Timing Unbalance at Meiosis in the Pollen-sterile *Lathyrus odoratus*

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A form of *Lathyrus odoratus* which has sterile pollen although the ovules are fertile was described by Bateson, Saunders and Punnett in 1905. The abnormality appeared in their breeding experiments along with factors determining flower colour or pollen shape. They showed that it was the result of the action of a factor now designated (s). Its inheritance and general cytological properties have already been described by Fabergé (1937).

The development of the male gamete is more liable to be upset by the action of genes than that of the female, owing, it is said, to its longer and more complicated life. A considerable number of such genes are known in plants, of which there are fifteen in *Zea mays* alone (Beadle 1932 b). Different genes affect the pollen of this plant at different stages of its development so that in some cases no visible abnormality is produced. Sterility may be due to "asynapsis", to the occurrence of supernumerary divisions following meiosis, or to the failure of cell-wall formation (Beadle 1930, 1931, 1932 a).

These and other cases in *Crepis* (Richardson 1935) etc., largely depend on abnormal timing relationships. The gene for pollen sterility in *Lathyrus* also alters these relationships, but in an entirely different way.

Material and Methods

The material was obtained from the genetical crop of *L. odoratus* growing in this Institution. Buds were taken from different plants and from different families, but no differences between them were detected.

The usual method of fixing in medium Flemming or some similar fixative is useless for the pollen-sterile plants, because the bivalents contract greatly and run together into an amorphous mass. The shape of the spindle however can be shown by this method. For observation of chromosome morphology, aceto-carmines was used exclusively. Anthers were teased out in aceto-carmines without pre-fixing, a little iron added, and the slide warmed slightly. These preparations cannot be made permanent as the cells collapse, pre-

sumably under the action of the xylol. By ringing the temporary preparations, however, they can be made to last about a week.

Duration of Meiosis

The time taken for meiosis to be completed can be measured directly only by observing the living cells. An estimate of the length of the different stages may be made indirectly by various means such as the number of anthers containing pollen-mother-cells at a given stage, or by the size of the anthers at different stages of development. I have used the latter measure in the present case.

Figs. 1 and 2. Outlines of anthers at various stages of development ($\times 30$). The stage of the pollen-mother-cells is represented diagrammatically on each anther. 1. Normal anthers. 2. Sterile anthers.

On this scale, meiosis in the normal *Lathyrus odoratus* is rapid since there is little growth between pachytene and the second division (Fig. 1, a and b). By the time the tetrads are formed, however, a marked increase in growth has occurred, and this continues until just before the flower opens when the anthers contain ripe binucleate pollen (Fig. 1, c and d).

In the sterile plants, the growth of the anthers proceeds at the usual rate, but the prophase stages of meiosis last much longer than in the normal. At pachytene there is little difference in size between the two (Fig. 2, a) but at diplotene the sterile anthers are equal in size to normals containing tetrads (Fig. 2, b). By the time the anthers have attained their maximum size, the pollen-mother-cells have reached metaphase or first anaphase (Fig. 2, d). Degeneration sets in at this stage and no further development occurs.

Using the growth of the anthers as a measure of time, we see therefore that the first division of meiosis in the sterile plant takes as long at the whole development in the normal, from the end of pachytene to the formation of binucleate pollen (Fig. 3).

As a result of this prolongation of the prophase stages it follows that after

pachytene every living anther of the sterile plant contains pollen-mother-cells at some stage of the first division. When the anthers reach their maximum size, that is to say at the first anaphase, the cells begin to die, so that when the flower opens the anthers are shrivelled and therefore smaller than the normal.

The stages which are most prolonged are those during which spiralsation and terminalisation take place. From this abnormality then, we may observe directly the effect of time upon these processes.

Fig. 3. Graph showing the relationship of growth of anther to development of pollen-mother-cells in the normal and sterile plants.

