews and Webster (1971). Pollen grains are not produced in the respective plants. They can be easily recognised by some additional features such as widely opened flowers and whitish anthers with reduced size. Female fertility is not influenced by the mutant gene. In crossings with *ms*-1 and *ms*-2 plants of *S. vulgare*, normal hybrids were obtained. Thus, all the three genes are non-allelic.

Dactylis glomerata

Male sterility in orchard grass was first reported by Stapledon (1931) and Jenkin (1931). The respective plants had a normal appearance and anthers were characteristically exerted out but indehiscent. A more detailed analysis of this phenomenon was carried out by Filion and Christie (1966) using the tetraploid clone OD-1 of the species. The plants of this clone were morphologically normal and had smaller, dark green and non-dehiscent anthers. In diakinesis, up to 6 quadrivalents per PMC were observed besides bivalents, univalents and laggards. This behaviour is normal for the autopolyploid species and has no connection with the phenomenon of male sterility. In the male sterile plants, microsporogenesis continues till to microspore formation but immediately after their release they degenerate rapidly. Pollen grains are not formed. The earliest differences between male fertile and male sterile plants are observed in the tapetal behaviour. In the initial stages of meiosis, the tapetum of the sterile anthers degenerates very rapidly while no similar effect is found in the fertile ones. Furthermore, in the male sterile anthers, no gradual elongation and periclinal division of epidermal and endothecial cells occur. According to Filion and Christie, the male sterility is either due to the abnormal tapetal behaviour or due to an insufficient nutrition provided by the tapetum to the sporogenous tissue. A third possibility could be a genetically controlled breakdown of microsporocytes along with

tapetal cells. Only when the last assumption is confirmed, this type of sterility can be regarded as genetically conditioned. Further investigations concerning the inheritance pattern and studies of female fertility are necessary for further clarification of the situation.

Avena strigosa, A. byzantina

In the diploid species *A. strigosa*, partially male sterile mutants were obtained after X-irradiation (Sadanaga 1965). The anthers of these plants were shrivelled and failed to dehisce. In most of the PMCs, 7 bivalents were observed but only 5-10 per cents of the pollen grains stained with potassium iodide solution. This kind of sterility seems to be controlled by a single recessive gene. According to Sadanaga, this gene belongs to the *ms*-group. Under greenhouse conditions, most of the partially male sterile plants are completely sterile. The fertile ones set a relatively few number of seeds. In the field, the plants produce more seeds but most of them are shrivelled. This increased seed production seems to be due to natural cross-pollination.

An impaired male fertility appears to be present in a nullisomic *A. byzantina* line (Ramage and Suneson 1958). Pollen is defective in many anthers of these plants and in many of them no pollen grains are produced at all. Even the pollen from most normal appearing anthers are small, shrivelled and non stainable with iodine. Selfing produced 10 per cent seeds; manual crossing yielded seeds in about one fourth of the florets. Thus, the female sex has a considerably better functionability than the male sex. Therefore, it may be justified to regard the mutant as belonging to the male sterile group. A detailed cytological analysis of this material is not yet available, however, a regular bivalent formation was observed indicating that the gene action does not become effective during the first meiotic division.

Hordeum vulgare

A voluminous collection of male sterile barley mutants intensively studied by Hockett and Eslick (1968, 1969, 1970a, b), Hockett *et al* (1968), Roath and Hockett (1971) and Hockett (1972) is available at Bozeman/USA. According to allelism tests, at least 24 different recessive genes of the barley genome are known to induce male sterility. Some of them have already been localized. Since these mutants do not differ morphologically from the non-mutated plants, their identification in the field is not possible during the early stages of ontogenetic development. However, when the normal plants at maturity lodge, the male sterile mutants remain erect because they are unable to produce seeds. A second possibility for their quick and reliable selection is by observing their foliage leaf colour. While the leaves of the non-mutated plants become yellow during maturity, the leaves of the mutants remain green. With the exception of *ms*-9, *ms*-15 and *ms*-17, all the other genotypes of this group do not produce any seed after selfing. The development of the anthers varies from a nearly normal situation (*ms*-6, *ms*-16) to the formation of strongly reduced rudimentary organs (*ms*-3, *ms*-7). The female sex organs of these plants are fully functionable. This is evidenced by a good seed set after natural cross- or hand-pollinations. From this behaviour, it has been concluded that the male sterility of this material is due to *ms*-genes and not to *ds*-genes causing desynapsis. The *ds*-genes would affect equally micro- and megasporogenesis. It is pertinent to mention that two *ms*-mutants of this group are able to produce small amount of seeds after selfing. The seed set of each of these genotypes differed at two locations obviously due to differences of specific environmental factors not yet known (Hockett and Eslick 1968).

Unfortunately, a detailed cytological analysis of the whole group is still lacking. Hence, it is only possible to give some details regarding the time and mode of action of a few genes and to compare them with similar genes of other species.

In line *ms*-7, there is a completely undisturbed meiosis up to quartet stage after which the microspores become shrivelled and periplasmodial. In a few pollen mother cells, the development of the microspores continues but there is obviously no formation of functionable pollen grains. In mutant *ms*-8, the action of the mutated gene becomes likewise operative after the end of microsporogenesis leading to the formation of shrivelled pollen grains. In contrary to other mutants of this group, a broad variability in anther and pollen development often within the same flower or even the same anther was observed. Barley has normally two pollen sacs. This mutant, however, possesses uniformly four lobes. Very rarely, normal pollen grains are formed in one of the four pollen sacs indicating an unstable expression of the mutated gene with regard to its action on the stages between microsporogenesis and pollen formation.

The most delayed action of a *ms*-gene known so far was found in mutant *ms*-6. In the plants of this genotype, meiosis proceeds normally and pollen grains also appear normal. The exact time of the breakdown could not be observed. From the male sterility of these plants, it can be concluded that the gene action becomes operative sometimes after flower anthesis. In the above mentioned three *ms*-mutants, there is not a very clear correlation between microspore or pollen grain degeneration and the breakdown of the tapetum tissue. In non-mutated barley plants, the binucleate tapetum cells degenerate when PMCs undergo interphase II. The whole tissue is reduced to a thin layer of cells when the microspores are released from PMCs. At the time of pollen dehiscence, only traces of the tapetum tissue are observed. In the sterile plants, there are only slight differences in the behaviour of tapetum degeneration in comparison to the fertile ones. Degeneration starts a little earlier; moreover, a slight separation of tapetum cells is observed in premeiosis in *ms*-6 and *ms*-8. In *ms*-7, they are normal until telophase II.

