Embryological studies on seed incompatibility in primula

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Abstract of Ph.D. thesis. S.R.J. Woodell.

Valentine termed the phenomenon of post-fertilization failure in interspecific crosses "seed incompatibility." On the basis of the results obtained from crossing them, he allotted different "genetic values" to the genomes of three species of Primula. The genetic ratio "R" (ratio of genomes of endosperm and maternal tissue) was calculated for each cross. When "R" is greater than the normal 1.5, so-called "type B" seeds, large and often empty, are produced. When R is less than 1.5, "type A" seeds, small and often well-filled, are formed.

The present study is a detailed histological investigation of seed development of some crosses in the genus Primula. "type A" seeds have been shown to have overdeveloped inner integuments but relatively normal endosperms; "type B" seeds have thin integuments and poor endosperms. In every case the condition of the embryo apparently depends on that of the endosperm.

In the cross between diploid P. veris and its autotetraploid, the 4n x 2n mating (R = 1.25) produces extreme "type A" seeds, the reciprocal (R = 2.0) gives extreme "type B". The results of these and other crosses involving various values of "R" all give support to the "genetic value" concept.

The nature of genetic value is discussed, and the hypothesis advanced that a diploid species may evolve toward the polyploid state by the accumulation of small duplications, giving it an increased amount of chromatic material, and thus increasing the "genetic value" without changing the chromosome number.

Important previous work on seed incompatibility is reviewed and critically discussed. Finally the hypothesis is advanced that in Primula crosses, the incoming pollen imparts an abnormal stimulus to the embryo sac, and, depending on the value of "R", there is more or less serious physiological unbalance between endosperm and maternal tissues.

EMBRYOLOGICAL STUDIES ON SEED INCOMPATIBILITY IN PRIMULA.

BY

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B.Sc. (DUNELM)

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NOTE

Throughout this thesis, whenever a cross of any kind is mentioned, the first parent to be named is the female parent.

I INTRODUCTION.

It has been known for a long time that one or more of a variety of causes may prevent two species from hybridising successfully. Ecological and geographical barriers are often sufficient to prevent them from ever having the opportunity in nature. Cain (1944) discussed these barriers in detail. In many cases, when two species that are so isolated are brought together in cultivation, the genetic barriers between them are found to be weak, and successful gene exchange can occur. Species that have been in contact for a long while under natural conditions, on the other hand, have generally evolved genetic barriers to crossing. Between the extremes of fully successful crossing and complete failure to cross there are many stages.

If two species are crossed artificially, failure of the cross can occur in either pre- or post-fertilization stages. Pre-fertilization breakdown can be attributed to one of several causes. Thompson (1940) has quoted at least one example of each of the following:

1. Failure of pollen to germinate on the stigma.
2. Slow growth of the pollen tube in the style.
3. Bursting of the pollen tube.
4. Death of the pollen tube for reasons other than bursting.
5. Inability of the sperm to fertilize the egg or endosperm nucleus.

In some cases there are means of overcoming these barriers; by splitting the style, for instance, or by the use of hormones. However, even where these aids to successful fertilization are used, the hybrid still often fails. Breakdown after successful fertilization has been classed by Thompson (loc. cit.) into three groups:

1. Death of the hybrid, at any time from a few-celled embryo to a nearly mature plant.
2. Failure of the endosperm to develop.
3. Abnormal development of the endosperm.

In the present author's opinion failure is not so simply classified into clear-cut groups, but Thompson's divisions give the broad outline. Death of the hybrid, his first group, is in many cases directly dependent on one of the other two.

This thesis is concerned with the phenomenon of post-fertilization breakdown, in particular the failure of seed to develop to full maturity, or the partial failure to do so.

The phenomenon of post-fertilization breakdown has been termed "seed-incompatibility" by Valentine (1953). Valentine coined this term in the course of his work on seed breakdown in the genus Primula. His work will be described in some detail in the historical review, since it formed the basis of the present study. The work discussed in this thesis is a detailed histological study of some of the Primula crosses. An attempt will be made to elucidate the process of seed incompatibility in the light of the behaviour of the tissues in the developing seed.

II HISTORY.

A complete review of all the literature relevant to the topic of seed breakdown would occupy a great deal of space, and in fact is unnecessary. Instead, the most important hypotheses that have come out of former work have been followed, and have been illustrated with brief accounts of the work that led to their proposal. Other work will be mentioned in passing, and the bibliography will include many relevant papers that will not find a place in this brief historical survey.

There are two obvious omissions; the physiology of seed and fruit development, which has been adequately reviewed by Nitsch (1953) and embryo culture, which in recent years has been widely used as a means for overcoming seed failure. This review is confined to the topic of the actual course of seed development, and to the abnormalities to be found in hybrid seed, since it is with this topic that we are mainly concerned.

Abnormalities in seed development were noted by early workers, not many of whom attempted to explain them. Renner (1914) and Riorth (1926) in Oenothera, Michaelis

(1925) in Epilobium, Sawyer (1925)in Iris, and Christoff (1928) in Nicotiana all noted seed breakdown in interspecific crosses. They also often found reciprocal differences in these crosses. Those that carried out histological studies found endosperm abnormalities of varying severity. Clausen (1931) found reciprocal differences in the cross involving Viola arvensis and V. rothomagensis. He suggested that disturbance of the genic equilibrium of the endosperm might be responsible. The first to formulate some sort of a general rule to account for reciprocal differences in seed development was Thompson (1930 a, b).

Thompson worked with various wheats, crossing types which had 14 and 21 chromosomes. When he used the 21 chromosome wheat as female, the endosperm was plump, whereas in the reciprocal cross it was shrivelled. He carried out a series of experiments which indicated that the condition of the endosperm depends on the seven vulgare chromosomes that behave as univalents. (He used Triticum vulgare, n = 21 and T. durum, T. dicoccum and T. persicum, all n = 14). When the endosperm contains three sets of the vulgare chromosomes, or none or very few of them, it is plump and large. It is plump and small

when it contains two sets, and shrivelled when it is haploid for all seven or diploid or triploid for a few only, ie. the farther the situation departs from complete absence or complete triploidy of these chromosomes, the more shrivelled is the endosperm. He later suggested as a general rule that crosses between species of different chromosome numbers would be more successful when the species with the higher number was used as female, on the grounds that the chromosomes that it possesses and the other species lacks will be duplicated in the endosperm, whereas in the reciprocal they will only occur once.

Watkins (1927) came independently to a similar conclusion, and in a later review (1932) surveyed the situation to date. He supported Thompson's conclusions. He suggested that, for instance, in a tetraploid - diploid cross, when the tetraploid is used as female, a 4x plant is nourishing a 5x endosperm and a 3x embryo, whereas in the reciprocal a 2x plant nourishes a 4x endosperm and a 3x embryo. In general the 5x endosperm in a 4x plant develops better than a 4x endosperm in a 2x plant, since it more closely corresponds to the normal situation where a 2x plant nourishes a 3x endosperm. (This is ignoring the consideration of qualitative factors).

This was also the first suggestion that the relationship of the endosperm and mother plant was important.

His suggestion that the hypothesis could be supported by reference to diploid - autotetraploid crosses was followed by comments on such crosses in Saccharum, Musa and Datura, but in none of these cases was it certain that fertilization had been successful in the cross using the diploid as female. However, the results of Gairdner and Darlington, (1932) from such crosses in Campanula persicifolia, reproduced in Table I, did shed some light on the problem.

TABLE I. The results of crosses made with diploid, autotetraploid and triploid plants of Campanula persicifolia.

Cross	Capsules	Seeds per capsule.	Percentage germination	Seedlings per capsule.
3n x 4n	10	46	23.9	6.4
3n x 2n	2	140	10.7	14.5
2n x 4n	22	76	0.6	0.5 *
4n x 2n	34	18	9.8	1.5 +
2n x 2n	-	325	55.3	-
4n x 4n	-	130	38.5	-

* 10 seedlings, 7 tetraploid and 2 diploid.
+ 32 seedlings, 8 tetraploid, 23 triploid and 1 parthenocarpic diploid.

This series of results is somewhat inconclusive, but the germination figures suggest that triploid seed develops better in a tetraploid parent than in a diploid one. The fact that tetraploid seeds can develop in a diploid plant is, however, disturbing.

Watkins also pointed out that qualitative, as distinct from the quantitative differences in these crossed species, may be of importance, and he quoted reciprocal differences in crosses between diploids as evidence. He suggested that in allopolyploids qualitative and quantitative differences might modify each other, but could not assess the relative importance of qualitative and quantitative differences from the evidence then at his disposal.

His general conclusions were:

1. A change from the usual quantitative relations of embryo, endosperm and maternal tissue can arrest seed development.

2. Development seems to be independent of the quantitative relations between the mother on the one hand and the embryo and endosperm on the other.

3. From this it seems that seed development depends on

the quantitative relation between endosperm and embryo.

4. When the chromosome ratio of endosperm:embryo is greater than 3:2 development is better than when it is less.

5. Plants of high chromosome number give better results as females than as males. In autopolyploids this effect is straightforward, but qualitative relations complicate the situation in allopolyploids.

These genetical explanations for seed failure, in terms of chromosome balance in the tissues of the developing seed, did not make it clear how the unbalance was reflected in the tissues. The first real attempt to do this was made by Kihara and Nishiyama (1932). Later workers have shown reluctance to accept their hypothesis. It was based on the seed development they obtained when they crossed different species of Avena, notably A. strigosa, a diploid and A. fatua, a hexaploid. When the diploid was used as female, early development was more rapid than in the intraspecific cross, but irregularities soon appeared, and the seeds were inviable. In the reciprocal, development was slower than normal, but a few of the seeds were viable.

In some respects their views were the opposite of Watkins', at least in terms of seed set, but in terms of viability of seeds their crosses fitted into his scheme. They quoted the results of Mangelsdorf and East (1927) and Yarnell (1931) on Fragaria in support of their case.

They found that seed set was often better where the female parent had the lower chromosome number than in the reciprocal. Germination, on the other hand, was either worse, or did not occur at all. They suggested that the relationship of the gametic chromosome numbers is the governing factor in the setting of seed. The male nucleus, they indicated, can be classified according to its "stimulative strength" and they classified the stimulation into overstrong, normal and weak. Where there was no union of male and female nuclei they stated that the male gamete had "no affinity." If the endosperm and embryo grow abnormally fast, as in Avena strigosa (2n) x A. fatua (6n), the stimulation has been overstrong. If growth is normal, stimulation is normal, and a weak stimulation results in weak growth. A moderate stimulus of the female nucleus by the male is necessary, they surmised, for normal growth of the seed. Overstrong

stimulation results in too rapid growth and subsequent failure, and a weak stimulus fails to initiate a sufficiently rapid growth rate in many cases, though occasionally such a stimulus can result in near normal but slow development, whereas overstrong stimuli are destructive. They could find no explanation for differences in reciprocal crosses between species of the same chromosome number.

This hypothesis was severely (and in this author's opinion, rather unfairly) criticised by Muntzing (1933). He commented; "as far as I know, there is no direct evidence to explain why a nucleus in the pollen grain with a higher chromosome number than normal should "stimulate" an egg cell to more rapid divisions than normally. This assumption seems to me to be rather gratuitous." Muntzing went on to suggest that "plasmatic differences" were responsible, one of the parents being a "better mother" for the hybrid seed than the other. He went on to say that the normal balances of three tissues, endosperm, embryo and mother, which are in intimate contact morphologically and physiologically, will be disturbed if the embryo sac is fertilized by

male gametes which have a different chromosome number from that of the egg cell, and suggested "it is reasonable to assume that this disturbed balance is the cause of disturbed embryo and endosperm development and results in bad seeds." In the present author's opinion Muntzing has departed verymlittle from the argument of Kihara and Nishiyama, who also stated that disturbed balance between gametes was the cause of faulty development. This paper, however, was of some significance, and we shall return to it later. Subsequent workers found that their results were similar to those of Kihara and Nishiyama, and put forward similar explanations. Notable among them were Katayama (1933), Wakakuwa (1934) and Ledingham (1940). Beasley (1940) obtained similar results but did not attempt to explain them in this way. Katayama used Aegilops and Triticum, and could not see any really consistent relation between chromosome number and seed set, or between seed set and germination. He pointed out that with closely related species quantitative relations would be important, whereas less closely related species would be more affected by qualitative differences. Excessively fast development (progressive abnormality), or excessively slow, (regressive abnormality) could both

cause seed failure. He summed up the possible disturbances, qualitative and quantitative, in tabular form, showing the inter-relationships of all tissues concerned, but did not state which he thought most important.