Meiosis in the Male-Sterile

The somatic complement of *Lathyrus odoratus* consists of seven pairs of chromosomes which differ slightly in length. Each of these can be recognised at mitosis but at meiosis only one pair is distinguishable. This one (A) carries a trabant on the short arm, at which point the nucleolus is attached (*cf* chromosome 6 in *Zea mays*, McClintock 1933, Darlington 1934).

The later stages of prophase in the sterile plants differ from the normals in yet another respect besides their abnormal length.

They react differently to aceto-carmin. In normal plants no analysis of chiasma frequency is possible before metaphase because the earlier stages fix so badly that individual bivalents are not distinguishable. In the sterile plants, however, the bivalents are clear and distinct and the chiasma frequency and movement may be analysed and compared at all stages.

Fig. 4. a) Mitotic division from the root tip showing different chromosome types ($\times 3000$) (Flemming-gentian violet preparation). b) Haploid complement at anaphase drawn separately.

At early diplotene the bivalents are long and show occasional relic coils which might be mistaken for chiasmata (Fig. 5a). The A bivalent is still attached to the nucleolus, the point of attachment being marked by two deeply staining trabants. The amount of condensation of this and the other bivalents is not equal throughout their length, but is greater at one end. Such differential condensation has been observed also in *Agapanthus* and *Zea* (*cf* Darlington 1937) where it is at a maximum next to the centromere and at a minimum at the ends. In *Lathyrus*, however, most of the centromeres are median so that it appears that condensation begins at one end in these bivalents and gradually extends along them until all are equally condensed, as we find at late diplotene and diakinesis.

The nucleolus disappears before diakinesis but the time of its disappearance is variable. The bivalents may be relatively long and as yet uncontracted after its disappearance, or it may still be visible when the bivalents have almost reached diakinesis (Fig. 5, b and c). A second bivalent often lies close to it, but does not appear to be actually attached.

As diplotene passes to diakinesis, the bivalents become shorter and more contracted, and the number of terminal chiasmata greater. The centromere loop increases in size at the expense of the distal loops or of the distal ends which become part of the loop as the chiasmata terminalise.

At metaphase (Fig. 6, b) terminalisation is complete. As compared with the normal at this stage (Fig. 6, a) the chromosomes are shorter and thicker and the centromeres more pulled out, owing probably to their greater mutual repulsion.

The reduction in chiasma number by terminalisation renders invalid a comparison of the chiasma frequency in sterile plants at metaphase with that of normals, in which there is probably little reduction, as shown by the number of interstitial chiasmata still persisting. We may however compare the chiasma frequency in sterile plants at diplotene and diakinesis with that in normals at metaphase.

At these stages, we find a slight difference between the two (Table 1), but this is not greater than that which might be caused by changes in the external conditions such as temperature. In the samples from which the data are taken, the variance is lower in the sterile plants than in the normal, but this difference is not necessarily significant. A comparison with the later stages shows that

Fig. 5. Diplotene and diakinesis in a male sterile plant ($\times 2000$). The bivalents show differential condensation in the early stages and considerable terminalisation as contraction proceeds. The total number of chiasmata and the number terminal is given for each nucleus. The chiasma frequency of the first nucleus is unusually high so that the reduction in number with terminalisation is probably not as great as would appear.

Table 1. Comparison of chiasma frequencies in the normal and sterile *Lathyrus odoratus*

	Nos. of bivs. with diff. Nos of Xta.				Total Xta.	Total bivs.	Xta per biv.	Cross- over units	Vari- ance
	1	2	3	4					
Normal* (met.)	12	37	27	8	200	84	2.38	119	0.8036
Sterile (dipl. & diak.)	9	51	17	7	191	84	2.27	113	0.5812

* These data were obtained by the late Mr. L. H. A. Stone.

while no univalents are recorded at diplotene and diakinesis, several occur at metaphase in the same plant. Since chiasmata are a condition of pairing at all stages after pachytene, we must assume that these univalents are the result of reduced pairing at pachytene, and since they have not been observed at the earlier stages, that the completeness of pairing at pachytene varies during the life of the plant. Probably therefore, the abnormality begins to act before this stage. Later we shall find another means of showing this to be the case.

