Cytology of megasporogenesis as a basis for sterility in autotetraploid sweetclover, Melilotus alba

Bidhan Chandra Das
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CYTOLOGY OF MEGASPOROGENESIS AS A BASIS FOR STERILITY
IN AUTOTETRAPLOID SWEETCLOVER, MELILATUS ALBA

by

Bidhan Chandra Das

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
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INTRODUCTION

The production of mature viable seed is determined by a complex series of morphological and cytological processes. Varying degrees of abnormality in one or more critical processes during the reproductive process may produce different levels of sterility.

Cytological studies of artificially produced autotetraploids are of theoretical and practical importance. A study of meiotic behavior may reveal correlations between fertility level and chromosomal behavior. Such information is particularly valuable for a comparison of the results of natural selection pressure on naturally-occurring autotetraploids, with the effects of experimental selection for fertility level on artificially produced autotetraploids.

From the standpoint of increasing seed production by raising fertility levels, a breeding program can be outlined more intelligently if the cytological behavior of the species is understood. The cytology of the male gametophyte of autotetraploids has been studied more extensively than the female gametophyte. It has been generally assumed that cytology of the latter is essentially the same as that of the male gametophyte.

An autotetraploid sweetclover, Melilotus alba, which was developed by colchicine treatment (13), was found to be low in fertility. The present study was undertaken to determine the basis for different levels of fertility in this autotetraploid sweetclover, particularly with respect to the cytology of megasporogenesis and embryo sac development.
REVIEW OF PERTINENT LITERATURE

Literature dealing with reduced fertility of artificially produced autotetraploids is very extensive. Reduction in fertility has been attributed to morphological and histological abnormalities during seed development, cytological irregularities, physiological conditions of the mother plant, presence of self-sterility genes, and various other factors. Only the most pertinent papers will be reviewed here.

Reduced fertility in induced autotetraploids has been attributed by Darlington and Kostoff (6, 15) to meiotic irregularities resulting from multivalent association of the chromosomes in synapsis. According to Kostoff (15, 16), chromosome size is an important factor in determining the fertility level of polyploids. Autotetraploids with small chromosomes have fewer chiasmata, form fewer multivalents, and for this reason are more fertile.

Baptopoulos (33) reported an inverse relationship between fertility and the mean number of quadrivalents in autotetraploid cherries. Similarly, Belling and Blakeslee (1) reported the formation of 12 quadrivalents in tetraploid Natura. Non-disjunction in diploids was rare but in tetraploids 25 percent non-disjunction was found in pollen mother cells.

Lesley and Lesley (18) found 7 to 12 quadrivalents at diakinesis in pollen mother cells of a tetraploid tomato which was derived from a cross between a double trisomic and a diploid. This tetraploid produced seed sparingly when selfed. Counts of second metaphase plates indicated
unequal chromosome distribution.

According to Matsuda (21) chromosomes of tetraploid Petunia showed a tendency to form tetravalents. Multivalent association also took place. Non-disjunction of chromosomes occurred at anaphase I, and lagging chromosomes were observed in both divisions.

Tanaka (36) reported that in tetraploid Carex, 30 percent of the gametes were unbalanced due to meiotic irregularity. The observed proportion of unbalanced gametes accounted for reduction in fertility. Tanaka also indicated that meiotic irregularities in tetraploid were due chiefly to irregular separation of the metaphase I multivalents.

Similarly, Upcott (37) found that in tetraploid tomato non-disjunction resulted in formation of more than 30 percent unbalanced gametes. It was also observed that the occurrence of non-disjunction was almost entirely dependent upon the formation of quadrivalents.

The occurrence of trivalents, tetravalents and multivalents was found in tetraploid apple by Vaarama (38). Lagging univalents also were observed in anaphase I. Elimination of univalents took place frequently. Lagging chromosomes also were common in anaphase II.