A similar lack of cytological analysis exists in male sterile barley mutants reported by many authors. For instance, Suneson (1940) found a single plant having rudimentary shrunken anthers but a normal ovary. This anomaly was certainly caused by the action of a recessive gene because a 3:1 segregation was observed in the offspring of heterozygous plants. However, it cannot reliably be concluded from these findings that the mutant gene in question does really belong to the group of the typical *ms*-genes. It is likewise conceivable that there was a lack of archespore differentiation in the anthers thus leading to male sterility as is known in a pea mutant.

Kasha and Walker (1960) have investigated three non-allelic male sterile barley mutants. In one of them (*ms*-3), male sterility is associated with dwarfness obviously due to a pleiotropic effect of the recessive gene. The mutant is completely sterile after selfing but it shows good seed set after cross-pollination. Also in this case, only the failure to produce viable pollen grains has been reported and no details on the specific action of the gene on microsporogenesis are available.

Interesting experiments conducted by Kasembe (1967) on two plants of a male sterile barley mutant show that the action of a *ms*-gene can be reduced by treating the plants with growth regulators. By spraying gibberellic acid in concentrations of 100 and 300 p.p.m. upon the leaves some seeds were obtained. The ears of these plants had carefully been bagged; therefore, the seeds were really produced as a result of selfing. Thus, a certain amount of functioning male germ cells had been produced in these male sterile mutants which are never formed in untreated plants of this genotypic constitution. From the seeds plants homozygous for male sterility developed. These findings show, that a certain degree of restoration of the fertility can occur under the influence of this growth regulator but it is not possible to suppress the action

of the *ms*-gene completely and permanently. 10 different *ms*-lines of barley were studied by Kaul and Singh (1966, 1968) and Singh and Kaul (1966). Male sterility of all of them appears to be genetically conditioned. All the anthers of the respective mutants do not dehisc. They are small and shrivelled containing empty pollen grains having no cytoplasmic contents. Their walls are shrunken. The plants are female fertile showing a seed set of about 95% when crossed or hand-pollinated. Some of these mutants were cytologically analysed. The lines *ms*-B3 and *ms*-B6 were isolated from two-row barley. In *ms*-3, two types of cytological anomalies concerning PMCs and tapetum cells are regularly observed within the same plants. In the uppermost 3-4 spikelets of the spikes, the tapetum tissue degenerates before meiosis begins. A little later, degeneration of the PMCs occurs. Anthers developed in the lower parts of the ear show a different action of the gene. The PMCs are arranged on the periphery of the pollen sacs. Meiosis begins, but there is no continuation of microsporogenesis because of the disintegration of the nuclei of the PMCs. Breakdown of the tapetum tissue occurs in these anthers considerably later, when the PMCs have completely degenerated.

In line *ms*-B6, there is obviously a normal meiotic behaviour up to tetrad stage. PMCs increase enormously; furthermore, a high amount of vacuolation takes place pushing the nucleus towards the periphery of the cell. Degeneration of the PMCs occurs approximately together along with that of the tapetum tissue at the end of the second meiotic division resulting in the formation of a mass of disintegrating tissue.

In six-row barley, the two male sterile lines *ms*-B1 and *ms*-B4 were isolated. Degeneration in the PMCs takes place at the end of microsporogenesis when microspores leave the PMCs. Also in these cases, tapetal degeneration follows immediately after the degeneration of microspores takes place. The tapetum cells are highly vacuolated having a reduced amount of cytoplasm. Moreover, a lack of acid phosphatase activity was observed. Obviously, the tapetum tissue is not fully functionable. This will probably be the cause of the breakdown of microsporogenesis.

Triticum aestivum, *T. durum*

Pugsley and Oram (1959) reported about male sterile plants of *Triticum aestivum* that they obtained in crossing-experiments in Australia. This male sterility is conditioned either by a single recessive gene or by two complementary loci. The data available do not permit definite conclusions. Moreover, the expression of the sterility is considerably influenced by seasonal and environmental factors. According to Waninge and Zeven (1968), Pugsley's male sterile plants are not completely euploid. Some plantlets investigated by the authors had chromosome numbers of 2n=41 or varying from 40-44 besides the euploid number of 42. This variation occurs obviously without regularity. The microsporogenesis has not been studied. The use of this material in cross breeding programmes was discussed by Suneson (1962). The gene or the genes of Pugsley's male sterile wheat were transferred by Briggle (1970) to "Chancellor" variety. In these experiments, the transmission of the recessive gene by the male gametes occurred at the same frequency as by the female gametes. Therefore, "Chancellor" seems to have a compatible genetic background for the expression of male sterility governed by these *ms*-genes.

Another male sterile mutant of *T. aestivum* appeared spontaneously in England. Plants homozygous for the recessive gene have a completely normal microsporogenesis but pollen development fails (Lupton and Bingham 1966). As the plants turned out to be female fertile, there is no doubt that this represents a true case of genetically controlled male sterility. A similar situation seems to be realized in a Russian

material. Also in this case, the female fertility is unimpaired (Krupnov 1968). These English and Russian mutants are incorporated in wheat breeding programmes in the field of heterosis breeding. Fossati and Ingold (1970) selected an X-ray induced male sterile mutant of *Triticum aestivum*. The plants do not differ morphologically from the fertile ones but their florets remain open at flowering. Details on the course of microsporogenesis are not given, but a microscopical examination revealed exclusively empty pollen grains in the anthers. Thus, gene action appears to become effective at the end of meiotic division or during the post meiotic stages. The male sterility of the mutant is due to a single recessive gene.

In *T. durum*, segregation for male sterility was observed in F_1-generation. The pollen grains of the respective plants were empty and agglutinated when stained by aceto-carmine. Isolated spikes did not set any seeds while open pollinations resulted in a good seed production. The full functionability of the female sex was likewise evidenced by hand pollinations of isolated spikes giving a very high seed set. Male sterility was inherited in a monogenic recessive manner; the respective gene was designated as *ms*-d1 by Bozzini and Scarascia-Mugnozza (1968). The analysis of microsporogenesis shows a normal behaviour up to tetrad stage. Consequently, the degeneration of the microspores sets in between microspore and pollen formation. The plants have obviously arisen by a spontaneous mutation.