Ledingham (1940) crossed Medicago sativa (4n) with a diploid form of M. falcata. With the diploid as female, development of the embryo goes on slowly for two weeks, and though the ovules abort the ovary forms a mature pod. In the reciprocal cross fertilization is delayed and endosperm and embryo development is quickly arrested. However, a few tetraploid hybrids are formed, presumably from unreduced pollen, and it was pointed out by Ledingham that divergence from normal balance of embryo and endosperm was greater here than for diploid hybrids, and yet these tetraploids are fertile. As pairing between the chromosomes of the two species is very good, he dismissed chromosome unbalance as being the cause of failure. He suggested that if the male has the higher chromosome number the rate of physiological activity and cell division seems to be increased, whereas in the reciprocal the endosperm and embryo nuclei are not stimulated to a sufficient rate of division, and the developing maternal tissues utilise the available nutrients, causing embryo abortion.

All the cases so far mentioned have led those investigating them to dwell on the quantitative relations of the chromosome complements of the species concerned, and several of them have come to the conclusion that the stimulus to development imparted by the incoming pollen is the vital factor in determining the rate and subsequent success of seed development. These we might call the "pollen stimulus" theories.

A theorytthat has excited a good deal of interest was that put forward by Brink and Cooper (1940) as a result of their studies on Medicago, and later developed by them in the light of the evidence provided by other groups. For convenience this will be called the "nutrient competition" hypothesis.

Medicago sativa is a self-incompatible species. When it is selfed, ovule collapse is frequent in the early stages. The inner integument (endothelium) is normally two cells thick, but in the selfed seeds it shows marked meristematic activity, and as the endosperm degenerates, the inner integument proliferates. This local hyperplasia of maternal tissue they called "somatoplastic sterility."

Their argument (1940) is worth quoting fairly fully, as it has been the basis of their later ideas, and of some other workers hypotheses.

In their discussion of the results obtained in Medicago, they said: "Fertilization initiates the development of endosperm and stimulates mitosis in the surrounding maternal tissues. The ovule springs into active growth. Visible reserves of food soon disappear, and development depends on food moving in from other parts of the plant. The critical factor seems to be the manner in which the translocated food is shared between the endosperm on the one hand and the inner integument on the other, and this partition of nutrients appears to depend on the rate of growth inside and outside the embryo sac. It may be assumed that the synthetic processes are essentially alike in the three tissues concerned and hence that the same raw materials are in concurrent demand. Under these conditions of parallel growth the available foods will be shared by the inner integument and the embryo sac structures in proportion to the rate at which growth is going on in the respective tissues. Successful development demands a balanced growth

rate between the endosperm, the dominant embryo sac tissue, and the adjacent maternal tissue, ensuring the nourishment of both. If this balance is upset in early development by failure of the endosperm to keep pace with the surrounding tissue the endosperm starves and collapse ensues. Following cross pollination in alfalfa the early endosperm growth rate is higher than that in selfed ovules, and the higher survival rate is attributed to this more active growth."

Brink and Cooper regarded the endosperm - maternal tissue relationship to be vital in seed development, and formulated the hypothesis that the conjugation of a male nucleus with the polar-fusion nucleus to form the primary endosperm nucleus is a mechanism whereby the physiological advantages of hybridity are conferred upon the endosperm, and the more successful development following cross-fertilization in alfalfa is due to endosperm heterosis.

They went on to show that their hypothesis could be applied to other groups. (Cooper & Brink 1940, Brink & Cooper 1941). In crosses between Nicotiana rustica and N. glutinosa, and between Petunia violacea and

Lycopersicon esculentum, the nucellus is hyperplastic and the endosperm degenerates. In Nicotiana rustica x N. tabacum they found that the process was less marked, hyperplasia of the nucellus was incomplete, and seed failure was not total. They regarded this as a case of incomplete somatoplastic sterility.

Their later papers need not be referred to in detail. They investigated failure in Hordeum and Secale (1944) and attributed failure in crossing to abnormal antipodals. Such failure they regarded as being peculiar to the Gramineae. Lycopersicon was their next subject; they used diploid and autotetraploid races of L. pimpinellifolium and a diploid, L. peruvianum. They again (1945) found varying degrees of inner integument hyperplasia. As far as crosses between different chromosome races of the same species were concerned, they attributed seed failure to variation in chromosome balance between endosperm and mother. Where two diploids were concerned, and there was no difference in chromosome number, the similar course of seed collapse was attributed to alteration in the physiological balance between endosperm and mother by genome substitution. Both numerical or genic unbalance precipitate the same series of histological changes. Finally, they found that tetraploid L. pimpinellifolium.

x diploid L. peruvianum was more successful than tetraploid L. pimpinellifolium x diploid L. pimpinellifolium. They suggested that here genic and numerical disturbances were acting in opposite directions, tending to cancel each other out. This points to the difficulty of analysing the causes of seed failure when species are crossed which differ both in genic complement and chromosome number.

In a review of the problem of seed development in general (1947b) they did not make a detailed analysis of the current hypotheses. They concluded that the endosperm is vital in maintaining the life cycle during the early stages of development of the seed, nursing the young embryo and taking over its functions in early life. They also suggested that the double contribution that it gets from the maternal parent weights the endosperm genetically in favour of its mother, and further that its triploid condition might have some physiological significance. Finally they suggested that the secondary fertilization confers the physiological advantage of hybridity on the endosperm in the competition for nutrients with the maternal plant.

They concluded by putting forward some suggestions for future work. The speculated on whether the embryo ever plays any part in seed failure; whether the antipodals are important in any group other than the Gramineae; whether there are any special metabolites involved in seed development, and whether the endosperm, when abnormal, can affect structures outside the seed.

Their approach was purely histological, and their conclusions were based solely on histological evidence. More recently their hypotheses have been criticised by several workers, and in general the nutrient competition hypothesis has not gained much acceptance. Fagerlind (1948) has attributed seed failure in Rosa to somatoplastic sterility. Beamish (1955) traced a similar course of breakdown in various crosses in which Solanum demissum (2n = 72) was pollinated by various diploids. Endosperm failure was accompanied by over-growth of maternal tissue. Other workers have found such maternal overgrowth, but have not attributed it to nutrient competition.

There are two other main lines of thought, one of which is based on the conclusion that physiological

disturbances are responsible for seed failure, and the other on the importance of genetical relationships. The chief champions of the physiological approach have been Blakeslee and his school, who have worked almost exclusively on Datura. The genetical explanation has been used by Stephens (1942), Howard (1947) and Valentine (1947 et seq.) Valentine's work led to the present investigation, and so consideration of the genetic approach will be left until the end of this review.

Such terms as "physiological unbalance" and "chemical regulation" had been used rather indiscriminately by some workers. The first papers on the topic from Blakeslee's school did not carry their consideration much further, but they laid the foundations for the later development of the ideas that they put forward, and they deserve brief mention in this context. They were themselves preceded by a decade of genetical and pre-fertilizations studies, which will not be brought into this discussion.

Sansome, Satina and Blakeslee (1942 a, b) discussed a series of incompatible species crosses and diploid-autotetraploid crosses. In the cross tetraploid x diploid fertilization is the rule, the endosperm develops

to a certain extent, but its eventual degeneration is followed by endothelial proliferation. In the reciprocal the frequency of fertilization is less, but when it is successful the behaviour of the young seed is essentially similar to that of the tetraploid x diploid. In incompatible species crosses development goes a little further, but the same type of breakdown pattern is followed. Blakeslee and his collaborators were at a loss to account for the breakdown of all three types of cross and the similarity of its course in each case. Quantitative changes in either direction as well as qualitative changes all have the same effect. The nutrient supply is not blocked by the hyperplastic endothelium. They could say no more than that they thought the early stages were critical, and that there might be some relation to chemical regulation.

Sachet (1948) as a result of a series of crosses involving six species of Datura, noted similar patterns of breakdown, and also pointed out that within a single capsule different rates of development and sometimes variations in the pattern of breakdown can occur. Her hypothesis was that the foreign pollen tube may bring some substance or substances into the ovules, in either

too great or insufficient quantity, or a precursor which may induce the exaggerated or insufficient formation of such substances within the ovule. In interspecific crosses there would be differences between the substances of the different species, and in diploid - polyploid crosses a difference in quantity. Sachet suggested enzyme relations as a possible further effect that might be unbalanced.

Sachet referred to some work by White & Braun (1942) who had shown that tumours arise on sunflower plants inoculated with the bacterium Phytomonas tumefaciens. These tumours are free of bacteria and often arise a long way from the original inoculation.. Further; implantation of these tumours into healthy plants induced more tumours on them. White & Braun compared these tumours with animal cancers. Sachet made the suggestion that the overgrowth of the endothelium in Datura might be due to a similar factor.

Sanders (1948) made a series of crosses with four Datura species, and came to the conclusion that variation within a single capsule was environmental. She pointed out that some of her results were explicable by Brink

and Cooper's theories, but others were not.

Swanson, Lavelle & Goodgal (1949) treated young developing seeds of Tradescantia with 2,4 dichlorphenoxyacetic acid. The earlier in development it was applied the more effective it was. It greatly reduced endosperm development, the chalazal region of the integument disintegrated, the seeds collapsed and then the nucellus and integuments broke down. They attributed the endosperm failure directly to the effect of the growth regulator.

The discovery that led to the most promising line of work was made by Rappaport, Satina & Blakeslee (1950a). This was the presence of so-called "ovular tumours" in incompatible species crosses in Datura. The endothelium in such crosses proliferates and penetrates into the embryo sac to form tumour tissue. Satina, Rappaport & Blakeslee (1950) described them in more detail. Instead of the normal degeneration of the endothelial cells, they proliferate and in many cases entirely absorb the embryo-sac contents. They sometimes incorporate embryos within them, these embryos being inviable. In a few cases they found that capsules contain two types of seed,

those with tumours, and others in which the endothelium disintegrates and the embryo-sac is swollen with a large amount of jelly-like substance.

A further discovery was that seeds from selfings have decreased starch and increased fat and aleurone during embryo development, whereas the reverse holds for incompatible crosses. The lack of fat and aleurone could be explained by the absence of endosperm in which they normally appear. The excess of starch suggests lack or inhibition of the enzyme which converts it.

They offered two explanations for these phenomena. Firstly, there might be two substances involved, one stimulating growth of endothelium and tumour tissue and the other inhibiting the embryo and endosperm, or there might be one substance doing both. They observed that ingrowths of maternal tissue had been recorded by Renner (1914) in Oenothera; Michaelis (1925) in Epilobium; Brink & Cooper (1940) in Medicago; Kostoff (1928) in Nicotiana and Cooper (1945) in Lycopersicon, and that there is no fundamental difference in seed breakdown between these and Datura.

They went on to quote the success of embryo culture

techniques as evidence of the importance of the maternal tissue in development, but added that none of the previous workers had adequately explained why the maternal tissues should be favoured over the embryo.

A variety of different treatments was tried in an attempt to prevent embryo abortion in these crosses. They treated the pollen and the ovaries, they injected various substances into the ovaries, and sprayed the plants with different extracts. None had any effect. They found on the other hand that tumour tissues and embryo sac extracts from incompatible ovules inhibited cultures of normal Datura embryos.

The obvious approach now was to study the extracts from the tumours, and Rappaport, Satina & Blakeslee (1950b) did this. They extracted a water soluble, thermostable substance, which inhibited and eventually killed embryos in culture. Their tests of the substance indicated that it was unrelated to auxins.

Injection of aqueous solutions of this substance into normal ovules caused inhibition. Extracts from the inhibited ovules inhibited a further set, and this process could be repeated three times, suggesting that the inhibitor was self-duplicating.

Ultraviolet absorption spectra revealed the presence of nucleic acids in the extracts; however selfed Datura ovules contain about six times as much of the same substance as do ovules from incompatible crosses. Injections of extracts of embryo-sac contents of selfed ovules, of commercial RNA and DNA into ovules of incompatible crosses failed to retard embryo abortion. RNA and DNA inhibited embryo growth in selfed D. stramonium ovules. However, it was demonstrated that embryo-sac contents of D. stramonium and D. meteloides both contain nucleic acids, whereas those of the incompatible hybrid between them do not, though these acids strongly inhibit embryo growth. They concluded that although nucleic acids inhibit embryo growth in a similar way to tumour extracts, they are probably not alike in their action.

Rietsema, Satina & Blakeslee (1953) studied the effect of indole-3-acetic acid on Datura embryos. It inhibits them very strongly in vitro. They showed that the endosperm of D. stramonium contains auxin, and they suggested that embryo growth may be related to the auxin content of the endosperm; further some factor also present in the endosperm regulates auxin inhibition in

vivo. The absence of this auxin inhibitor in their in vitro cultures could explain the embryo inhibition caused by auxin in such cultures.

Their work continued on similar lines. Paris, Rietsema, Satina and Blakeslee (1953) investigated the effects of amino acids, and found that the addition of a mixture of these to cultures stimulated growth. Rietsema, Satina & Blakeslee (1944) made extracts from ovules of the cross D. inoxia x D. discolor, and these inhibited seeds of D. stramonium. They showed that the inhibitor in the extract is identical with indole 3-acetic acid.