Myers (25), in a study of meiosis in autotetraploid Lolium perenne, observed the absence of orderly orientation of chromosomes on the equatorial plate prior to the initiation of anaphase I. Frequently, members of quadrivalents disjoined unequally at anaphase I. Lagging univalents, which resulted in some cases from incomplete disjunction of quadrivalents, also were found at anaphase I. The frequency of univalents at metaphase I was high and these univalents tended to lag and
divide equationaly at anaphase I and to remain in the cytoplasm at telophase I and II. The frequency of metaphase I univalents in this artificially induced autotetraploid was higher than in naturally-occurring autotetraploid grasses, such as *Dactylis glomerata*.

Relationships between chromosome behavior and seed setting have been found in orchard grass, tall oat grass, and crested wheat grass by Myers (24) and Myers and Hill (26, 27, 28). Significant negative correlations were found between seed set and increased percentages of univalents, laggards and quartets with micromolecules. Significant differences in chromosomal behavior during meiosis were found among different plants within a species and between plants of the first inbred generation of orchard grass. This indicated heritable differences in meiotic behavior among lines. The authors suggested the possibility of selection for increased fertility in these autotetraploid grasses.

Cooper (5) reported lagging chromosomes in both heterotypic and homotypic divisions of autotetraploid alfalfa (2n = 64). Chromosome behavior in macrosporogenesis was similar to that of microsporogenesis.

Meiosis in autotetraploid rye was studied by O'Mara (29), who described multivalent association, inversion or duplication bridges, univalent bridges, univalent laggards and non-laggards, irregular anaphase distribution and chromosome loss.

Jørgensen (14) found that meiosis was very regular in tetraploid *Solanum* and the chromosomes were associated as bivalents in most cases. No correlation between reduced fertility and meiotic irregularities or number of quadrivalents was found by Lindstrom and Humphrey (19),
Lindstrom and Koos (20) and Humphrey (12) in autotetraploid tomato, and by Randolph (30, 31) in autotetraploid maize. According to Randolph (32), homozygosity per se seemed to be responsible for reduced vigor and fertility of the more homozygous tetraploid maize. Randolph (32) also indicated that although meiotic irregularities may account for 10 to 15 percent reduction in fertility of tetraploids, physiological and genic disturbances resulting from chromosome doubling are more important causes of sterility.

Sparrow et al. (35) found no indication of correlation between sterility and the number of quadrivalents in tetraploid snapdragons. They also found that low fertility could not be attributed to lack of pollen. They suggested that incompatibility and female or zygotic sterility act as barriers to fertility.

According to Fischer (10) genetic factors were more important causes of high sterility in tetraploid maize than chromosomal irregularities during meiosis.

Muntzing (22), assumed that the degree of fertility in experimental autotetraploids was not necessarily correlated with the frequency and behavior of quadrivalents. Physiological condition of the mother plant seemed to be a more important factor than the constitution of the spores.

Aneuploidy was regarded to be an important factor in causing a high degree of sterility in tetraploid rye by Muntzing (23) and in tetraploid lettuce by Einset (9).

Increased sterility of autotetraploid rye, as compared with the diploid, was found to be due to increased failure of fertilization and
increased frequency of slow and interrupted development of endosperm in the tetraploid (11). From a study of reciprocal crosses between *Medicago falcata* (2n) and common *M. sativa* (4n), Ledingham (17) observed that difference in seed development in reciprocal crosses was due to the rate of physiological activity and cell division initiated in the endosperm at the time of fertilization. Relationship between the chromosome number of the male and female nuclei seemed to be of considerable importance. Embryological study of autotetraploid sweetclover (7) revealed that the collapse of a high proportion of fertile ovules was due mainly to the failure or retarded rate of endosperm growth. Failure of fertilization and abortion of embryo sacs also were observed.

From a cytological study of megasporogenesis of autotetraploid lettuce, Einset reported that the chief causes of sterility were ovule abortion, due to extreme meiotic irregularities, and failure of pollen germination and growth of the pollen tube (8). Cytological studies of the ovules revealed gametophyte abortion, and this was found to account for 65 to 86 percent of the sterility in *Hilaria* (2).