Fig. 6. Metaphase I in the normal (a) and pollen sterile plants (b) ($\times 2000$). The latter shows complete terminalisation.

A considerable amount of data has been and is being collected on linkage in this plant (Punnett 1932, and various workers at this Institution, unpublished). I have therefore thought it worth while to give the chiasma-frequency in terms of cross-over units. On the assumption that one chiasma is equivalent to 50 cross-over units, the average length of the chromosomes in the normal and in the sterile plants is 119 and 113 units respectively.

From metaphase onwards, the behaviour is increasingly variable. In relation to the congression on the spindle, the nuclear membrane sometimes breaks down prematurely so that the bivalents are left for some time unorientated yet not visibly separated from the cytoplasm. Congression on the plate is often incomplete, one or more bivalents or pairs of univalents being left off it (Fig. 7). In aceto-carmine, the limits of the spindle are not visible, but in Flemming-gentian violet preparations made for this purpose, they are distinct

although the shapes and configurations of the chromosomes are not. From these it is clear that the spindle is unusually long and narrow, reaching across the pollen-mother-cell, from wall to wall. In many cases it is straight but it may also be bent, in which case the bivalents seem to be crowded on to the inside of the curve. This length and narrowness are characteristic of the spindle at a normal anaphase rather than at metaphase.

Fig. 7. Side views of metaphase I in sterile plants ($\times 1000$) showing failure of orientation and the occurrence of univalents. The probable position of the spindle is marked.

The similarity in shape between these spindles and those found in organisms with failure of pairing or large number of univalents also suggests that the time relations of the bivalents and of the spindle or, as Darlington (1936) assumes, of the centromere and the centrosome, are out of step. Since the time relations of the whole process are abnormal this is not unexpected.

Fig. 8. Side views of metaphase ($\times 2000$) showing a very narrow and a bent spindle (Flemming-gentian violet preparation).

The lack of co-ordination between the spindle and the bivalents accounts also for the lag in congression at diakinesis after breakdown of the nuclear membrane, and in extreme cases, for the complete failure of congression of one or more bivalents.

The anaphase separation depends, of course, upon the degree of regularity of congression at the previous metaphase. Normal anaphases occur in which each nucleus contains seven chromosomes, but there may be non-disjunction of one or more pairs, or irregular separation with no clearly defined poles (Fig. 9). As a result of these

Fig. 9. Anaphases ($\times 1000$) showing irregular separation. The two chromatids in outline have been divided at the centromere by pressure in smearing.

anaphases we find interphases containing two, three or more nuclei of varying sizes and at various stages of degeneration (Fig. 10). For at this stage, the process evidently becomes completely dislocated. It breaks down and the cells die before the second division occurs. Occasionally two fairly normal looking nuclei appear to be in prophase of the second division, but they rarely get beyond this stage. The pollen-mother-cell wall degenerates, the cytoplasm stains more

Fig. 10. Pollen-mother-cells ($\times 1000$) at various stages of degeneration after first anaphase.

deeply, and the nuclei become more and more indistinct, until they finally merge into the cytoplasm. When the flower opens the individual cells are no longer recognisable.

Exceptional Pairing in Reduplicated Segments

The material examined came from genetical stock which has been somewhat inbred for many generations. Nevertheless, in four pollen-sterile individuals, selected at random from three families in which this factor was segregating, chromatid bridges at anaphase showed that the plants were structurally heterozygous. Normal bivalent bridges and fragments were observed both at anaphase and telophase in approximately 5% of cells (Fig. 11 and 12) and in a few cases two bridges occurred in the same cell (Fig. 12, b). One case of a univalent bridge (Fig. 11, b) was observed such as would normally occur only

Fig. 11. Anaphases showing chromatid bridges ($\times 2000$). a) Bivalent bridge and fragment. b) Univalent bridge and fragment.

in a triploid. The bridge itself must have been a loop which has become a bridge at the first division owing to the precocious division of its centromere probably brought on by its lagging on the plate at anaphase, as happens with univalents of whatever origin.