(b) The action of dominant *Ms*-genes on microsporogenesis

So far, only very few cases are known in which male sterility is controlled by dominant genes. An elaborate example is the *Ms*-8 gene of *Gossypium hirsutum*. It causes formation of anthers of reduced size containing a small amount of non-viable pollen grains (Weaver and Ashley 1971). There is a normal course of microsporogenesis up to microspore formation. Some pollen grains are produced which resemble the normal pollen in gross morphology. It was obviously not possible to state any degenerative alterations in the microspores or pollen grains; nevertheless, the plants are completely male sterile. Another dominant gene, *Ms*-4, causes male sterility in a completely different manner. The archespore tissue is not differentiated in the rudimentary anthers; neither PMCs nor pollen grains were observed in all the plants containing this gene. Hence, it does not specifically belong to the group of genes being discussed in the present paper.

The dominant gene *F* of the genome of *Origanum vulgare* causes anther abortion. Its action can be suppressed by another dominant gene *H* which is epistatic to the sterility gene. That means, that only plants having the genetic constitution *FFhh* are male sterile. Plants containing both these genes in the recessive state are lethal (Lewis and Crowe 1952). In the orchard grass (*Dactylis glomerata*), a dominant *Ms*-gene is known showing a certain interaction with a specific cytoplasmic condition (Myers 1946). Cytological details of this material are not available.

(c) The combined action of several recessive *ms*-genes on microsporogenesis

The findings discussed so far refer to examples where male sterility is controlled by single genes. But there are cases in which the sterility is governed by a joint action of two or more genes or by an additive action of several genes. These cases are described in the following section.

Arachis hypogaea

In the ground nut, two genes are known which possibly belong to the group of *ms*-genes. Plants homozygous for both these genes are dwarf and show some other additional morphological anomalies. They develop only a few flowers having empty pollen grains (Patel *et al* 1936).

Unfortunately, no details on the course of microsporogenesis are given; therefore, it is not possible to ascertain whether the respective genes are comparable with the typical *ms*-genes of other species. Moreover, these genes influence also the differentiation of the female sex organs, the stigmas being undeveloped. According to the segregations, two different recessive genes *n-1* and *n-2* are responsible for the sterility. They are possibly identical with two genes causing sterility reported by Hayes (1933). A detailed description of these West African sterile plants is not given. A similar situation is reported by Katayama and Nagamoto (see Ashri 1968). It would be necessary to know some details about the macrosporogenesis of the plants in order to classify specifically the genes involved. The male sterility could be due to a pleiotropic *ms*-gene influencing not only microsporogenesis but also the development of the stigma. This interpretation is only valid if macrosporogenesis remains undisturbed. The same effect is expected as a consequence of the action of *as*- or *ds*-genes with an additional pleiotropic influence on stigma development. In this case, the situation could not be considered as a typical example of male sterility. Hence, an analysis of the macrosporogenesis is necessary to ascertain the cause of sterility. The usual test by utilizing the plants as female parents in cross pollinations cannot be used in the present material because of the misdifferentiation of the gynaecium.

In crossing experiments, Ashri (1968) found some dwarf F_2-plants with certain morphological anomalies showing complete male sterility. Their anthers were reduced in size and contained no pollen grains. However, the gene action is also in this case not limited to the male sex, it influences also the female sex. The plants were either female sterile or were unable to support embryo development. According to Ashri, two duplicate genes control the inheritance of this type of sterility in majority of the crosses he made. However, in one case, a third locus was found which seems likewise to be associated with this sterility. The nature of this gene, whether it operates independently or as a modifier for the genes mentioned above is not yet clear. Unfortunately, also in this case no details on microsporogenesis are available so far. Therefore, it is difficult to judge the genetic basis of this anomaly. The exact classification of this type of sterility remains undecided due to the lack of relevant information.

Another male sterile dwarf genotype showing recessive inheritance was found by Hull (1937) in a F_2-family obtained from a cross of "Virginia Runner" × "Tennessee Red". In other families of the same cross, the male sterile plants did not appear. This indicates the possibility that a gene not directly related with male sterility influences the action of the *ms*-gene. Cytological details on the meiotic behaviour of this genotype are not available.

The above given findings demonstrate that the genetic and cytological background of the phenomenon of male sterility in *Arachis hypogaea* is not clear. This may partly be due to the complicated phylogenetic status of this tetraploid species. A thorough cytological analysis of the material would certainly contribute in understanding the nature of this type of sterility.

Medicago sativa

In *M. sativa*, male sterility was found to be associated with certain tapetal anomalies. Childers (1952) used a male sterile mutant (*P-1-3*) in crosses with fertile counterparts. In F_2, he obtained two different types of steriles: plants having complete male sterility and plants with partial sterility. The complete sterile plants exhibit an extremely irregular microsporogenesis coupled with specific anomalies in tapetal growth and development. Prior to the initiation of microsporogenesis, the tapetal cells enlarge considerably and become richly cytoplasmic. Simultaneously, a

strong vacuolation of the cytoplasm of the archespore cells is observed. The degeneration of most of the PMCs takes place during leptotene and zygotene.

At this time, nucleoli and weakly staining chromatin material are observed as remnants of the microsporocytes. The action of the gene or the genes, respectively, on the functioning ability of the tapetal tissue varies considerably as far as the time and intensity is concerned. In anthers showing only a slight degree of tapetal alteration, a few PMCs undergoing MI were observed. Some of the microsporocytes were able to go through the whole course of meiosis forming thick walled pollen grains. However, they cannot be used for pollination because the shrunken anthers do not dehisc. It is not mentioned whether these pollen grains are capable for fertilization if applied to the stigma artificially. The anomalies of the fully male sterile plants are obviously due to the action of two recessive genes giving either a 3:1 or a 15:1 segregation. The genes are designated as *ms-1* and *ms-2*.