Chen (3) studied the inheritance of self-fertility in autotetraploid sweetclover, and found that average self-fertility of the progenies was in good agreement with the parents from which they were derived. Parent-progeny correlation was 0.675, which was highly significant.

Cytology of autotetraploid sweetclover was studied by Johnson and Sass (13). First meiotic division was characterized by chromosome lagging and markedly irregular distribution. Univalent, bivalent, quadrivalent and occasional trivalent associations were observed.
However, the reduced fertility of this autotetraploid could not be explained entirely on the basis of pollen condition.

From a study of interspecific hybrids of *Melilotus*, Webster (39) showed a positive correlation between the percentage of stainable pollen and self-fertility in the F₁ plants. Ovule abortion also was noted. It was further concluded that pollen abortion was conditioned by factors other than meiotic irregularities. The author suggested a hypothesis of pollen degeneration resulting from certain lethal gamete combinations.
MATERIALS AND METHODS

The lines of autotetraploid *M. alba* used in this study were developed in the sweetclover improvement program of the Iowa Agricultural Experiment Station. Four lines were used, two of relatively high self-fertility and two of low self-fertility. The same four lines were also used in a previous embryological study (7). The rating of self-fertility was based on the percentage of selfed-seed set, after hand-pollination under controlled greenhouse condition.

Cuttings were made from the previously used plants (7) and rooted in vermiculite during the first week of June. The cuttings were transplanted later to four-inch clay pots in a sterilized mixture of soil, peat and sand. Each pot was given nutrient solution as needed.

Early in October, the photoperiod was extended to 18 hours by the use of 200 watt Mazda lamps. With this treatment, flowering began in early November.

The period of collection of material extended from July to March. Meiotic stages were most abundant in young flower-buds in which pollen was just changing color from green to yellow, a change which was easily detected with a magnifying glass. The most numerous figures were obtained from the materials collected between 9:30 A.M. and 10:30 A.M. in full sunlight, in January and February.

A Namaschin type formula, Graf III, was used for killing the floral buds, and a dioxan-normal butyl alcohol series was used for dehydration (34). Materials were embedded in paraffin. Proper orientation in the
paraffin block was necessary to permit sectioning in the correct plane. Four to five floral buds were put side by side in the block in order to section the group in one operation. Sections were cut eight microns in thickness. Three to four ribbons totaling 12 to 20 flowers were mounted on a slide. The sections were stained with iron-hematoxylin (34).
EXPERIMENTAL RESULTS

Meiotic Prophase in the Megasporocyte

The megasporocyte is evident in an ovule in which the integument primordia have just begun to grow out. The sporocyte, of hypodermal derivation, becomes distinguishable from the cells of the enveloping nucellus by its size, its large nucleus, and by its staining properties (Figure 1).

The sporocyte seems to be normal during prophase I. Early prophase can not be analyzed profitably because of the large number of very small chromosomes. In late prophase the chromosomes become shortened and thickened, and typical diakinesis figures are evident (Figure 2). In these figures, bivalent, quadrivalent, and occasional univalent and trivalent associations have been observed.

Metaphase I

Chromosome counts can be made in metaphase plates, but chromosome associations can be determined only in favorable preparations (Figure 3). In normal metaphase figures, all the chromosomes are oriented in a regular manner on the plate (Figure 3). More commonly, metaphase figures exhibit various abnormalities. Chromosomes are not always oriented on the metaphase plate, but are scattered irregularly toward the polar regions of the spindle, or they lag outside the spindle (Figures 4, 5, 6). In
Figure 1. A young ovule showing megasporocyte in prophase. (400X).

Figure 2. Megasporocyte at diakinesis. (1000X).

Figure 3. Normal metaphase I (highest-fertility line). (1000X).

Figure 4. Abnormal metaphase I, with some chromosomes outside the spindle (highest-fertility line). (1000X).

