MALE STERILITY AND POLLEN SIZE IN THE GENUS MENTHA (MINT)*

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Sterility and pollen size, in five pure species namely: *Mentha pulegium L., Mentha aquatica L., Mentha longifolia* (L.) *Huds., Mentha spicata L., Mentha arvensis L.*, and six interspecific hybrids namely: *M. piperita L. (M. aquatica X M. spicata); M. niliaca Juss. ex Jacq. (M. longifolia X M. rotundifolia); M. alopecuroides Hull (M. rotundifolia X M. longifolia); M. verticillata L. (M. aquatica X. M. arvensis); M. dumetorum Schult (M. aquatica X M. longifolia)*; *M. gentilis L.* M. (spicata *X.M. arvensis*) have been studied. All the hybrids show a complete male sterility while fertility in the various pure species varies from 20 percent to 80 percent. Degeneration of male reproductive organ was found to be a constant feature in the hybrids. Stages from complete anther-abortion to those bearing normal-looking but unstainable pollen grains were noted in the hybrids. In all those hybrids where anthers were at all formed, pollen grains were found to be invariably of a dwarf size.

Introduction

Mentha is an important genus both medicinally and economically. For this reason, it is under wide cultivation wherever the climatic requirements are met, besides occurring wild almost throughout the world. Its polymorphic nature accompanied by high polyploidy and varying essential oil contents provides an interesting material for cytological and pharmacognostic studies.

Material and Method

The plant material used for the present study was collected from Schleswig-Holstein (N.W. Germany) and 415 plants distributed over 110 population were transplanted and cultivated under uniform conditions to avoid variations caused through environmental factors. All the pots before bringing them out to the plot of cultivation in the garden were kept in the green house for 30 days at a temperature of 15°C.

For the study of the pollen grains, the fluid used by Reese [1] was tried with good results. The pollen grains were kept on a slide having this fluid (4 parts of alcohol-acetic acid-chloroform-mixture in the ratio of 6:3:1; 7 parts of rhenohistol and 1 part of carmine stock solution). The fluid works at the same time as a fixative, staining and mounting material and produces nice permanent mounts. Pollen grains which took deep stain have been considered as fertile.

Results and Discussion

The genus *Mentha* displays a very high percentage of sterility. The exact cause of this sterility is only partly known, but all of them suggest some kind of genetic unbalance which affects hybrid inviability. The irregular meiotic behaviour of the chromosomes in the hybrid species of this genus resulting in inversion bridges and laggards suggest chromosomal type of sterility. Such sterility results from the structural difference between chromosomes which reduces the probability of pairing and thus causes various kinds of irregularity as defeciency, duplication and inversion. Chromosomal sterility is more common amongst the hybrids of the related species than the genic sterility (Stebbins).[2] However, it is not always easy to decide whether the primary cause of sterility is a disharmonious interaction of genes or the structural differences in the chromosomes. Ofcourse in some cases both causes might be acting simultaneously. According to Gajewski [3] the failure of pairing in plant hybrids is often due to the combined effects of structural differences and genic unbalance.

The sterility in the interspecific hybrids may be associated with degeneration of sexual organs (male, female or both) or of the entire floral parts. In Angiosperms, male sterility is generally more common than the female sterility. A typical example of male sterility can be found in the interspecific and intraspecific hybrids of the genus *Mentha*. In practically all the hybrids studied such as *M. piperita L., M. niliaca Juss. ex Jacq., M. alopecuroides Hull, M. verticillata L., M. gentilis L.* and *M. dumetorum Schult*, the male sterility was found to be 100 percent. The hybrids studied comprised not only the crosses of the first order but appeared to be intercrosses and back crosses with either of the two parents and showed a variety of intermediate forms. In almost all cases female reproductive organ appeared to develop normally. An embryological study however has not been pursued in the present work.

Developmental Variation in Degeneration of Male Reproductive Organs.—Degeneration of the male reproductive organ of the hybrids which was a consistent feature varied from one type to another.

*This work was carried out in the Department of Botany, University of Kiel, West Germany.

It was found to occur at different stages of the development of anthers and has been classified as follows. (1) No stamens were formed at all; (2) Anthers develop but they do not show any differentiation of archesporial or tapetal tissue; (3) Anthers develop but the division of the pollen mother cells does not proceed beyond diplotene stage; (4) Development follows upto tetrad formation but pollen does not mature further and degenerates; (5) Pollen grains are formed but there is a preponderance of dwarf pollen grains which are unstainable, thick-walled and without nucleus; only a very small percentage being normal-looking.

