THE CYTOLOGY OF THE MALE STERILE LATHYRUS ODORATUS

by

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With 2 plates

INTRODUCTION

A number of cases in which meiosis is affected by a definite gene are known. It is believed however that the present case differs from all previous ones in several important respects, and as such merits a full description.

The recessive gene for male sterility, or contabescent anthers, in the Sweet Pea has been known since 1903 (BATESON, SAUNDERS and PUNNETT 1903). PUNNETT (1925) called the factor b_2 and showed it to be linked with the factors for l i g h t a x i l and for „c r é t i n". A coupling back cross was made by the present author for m a l e s t e r i l e and l i g h t a x i l, and the following results obtained:

Red Axil-fertile	88
Red axil-sterile	4
Light axil-fertile	6
Light axil-sterile	84

Thus the crossing over between m a l e s t e r i l e and l i g h t a x i l is 5.8% with a standard error of 1.8%. The material for the present study was mainly obtained from this back cross and from its parents.

It has been standard practice in Sweet Pea genetics to use m a l e s t e r i l e as a female parent, thus avoiding emasculation. The extensive genetic results show that there is nothing very abnormal in

the female gametogenesis of m a l e s t e r i l e plants, and that the sterility of the pollen is absolute. (PUNNETT 1923, 1925, 1927, 1932.)

The cytology of the m a l e s t e r i l e has been described by GREGORY (1905). He established that the main abnormality takes place during meiosis, although there is a stage before that during which archesporial cells fail to divide. He also ascertained that meiosis is normal on the female side, which is confirmed by the genetic data mentioned above.

It appeared of interest to reexamine the m a l e s t e r i l e Sweet Pea in the light of present day cytological knowledge. This was carried out by the author in 1934 and 1935.

TECHNIQUE

Nearly all the observations have been made on Carmine Acetic smears. All attempts to make permanent preparations by standard methods have been unsuccessful, as the conditions of fixation are evidently quite different in the m a l e s t e r i l e Sweet Pea than in the normal.

Medium FLEMMING, 2BE (LA COUR 1931), NAVASHIN, GILSON and BOUIN fixatives were tried. Moreover the first three were used both directly on the buds whose calyces had been removed, and also after one minute prefixation with chloroform CARNOY, — without removing the calyx in the latter case. The material was dehydrated and embedded in paraffine in the standard manner. Without exception fixation was so bad that the preparations were not used for observations.

Permanent smears gave fairly good, and occasionally very good preparations. Medium FLEMMING, 2BE and NAVASHIN solutions were used, all giving very similar results.

Smears in Carmine Acetic gave consistently good preparations which last some weeks after sealing. It is from Carmine Acetic preparations that all the drawings and photographs were made. There can be little doubt that had the Carmine Acetic technique been known in GREGORY's time, some of his conclusions would have been different.

All the text figures are reproduced at a uniform magnification of $\times$ 1270.

OBSERVATIONS

Meiosis in the normal *Lathyrus odoratus* has been described on several occasions. (MAEDA 1928, 1930). It will therefore not be described again here, and will only be refered to when necessary for comparison.

As the result of a considerable number of observations of different stages, a cycle of events was worked out. This consists essentially of two parts: abnormalities during meiosis, and the final arrest of all division by granulation of the cytoplasm. The precise stage at which the arrest takes place is variable; consequently meiosis will first be described up to the latest stage to which it has been observed to go.

No observations have been made on divisions of the archesporial cells. But the much smaller bulk of the contents of the pollen sac, coupled with the fact that individual P.M.C. are of the same size as in the normal, certainly confirms GREGORY'S observation that fewer P.M.C. are formed.

Detailed observations comparing diakinesis and preceeding stages in the male sterile and normal have been made by Miss UPCOTT (UPCOTT, in press). In the normal Sweet Pea no (or very little) terminalisation takes place, the mean number of chiasmata at metaphase being greater than 2.5. Free ends are frequent. In the male sterile terminalisation and contraction of the chromosomes proceeds, the nuclear wall being intact. Diakinesis is shown in Fig. 1, and two subsequent stages exhibiting degrees in the amount of terminalisation are shown in Figs. 2 and 3. In the end one sees highly contracted chromosomes with completely terminalised chiasmata still within a nucleus, and scattered at random as at diakinesis. When the nuclear wall does eventually disappear, the chromosomes are still scattered at random. This stage is illustrated in Figs. 4 and 5. A polar view of normal metaphase is given in Fig. 6 for comparison. The same stage can be seen in the photographs (Fig. 9 and 11). This stage can evidently last for an extraordinarily long time, for it is not unusual to find every bud between 4 mm. and 6 mm. long on any given plant in this condition. It is occasionally possible to distinguish the double nature of the chiasma in the ring shaped bivalents.