Figure 5. Abnormal metaphase I, showing univalent, non-oriented bivalents one of which is outside the spindle (medium-low-fertility line). (1000X).

Figure 6. Abnormal metaphase I, with univalents, non-oriented bivalents and lagging chromosomes outside the spindle (medium-low-fertility line). (1000X).

Figure 7. Abnormal metaphase I, showing chromosome clumping (lowest-fertility line). (1000X).

Figure 8. Polar view of metaphase I. Note the univalent chromosome outside the plate (lowest-fertility line). (1000X).

Figure 9. Normal anaphase I (highest-fertility line). (1000X).

Figure 10. Anaphase I, showing univalent laggard (highest-fertility line). (1000X).

Figure 11. Anaphase I, with lagging chromosomes (highest-fertility line). (1000X).

Figure 12. Anaphase I, showing laggards (medium-high-fertility line). (1000X).

Figure 13. Anaphase I, with two lagging chromosomes (medium-high-fertility line). (1000X).

Figure 14. Anaphase I, with laggards. Note the lagging chromosomes near the polar groups (medium-low-fertility line). (1000X).

Figure 15. Anaphase I, showing laggards and chromosomes of one pole scattered irregularly (medium-low-fertility line). (1000X).

Figure 16. Anaphase I, showing very irregular distribution of chromosomes with six or seven lagging chromosomes (medium-low-fertility line). (1000X).
addition to the more common bivalents, univalents also occur frequently at metaphase I (Figures 4, 5, 6, 8). In some other cases, chromosomes are clumped into a deeply stained mass (Figures 4, 7).

Anaphase I

Normal anaphase separation is comparatively rare, especially in low-fertility lines (Figures 9, 17). The presence of lagging chromosomes characterizes most of the anaphase figures examined in this study. The extent of lagging varies greatly. In some cells one or two laggers are present (Figures 10, 13, 20, 46), whereas in other cells three or more laggers occur (Figures 11, 12, 14, 16, 18, 19). Some of the laggers may be near the polar groups (Figures 11, 14, 19, 20), or they may be outside the spindle (Figures 15, 18). In most cases these laggers are univalent or possibly dividing univalent chromosomes (Figures 10, 11, 12, 13, 14, 18, 19), but in relatively rare cases lagging chromosomes seem to be bivalent, on the basis of mass, when compared with the frequent univalents (Figures 16, 18, 19, 20).

In addition to frequent lagging in anaphase I, other chromosome aberrations have been observed. In low-fertility lines, irregular scattering of the chromosomes of one of the polar groups is frequent (Figure 15). The chromosomes of one polar group of a cell may be spread out along the spindle and in the surrounding cytoplasm, whereas the other polar group seems to be in the process of interphase reconstitution. Chromosome clumping is also common in anaphase I. This condition is more pronounced in low-fertility than in high-fertility lines.
Figure 17. Normal anaphase I (lowest-fertility line). (1000X).

Figure 18. Anaphase I, showing very irregular distribution of chromosomes. Note the laggards outside the spindle (lowest-fertility line). (1000X).

Figure 19. Abnormal anaphase I, showing laggards and clumping of chromosomes at one pole (lowest-fertility line). (1000X).

Figure 20. Anaphase I, possibly with a bivalent laggrand (lowest-fertility line). (1000X).

Figure 21. Very irregular anaphase I separation. Note the clumping of chromosomes (lowest-fertility line). (1000X).

Figure 22. Normal telophase I (highest-fertility line). (1000X).

Figure 23. Telophase I, with lagging chromosomes. Note one laggard beyond a polar group (highest-fertility line). (1000X).

Figure 24. Telophase I, showing five or six laggards (highest-fertility line). (1000X).

Figure 25. Telophase I, with laggards (highest-fertility line). (1000X).

Figure 26. Telophase I, with two lagging chromosomes (medium-low-fertility line). (1000X).

Figure 27. Telophase I, showing five or six laggards (lowest-fertility line). (1000X).