Sterility and Pollen Size in Pure Species.—The so called pure species as *M. aquatica L.*, *M. arvensis L.*, and *M. longifolia* (*L.*) *Huds.* show a sufficiently high percentage of fertility as compared to hybrids although in no case was it found to be over 80 percent *M. pulegium L.*, which too is considered to be a pure species and is said not to hybridize easily with other *Mentha* species, showed surprisingly as low as 20 percent fertility although the meiotic cycle and the tetrad formation appeared normal. No dwarf pollen grains were observed which suggests the purity of this species. The size of the pollen grains in *M. pulegium L.* varied between 21μ-38μ, the modal value lying between 27μ-29μ (Fig. 1).

A very characteristic feature of the hybrids was the presence of dwarf pollen grains. However, the size and the percentage varied from plant to plant. Such dwarf pollen grains, which are presumed to originate from laggards in the anaphase I and II, were also reported by Schurhoff,[4] Wolf [5] and Ruttle.[6] The occurrence of such pollen grains, in the author's opinion appears to be purely due to genetical and chromosomal unbalance. Had any other factor been responsible for such anomaly it would have been constant for other pure species also but as no dwarf pollen grains have been reported from pure species, such possibility does not seem probable. The plants cultivated in the garden could stand as low a temperature as-15°C. in winter (1962) and if any outer factor is the cause, all *M. aquatica* plants would have produced dwarf pollen grains, but out of 230 *M. aquatica* plants studied only three cases with such pollen grains were noted, which in the author's opinion are not pure species but a very closely resembling hybrid of *M. aquatica*. Ruttle[6] found also a great uniformity in the size of the pollen grains from *M. aquatica.* She reported only five cases with one extra microcytes

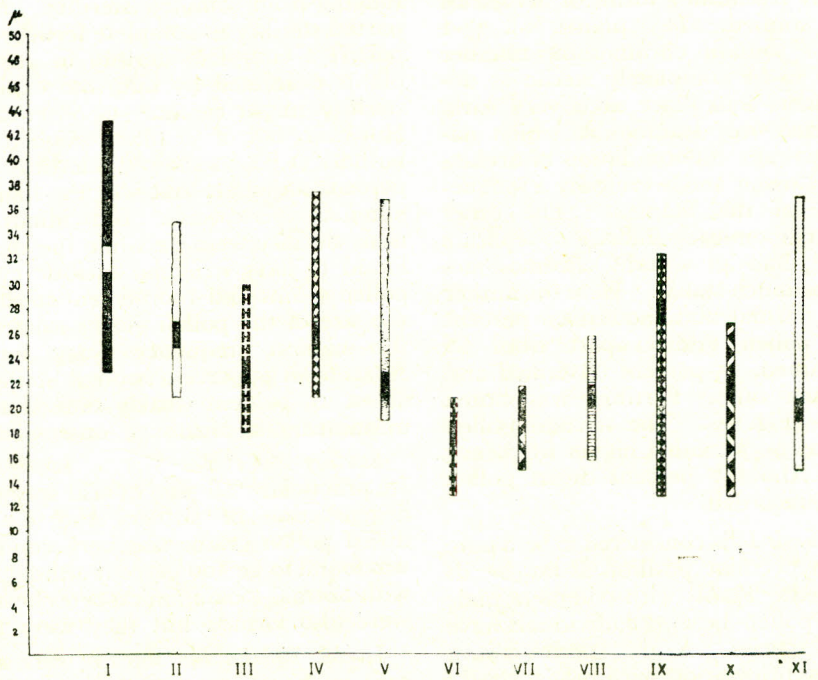

Fig. 1.—Showing the range and pollen size and frequency in Mentha sp. I, M. aquatica; II, M. arvensis; III, M. longifolia; IV, M. pulegium; V, M. spicata variety crispata (variety II); VI, M. alopecuroides; VII, M. niliaca; VIII, M. piperita; IX, M. verticillata (variety I); X, M. verticillata (variety II); XI, M. gentilis variety cardiaca variety II).

out of 200 quartets examined by her. The fertility *in M. aquatica*, on an average, was pretty high as compared to other species and was found to be fluctuating between 60-80 percent from plant to plant. The size of the pollen grains varied from 23-43μ with a higher frequency between 31-33μ. Shrunken pollen grains were found to be between 2-5 percent varying from plant to plant.