It is at this stage that the arrest of meiosis by granulation most frequently takes place. Consequently observations on subsequent

stages are more fragmentary, owing to the difficulty of getting sufficient material.

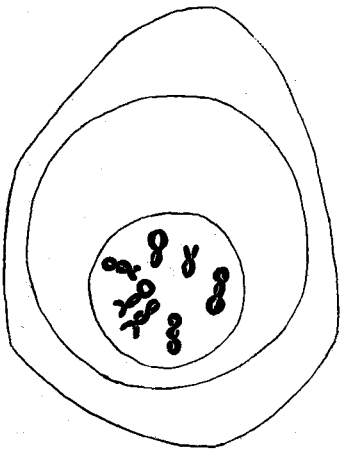


FIG. 1. Diakinesis in the *Male Sterile*, identical with te normal.

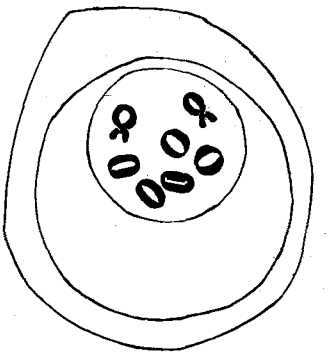


FIG. 2. *Male Sterile* at a stage when terminalisation is greater than in the normal, but not yet complete.

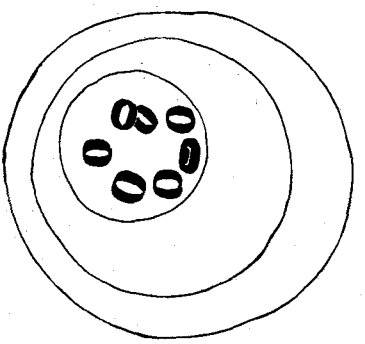


FIG. 3. *Male Sterile* showing complete terminalisation, with the chromosomes still within a nucleus.

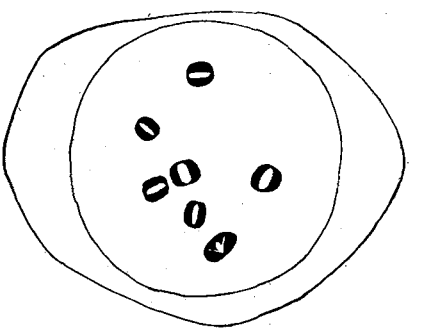


FIG. 4. *Male Sterile* with completely terminalised chiasmata and the chromosomes scattered at random in the cell.

The separation of the chromosomes occasionally takes place. Most frequently no metaphase plate is formed, the chromosomes sepa-

rating whilst scattered at random throughout the cell; in such a case the two members of a bivalent never get very far from one another.

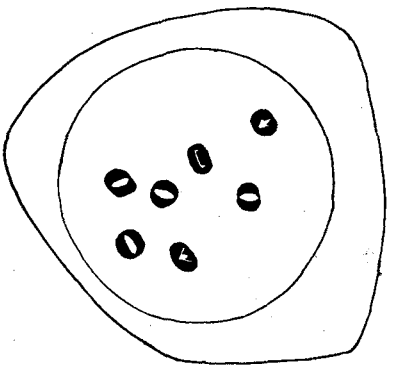


FIG. 5. *Male Sterile* with completely terminalised chiasmata and the chromosomes scattered at random in the cell.

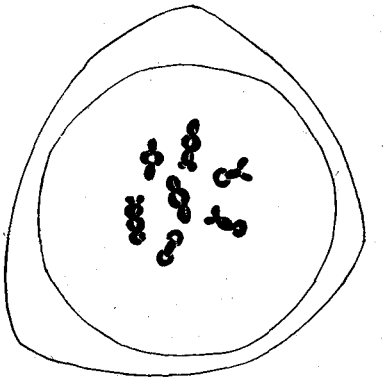


FIG. 6. Metaphase in the normal *Lathyrus odoratus*.

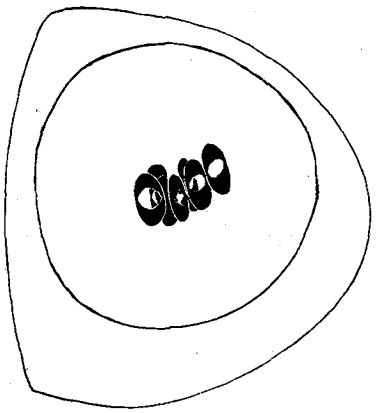


FIG. 7. One of the rare metaphase plates formed in the *Male Sterile*.