At this stage the work, with many questions left unanswered and controversies left unresolved, was abandoned with the death of Blakeslee. It is to be hoped that this line of attack on the problem of seed failure, in many ways the most promising, will be resumed.

Other workers who invoked physiological phenomena in explanations of their results, sometimes somewhat vaguely, include Modilewski (1945, 1950); Britten (1950) and Buell (1953). Ziebur & Brink (1951) and Pieczer (1952) have carried out embryo culture experiments that have a bearing on the problem.

Before turning to a consideration of the genetical hypothesis, there is one further theory that need be only briefly considered. Many workers have suggested that the embryo may play a part in seed failure, but there has been little evidence in support of this. Weaver (1955, 1957) deduced from the results of crosses between different species of Gossypium that the hybrid embryo sometimes has an adverse effect on the endosperm. His evidence for this is that in some cases the egg cell is apparently not fertilized, and when this occurs the endosperm develops very well. It seems also that the near-normal endosperm in the embryo-less seeds stimulates embryo growth in adjacent degenerating seeds. He could not show this to occur in every cross, and it is the only evidence that supports the thesis that the embryo is active in seed failure.

The genetical hypothesis, which will now be discussed, is rather an attempt to explain the genetic relationships of species involved in crosses than to explain the basic causes of failure. They are, however, intimately related, and the latter may be to a greater or lesser extent dependent on the former.

Stephens (1942) was the first to attempt to explain the results of species crosses in terms of the "strengths" of the genomes involved. These "strengths" are not necessarily correlated with chromosome numbers. His arguments are of some importance and are therefore dealt with fairly fully here. His lead was important in that it enables us to suggest possible explanations of the relationships in crosses between diploid species.

His material was an artificial tetraploid of Gossypium arboreum (N14), which he crossed with several wild diploids. It is rather male sterile, but when used as female, fertilization always occurred except in crosses with G. thurberi. The failure of the pollen of this species to fertilize the ovule of the tetraploid was attributed to some physiological factor not operating in the other species, and is outside the scope of our present discussion.

Stephens started with the assumption that the important relationship in seed development is that between the endosperm and the zygote, and set out to show how his results emphasized this importance. Crosses between the diploid N14 and wild diploids are incompatible,

whereas crosses between tetraploid N14 and wild diploids give up to 100% compatibility. Denoting the N14 genome by 'n' and the wild diploid by 'm', the tissue relations of these two crosses are, respectively (endosperm : embryo) 2n + m : n + m and 4n + m : 2n + m. Thus quantitative differences in cytological balance between endosperm and zygote are associated with the differences in compatibility. On the other hand, diploid N14 seldom shows less than complete viability, whereas, as already stated, diploid N14 x wild diploid is inviable. Here the ratios are respectively 3n : 2n and 2n + m : n + m. Here there are qualitative but not quantitative differences.

In the diploid N14 selfed and the tetraploid selfed the endosperm : zygote ratio is 3:2 and fertility is 100%. In tetraploid x diploid and the reciprocal, both infertile, the ratios are respectively 5:3 and 4:3. Thus the normal ratio is intermediate, deviations in either direction leading to decreased fertility. In interspecific hybrids this normal balance is also upset, and to account for this Stephens introduced his concept of genetic "strength."

He argued that if this endosperm : zygote balance

was really the vital one, it should be possible to tabulate his results on the basis of the size of the deviation above and below the normal "provided it is possible to take account of the differences in "strengths" between different genomes."

Stephens constructed a table of results, which is reproduced here. It can be seen from this table that

Table 2. *The relation between endosperm : zygote ratio and compatibility in diploid and tetraploid* N 14 *crosses*

Percentage viability	Type of cross	Endosperm/zygote ratios	Quantitative ratios	Adjusted ratios ($m = 2n$)
0	Tetraploid N 14 (♀) × diploid N 14 (♂)	$5n : 3n$	5 : 3	5 : 3 (=1·67)
	Tetraploid N 14 (♀) × *G. davidsonii* (♂)	$4n+m : 2n+m$	5 : 3	3 : 2 (=1·50)
Below 20	—	—	—	—
20–40	Tetraploid N 14 (♀) × *stocksii* (♂)	$4n+m : 2n+m$	5 : 3	3 : 2 (=1·50)
40–60	—	—	—	—
60–80	—	—	—	—
Above 80	Tetraploid N 14 (♀) × *armourianum* (♂)	$4n+m : 2n+m$	5 : 3	3 : 2 (=1·50)
	Tetraploid N 14 (♀) × *aridum* (♂)	$4n+m : 2n+m$	5 : 3	3 : 2 (=1·50)
100	Tetraploid N 14 (♀) × *sturtii* (♂)	$4n+m : 2n+m$	5 : 3	3 : 2 (=1·50)
	Tetraploid N 14 (♀) × *raimondii* (♂)	$4n+m : 2n+m$	5 : 3	3 : 2 (=1·50)
	Diploid selfed, tetraploid selfed	$3n : 2n$	3 : 2	3 : 2 (=1·50)
80	—	—	—	—
60–80	—	—	—	—
40–60	—	—	—	—
20–40	Tetraploid N 14 (♀) × *hirsutum* (♂)	$5n+m : 3n+m$	3 : 2	7 : 5 (=1·40)
Below 20	Tetraploid N 14 (♀) × *barbadense* (♂)	$5n+m : 3n+m$	3 : 2	7 : 5 (=1·40)
0	Diploid N 14 (♀) × wild diploid (♂)	$2n+m : n+m$	3 : 2	4 : 3 (=1·33)
	Diploid N 14 (♀) × tetraploid N 14 (♂)	$4n : 3n$	4 : 3	4 : 3 (=1·33)

instead of the crosses falling into three fertility groups corresponding to ratios of 5:3, 3:2 and 4:3, all crosses containing the wild genome 'm' with the

exception of tetraploid N14 x G. davidsonii occur in lower quantitative ratio groups than their fertilities warrant, ie. in the cross diploid N14 x wild diploid, where the ratio is 3:2, fertility is zero, whereas in the cross tetraploid N14 x wild diploid, where the ratio is 5:3, 100% fertility is obtained.

Stephens obtained an estimate of the relative "strength" of the 'm' genome of G. raimondii by equating the endosperm : zygote ratio of the cross: tetraploid N14 x G. raimondii with the normal selfed diploid ratio, since both were 100% fertile. Thus:

$$4n + m : 2n + m = 3n : 2n$$

$$\text{then } m = 2n$$

Similarly when the ratios of the cross: diploid N14 x wild diploid are equated with those of diploid N14 x tetraploid N14:

$$2n + m : n + m = 4n : 3n$$

$$\text{then } m = 2n$$

Inserting m = 2n in the table, it will be seen, (last column) clarifies the relation between fertility and endosperm:zygote ratio, though there are still some anomalies.

Stephens has agreed (personal communication) that this hypothesis could be equally well applied using the ratio between endosperm and maternal tissues rather than endosperm:zygote ratio. The significance of this will be evident later.

Howard (1947) was the next to use this concept of "genetic strength." He measured seed size in crosses between diploid and autotetraploid Nasturtium officinale and allotetraploid N. uniseriatum. The autotetraploid has a seed fertility 44% that of the diploid, and its seed weight is 138% that of the diploid.

Table 3. Seed types obtained from a series of crosses in Nasturtium (Howard, 1947).

Large full seeds.	The three self pollinations
Small full seeds.	N. uniseriatum x 2n N. officinale 4n N. officinale x N. uniseriatum
Large empty seeds.	2n N. officinale x N. uniseriatum N. uniseriatum x 4n N. officinale
Small empty seeds.	2n x 4n N. officinale 4n x 2n N. officinale

Howard's results show various types of seed, and at first there seems to be no relation between different crosses. Howard suggested that N. uniseriatum has a "physiology of seed production" intermediate between those of the diploid and autotetraploid N. officinale.

The genome of N. uniseriatum was calculated to have a "strength" of 1.41 as compared with 1.0 for N. officinale. N. uniseriatum is a tetraploid, and we might therefore expect its "strength" to be 2.0, but Howard has suggested that it has evolved part way toward the diploid condition.

Using this value, and those of 1.0 and 2.0 for the diploid and autotetraploid N. officinale respectively, the endosperm : embryo ratios of the crosses can be calculated. Howard worked them out and arranged them in order of ascending values.

Table 4. The endosperm : embryo ratios in various crosses in Nasturtium (after Howard 1947)

Cross	Endosperm: embryo ratio.	Type of seed.
2n x 4n N., officinale	1.30	small empty
2n N. officinale x N. uniseriatum	1.41	large empty
N. uniseriatum x 4n N. officinale	1.42	large empty
2n x 2n N. officinale	1.50	large full
4n x 4n N. officinale	1.50	large full
N. uniseriatum selfed	1.50	large full
4n N. officinale x N. uniseriatum	1.58	small full
N. uniseriatum x 2n N. officinale	1.59	small full
4n x 2n N. officinale	1.67	small empty

The results of arranging the ratios in ascending order are presented in Table 4, and it will be seen that the allotment of an intermediate value to N. uniseriatum does make for a logical classification of the results.

Valentine's work on Primula provided the basis for the present study, and will be examined in some detail. The section Vernales of the genus Primula has three British representatives, P. vulgaris (the primrose), P. elatior (the oxlip) and P. veris (the cowslip). There are other European members of the Section, which do not concern us.

Valentine made all the possible crosses between the three species, and found that in every case fertilization is successful and seed development begins. He found, however, that the crosses produced very different types of seed, both in size and in contents. Their "seed compatibility" differed. The types of seed clearly vary according to the direction in which a particular cross is made. With each pairing, if the cross is made in one direction the seeds are small and generally well filled with endosperm, whereas in the reciprocal they are often as large as normal seeds, but are usually empty or sparsely filled. The results can be arranged in order of decreasing success of the cross, and the order is the same on both sides of normal. The results are shown in Table 5.

Table 5. Results of crossing three species of Primula (after Valentine 1954)

Cross: Maternal parent first.	mean seed length* adjusted.	% seeds with embryo at maturity.	Highest % germtn. recorded.	Genetic Ratio R
P.veris x P.elatior	17.5	0-10	0.5	1.28
P. veris x P.vulgaris	25.5	90	37.0	1.36
P.vulgaris x P.elatior	23.0	90	64.0	1.385
Intraspecific	40.0	100	100.0	1.50
P.elatior x P.vulgaris	37.5	20-40	39.0	1.65
P.vulgaris x P.veris	39½0	0-15	0	1.69
P.elatior x P.veris	37.0	0	0	1.90

* 25 units = 1mm.

Valentine allotted "genetic values" to the genomes of the three species. At first he considered, as had Stephens, that the relation between endosperm and embryo governed seed failure or success, but later he revised this view and inclined to the view that the relation between endosperm and maternal tissue was more important.

He gave the name "Type A" to seeds that are of small size. These seeds occur when the endosperm : maternal tissue ratio is less than normal. The less extreme type A seeds have a fair quantity of endosperm, and even extreme types, such as P. veris x P. elatior,

sometimes contain a little endosperm. Type B seeds are produced by the reciprocal crosses. R is greater than normal, and the seeds are almost as large as normal seeds. Even the less extreme deviants, however, are often empty, and the extremes are all empty or nearly so at maturity.

The calculations for genetic ratio (R) are made from the genetic "values" that Valentine allotted to the genomes of the species. He chose values of 1.0 for P. elatior, 1.3 for P. vulgaris and 1.8 for P. veris. He derived these values on the grounds that:

(a) The evidence from the crosses suggests that the value for P. vulgaris is intermediate between that of P. veris and that of P. elatior.

(b) P. vulgaris appears to be more similar to P. elatior than to P. veris.

(c) they offer a convenient basis on which to compare tetraploids.

It must be emphasized that these are arbitrary values, selected as a result of the data obtained from crossing the species, and although Valentine has found them to work effectively, they have not been actually demonstrated to represent the situation precisely.

Valentine has more recently (1956) discussed the results he obtained from using the F_1 hybrids as parents in crosses with each other and in backcrosses to the parents. Taking the genetic value of a hybrid to be intermediate between its parents, he has calculated the genetic ratios of the products of such second crosses, and fitted them into the sequence, and in the majority of cases the type of seed that would be expected has in fact been produced, thus confirming that the genetic values are to some extent representing the true relationships of the species. He has made an attempt to establish the genetic basis of the values. He has shown that genetic value is controlled by more than one gene, and given evidence that more than two genes are concerned in the control of seed contents.

Without more detailed evidence, Valentine could not speculate too deeply on the causes of seed failure, but he has suggested that type A seeds may result from a general slowing down of developmental processes, and type B seeds from a stimulation and non-synchronization of stages. This is a faint echo of the proposals put forward by Kihara and Nishiyama (1932).