In *M. longifolia* (*L.*) *Huds.* fertility was found to be 70 percent although with a slight variation from plant to plant. In plant No. 63-4 a degeneration of anther was also noted in diplotene stage. In other plants tetrad condition was quite normal. Pollen grain was found to range between 18-30μ with a higher frequency between 22-24μ. In plant No. 31-3 and 63-3 few pollen grains with 8 Kolpa instead of normal 6 and about 5 percent dwarf pollen grains were noted. These pollen grains are of the same type as in other hybrids which suggests that these two plants are *M. longifolia* resembling hybrids. These dwarf pollen grains were about 10μ in diameter with 2 or sometimes 4 Kolpa. These plants are morphologically difficult to separate from *M. longifolia*. Morton 7 also reported some difficulty in separating the *M. longifolia* plants, he studied, from its hybrid *M. nemorosa*.

Two very closely resembling forms of *M. spicata var. crispata* were studied. Two plants No. 37-1 and 37-2 having a somatic chromosome number of 54 were found to be completely sterile as degeneration of anthers took place at a very early stage. They formed only staminodes which naturally did not have any differentiation of archesporial and tapetal tissue and were only a tongue-like prolongation of the filament. The other form, which was taxonomically difficult to separate from the first one, had 48 somatic chromosomes and was found to be much stable. Here the anther development was normal and showed 20 percent strongly stained pollen grains apart from 60 percent weakly stained, 15 percent unstained and 5 percent shrunken ones. Fertility was noted to be 20 percent (Fig. 2). The normal pollen grains ranged from 19-37μ with a higher frequency between 21-23μ. About 1 percent dwarf pollen grains were also recorded.

Although *M. arvensis L.* is considered to be a pure species, it takes a peculiar position as far as its fertility is concerned. Plants with complete male sterility to normal pollen-bearing individuals have been noted in this species. As this species is in a habit of frequent hybridization with other *Mentha* species and produces hybrids which are sometimes indistinguishable from *M. arvensis*, it is at times difficult to separate taxonomically a pure species from its hybrid. Probably it is because of this

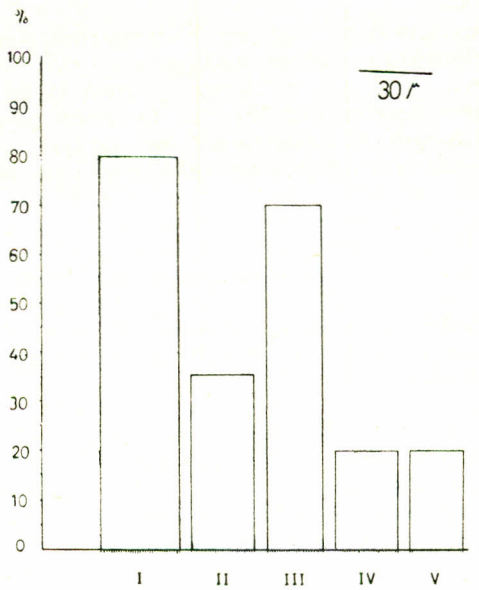

Fig. 2.—Histogram showing fertility in Mentha sp. I, M. aquatica; II, M. arvensis; III, M. longifolia; IV, M. pulegium; V, M. spicata variety crispata(variety II). The other six species, VI, M. alopecuroides; VII, M. nilaca; VIII, M. piperita; IX, M. verticillata (variety I); X, M. verticillata (variety II); XI, M. gentilis variety cardiaca (variety II) show complete sterility.

confusion, that this species has been regarded to be ranging from complete sterility—partial fertility—partial sterility to complete fertility. Schurhoff[8] reported a complete sterility in all the *M. arvensis* plants examined by him and explained that this sterility might be due to inter-racial hybridity. However, out of 52 plants studied by the present author, 31 plants showed a fertility more or less 35 percent although that was the highest percentage noted in this species. Individuals with complete male sterility were 12 while the remaining 9 were found to have irregular meiotic cycle with dwarf pollen grains and 100 percent sterility. Normally the size of the pollen grains ranged from 21-35μ, the highest frequency lying between 25-27μ. Apart from 35 percent strongly stained pollengrains about 15 percent weakly stained and 50 percent unstained and shrunken ones were also noted.

Sterility and Pollen Size in Interspecific Hybrids.—In practically all the hybrid species studied, the degeneration of anthers and the formation of dwarf pollen grains was very common. Sterility was found to be 100 percent although some hybrids with normal-looking microcytes bearing 1-2 nuclei were also formed but they were unstainable.

In *M. piperita L.* tetrads were seen in 10 percent with 1-4 extra microcytes which measured 7-8μ in diameter and were about 1/6 of the size of the bigger pollen grains. Apart from 70 percent normal-looking but unstainable pollen grains which ranged from 16-26μ with a higher frequency

between 20-22μ, 30 percent unstained and shrunken ones were also observed.