In the partially male sterile plants, a normal course of meiosis up to the formation of young microspores is observed. Degeneration occurs at this stage during which the microspores become vacuolated and shrunken. Finally, they are completely devoid of cell contents. This anomaly is not observed uniformly in all the plants belonging to this group. Microsporogenesis seems to be normal in a certain proportion of PMCs varying from plant to plant. Thus, their pollen sterility ranged from 5·5 to 81 per cent. In these plants, an atypical tapetal behaviour is found. At the initial stages of microspore growth, the tapetal cells do not elongate and touch the PMCs. Microspore degeneration initiates immediately after this abnormal tapetal behaviour. In some cases, however, the tapetum behaves normally probably resulting in the formation of functionable male germ cells. The partial sterility appears to be controlled by a group of at least three genes A_1, A_2 and A_3, the action of which is highly influenced by environmental factors.

Furthermore, another three *ms*-genes of the genome were isolated by Childers and McLennan (1960) in segregating families following hybridizations and back crossing of two fertile strains. The anthers of these plants were brown and shrunken and did not contain any pollen grains. Microsporogenesis was normal till to the end of the second meiotic division. Degeneration was set in at a very rapid pace after microspore formation. Later, the microspore walls, nuclei and the whole cell organisation got disintegrated. At the same time, the tapetum became densely cytoplasmic and a separation of the tapetum cells from the inner walls of the anther locules as well as the separation of the cells from one another was observed in contrast to male fertile plants. Finally, the whole tapetal tissue degenerated and was absorbed by other tissues of the anther. From the findings, Childers and McLennan concluded that three genes (*ms-3*, *ms-4*, *ms-5*) are involved in the male sterility of the material studied.

Vaccinium angustifolium

Out of the 24 plants of *Vaccinium angustifolium* investigated by Hall and Aalders (1961), three plants were found to be male sterile. Their flowers were similar to those of the male fertile ones but their stamens were shorter having shrunken pollen sacs that contained no pollen grains. Cross pollinations by hand gave 80-90% seed set indicating the full functionability of the female sex organs. Later, these plants were developed into clones designated as Q_2, *N68* and TH_8. The cytological examinations of the material revealed certain differences in the time and type of gene action inducing male sterility (Aalders and Hall 1963).

In Q_2 strain, the microsporocytes and the tapetum cells degenerate more or less simultaneously. In mature flowers, no trace of pollen is observed. Obviously, the action of the gene responsible for this

breakdown becomes effective at the end of microsporogenesis. In clone *N68*, the earliest signs of abnormalities in the male sex appear somewhat later when pollen tetrads are already formed. The pollen immediately degenerates and collapses to a darkly stained mass. Both, the middle wall layers and the enlarged tapetal cells begin to dissolve out. Finally, in clone *TH8*, the development of PMCs is normal up to tetrad formation. In this stage, the PMCs degenerate and not a single viable pollen grain is observed. The genetic situation of this type of male sterility is not yet elucidated. It appears that either the male sterility is due to more than one gene or that there are different types of male sterility inherited in a different manner.

Plantago lanceolata, coronopus, lagopus, ovata, major

In *P. lanceolata*, male sterility was found to be controlled by two recessive genes (Ross 1969). A male sterile plant was found in a natural population in Berkshire, England. In its descendants, segregation ratios of 1:1, 3:1, 7:1 and 15:1 were observed. Furthermore, divergent results were obtained in the offspring of other male sterile plants indicating the presence of some other additional factors influencing the inheritance of male sterility in this species.

The phenomenon of male sterility is also known in *P. coronopus*. Paliwal and Hyde (1959) studied the mitotic and meiotic behaviour of this material in three successive generations. Microsporogenesis was normal but a B-chromosome was invariably observed in the somatic cells and the microsporocytes. Not all the microspores received the B-chromosome, but in every case they degenerated before pollen mitosis took place. The shrivelled anthers contained only the walls of the PMCs. The degeneration of the microsporocytes hindered fertility, but it did not influence the propagation, because the male steriles turned out to be apomictic. It is not yet known whether the male fertile plants are also apomictic. Ross (1970) investigated the material of *P. coronopus* obtained from Hyde. He found two types of male sterile plants. In one type, the anthers were extremely small and were either not at all or only to a small extent exserted. They produced very little or no pollen grains. This is obviously a case of functional male sterility. As no meiotic studies were carried out, a definite decision regarding this type of sterility cannot yet be made. In the second type, the anthers were exserted but they produced likewise very little pollen as compared to the fertile plants. The genetic base is not clear. From the available crossing data, the action of cytoplasmic factors cannot be ruled out.

Furthermore, male sterility has been observed in *Plantago lagopus* (Knuth 1909), *P. ovata* (Chandler 1954) and *P. major* (Ross 1970). As the sterility of the above material has not been cytogenetically analysed in detail, it is not possible to make reliable conclusions on the genetic systems controlling the anomaly.

Solanum verrucosum

In the diploid Mexican wild potato *S. verrucosum*, an interesting type of gene-controlled male sterility was found by Abdalla and Hermsen (1972). Independent, isolated pollen grains are not formed in these plants; on the contrary, the pollen-like structures produced represent the whole microsporocytes. In the cell walls surrounding these structures, four "points" resembling the pores of normal pollen grains are observed. This anomaly is designated as "undivided microsporocyte sterility" by the authors and is probably conditioned by the two recessive genes *um-1* and *um-2*. This gene action is similar to that described for mutants no. 71A, 78 and 98A of *Pisum sativum*. This is obviously a case of homologous mutations: homologous genes belonging to the genomes of different genera that cause similar anomalies in microsporogenesis when present in the mutated state.

In the same species, two more types of male sterility have been found by Abdalla and

Hermsen (1972). The "ordinary sterility" is characterized by the formation of shrunken, irregularly shaped pollen grains. In plants showing "bubble sterility", the cytoplasm of the pollen degenerates. For both these cases a polygenic control is assumed.