Figure 28. Telophase I, indicating irregular chromosome distribution (lowest-fertility line). (1000X).

In the Figures 29 to 40, chalazal cell of the diad is towards the top and micropylar one is towards the bottom of the page.

Figure 29. Metaphase II, with lagging chromosomes. Note that one nucleus is still in prophase II (highest-fertility line). (1000X).

Figure 30. Metaphase II, with laggard in the chalazal nucleus (medium-low-fertility line). (1000X).

Figure 31. Anaphase II, showing lagging chromosomes. Chalazal nucleus is more disturbed than the micropylar one (highest-fertility line). (1000X).

Figure 32. Anaphase II, showing normal division in the chalazal nucleus and chromosome clumping in the micropylar nucleus (highest-fertility line). (1000X).
Telophase I

In telophase I, normal figures are relatively more frequent than normal anaphase figures (Figure 22). During this telophase, the spindle becomes flattened laterally, and the chromosomes appear to be smaller than in metaphase I or anaphase I (Figures 25, 27, 28). Lagging chromosomes, which appear to be univalent in most cases, are also evident in this stage (Figures 23, 26, 27, 47, 48). Highly abnormal chromosome distribution may produce as many as six to seven laggards (Figures 24, 25, 27, 28). Other cells exhibit only one or two lagging chromosomes (Figures 23, 26). In a few cases stray chromosomes occur beyond an interphase nucleus, outside the spindle (Figure 23). This situation seems to arise from the frequent presence of non-oriented chromosomes at metaphase. During late telophase a clearly visible cell plate is laid down and the polar groups develop into well-defined interphase nuclei (Figures 23, 27, 28).

Metaphase to Telophase II

Lagging or non-orientation of chromosomes also are evident in metaphase II, (Figures 29, 30). One of the nuclei of the diad may be in metaphase II, whereas the other nucleus may still be in prophase II (Figure 29).

Various chromosome aberrations also occur in anaphase II. Laggards occur in one of the cells (Figure 35), or in both cells (Figures 31, 33, 34, 49). Some of the anaphase II cells have very abnormal chromosome
Figure 33. Anaphase II, with laggards. Note the plane of first division (medium-low-fertility line). (1000X).

Figure 34. Anaphase II, showing laggards in both nuclei (medium-low-fertility line). (1000X).

Figure 35. Anaphase II, with normal division in chalazal nucleus and abnormal separation in the micropylar one (medium-low-fertility line). (1000X).

Figure 36. Abnormal anaphase II. Note one laggard in the chalazal nucleus and the inactive condition of the micropylar nucleus (lowest-fertility line). (1000X).

Figure 37. One nucleus is in anaphase II with one laggard near a pole whereas other nucleus in metaphase II with chromosome outside the spindle (lowest-fertility line). (1000X).

Figure 38. Normal anaphase II division in chalazal nucleus and chromosomes of micropylar nucleus is clumped in three compact groups (lowest-fertility line). (1000X).

Figure 39. Telophase II, with laggards in one nucleus (highest-fertility line). (1000X).

Figure 40. Abnormal telophase II showing several laggards (lowest-fertility line). (1000X).

Figure 41. Linear row of four megaspores. (400X).

Figure 42. Four megaspores that are not strictly linear. (400X).

Figure 43. Ovule showing functional megaspore and three disintegrating megaspores at the micropylar end. (400X).

Figure 44. Anaphase in somatic cell of ovary showing lagging chromosomes. (2400X).

Figure 45. Same as Figure 44.
distributions (Figures 31, 36). The chalazal nucleus may undergo normal separation whereas the micropylar nucleus may exhibit considerable irregularity (Figures 32, 35, 38). In some ovules one of the nuclei of the diad may undergo normal anaphase II division but the other nucleus may fail to divide (Figures 32, 36, 38). This inactive nucleus may be clumped in a single heavily stained mass (Figure 32) or its chromosome complement may be in three to four compact, deeply stained groups (Figures 36, 38). Anaphase separation in two nuclei of the diad is not always simultaneous (Figure 37). One of the cells may be in anaphase II and the other in metaphase II (Figure 37) or in telophase II.