The hypotheses of Stephens, Howard and Valentine are all, in a sense, logical descendants of that of Watkins (1932) who first suggested that the numerical relations of the genomes might be important in diploid-polyploid crosses. Valentine's use of the maternal : endosperm tissue ratio rather than that of the zygote and endosperm springs from Muntzing's (1933) suggestion that this must be the vital relationship.

This brief and necessarily incomplete review has been so arranged as to follow the main trends of thought on the problem of seed incompatibility. Most other workers have come to similar conclusions to one or other of those outlined.

It would, I think, be valuable here to briefly summarize the main conclusions discussed. After describing the results obtained in the present study, I shall discuss them in the light of these hypotheses, and comment on their validity, not only to the case of Primula, but also to the problem of seed incompatibility as a whole.

There are five main lines of thought, and they can be summarized as follows:

1. The Pollen Stimulation hypothesis.

This, as stated by Kihara and Nishiyama (1932) emphasizes that the stimulation imparted to the young seed by the pollen that has initiated its development is of vital importance. An overstrong stimulus can cause development to be too rapid, breakdown ultimately ensuing, and a weak stimulus initiates such a slow rate of development that many seeds fail to reach maturity. Seed set is generally better with a strong stimulus, but germination is better when the stimulus is weak.

2. The Nutrient Competition hypothesis.

The propounders of this theory, Brink and Cooper (1940) believe that seed development is dependent on the balance of nutrient metabolism between the tissues within the embryo sac, notably the endosperm, and those outside, in particular the nucellus or the inner integument. They were led to this conclusion by the fact that seed breakdown is so often accompanied by gross hypertrophy of the maternal tissue, apparently at the expense of the endosperm.

3. The Physiological Unbalance hypothesis.

It is difficult to give a clear-cut summary of this

hypothesis, but the line of thought that most of its followers have put forward is that in hybrid seed there is some dislocation of chemical regulation. Blakeslee and his collaborators have most successfully tackled the problem from this angle, and they have indicated that indole 3-acetic acid is one of the substances most concerned.

4. The Embryo Control hypothesis.

Though it has often been suggested that the embryo may affect the course of seed development, very little evidence has been forthcoming. Weaver (1957) has shown that hybrid endosperm can develop in the absence of the embryo, and on the basis of this has attributed seed failure in the cross concerned to the hybrid embryo.

5. The Genetic Value hypothesis.

This is less an attempt to explain the causes of breakdown than an effort to explain the genetic basis of species differences that initiate failure. The failure of interspecific crosses is interpreted in terms of the quantitative relations of the genomes of the species concerned, and of the tissues in the seed.

Crosses between plants of different chromosome numbers, and crosses between different species with the same chromosome numbers can both be explained in this way, the latter on the assumption that genomes of different species may have different "genetic strengths" or "genetic values", the nature of which is obscure.

The one other hypothesis, which need not concern us further, was that put forward by Brink and Cooper (1944) to explain the failure of certain cereal crosses. This involved the antipodals as the cause of failure. The idea has been opposed since, and in any case the phenomena they observed appear to be confined to the Gramineae.

These hypotheses have, in the interests of some kind of order, been arbitrarily separated, but it would be attributing to any one of them more than its proposer did to suggest that they were intended to be complete explanations of seed incompatibility. They are all inter-related to some extent, and are certainly not to be considered mutually exclusive alternatives. They are attempts to explain some aspect of the problem, and what is needed now is a synthesis of the different

approaches to give a comprehensive picture. Without a great deal more evidence this is unlikely to be achieved.

In the discussion that will follow the results an attempt will be made to commence such a synthesis. An incomplete picture can be composed, and a possible explanation of seed failure involving more than one of the hypotheses outlined above will be suggested.

III. MATERIALS AND METHODS.

The plants used in this work were from the collection maintained at Durham by Professor D.H. Valentine, and were of British origin. They were grown in pots out of doors and brought into an insect-proof greenhouse when required, after removing any open flowers. The greenhouse was unheated.

Emasculation has been found to be unnecessary in the long-styled "pin" plants, since insect visitors were excluded. The short-styled "thrum" plants were emasculated by the removal of the corolla-tube, corolla and stamens before the anthers were mature.

The crosses were made in three successive years, 1954/5/6. Fixations were made of unpollinated ovules, and then of young seeds at intervals after fertilization. After preliminary runs it was decided that fixations would be made 10 and 20 days after pollination, and thereafter at four day intervals.

In the first season the seeds were fixed in Karpechenko under reduced pressure, and infiltrated by means of a chloroform - ethyl alcohol method. This resulted in severe shrinkage, so in the succeeding

seasons another method was adopted. (I am indebted to Dr. Sophie Satina, late of Smith College, Northampton, Massachusetts, U.S.A. for the details of this method). The young capsules were carefully split at the apex, to ensure rapid penetration of the fixative to the ovules or young seeds. The fixative used was F.A.A. (85 cc. ethyl alcohol 70% : 5cc. commercial formalin : 5cc. glacial acetic acid). Infiltration was effected by means of a normal butyl alcohol series. The results obtained from this method were far more satisfactory.

Sections were cut at 15μ on a rotary microtome, and mounted serially. They were stained either in Heidenhain's iron haematoxylin, or in safranin and fast green (Maheshwari 1939). The latter stain was particularly useful for showing the structure of the integuments.

Measurements were made of seed and embryo lengths with a micrometer eyepiece, as large a sample as possible being measured. Drawings were made with the aid of a camera lucida.

IV. THE CROSSES.

In order to obtain an understanding of the course of development in hybrid seeds it is necessary first to have a picture of normal development in intraspecific crosses, and to use this as a basis for the comparison of the hybrids. For reasons of readability detailed descriptions of the hybrid seed development are included in an Appendix, and in this section brief outlines only of development are included. Together with the tables summarizing development, illustrations and graphs of seed and ovule length, they should enable the reader to grasp the differences between normal and abnormal seeds without reference to finer details.

The development of the seeds of the three species is, apart from minor variations, very similar. The main differences are in timing and final seed size; the latter would be expected to be variable in any case, within certain limits. Between development of seeds on thrum and pin parents there appears to be little or no difference.

A diagrammatic representation of an unpollinated ovule is shown in Fig. 1. (p.46). The names which

Fig. 1. Diagrammatic representation of an unpollinated ovule of Primula (x approx 250).

a: Outer integument 1. b: Outer integument 2.

c: Inner integument 1. d: Inner integument 2.

e: Chalazal region. f: Chalazal pocket. g: Micropyle.

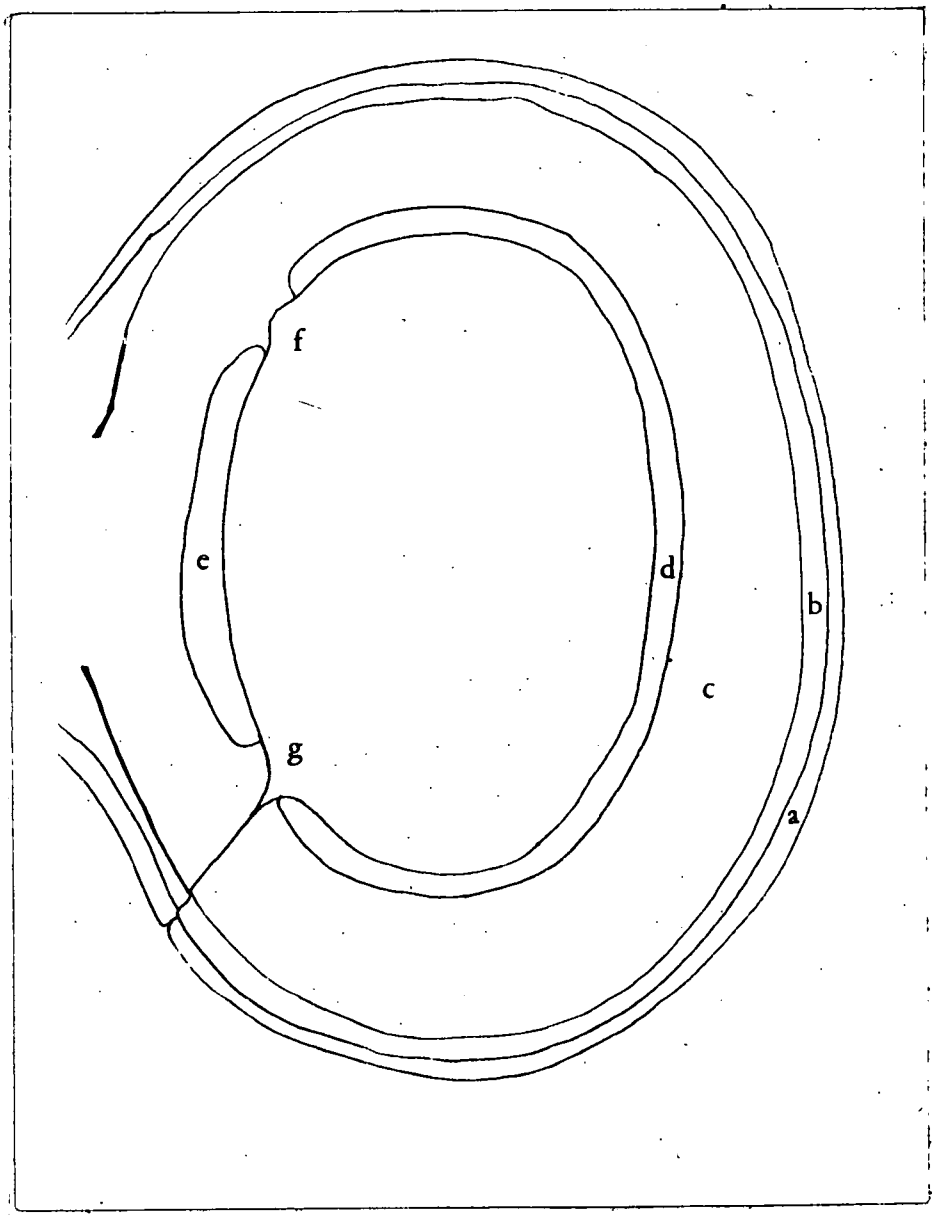
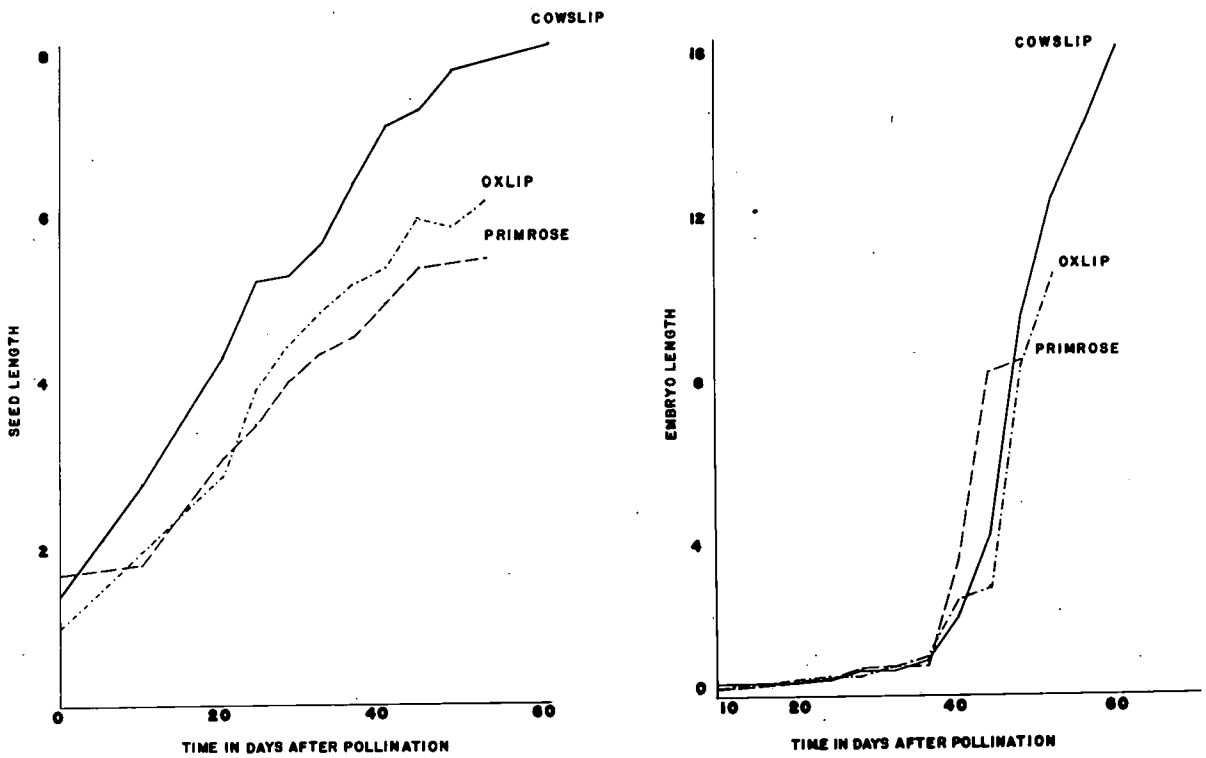


Fig. 2. Graphs of a: Increase of seed length with time, b: Increase of embryo length with time. Lengths are in arbitrary units.



have been indicated on this diagram for the various tissues will be used henceforth when referring to them. The graphs in Figs. 2a and B (p47) show the pattern of increase of seed and embryo lengths in the three species. The graph of seed length indicates the differences observed in seed size between the species.