Triticum aestivum

Male sterility in different *Triticum* species has already been discussed in Section "1a" of the present paper. In these cases, the anomaly is due to the action of single recessive genes. Furthermore, a specific type of male sterility in *T. aestivum* seems to be due to the joint action of several genes. In India, male sterile wheat plants were found in an F_2 population showing a wide range in the expression of this anomaly (Athwal *et al* 1967). In the PMCs, there was a normal course of meiosis. Nevertheless, many mature anthers possessed only few shrivelled and non-stainable pollen grains. This can, however, not be generalized for the whole material because the pollen fertility of different male sterile plants in segregating F_2-families varied between 1·5 and 43·8 per cent. No meiotic anomalies or chromosomal aberrations were observed which could be the cause of the sterility or extremely reduced fertility. On the other hand, the degeneration of PMCs, microspores or pollen grains characteristic for most of the *ms*-genes was not observed in this material. From the findings reported, it can be concluded that degeneration occurs probably after the end of microsporogenesis. The male sterile plants show normal seed set when pollinated by fertile varieties. Thus, the female sex organs of the plants are fully functioning. After selfing, a seed set ranging from 1-8 per cent was observed while the corresponding values for open pollination varied between 45-71 per cent. According to the segregation ratios, only one recessive gene seems to be involved. However, the genetic basis of this type of male sterility is not so simple. It is obvious, that the male sterile plants studied are not genetically uniform; on the contrary, they represent different classes having a different genetic background. Moreover, the action of the genes involved is highly influenced by environmental factors not yet analysed. According to a hypothesis given by Athwal *et al* (1967), three major genes with additive effects are operating in these groups. Plants homozygous for all the three genes show a high amount of sterility. The smaller the number of genes present, the less is the expression of sterility. This indicates that the phenomenon of male sterility is controlled by an additive polymeric system in this case.

(2) THE INTERACTION OF *ms*-GENES AND CYTOPLASM

All the examples given in the first part of the present paper refer to cases in which male sterility seems to be exclusively controlled by genes. In some of these cases, there may be a co-operation of the respective genes with a specific cytoplasm but this interaction has not yet been evidenced. In those cases, in which the cytoplasmic influence is very weak it will be arduous to discern this type of co-operation. On the other hand, this interaction is distinct in certain species that are being discussed in the following.

Allium cepa

In the common onion, three different aspects concerning the problem of male sterility have been studied, namely, the cytogenetics of male sterile genotypes, the frequency and distribution of these genes in various cultivars and the effect of temperature on the expression of male sterility.

The cytogenetics of male sterile onions has been studied by Monosmith (1926), Jones and Emsweller (1936), Jones and Clarke (1943), Tatabe (1952), Kobabe (1958), Lichter and Mündler (1961). Early in 1925, a sterile plant of an Italian Red Onion was investigated in California having a normally functioning female sex (Jones and Emsweller 1936). The meiosis of this genotype was regular. The first indication of abnormality was the appearance of hypertrophy in a few centrally located tapetal cells; they degenerated in an abnormal way. Immediately after this, the microspores degenerated. At the time of anther

dehiscence, the contents of the pollen sacs remained cemented together (Monosmith 1926). The results of crossing experiments demonstrated that male sterility results from an interaction between a recessive gene and a specific cytoplasmic factor Jones and Clark 1943). All plants with normal *N*-cytoplasm irrespective of their genetic constitution produce viable pollen. Plants homozygous for the *ms*-gene in combination with the *S*-cytoplasm are male sterile. Thus, the relations between the genomic and cytoplasmic factors in this material are as follows:

N Ms Ms:	male	fertile,
N Ms ms:	"	"
N ms ms:	"	"
S Ms Ms:	"	"
S Ms ms:	"	"
S ms ms:	male	sterile

Biochemical analyses of the male sterile line Italian Red 13-53 and of their hybrids with some other *Allium* species revealed clear differences between fertile and sterile anthers with regard to the synthesis of nucleic acids (Saini and Davis 1969). According to their results, the degeneration of the microspores is due to an insufficient RNA and DNA content in the anther loculi. Furthermore, the anthers of the *ms*-plants are unable to utilize the specific nucleotides thymidine and uridine required for RNA and DNA synthesis.

Tatabe (1952), who analysed Japanese material, found a similar meiotic behaviour. The *ms*-gene becomes effective at a somewhat later stage. Microsporogenesis is normal up to the liberation of the microspores from tetrads but pollen mitosis does not take place. Simultaneously, the tapetal cells behave abnormally. Their walls break down and the cell contents fuse to form a periplasmodium that soon degenerates. At this stage of development the degeneration of the microspores takes place. In three New Zealand selections, recessive genes for male sterility interacting with *S*-cytoplasm have been studied by Yen (1959). The plants produce a small amount of stainable pollen grains whose nuclei were undergoing degeneration. These plants when selfed were unable to produce any seeds. As no detailed cytological analysis has been made, it is not known whether the majority of the PMCs degenerate in earlier stages of microsporogenesis or whether the total number of PMCs in the anthers is extremely reduced under the influence of the gene. The interaction of the specific genes and the cytoplasm is similar to that found by Jones and Clarke (1943).

A similar cytogenetic behaviour was found in some American and German mutants. Microsporogenesis of these male sterile plants is normal. Degeneration takes place in the young pollen grains. At that time, the tapetum is still normal in appearance. A very careful work on the cytogenetics of male sterile onion plants has been done by Kobabe (1958). He investigated the German variety "Zittauer Gelbe". The microsporogenesis of the male sterile plants is normal up to the tetrad stage and the early stages of pollen formation. The pollen grains shrivel when they get freed from the wall of the PMC. Occasionally, a small proportion of viable pollen grains is indicating that the phenomenon of male sterility is influenced to a certain extent by environmental factors in this material. Selfing of these flowers leads to a small seed set while abundant seed production occurs when cross pollinated.

Comparative investigations of the tapetum of sterile and fertile plants revealed distinct differences in the tapetal behaviour. In the fertile plants, the dissolution of the tapetal cells begins after tetrad formation in the PMCs. When the pollen grains have been liberated from the PMC wall, only the remnants of the tapetum tissue are present. This degeneration is obviously a necessary prerequisite for the nutrition of the PMCs and the normal course of microsporogenesis. In the male sterile plants, the tapetum remains intact for a longer period. Its degeneration begins only when the pollen grains begin to shrivel and when their cytoplasmic contents are

dead. Also the endothecium shows an abnormal behaviour in so far as the formation of the opening mechanism of the thecae is delayed. According to Kobabe, male sterility of this material is not a consequence of a missecretion of the tapetum but of the delay of an important nutritional function of this tissue. American male sterile onions show the same behaviour in all the details.