M. niliaca Juss. ex. Jacq. and *M. alopecuroides Hull*, which are regarded as reciprocal hybrids by Morton,[7] showed very little difference in pollen size and sterility. Average *M. niliaca* pollens ranged between 15-22μ with 19-21μ as higher frequency while in *M. alopecuroides* the variation was between 13-21μ. The only difference noted between the two hybrids was in the formation of degenerated unstained and shrunken pollen grains. In *M. niliaca* such pollen grains (Fig. 3) were found to be as high as 95 percent in contrast to *M. alopecuroides* which showed only 75 percent. Dwarf pollen grains in both hybrids were about 20 percent. Anther degeneration took place generally after tetrad formation or at uni-nucleate stage of the pollen grain. Dwarf pollens were mostly thick-walled and without nucleus.

In *M. gentilis L.* two varieties of plants were studied. Variety II is referable to *M. gentilis var. cardiaca*. In variety I (2n= 72) Pl. No. 56-2, no anthers were formed at all whereas in Pl. No. 56-1 and 56-3 only staminodes were formed which did not show any differentiation of archesporial and tapetal tissue. In variety II (2n= 84) anthers were fully developed and formed 70 percent normal-looking pollen ranging between 15-37μ in size besides 30 percent shrunken unstained pollen grains. The dwarf microcytes were also common (50 percent) because of the irregular meiotic cycle.

In *M. dumetorum Schult* (2n= 84) anthers were formed which were comparatively small and showed irregular meiotic behaviour. The division did not proceed beyond tetrad formation. No mature pollens were seen in any of the nine plants examined because of their degeneration in immature stage.

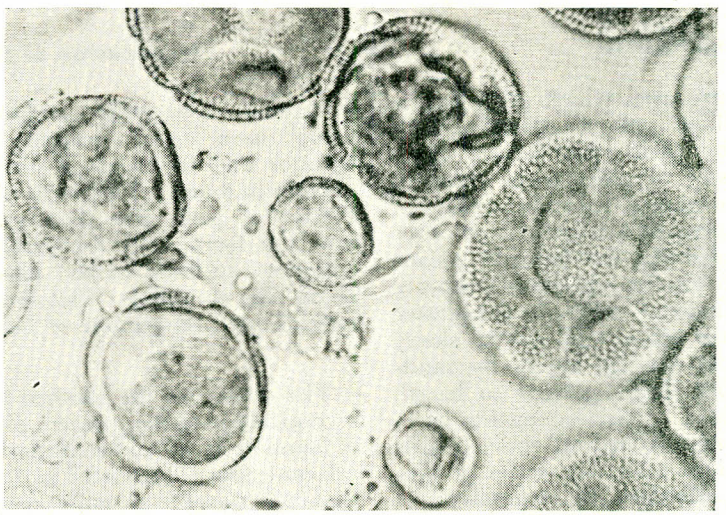

Fig. 3.—Showing comparative size of normal and dwarf pollen grains (Pl. No. 25-2) in M. niliaca.

In *M. verticillata L.* degeneration of anthers took place at various stages of its development ranging from early prophase to uni-nucleate stage of the pollen grain but this degeneration was more prominent in variety II with 120 somatic chromosomes and the percentage of dwarf pollen grains was also as high as 60 percent. Another important feature of variety II was the occurrence of 70 percent degenerated unstained and shrunken pollen grains in contrast to variety I, with 84 somatic chromosomes, where it was more or less 10 percent. These numbers were determined by Baquar and Reese.[9] The pollen in variety I ranged from 13-33μ with a higher frequency between 27-29μ, whereas in variety II the variation was much smaller being 13-27μ only with a greater frequency between 21-23μ.

References

1. G. Reese, Planta, **44,** 203 (1954).
2. G.L. Stebbins, Advan. Genet., **9,** 147 (1958).
3. W. Gajewski, Panc. Acta Soc. Botan. Polon., **23,** 259 (1954).
4. P.N. Schurhoff, Beitr. Biol. Pflanz., **15,** 129 (1927).
5. P. Wolf, Beitr. Biol. Pflanz., **17,** 351 (1929).
6. M.L. Ruttle, Gartenbauwissenschaft, **4,** 428 (1931).
7. J.K. Morton, Watsonia, **3,** 244 (1956).
8. P.N. Schurhoff, Arch. Pharm., **267,**515(1929).
9. S.R. Baquar and G. Reese, Pharmazie, **20,** (3), 159 (1965).