The following description refers to Primula veris, but could be equally well applied to either of the other species. The development of P. veris has been described by Dahlgren (1916) in some detail, with particular reference to pre-fertilization stages.

The ovules are anatropous, arranged on a free central placenta. They have two integuments (fig 1). The outer integument consists of two layers, each one cell thick; the outermost consists of cubical cells which contain large amounts of brown pigment. This colouration is due to tannins (Decrock 1901). The inner of the two layers (outer integument 2) is a single layer of cubical cells with dense contents.

The inner integument also has two layers. Inner integument 1 may have from 3 - 6 layers of cells with dense contents, whereas inner integument 2 is a single

layer of tannin containing cells. The nucellus is ephemeral, so the embryo sac is in contact with this layer from an early stage. The layer has been given some misleading titles in the past, including "tapetum" and "nutritive layer". Brink and Cooper called it the "endothelium" In this account it will be called "Inner integument 2" This presupposes no special functions for the cells.

After fertilization the zygotic division is delayed, and after it has occurred growth is slow (fig 2b, p. 47). Dahlgren has counted over 1000 endosperm nuclei in an embryo sac in which the zygote had not divided. He states that the primary endosperm nucleus remains adjacent to the egg apparatus at its first division; after the division one daughter nucleus migrates in the thin cytoplasmic layer towards the chalazal end of the embryo sac, the other remaining in its original position. A large number of free endosperm nuclei arise, the first few divisions being simultaneous.

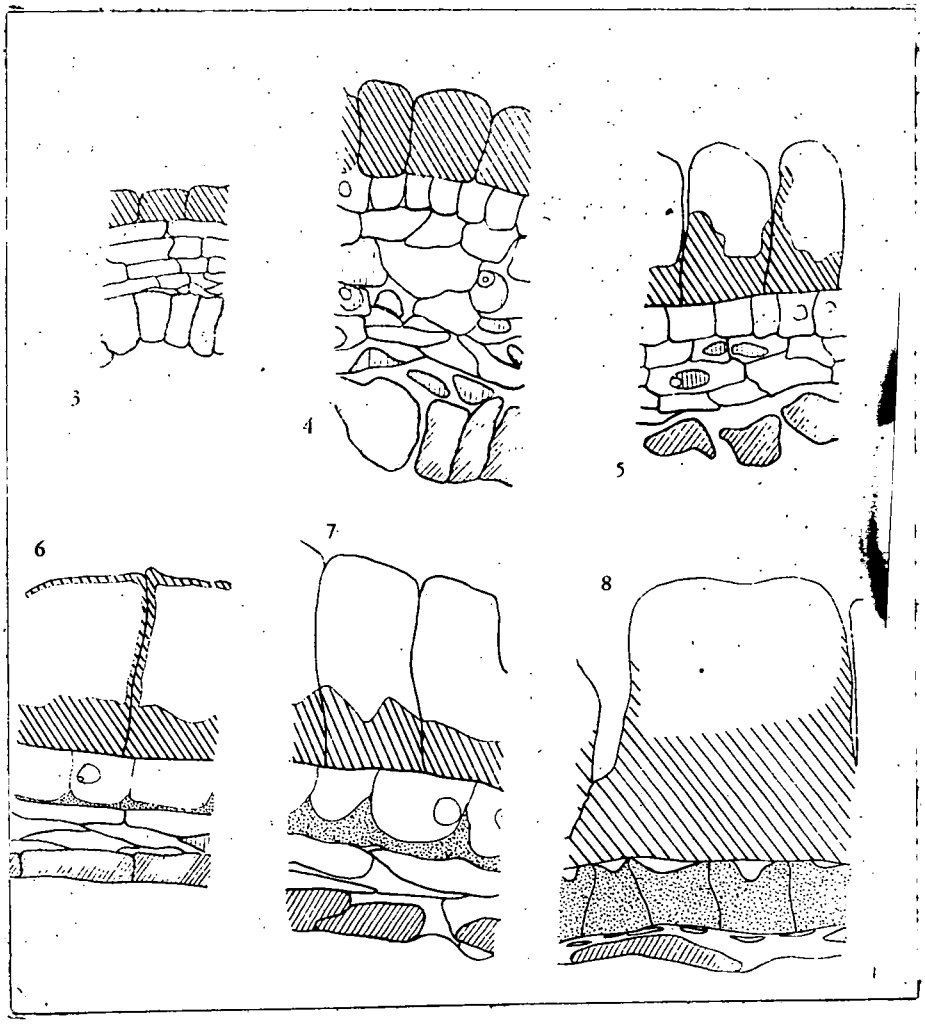
Since the process of normal development is to be used as the basis for comparison of the hybrids, it is described in more detail here than the others. The tables

which summarize development in all the crosses can be used to give a picture of any one tissue at a particular stage, to follow one tissue through all stages, or to see the state of all tissues at one stage.

The outer integument goes through a period of very rapid growth in the early stages of development in the normal seed, and by 20 days after pollination the outer integument 1 cells have elongated outwards, their outer surfaces becoming rounded (they will later form the numerous papillae on the surface of the mature seed). Thickening of the inner surface of outer integument 1 commences before the 20th day, and continues hereafter. by 24 days the cells of the outer integument 2 show a thin layer of thickening on their inner walls, extending up the lateral walls. (Fig.6 p. 51). This thickening increases steadily, about half-filling the cells by the 36th day, and almost completely filling them at 44 days (Fig. 8 p.51).

Inner integuments 1 and 2 undergo much more marked changes. The picture is one of progressive degeneration of first inner integument 1 and then inner integument 2. Inner integument 1, by 20 days, has all but the outermost

Figs. 3-8. Sections of integuments of P. veris. x 340. Outer integument uppermost in each case. 3: unpollinated ovule. 4: 10 days, inner integument showing early signs of breakdown. 5: 20 days, further breakdown apparent. 6: 24 days, thickening of outer integument 2 beginning. 7: 32 days, thickening increasing, inner integument nearly fully degenerated. 8: 44 days, thickening nearly complete, inner integument a thin layer of cell debris.



layer of cells disintegrating (Fig. 5, p.51) and inner integument 2 shows signs of breakdown by this time. Breakdown is less rapid in the chalazal region than elsewhere. At 28 days the inner integument 1 is completely reduced to cell debris except at the chalaza, and inner integument 2 is in an advanced state of degeneration, the cells being flattened and irregular. At 44 days the inner integument 1 has broken down even in the chalazal region, but inner integument 2 persists to a slight extent. The result of this degeneration of the inner integument is that the mature seed is lined with a thin dark layer of cell debris, between the contents of the embryo sac and the outer integument.

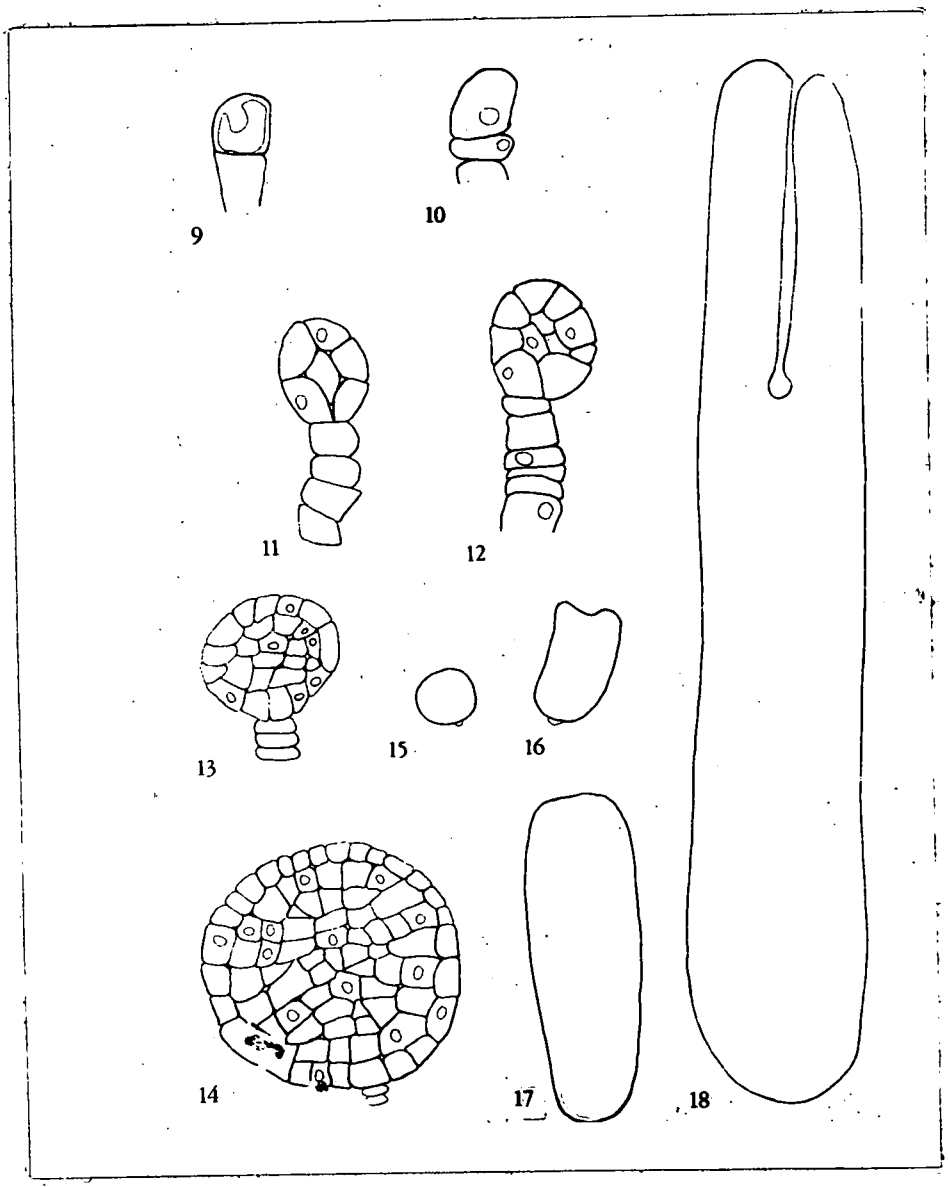
The endosperm in the early stages is a thin layer of cytoplasm lining the seed cavity, with a rapidly increasing number of free nuclei. Cell formation begins between 20 and 24 days after pollination, commencing on the outside with a single layer; successive layers being built up on the first to give rows of cells. Cell formation is most rapid in the chalazal region, and this is not unexpected since the part of the endosperm in this region will be the first to receive nutrients.

The seed is loosely filled with endosperm by about 36 days (Fig.22 p. 57), and it becomes more closely packed after this. The presence of oil droplets in the endosperm is apparent between about 40 and 44 days, the amount rapidly increases from this time on.

The embryo, as Dahlgren pointed out, gets off to a very slow start, and remains in the one-two celled state until about 20 days after pollination, and then begins a more rapid growth. From being 4 celled at 24 days it is 64 celled at 28 days, and then grows even more rapidly, starting to elongate by the 40th day. Cotyledons have appeared by 44 days. Further elongation follows, and food materials accumulate. (Fig 2b, p.47; 9 - 18, p. 54).

The overall picture of development in the normal seed is one of a primary phase of rapidly growing integuments, which is followed by a period of intense endosperm activity; accompanied by degeneration of the inner integument, and finally the sudden access of activity by the embryo, which undergoes a period of rapid growth and differentiation at the expense of the endosperm. Against this background must be viewed the developmental pattern of the hybrid seeds.

Figs. 9-18. Embryos of P. veris in longitudinal section. (9 - 14 x 390, 15 - 18 x 90). 9: 10 days, 10: 20 days. 11: 24 days. 12: 28 days. 13: 32 days. 14: 36 days. 15: 36 days. 16: 40 days. 17: 44 days. 18: 60 days.