Irregular chromosome distribution also occurs in telophase II (Figures 39, 40).

Comparison of Meiotic Irregularities in Different Lines

Irregular chromosome distribution is of more frequent occurrence in low-fertility than in high-fertility lines. The low-fertility lines commonly exhibit extreme meiotic irregularity, which seems to be associated with subsequent embryo sac abortion (Figures 7, 15, 18, 19, 21). Scattering of chromosomes of one polar group during anaphase I and telophase I also was observed in the two low-fertility lines studied (Figure 15). Abnormality in metaphase, primarily the presence of univalents and non-oriented bivalents, seems to be of common occurrence in both low- and high-fertility lines (Figures 4, 5, 6, 8). Clumping of chromosomes was more frequent in the low-fertility lines (Figures 7, 18, 19, 21, 36, 38), than in high-fertility lines (Figures 4, 32).
A compilation of the observations of megasporocytes is given below:

<table>
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<tr>
<th>Dividing cells examined</th>
<th>Normal</th>
<th>Abnormal</th>
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<tbody>
<tr>
<td>highest-fertility line</td>
<td>91</td>
<td>41</td>
</tr>
<tr>
<td>medium-high-fertility line</td>
<td>11</td>
<td>4</td>
</tr>
<tr>
<td>medium-low-fertility line</td>
<td>98</td>
<td>17</td>
</tr>
<tr>
<td>lowest-fertility line</td>
<td>55</td>
<td>10</td>
</tr>
<tr>
<td>total</td>
<td>255</td>
<td>72</td>
</tr>
</tbody>
</table>

It is evident from the above list that the majority of the irregular and abnormal separations at anaphase I and II and telophase I and II were observed in the two low-fertility lines. Only a few cells of the medium-high-fertility line were examined, because this line did not flower regularly under greenhouse conditions.

Development of the Female Gametophyte

After the second meiotic division, a row of four megaspores is formed (Figures 41, 42). The plane of the second division is frequently oblique, in which case the megaspores may not be strictly linear (Figure 42). Four apparently normal megaspores were observed in most of the ovules examined, except in some ovules of the low-fertility lines, in which abortion occurred after meiosis.

Subsequent to the formation of the megaspore quartet, the chalazal megaspore enlarges, whereas the micropylar ones degenerate (Figure 43). The chalazal megaspore gives rise to the 3-nucleate embryo sac.
Mitotic Abnormality

Mitotic anaphases of somatic cells of the ovule and ovary also exhibit lagging chromosomes (Figures 44, 45). This mitotic abnormality leads to the exclusion and irregular distribution of chromosomes in the dividing somatic cells. In some young ovules, hypodermal cells, from which the a sporocyte is derived, were found to have mitotic irregularities.

Periodicity of Meiosis

The frequency of meiotic figures exhibited diurnal periodicity. Collections were made at four-hour intervals over a 24-hour period. Collections made in the morning yielded more abundant meiotic figures than those collected in other periods. The best results were obtained from materials collected between 9:30 A.M. and 10:30 A.M., in full sunlight in January and February.
Figure 46. Anaphase I, showing one laggard (lowest-fertility line). (960X).
Figure 47. Telophase I, showing laggards. Note the laggards near polar groups (lowest-fertility line). (960X).

Figure 48. Same ovule as Figure 47, photographed at a different depth of focus showing laggards in other positions. (lowest-fertility line). (960X).
Figure 49. Anaphase II with laggards in both nuclei (highest-fertility line). (960X).
DISCUSSION

Meiotic irregularities in the microsporocyte of autotetraploid Melilotus alba were found in a previous study to contribute to low fertility (13). Cytological studies of the male gametophyte of other artificially produced autotetraploids also indicate that unbalanced gametes, resulting from irregular chromosome distribution during meiosis, account for their low fertility (1, 21, 36, 37).