The male sterility of these plants is due to the action of a single recessive gene acting in co-operation with *S*-cytoplasm. However, the frequency of the *ms*-allele in the crossing populations is very high in relation to that of the dominant *Ms*-allele. The same situation was found and interpreted by Jones and Clarke (1947) for American male sterile onions. The increased frequency of the *ms*-allele is possibly due to a slight inbreeding effect. Male sterile plants seem to be of wide occurrence in German as well as in American varieties. Their origin is the result of two independent mutational events: namely, (*i*) the cytoplasmic factor *N* mutates to *S* and (*ii*) a nuclear gene *Ms* mutates to *ms*. The chance combination of both these events results in male sterility.

Similar findings were obtained by Lichter and Mündler (1961). They found the gene *ms* to be present in 7 out of 8 German and American varieties tested. Two types of plants among the male steriles were observed. The first group comprises of completely sterile plants having shrivelled pollen grains which are not stained by acetocarmine. The second group consists of plants in which a small proportion of pollen grains are more or less stainable. Sometimes, the remnants of the nuclei are discerned, but they are not viable and are not comparable with the "viable appearing pollen grains" in the material described by Barham and Munger (1950). The authors are of the opinion, that the two groups are not fundamentally different from each other. The differences just mentioned are obviously only due to a delayed time of degeneration in the second group. Furthermore, the authors found in male fertile plants a clear dependence of the degree of pollen fertility upon environmental factors, particularly upon the temperature. In the male sterile plants, such a dependence could be observed only to a very small extent. Considering the findings obtained by Lichter and Mündler 1961), it is conceivable that apart from the combination

S-cytoplasm/*ms ms*-genes

there may be still other factors existing that influence the degree of male fertility.

The spontaneous occurrence of male sterile plants in different varieties of the species was studied by a number of investigators who obtained widely varying results. Little *et al* (1944) found that 25 out of 29 commercial varieties possessed genes of this group. In the German variety "Zittauer Gelbe", the proportion of male sterile plants varied between 1 and 2·9 per cent (Kobabe 1958). In contrast to this relatively high frequency, the proportion of male sterile onions was only 0·03 per cent in the Dutch varieties "Primeur" and "Wijbo" (Banga and Petiet 1958). In the variety "Scott County Globe", frequencies of 0·84 and 0·96 per cent of male sterile plants were found in field trials (Peterson and Foskett 1953). In the last mentioned material, obviously no cytoplasmic factors are involved. Davis (1957) collected onions from France, The Netherlands, England, Russia, Turkey, Syria, India, Japan, Korea, New Zealand and South Africa. He found that genes for male sterility are present in the material collected from all the above mentioned countries except that of South Africa. Most of the introductions were not homozygous for the *ms*-genes. The material from Turkey shows more genetic variability than that from any other country investigated so far (Davis 1958). The great frequency of *ms*-genes even in small samples indicates that the spontaneously mutated genes of this group are widespread. The type of sterility is the same than that reported by Jones and Clarke (1943). It is not yet known, whether the same gene is present in these populations or whether the genetic diversity mentioned above is due to a relatively high number of different *ms*-genes of the

genome as known in tomato and pea. According to Davis (1958), some of the genes seem to be identical with those reported by Jones and Clarke (1943). Furthermore, the frequency of genetically controlled male sterility was studied in other selections of some commonly grown onion varieties in The Netherlands. In the widespread variety "Rijnsburger", the frequency of the genetic factors governing male sterility was 0·95 per cent while the cytoplasmic factor *S* was found in a frequency of less than 0·01 per cent (van der Meer and van Bennekom 1971). According to van der Meer (1970), the frequency of *ms*-genes in the Dutch varieties "Rijnsburger" and "Noordhollandse Strogel" is essentially higher than that reported by Berninger (1956) for French and by Pienaar (1958) for South African material.

Investigations concerning the expression of the *ms*-genes were carried out by Barham and Munger (1950) using different commercial strains and the male sterile line already studied by Jones and Emsweller (1937). The male sterile plants were grown at temperatures ranging between 21-26·5°C. The experiments show, that the temperature has a profound influence upon the male sterility of the plants. It acts during the developmental stage between microspore formation and pollen mitosis. Below 21°C, no viable pollen grains are formed. At 21-26·5°C a few pollen are produced which germinated on sugar-agar medium. However, no seeds were obtained when these plants were selfed. This failure could either be due to a physiological weakness of the pollen or due to non-deshiscence of the anthers. Differences in the genetic background do not influence the expressivity of the *ms*-genes studied. The effect of temperature on the expression of male sterility was likewise studied by van der Meer and van Bennekom (1969). Moreover, these authors grafted male sterile flower stalks on male fertile plants in order to determine whether "cytoplasmic male sterilizing substances" can be transmitted. This transmission was not observed (van der Meer and van Bennekom 1970).

Allium porrum

In a frequency of 10^{-4}, male sterile plants were found in *Allium porrum* by Schweisguth (1970). The genetic situation of this male sterility is especially complex because the species is basically a tetraploid. The genetic background is not yet completely clear. According to some segregations, single recessive genes appear to be involved, while in others, 2-3 genes, not linked with each other, were found to control the sterility.

Daucus carota

Genes influencing the male fertility of carrots have already been described in section "1a" of the present paper. An intensive study of the inheritance of male sterility in wild and cultivated carrots has been made by Thompson (1961) using diverse sources. He found an interaction of two dominant genes designated as *Ms-1* and *Ms-2* that interact with a cytoplasmic factor *S* influencing the male fertility in the following manner:

S/*Ms Ms*:	male sterile,
S/*Ms ms*:	" "
S/ *ms ms*:	male fertile,
N/*Ms Ms*:	" "
N/*Ms ms*:	" "
N/*ms ms*:	" "

The *Ms*-genes can only act in the presence of *S*-cytoplasm while they are rendered ineffective with *N*-cytoplasm. According to the segregation ratios, the possibility of involvement of three or four dominant genes cannot be ruled out. Cytological details concerning the meiotic behaviour of the plants are not available.