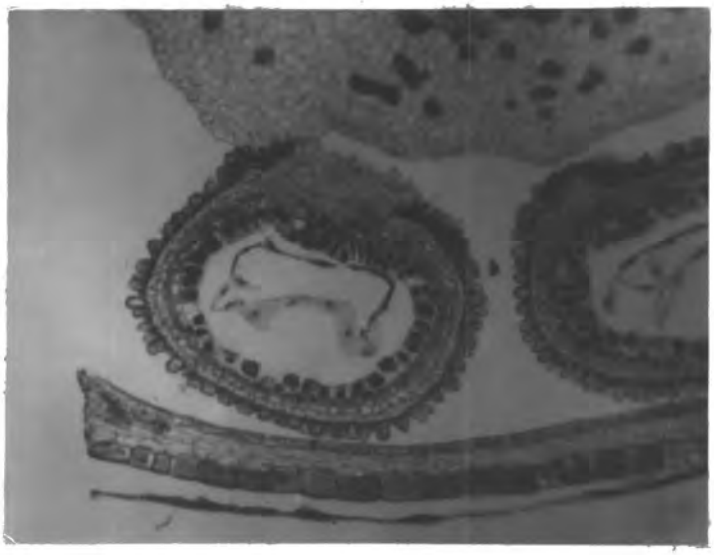
In any one of the species there is a good deal of variation between seeds in the same capsule, and between different capsules on the same plant or different plants. These differences are not likely to affect the problem with which we are concerned very much. They are probably caused in part by minor differences in fertilization time; all ovules in a capsule are not likely to be simultaneously fertilized. Those which are fertilized first will get off to a better start than the rest, and the difference between them will probably be enhanced by the ability of the older seeds to compete for nutrients more effectively. Between different plants, some genetical and environmental variation would be expected.

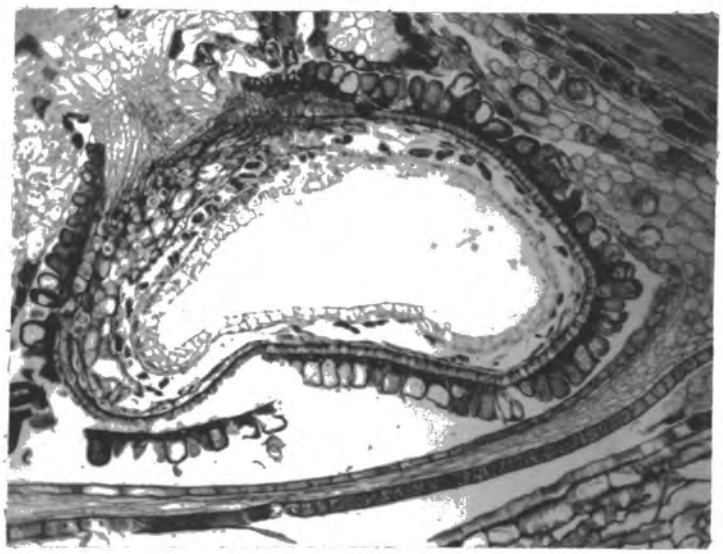
Figs 19 - 21. Longitudinal sections of seeds of Primula veris (Figs 19 & 20 x 75, 21 x 48).

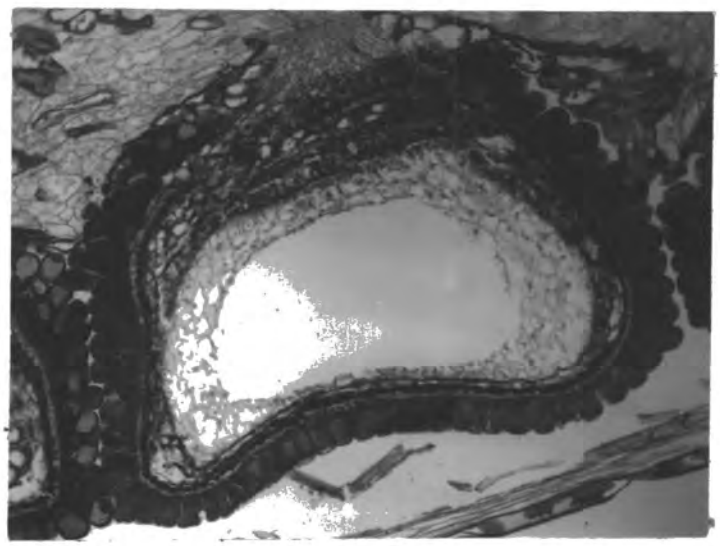
19: 10 days, inner integument showing signs of breakdown, endosperm a non-cellular layer with free nuclei.

20: 24 days, endosperm becoming cellular, outer integument 2 becoming thickened.

21: 32 days, inner integument almost completely absorbed.



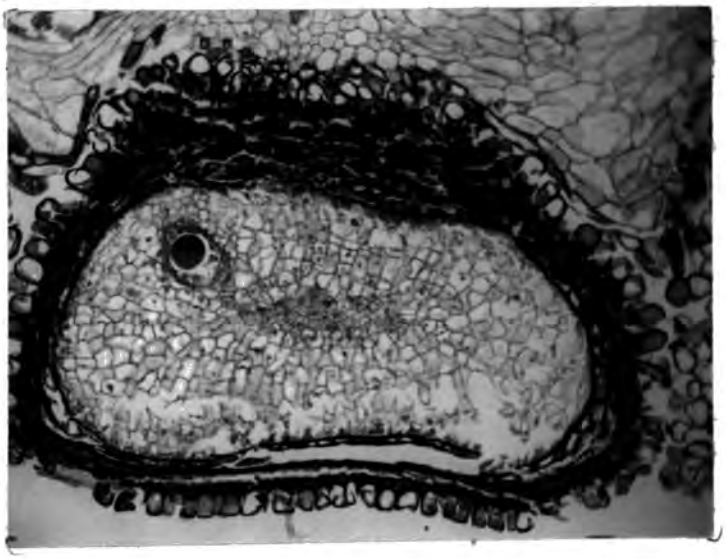


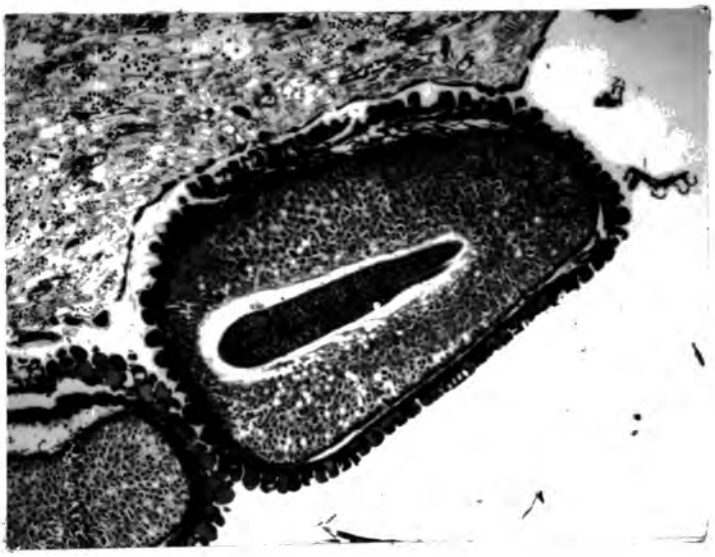


Figs 22 - 23. Longitudinal sections of seeds of P. veris. (fig. 22 x 48, fig. 23 x 25)

22: 36 days, inner integument completely flattened. Endosperm now fills seed, embryo still small and spherical.

23: 60 days. Endosperm packed with reserve foods, embryo fully elongated. Integuments hardened.





COWSLIP x COWSLIP

Time	Outer layer of outer integument	Inner layer of outer integument	Outer layer of inner integument	Inner layer of inner integument	Endosperm	Embryo
Unpollinated	Cells cubical and regular, dark brown	Cells tangentially elongated, dense cytoplasmic contents. More than one layer at chalaza.	Several cells thick, dense cytoplasmic contents, outer layer regular, inner ones progressively less so.	Large cells, elongated inwards, brown and irregular, large at chalaza. Interrupted at micropyle and chalazal pocket.	Wall of the embryo sac is a thin membrane lining the ovule cavity.	
10 days	Cells larger, walls becoming thickened.	Cells smaller, owing to repeated division.	Outermost layer unchanged, others more irregular, with dense shrunken contents.	Elongated inwards, giving 'dumb-bell' appearance, especially at chalaza.	Thin layer of cytoplasm with scattered nuclei. Thicker at chalaza.	Apparently one-celled.
20 days	Cells elongated outwards. Inner walls becoming thickened.	Cells cubical, unchanged in appearance	All but outer layer degenerating, losing contents.	Degenerating, becoming flattened except at chalaza.	Thicker, containing more nuclei.	One-celled.
24 days	Thickening greater, otherwise little change.	Inner wall becoming thickened. Thickening extending up lateral walls.	Contents of outer layer disappearing, other layers now completely flattened.	Very much flattened, except at chalaza.	Becoming cellular, one cell thick, 2 cells at chalaza, here cells denser.	About 4 cells
28 days	Little change.	Thickening increasing up lateral walls, forming a cup inside cell.	Completely degenerated except at chalaza.	Completely degenerated except at chalaza.	2-3 cells thick, very dense next to chalaza.	About 64 cells
32 days	Thickening on inner walls increasing.	Thickening filling about a quarter of the cell.	As above.	As above.	Several cells thick.	Growing rapidly, still spherical.
36 days	As above.	About half thickened.	As above.	As above.	Fills ovule, but cells loosely packed.	Much larger, spherical.
40 days	As above.	About two-thirds thickened.	Degeneration extending to chalaza.	As above.	Cells in regular rows, perpendicular to the chalaza, those near to it small and dense.	Egg-shaped, much larger.
44 days	As above.	Almost completely thickened. In chalazal region, forming a thick wad.	Almost degenerated in chalazal region.	As above.	Number of dense cells increasing, stored food becoming apparent.	Elongated, cotyledons apparent.
48 days onwards	Integument hardens off in the mature seed.				Stored food materials increase in quantity.	Grows rather more, food materials accumulate.

The hybrid seeds.

Valentine's classification of the hybrid seeds into two groups, type A and type B has already been mentioned. Each pair of species, when crossed in one direction produces type A seed, and in the reciprocal cross type B seeds are formed. The species pairs are here considered together, since in this way the results of reciprocal crosses can be immediately compared. After the three species pairs have been considered, the characteristics of type A and type B seeds will be summarised.

Crosses involving tetraploids have been described separately, as they are in a different category. It is convenient to discuss them in the light of the species crosses. There are two types of crosses involving autotetraploids: Crosses between diploids and their autotetraploids, and crosses between diploids of one species and autotetraploids of another. Their significance will become evident later, and will be commented upon in the discussion.

1. P. elatior and P. vulgaris.

The crosses involving these two species are those which produce seeds whose genetic ratios are the least different from normal ("normal", here and in the rest of this thesis refers to the seeds produced by intraspecific matings.) In considering each species pair, the type A cross will be described first.

A. Primrose x Oxlip (P.vulgaris x P. elatior). R = 1.385.

integuments. Outer integument 1 is slightly less thickened than normal. Outer integument 2 is later than normal in starting thickening (28 days as against 24) and has it laid down more slowly. Inner integument 1 is less rapid in its degeneration, complete breakdown being delayed until after 44 days. The inner integument 2 shows most marked differences from normal. Even at 10 days it is rather thick (Fig. 24, p.60) and it becomes progressively thicker as development proceeds, especially in the chalazal region, where there may be two layers of cells. (Figs 25, p.60, 26, p.61). In the more mature seeds the inner integument 2 may occupy about one third of the seed cavity (Fig. 26) but in others it is less extensive (Fig. 27).

P. vulgaris x *P. elatior* developing seeds (x 80)

Fig. 24. 20 days. Inner integument 2 showing signs of hypertrophy already. Endosperm has rather few nuclei, embryo minute. Note that inner integument is especially thick in chalazal region.

Fig. 25. 40 days. Inner integument 2 very thick, especially in chalazal region. Inner integument 1 not yet fully degenerated. Endosperm shows some irregularity, and does not yet fill seed. Embryo still small, very deeply staining.