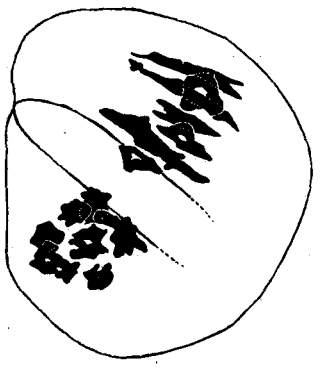


FIG. 8. Second anaphase in the *Male Sterile*; the cell wall only extends across two thirds of the cell.

Very occasionally a metaphase plate of normal aspect is formed; this is illustrated on Figs. 7 and 10.

Only very few second divisions were seen. The chromosomes then

have a very ragged appearance and indefinite outline. Nevertheless, seven bodies can always be recognised going to each pole. In the second division illustrated in Fig. 8 the cell wall only extended across about 2/3 of the cell. In Figs. 12 and 13 will be seen a group of cells at second division. Furrowing and wall formation are very irregular. Often more than four chambers are partitioned off, some being left without any nuclear material whatever. This stage reminds one of tetrads which had been heavily X-rayed during meiosis (e.g. MATHER 1934) though here there is no fragmentation of the chromatin.

The final arrest of meiosis by a change in the colloidal state of the cytoplasm can take place as early as diakinesis, or as late as second anaphase. There is reason to believe that the exact stage at which granulation takes place is to some extent governed by external conditions, for plants raised in the green house and flowering in May consistently showed it at diakinesis. On the other hand, the very late onset of the change in the cytoplasm (second division) was only observed in late summer. It was not found possible to establish the exact nature of this granulation. The highly refractive particles in the cytoplasm (Fig. 14) suggest oil droplets, but fat solvents and osmic acid are without action on them. They can in fact best be seen in permanent preparations which had been embedded in paraffine. The granules stain deeply with Gentian Violet, but not appreciably with Carmine Acetic. The process of granulation is evidently rapid, for no intermediate stages have ever been seen. It does not spread from cell to cell, but pollen mother cells are found to be affected at random throughout the pollen sac at the onset of the process. This is illustrated in Fig. 11. It will be seen in that photograph that unaffected cells show ring shaped bivalents with completely terminal chiasmata.

DISCUSSION

Leaving out for the time being the arrest of meiosis by granulation, the whole cycle of events can be most conveniently described as follows.

For the purpose of this discussion meiosis may be looked upon as consisting of two separate but strictly coordinated processes, namely the cell division process and the chromosome cycle. It must be emphasised that this distinction is made here solely for the purpose of

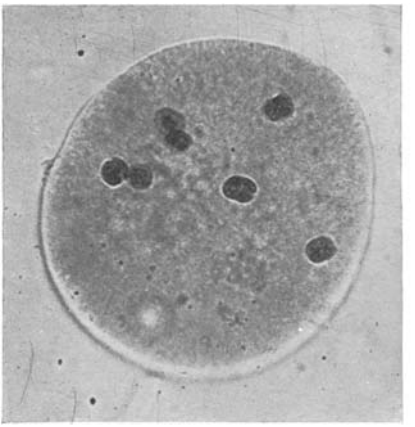


Fig. 9

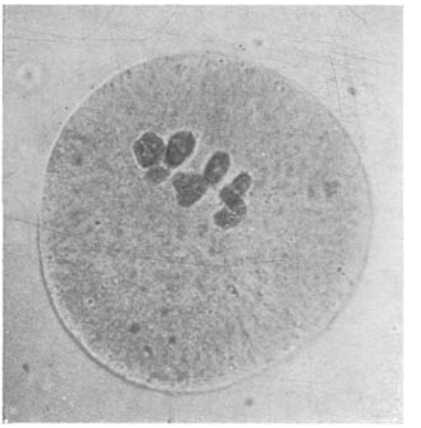


Fig. 10

9. *Male Sterile* with completly terminalised chiasmata and the chromosomes scattered at random.
10. *Male Sterile* with apparently normal metaphase plate.