Sorghum vulgare

Male sterility in *S. vulgare* is not only genetically conditioned but like in other species it is also a cytoplasmic phenomenon which will not be discussed in the present paper. However, it seems, that specific genes of the *Sorghum* genome influence the cytoplasmic male sterility. The exact mechanisms of this interaction is not yet fully understood, but it appears that more than

two genes are involved (Stephens *et al* 1952, Stephens and Holland 1954). Also Maunder and Pickett (1959) found a gene *ms-c* interacting with "factors" causing cytoplasmic male sterility. Plants homozygous for *ms-c* undergo normal meiosis and pollen grains are formed. They are, however, non-viable and cannot be used for fertilization. A similar behaviour has been reported by Mittal *et al* (1958) in a single *Sorghum* plant of an Indian variety.

Beta vulgaris

In sugar beets, the interaction between cytoplasm and *ms*-genes is even more complicated because two recessive genes are involved. Investigations carried out by Owen (1942) show that two different types of cytoplasm are present in the material studied. *N*-cytoplasm permits the production of functionable pollen grains while *S*-cytoplasm renders the plants male sterile. The pollen development in *S*-plants is influenced by the two recessive genes *x* and *z* showing complementary effects. The possibilities of interaction and their effects can be visualized from the following data:

S/xx zz: Male sterility with white empty anthers.

S/Xx zz, S/xx Zz, S/XX zz, S/xx ZZ: Partially male sterility with yellow anthers; little or no viable pollen.

S/Xx Zz, S/XX Zz, S/Xx ZZ, S/XX ZZ: Male fertility; more or less normal pollen development influenced by environmental conditions.

From the crossings of Owen's material with other male fertile genotypes, a more detailed analysis of genes *x* and *z* was carried out by Bliss and Gabelman (1965). They confirmed Owen's findings with regard to gene-cytoplasm co-operation. Furthermore, they found that the two genes are not linked with each other. The dominant allele *Z* is hypostatic to *X* and restores partial fertility to plants having *S*-cytoplasm. Plants containing *N*-cytoplasm are male fertile regardless whether they contain the male sterility genes or not.

Later, Owen (1952) found another recessive gene designated *a* responsible for male sterility in *Beta vulgaris*. In the male sterile plants at bud stage, the flowers have white to greenish shrunken anthers that turn brown at anthesis. They are completely devoid of pollen. The female parts of the flowers are functionally normal. Theurer (1968) studied the linkage of this gene with 8 other genes of the genome.

Crotolaria mucronata

Kempana and Sastry (1958) found a male sterile plant in a normal population of *Crotolaria mucronata*. The progeny of this genotype was studied by Edwardson (1967), who found a normal course of meiosis but the pollen produced were non-viable. The male sterility was found to be controlled by two recessive genes, *ms-1* and *ms-2*, that interact with a specific type of cytoplasm. The restoration of the fertility is already obtained when one of the two *ms*-genes are present in the dominant state.

Discussion

The manifold findings described in the empirical part of the present paper demonstrate clearly that the genetically conditioned male sterility is not a uniform phenomenon. The original concept that all the *ms*-genes act principally in a similar way and distinguish each other only with regard to their time of action during microsporogenesis appears to be too narrow. So far, at least four different groups of male sterile genotypes are known. Male sterility caused by a breakdown of microsporogenesis or of post meiotic stages can be due to: (*i*) the action of single recessive genes; (*ii*) the action of single dominant genes; (*iii*) the joint action of several recessive genes; (*iv*) the co-operation of recessive *ms*-genes with a specific type of cytoplasm.

Instances for these four groups are given in the empirical part.

The genetic control of the whole process becomes particularly clear when a very large number of genes belonging to these four groups is considered. Unfortunately, many of the male sterile genotypes of different species are not analysed cytologically and/or genetically. Therefore, many *ms*-

genes existing since several decades in plant populations cannot reliably be arranged in the whole system because their time of action during the course of microsporogenesis is not exactly known. Similar uncertainties exist with regard to the joint action of *ms*-genes. Nevertheless, 99 *ms*-genes of 48 species belonging to 12 families have been analysed in detail with regard to their influence on microsporogenesis. They are diagrammatically represented in figure 3 in which they are arranged according to their time of action during microsporogenesis. The individual names of the species are not given since the main purpose of the figure is to give a general survey on the action of the majority of the *ms*-genes known so far. The species are grouped together according to their taxonomic position. It is obvious that most of the *ms*-genes influence the final stages of meiosis between interphase II and pollen formation. In all these genotypes there is a completely normal course of microsporogenesis till to the stage at which the degeneration of nuclei, microspores or pollen grains sets in. A second, smaller group of *ms*-genes acts during the early and midd