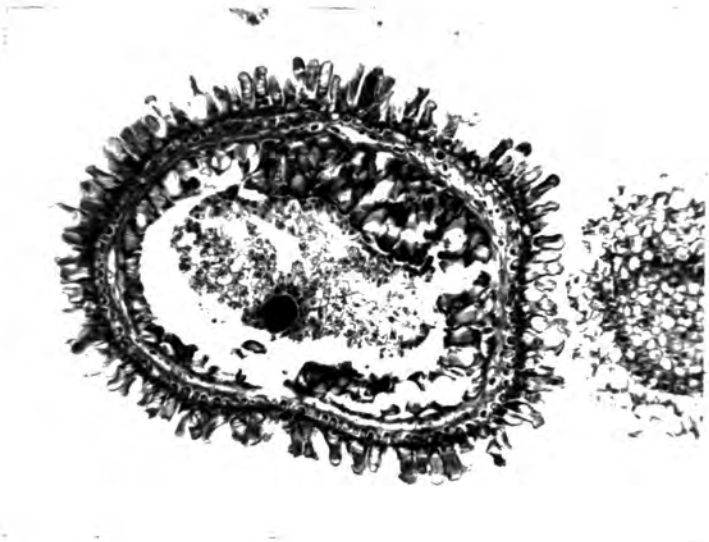
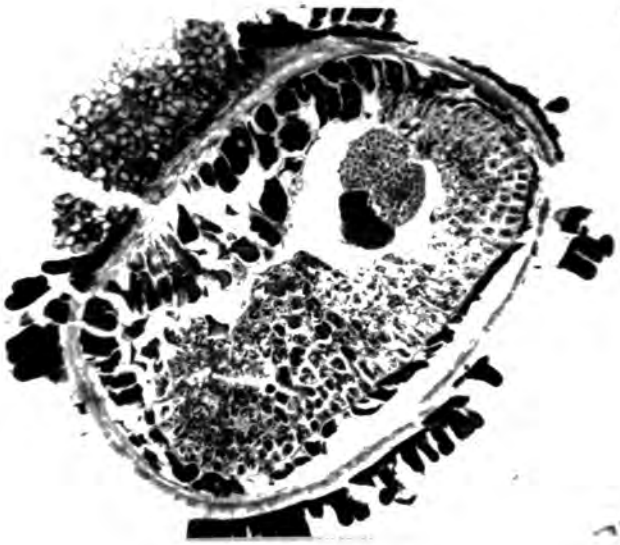


Fig. 26. 44 days. Inner integument 2 thickening most pronounced in chalazal region. Note looseness of endosperm; deep staining material in the interstices.
Fig. 27. 48 days. Seed of type with rather less over-developed integument, endosperm more regular, embryo better developed, much food material present.



Endosperm development is rather slow, cell wall formation begins late and the cells are irregular in shape, and are not in the rows that are seen in normal seeds. The seed is not fully filled until about 44 days after fertilization, despite its smaller size. Food material is less rapid in its accumulation, but in the nearly mature seed the endosperm is not highly abnormal. The embryo is delayed somewhat, and occasionally is distorted (Fig 27).

Two types of seed can be distinguished, one of which shows rather more severe overgrowth of the inner integument (Fig. 26) and the other with less hypertrophy and correspondingly more rapid endosperm and embryo growth (Fig. 27).

The seeds from this cross have, then, a relatively normal outer integument, a greatly overdeveloped inner integument, and a slow-growing but not very abnormal endosperm and embryo. There are two types, one more severely hypertrophied than the other.

PRIMROSE x OXLIP.

Time	Outer layer of outer integument.	Inner layer of outer integument	Outer layer of inner integument	Inner layer of inner integument	Endosperm	Embryo
10 days	Cell walls becoming thickened.	Cells small and cubical with dense contents.	Outer two layers unchanged, others degenerating.	Rather thicker than normal.	Fewer nuclei than in the intraspecific cross.	Single celled.
20 days	Cells elongating outwards, inner walls becoming thickened.	Cells unchanged in appearance.	Outermost layer intact, others degenerating except at chalaza.	Very thick, cell contents degenerating slightly.	Non-cellular, still fewer nuclei than normal.	One celled.
24 days	Thickening somewhat less than normal.	No change in general appearance.	Not yet completely degenerated.	Still very thick, especially at chalaza.	Variable in quantity, sometimes cell walls developing; abnormal in appearance.	One celled.
28 days	Little change.	Thickening beginning on inner walls of cells.	One layer still persisting to a variable extent, especially at chalaza.	Much thicker than normal, chalazal pocket still open.	Single or double layer of cells, best developed when inner integument most degenerated.	Two celled.
32 days	Slightly more thickened.	Thickening increasing.	Outer layer still slowly degenerating, with shrunken contents, less degenerate than normal at chalaza.	Cells very large, longer and two cells thick at chalaza on occasions.	Several layers of cells, irregular in shape, contents dense adjacent to chalaza.	Up to 50 - 60 cells.
40 days	Thickening ceased, cell walls hardening and becoming brittle.	About 1/3 thickened.	One layer still not entirely broken down.	Cells losing their individuality.	Up to about half filling ovule, cells granular at chalaza.	Spherical, rapidly growing.
44 days	As above.	Nearly ½ thickened.	Now fully degenerated.	Very thick, usually in chalazal region filling about 1/3 of ovule cavity, sometimes rather less.	Fills remainder of cavity. Not wholly regular. Contains some food material.	About twice as long as broad.
48 days	As above.	Over ½ thickened.	As above.	As above.	Variable. Still more stored food material.	Increasing in size rapidly, stored food apparent.
52 days	As above.	2/3 thickened.	As above.	As above.	As above.	Still growing, variable in size.

B. Oxlip x Primrose (P. elatior x P. vulgaris) R = 1.65

integuments. Outer integument 1 does not become very much thickened, and even in the mature seed is flimsy. Outer integument 2 is less rapidly thickened than normal, but the process is complete by 48 days.

Inner integuments 1 and 2 degenerate slowly, being fully broken down by 32 days.

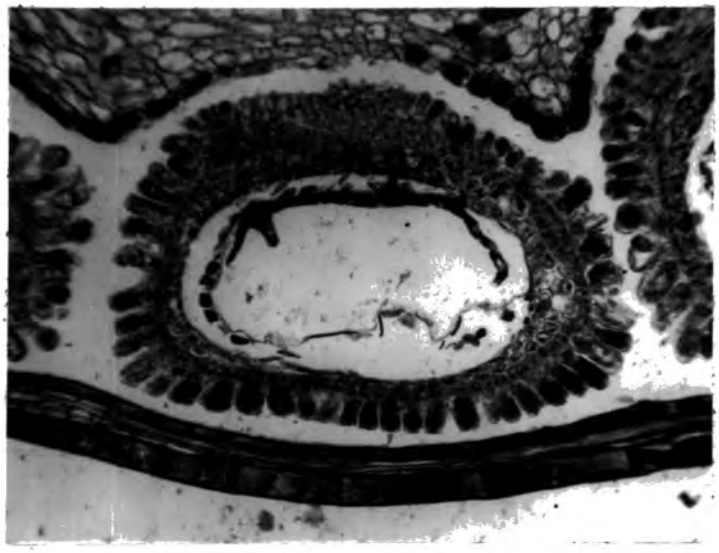
embryo sac. The endosperm shows very marked abnormalities in this cross. The seeds can be classed into two groups on an endosperm basis. In neither class do cell walls appear until the 32nd day, but by this time one type is quite extensive and cellular, the other small in quantity and non-cellular. There are scattered large nuclei in each type, and vacuoles are present, both these nuclei and vacuoles are more prominent in the non-cellular endosperm, which begins to degenerate after about 40 days (Fig. 31. lower seed). The other group of seeds, in contrast, goes on to maturity (Fig. 31 upper seed). They contain a moderate amount of endosperm, and a healthy embryo.

In contrast to the type A seed, these have nearly normal integuments, and either very poor endosperm and embryo, or relatively well developed one. Graphs of seed and embryo lengths are shown in Fig. 32, (P. 67).

P. elatior x P. vulgaris. Figs 28 - 30 x 80, Fig. 31 x 56.

Fig. 28. 10 days. Integuments relatively normal. Endosperm dense and deeply staining, embryo larger than at this age in normal seeds.

Fig. 29. 20 days. Inner integuments practically disappeared. Endosperm with large nuclei and vacuoles. This seed is of the more abnormal type.



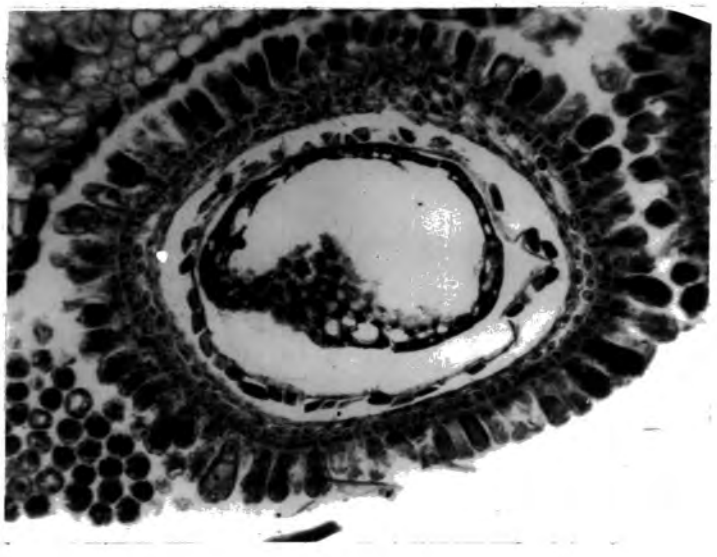
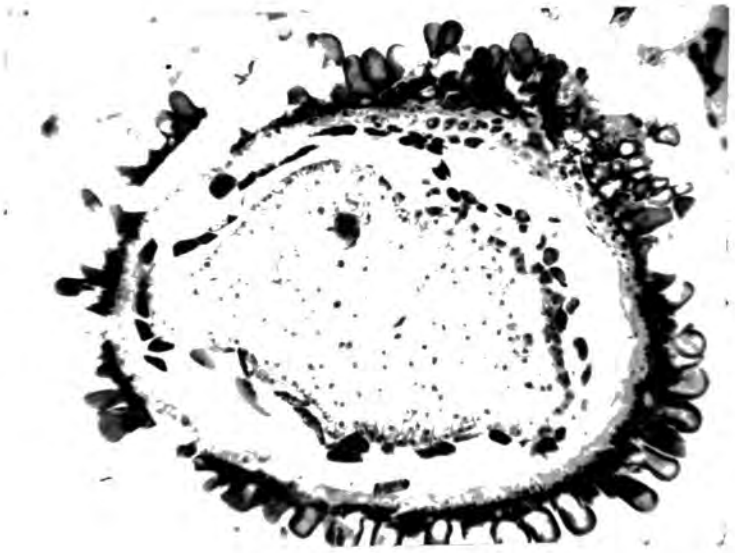


Fig. 30. 36 days. Inner integuments now a thin layer of cell remnants. Endosperm sparse, with scattered large nuclei. Embryo starting rapid growth. This seed is of the more normal type.

Fig. 31. 40 days. Seeds of both types represented in this photograph. The upper seed is of the more normal type, and will develop to maturity. Note presence of scattered large nuclei. Lower seed contains dense endosperm, which is beginning to degenerate. Note that there is little difference between the integuments of the two types of seed.