Fig. 12. Bridges at telophase ($\times 2000$). a) Fragment coiled round the bridge. b) Two bridges. Fragments not visible.

Since these bridges do not occur in the normal sisters of the sterile plants, they must be due to crossing-over, not in simple inversions, but rather in inverted segments which have been translocated and reduplicated and do not therefore usually pair. These segments may lie in non-homologous chromosomes. Their

existence has been revealed by the occurrence of a certain amount of pairing at meiosis in the haploids of most organisms where these have been examined (e.g. Katayama 1935, *cf.* Darlington 1937). Such intra-haploid pairing probably occurs also in triploids and other odd-multiple polyploids where complete pairing with normal homologues is impossible, but here it cannot always be distinguished from normal crossing-over (Upcott 1937). In diploids and even-multiple polyploids, it can occur only when normal pairing is interrupted or prevented.

A case comparable to the present one has been found by Lamm (1936), in *Secale*. In certain strains which had been inbred for several generations, bridges and fragments occurred in the pollen-mother-cells, although they are not present in the out-crossed plants of the same variety. In these strains, metaphase pairing and spiralisation are reduced, so that the meiosis may be described as semi-precocious. Evidently, normal pachytene pairing has been interrupted and in part replaced by intra-haploid pairing. The fact that bridges occur also in the sterile and similarly homozygous *Lathyrus* is an indication not only that similar reduplicated segments exist, but also that the timing abnormality begins before pachytene, a conclusion which had been reached on other grounds.

Correlated Effects of Delay on Meiosis

The present observations provide for the first time experimental evidence of the genotypic control of spiralisation and terminalisation

which has been indirectly evident for some time (Darlington 1932). A similar case of this has been found among the Neuropterans by Naville and de Beaumont (1933). They illustrate meiosis in the male and in the female *Macronemurus appendiculatus*. From their illustrations, it appears that in the male the chromosomes are more contracted and the chiasmata more completely terminalised than in the female. The difference between the two sexes corresponds in a less degree to that between the sterile and the normal *Lathyrus*. In the one case the difference is due to the different conditions of development of the eggs and sperm, in the other to change in a single gene. The significance of this parallel is increased by the remoteness of the groups to which these organisms belong.

It is now possible to show directly what are the effects of delay in the later prophase stages of meiosis. We see that a greater degree of terminalisation occurs and associated with this, a greater degree of spiralisation. Terminalisation is presumably determined by the mutual repulsions of the chromosomes forming the bivalent and also by the length of time during which this repulsion is allowed to act. On the precocity theory, it is understandable that the greater spiralisation observed at meiosis should be due also to the greater length of time available, provided that it is a function of time. If more than the usual length of time is available, the degree of terminalisation and of spiralisation should be increased.

The present observations show that this is so, and that the two processes are correlated, the one being an internal, the other an external adjustment to the electrical surface conditions of the chromosomes.

Summary

Pollen sterility in *Lathyrus odoratus* is known to behave as a simple Mendelian recessive.

As compared with the normal meiosis, chromosome development in the sterile is delayed in relation to the growth of the anthers. This delay begins to take effect before pachytene.

That the timing is abnormal is shown by the fact that the sterile anthers containing pollen-mother-cells at the first metaphase are equal in size to normals containing binucleate pollen and by the fact that the spindle at metaphase is elongated as it is at a normal anaphase. The development of the chromosomes is delayed relative to that of the spindle. Associated with this delay is a difference of substrate conditions in the nucleus as shown by (i) its reaction to the fixative in prophase (ii) greater spiralisation (iii) greater terminalisation, indicating that these are all a function of rate of development.

After the first division, the timing disharmony apparently becomes so acute that further development ceases.

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