Low self-fertility in autotetraploid sweetclover, M. alba, is due only in part to failure of fertilization. Other important factors in the reduction of fertility are the presence of aborted embryo sacs and the frequent collapse of fertilized ovules during various stages of embryonic growth (7). The present study indicated that meiotic irregularity in the ovule is also a contributing factor in the observed reduction of fertility. Various meiotic abnormalities, primarily lagging and exclusion of chromosomes, lead to the formation of unbalanced gametes, and cause different degrees of sterility. The abnormal embryo sacs in autotetraploid lettuce reported by Einset (8) were believed to be due to a complete break-down of the meiotic process. Abnormal development of the female gametophyte in Hilaria was found to account for 65 to 85 percent of the sterility in different species of Hilaria, but the underlying cause of this anomaly still is not known (2).

Relatively few studies have been made dealing with the cytology of the megasporocyte and female gametophyte of autotetraploids. This largely has been due to the difficulty involved in cytological studies of
the ovule. Meiosis in the microsporocyte can be studied with relative ease by means of smears, but the study of similar divisions in the megasporocyte necessitates precise sectioning of minute ovaries. It has been assumed that the cytological behavior of the female gametophyte is essentially similar to that of male gametophyte (22), but this may not always be true. Brown and Coe (2) observed normal segregation of chromosomes in microsporogenesis of Hilaria belangeri, but 65 to 85 percent of the sterility in this species was found to be due to abnormality in macrosporogenesis. Some workers have suggested that low-fertility in autotetraploids may be caused by female or syngenic sterility (35) or to the formation of aneuploid ovules (23).

Lindstrom and his co-workers (12,19, 20) found that meiosis in the microsporocyte of autotetraploid tomato was very regular. The high sterility of this autotetraploid was unexpected and no explanation was advanced. However, Upcott (37) re-investigated the cytology of tetraploid tomato and found that low seed production could be explained by the observed frequency of non-disjunction from quadrivalents. It was further assumed that a part of the numerically balanced gametes were genetically unbalanced. On this basis Upcott estimated the proportion of functional male and female gametes at about 40 percent and stated that the observed level of fertility was determined by the combination of these gametes. Muntzing (22) discussed the findings of the above study and stated that (p. 319)

This conclusion, however, can scarcely be accepted, firstly because the proportion of functional pollen grains would suffice to fertilize all functional ovules, secondly because haplontic sterility is generally less pronounced in the ovules
than in the pollen. . . . Consequently, the only possible explanation seems to be that physiological disturbances and diplontic sterility are also, and perhaps chiefly responsible for the low fertility in this case, just as in the other cases mentioned above.

Randolph (31) found that not more than 15 percent of the observed sterility in autotetraploid maize could be attributed to meiotic irregularities, and suggested that physiological and genic disturbances resulting from chromosome doubling may account for the high degree of sterility. Sterility in autotetraploid Melilotus alba could not be explained solely on the basis of meiotic irregularities (13).

In order to determine the basis for sterility in experimental autotetraploids, developmental studies should be conducted of male as well as of female gametophytes, including subsequent embryonic growth. These studies preferably should be with the same cultures.

From the presence of numerous apparently normal embryo sacs observed in autotetraploid sweetclover (7), it seems that a certain degree of meiotic irregularity can be tolerated in the development of a mature female gametophyte. The formation of microspore quartets was not necessarily influenced by lagging or excluded chromosomes (38). Therefore, it is quite probable that in some species aneuploid gametes are formed. Ultimate expression of different levels of sterility may be a manifestation of this aneuploid condition of the female gametophyte. Aneuploid plants of autotetraploid lettuce are extremely sterile when compared with their euploid sibs (8). Muntzing (23) contended that aneuploidy may cause "disturbed quantitative relation" between the chromosome numbers of embryo, endosperm and surrounding maternal tissues. The
normal quantitative relation between these tissues is 2n : 3n : 2n, and any disturbance in this normal relation has severe effects on the development of seed.