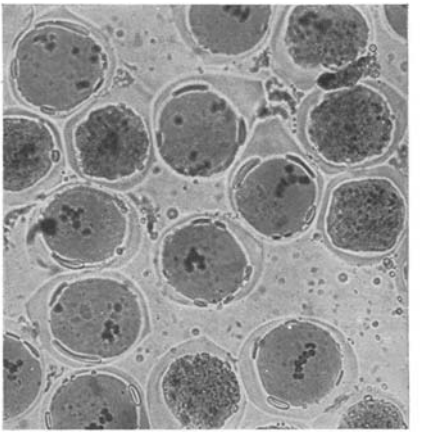


Fig. 11

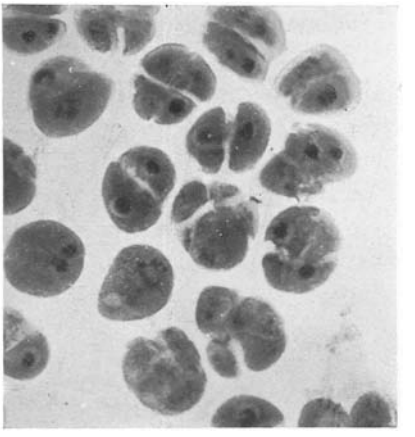
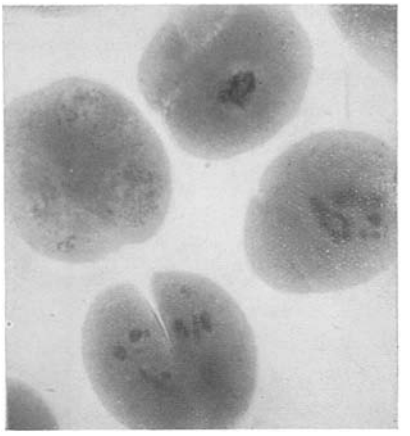


Fig. 12

11. Pollen mother cells of the *Male Sterile* at the same stage as those in Fig. 9, with some cells showing granulation of the cytoplasm.
12. Cells of the *Male Sterile* which have undergone abortive second divisions, showing irregular furrowing.



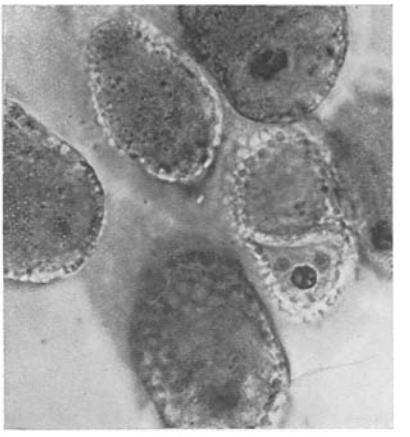


Fig. 13 Fig. 14

13. Second anaphase in the *Male Sterile*.
14. Granulated cells of the *Male Sterile*, showing refractive particles in the cytoplasm.

description, and is not intended to represent a reality intrinsic in the nature of meiosis. In these terms one can describe meiosis in the male sterile Sweet Pea by assuming that the process of cell division ceases to keep pace with the nuclear cycle. Thus we see chromosomes in a stage of contraction corresponding to normal metaphase, but still enclosed in a nuclear wall. Later chromosomes are seen with completely terminalised chiasmata and a degree of contraction greater than normal, but lying in the cell as at metaphase.

It is of interest to point out that the two processes considered in the above description are in fact similar to those postulated by DARLINGTON in the Precocity Theory of the relationship between meiosis and mitosis. (DARLINGTON 1931, 1932). It cannot of course be asserted that there is any real similarity between the two cases. Nevertheless the comparison seems sufficiently suggestive to be worth noting.

Two other points of general interest arise. It is of course always difficult to compare an „abnormal" case like the one described here with events in normal organisms, and the statements below are made with that p r o v i s o.

The fact that complete terminalisation can occur if the chromosomes „are given sufficient time" is of considerable interest. It shows that the difference between organisms with complete terminalisation and those with none may, at least in some cases, be a matter of different time relationships.

The fact that the two members of a bivalent can separate without lying on a plate shows that the latter is not indispensable for separation. It was seen on the other hand that bivalents which separate under those conditions never get very far from one another. The observation that bivalents separate at two different stages of terminalisation and contraction in the normal and in the m a l e s t e r i l e suggests that the moment of separation is not determined by the state of the chromosomes, but by an influence outside them.

SUMMARY

The cytological abnormalities caused by the genetic factor for m a l e s t e r i l i t y in *Lathyrus odoratus* are described and discussed. Data from a small backcross showing the amount of recombination between m a l e s t e r i l e and l i g h t a x i l are given.

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