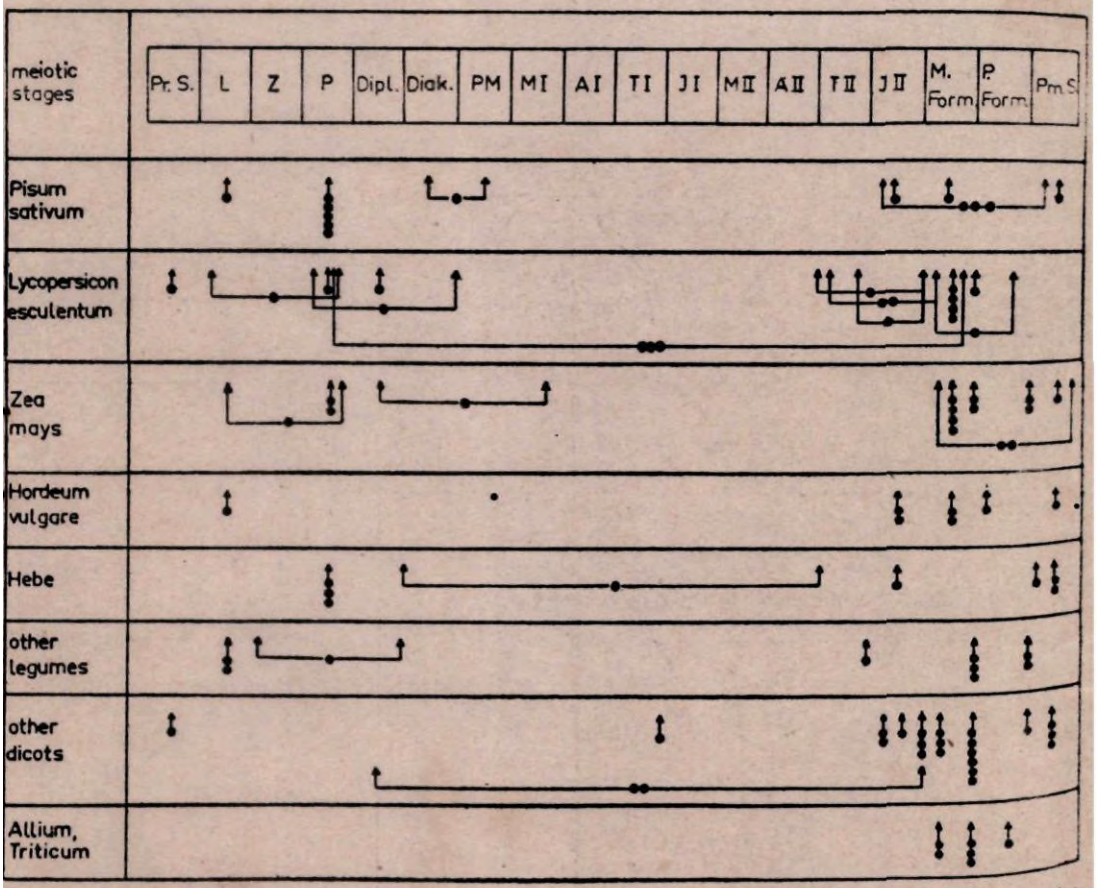

Fig. 3: The time of action of 99 different *ms* genes of different species during microsporogenesis. Each dot represents one specific gene and the arrows the range during which it is effective. Pr. S = premeiotic stages, M. Form = microspore formation; P. Form = Pollen formation, Pm. S. = Postmeiotic stages.

stages of the first meiotic prophase preventing further meiotic process and pollen formation. It is surprising, that only a very small number of *ms*-genes becomes effective between diakinesis and anaphase II. Undoubtedly, this distribution of the action of the *ms*-genes represents a specific regularity, but at the present state of the analysis of the whole phenomenon no well founded explanation can be given. The distribution of the action of the *ms*-genes upon the various stages of microsporogenesis becomes especially clear from figure 4.

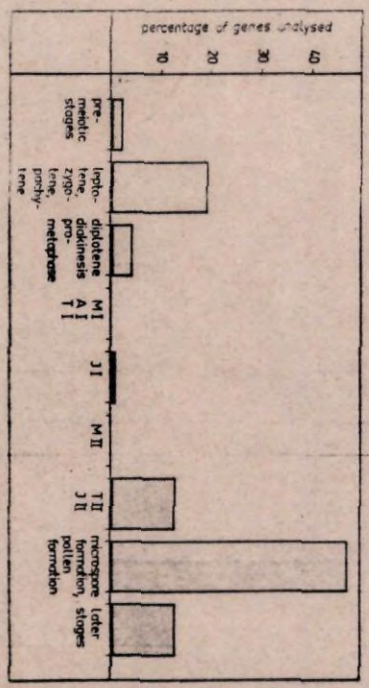

Fig. 4: Distribution of time of action of 93 *ms*-genes of different species during microsporogenesis.

It can be assumed, that *ms*-genes are present in the genomes of all the flowering plants irrespective of their taxonomic position. Moreover, there is no doubt, that some of these genes represent "homologous" genes in an evolutionary sense leading to similar meiotic anomalies in different species when present in the mutated state. The "homology" can clearly be discerned in those cases in which the respective genes express a very specific pleiotropic spectrum.

From comparative developmental and biochemical studies of the anthers of male sterile and fertile plants it has been concluded, that there are close physiological correlations between the degeneration of the tapetum and the course of microsporogenesis. However, the exact nature of this dependence is not yet fully known (Singh 1965, Echlin 1971). It was already assumed by Rick and Butler (1956) that the primary action of the *ms* genes is not directed to the pollen mother cells but to the tapetum. In plants having genetically controlled male sterility, much diversity is exhibited in the time and type of tapetal degeneration. The correlative breakdown of tapetum, microsporocytes, microspores or pollen grains is not realized in all the cases analysed so far. In male sterile *Dactylis glomerata* mutants, for instance, tapetum degenerates at early stages of meiosis but microsporogenesis continues till to microspore formation. In *Brassica oleracea*, tapetal breakdown does not occur even after the pollen breakdown is completed. In partially male sterile plants of *Medicago sativa*, microspore degeneration initiates immediately after abnormal behaviour of tapetum is observed. The *ms*-mutants of *Hordeum vulgare*, finally, show a strikingly variable behaviour with regard to the degeneration of the two tissues. Already these few examples show a very heterogeneous situation in male sterile plants with regard to the correlative breakdown of sporogenous and tapetal tissue. Hence, a more detailed analysis of the two phenomena in a large number of different male sterile mutants of different species is necessary before well founded interpretations can be given.

The genetically conditioned male sterility in higher plants can also be due to genes which influence the functioning ability of the male sex organs without influencing microsporogenesis. The dominant gene *Ms-4* of *Gossypium hirsutum*, for instance,

causes complete male sterility due to the formation of rudimentary anthers or to failure of anther formation (Allison and Fisher 1964). In the rudimentary anthers, the archespore tissue is not differentiated. As no other tissues are able to take the function of the archespore, microsporogenesis cannot take place and no male germ cells are formed. The action of this dominant gene is comparable to the action of a recessive gene of *Pisum sativum* (mutant 2228; Klein and Milutinovic 1971). The lack of stamen differentiation or of the formation of rudimentary anthers as the consequence of the presence of a mutated gene is also known in *Lycopersicon esculentum* (Larson and Paur 1948, Bishop 1954, Rick 1966, Phatak *et al* 1966), *Cucurbita pepo* (Shifriss 1945), *Cucumis sativus* (Barnes 1961), *Nicotiana tabacum* (Raeber and Bolton 1955), *Origanum vulgare* (Lewis and Crowe 1952) among others. Moreover, a "functional male sterility" can be realized by a transformation of male into female sex organs known in *Pisum sativum* (mutant 192; Gottschalk 1961); further examples are given by Gottschalk (1971). Since we are primarily interested in the *ms*-genes influencing microsporogenesis, the phenomenon of "functional male sterility", was not considered in the present paper.

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