The frequent occurrence of shrivelled seeds in autotetraploid rye was explained on the basis of this "disturbed quantitative relation" (23). If this hypothesis is tenable, then aneuploid gametes in autotetraploid sweetclover, resulting from meiotic irregularities, may cause disturbances during the development of seed. A functional megasporocyte, normal in its chromosome complement, may still yield an abnormal embryo sac by subsequent mitotic irregularities.

Collapse of fertile ovules has been reported to occur during various stages of embryo development (7). Collapse of this type was due mainly to the failure or retarded rate of endosperm growth. Thus, it becomes evident that meiotic irregularity in the development of female gametophyte, followed by embryonic failure, associated with, and possibly caused by endosperm abnormality, all may contribute to low seed production. The endosperm seems to be more sensitive to the aneuploid condition than the embryo, and therefore, ultimate break-down of seed development occurs.

Similar conditioning of the development of seed by endosperm abnormality was reported in autotetraploid rye (11) and in reciprocal crosses between Medicago falcata (2n) and M. sativa (4n) (17).

Irregular meiotic behavior appeared to be correlated with different levels of sterility. This relationship suggests the possibility of selection for increased fertility in autotetraploid sweetclover. Such relationships have been reported in other autotetraploids (3, 23, 26,
The present study is in agreement with the report by Cooper (4) that a row of four megaspores is formed, and is not in accord with the report of Young (40) that the megasporocyte of *M. alba* develops directly into the female gametophyte.

The observed mitotic abnormalities, primarily lagging and exclusion of chromosomes, may give rise to abnormal sporocytes. The sporocyte is derived from a hypodermal cell of the ovule, and mitotic irregularities in somatic cells of the ovule may ultimately produce unbalanced megaspores. Subsequent meiotic irregularities increase the opportunities for the formation of aborted embryo sacs and unbalanced gametes. All of these factors lead to the ultimate expression of different degrees of sterility in autotetraploid sweetclover.
SUMMARY

1. A study of megasporogenesis and embryo sac development of autotetraploid *Melilotus alba* was made to determine the cytological basis of self-sterility. Two lines that had relatively high self-fertility and two lines of low self-fertility were used.

2. Irregularities at metaphase I consist of non-orientation of lagging bivalents on the metaphase plate and frequent occurrence of univalents. These irregularities are common in both high- and low-fertility lines.

3. Three to four lagging chromosomes may occur at anaphase I in both high- and low-fertility lines. In low-fertility lines, the chromosomes of one polar group may be scattered irregularly in the surrounding cytoplasm, whereas the other polar group develops a typical interphase nucleus. Irregular chromosome distribution is more frequent in low-fertility than in high-fertility lines.

4. Lagging univalents are also evident in telophase I; as many as six to seven lagging chromosomes may occur, especially in the two low-fertility lines.

5. Considerable irregularity, primarily lagging and non-orientation of chromosomes, also occurs in metaphase II.

6. Irregularities also occur in anaphase II. The chromosomes of
one nucleus of the diad may undergo normal separation, whereas separation in the other nucleus lags or fails. The inactive nucleus may be clumped in a single heavily stained mass or its chromosome complement may be in three to four compact, deeply stained groups. The anaphase separation of the two nuclei of the diad is not always synchronous.

7. Irregular chromosome distribution also occurs in telophase II.

8. A total of 255 dividing cells were examined at anaphase and telophase of I and II, and 185 cells (71.76 percent), were found to have meiotic irregularities.

9. Dividing somatic cells of the ovule and ovary also exhibit lagging and exclusion of chromosomes.

10. Aberrant somatic divisions in the ovule may give rise to abnormal megasporocytes. Subsequent meiotic irregularities bring about further abnormalities in the chromosome complements of gametophytic nuclei. The foregoing irregularities of chromosome behavior provide the physical basis for embryo sac abortion and the subsequent sterility.
LITERATURE CITED


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