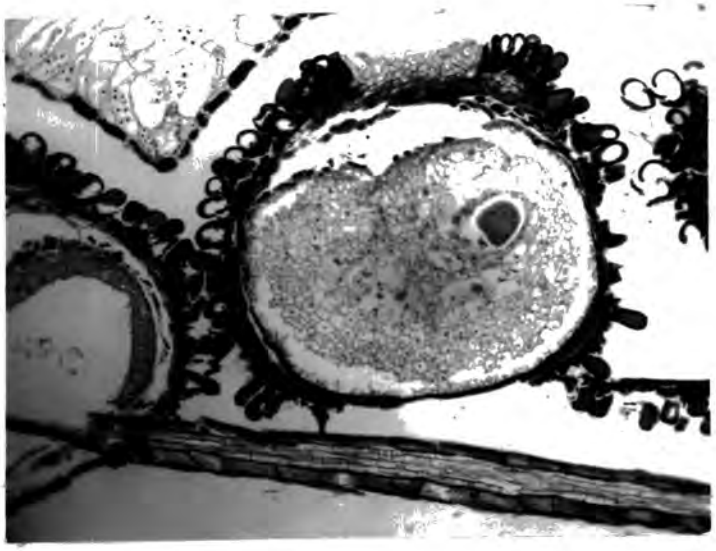
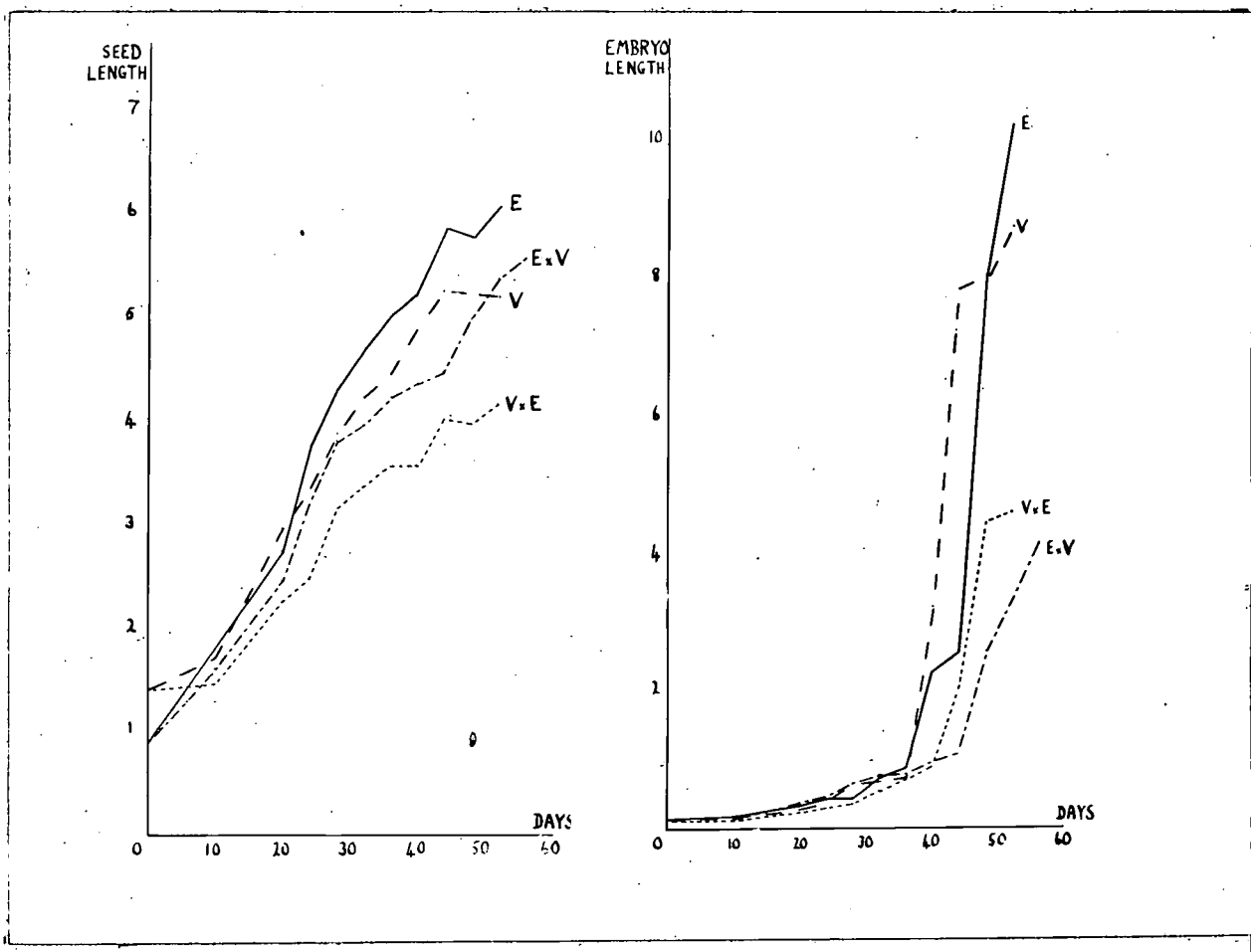


Fig. 32. Graphs of (a) seed length and (b) embryo length increase with time. P. elatior (E); P. vulgaris (V); P. vulgaris x P. elatior (V x E) and P. elatior x P. vulgaris (E x V). Scales for seed and embryo lengths in arbitrary units.



OXLIP x PRIMROSE.

Time	Outer layer of outer integument	Inner layer of outer integument	Outer layer of inner integument	Inner layer of inner integument	Endosperm	Embryo
10 days	Slight increase in cell size.	Small dense cubical cells.	Innermost layers have signs of degeneration.	Fairly normal in appearance.	Thin layer, with rather less nuclei than normal.	Not seen.
20 days	Growing rapidly, inner wall thickening.	Still small cubical cells with dense contents.	Cell contents of remaining layers shrinking.	Cells in all but chalazal region shrinking slightly.	Non-cellular, variable in quantity, with many 'giant' nuclei.	One celled.
24 days	Growing steadily.	Normal in appearance.	Completely broken down except at chalaza.	Fast degenerating except at chalaza.	Increasing in quantity, variable in appearance.	2 - 4 cells.
28 days	Less thickened than normal.	Starting thickening on inner wall.	Degenerating even at chalaza.	Degenerating even at chalaza.	Still non-cellular, with gross nuclear abnormalities.	Up to about 8 cells.
32 days	Rather flimsy.	Thickening going up lateral walls. Layer rather flimsy.	Completely degenerated, merely compacted remains of the cell layers now left.		Increasing but still non-cellular.	Up to about 32-celled.
36 days	Outer walls of cells becoming rounded.	About 1/4 thickened.	As above.	As above.	Sometimes a fair quantity, loosely cellular, often less, non-cellular.	Where much endosperm, spherical. Otherwise even smaller.
40 days	Still flimsy, changes little from now on.	About 1/3 - 1/2 thickened.	As above.	As above.	Two distinct classes: well developed and cellular; poorly developed and non-cellular, both with abnormal nuclei.	Where endosperm good, heart-shaped, otherwise no change.
44 days	As above.	About 2/3 thickened.	As above.	As above.	Good endosperm growing, others not.	Growing well where endosperm good.
48 days	As above.	Fully thickened.	As above.	As above.	Where good, rather loosely packed; where poor, beginning to degenerate.	Elongating rapidly.
52 days on	The ovules with good endosperm continue to maturity, and stored food materials accumulate in endosperm and embryo. The ovules of the second type, with non-cellular, very abnormal endosperm, slowly degenerate from now on.					

2. P. veris and P. vulgaris.

In the two crosses involving these species the deviation of R from 1.5 is little greater than that of the previous crosses; on these grounds one would not expect the seeds to be very much more abnormal. Valentine's germination figures show, however, that it is a considerably less successful cross than that between P. elatior and P. vulgaris, since the germination when P. veris is the female is only 37% and it is nil in the reciprocal.

A. Cowslip x Primrose. (P. veris x P. vulgaris).

integuments. The outer integument is a little more delayed in development, thickening being completed a few days later than in the normal seed. Inner integument 1 has degenerated by the 44th day. As in the P. vulgaris x P. elatior seeds, inner integument 2 is abnormally thick, but although the thickening is similar in early stages, it is distributed more evenly than in that cross. It is not so grossly thickened, and instead of being mainly concentrated in the chalazal region, it extends round the whole of the seed (Figs. 35 & 36, p.72). In addition the

cells are compressed into a compact layer, and are not so large and apparently active as in the previous type A seed.

embryo sac. The endosperm shows signs of cell wall formation quite early in development, but the actual growth rate of the endosperm is slow, and even in the most well developed seeds it barely fills the cavity. Food material has begun to accumulate by the 44th day, but not all the seeds are so advanced by this time.

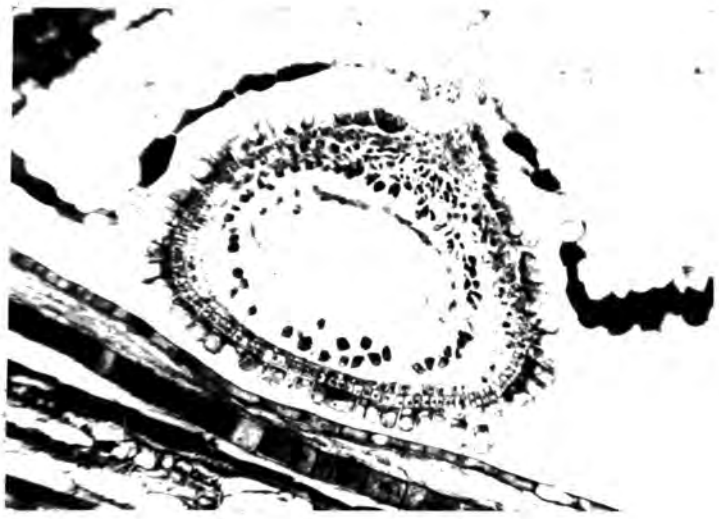
The embryo is again apparently dependent on the growth rate of the endosperm for its own rate of development, as its size in all cases is proportional to the state of the endosperm.

This cross produces seed of the general type A pattern, but it differs from that of the previous cross in that the thickened cells of the inner integument are evenly distributed, instead of being much more concentrated in the chalazal region. At any stage there is a great range of variation from relatively well-developed to rather poorly developed seed.

P. veris x P. vulgaris. All x 80.

Fig. 33: 24 days. Outer layer of inner integument 1 still present, inner integument 2 thicker than normal. Endosperm is still small in quantity, and the embryo is minute.

Fig. 34: 36 days. Inner integument 1 still persisting, inner integument 2 now a well-defined thick layer. Note commencement of thickening in outer integument 1. Endosperm somewhat shrunken, but embryo beginning rapid growth.



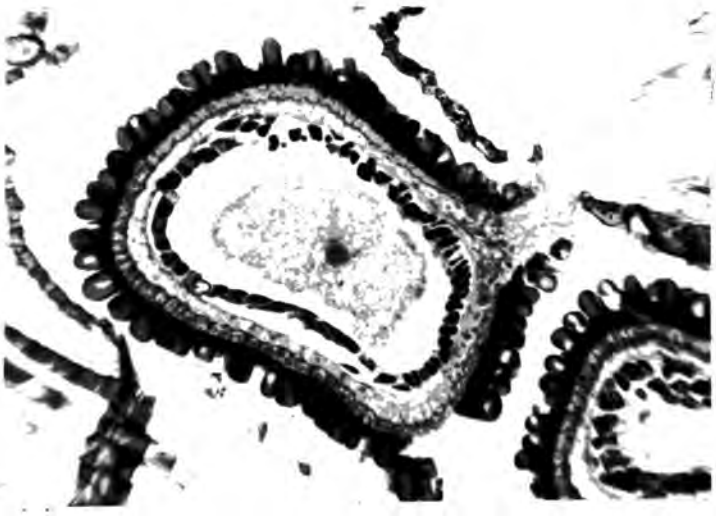
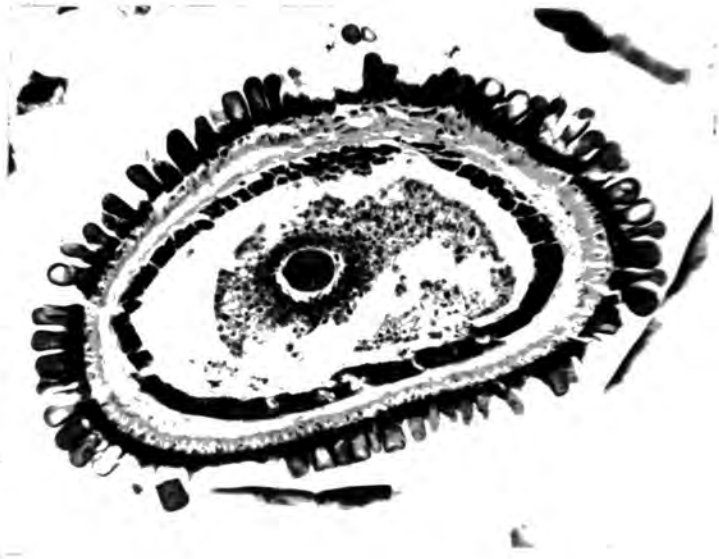
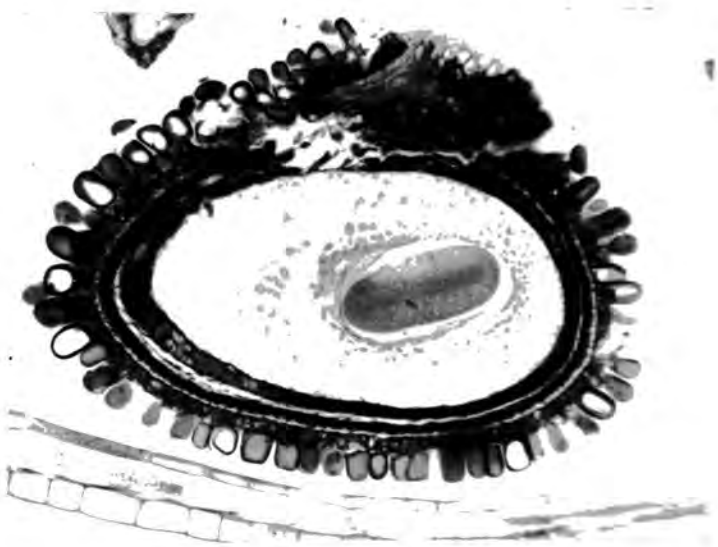


Fig. 35: 40 days. Inner integument 1 now broken down, inner integument 2 compacted. Endosperm shrunken and densely staining, but embryo beginning to lengthen.

Fig. 36: 48 days. Note the even thickening of inner integument 2. Note also that inner integument 1 persisted long enough to form a gap between the thickened innermost layer and outer integuments. The endosperm is a little loose, but contains a quantity of food material, and the embryo is rapidly elongating.





COWSLIP x PRIMROSE.

Time	Outer layer of outer integument	Inner layer of outer integument	Outer layer of inner integument	Inner layer of inner integument	Endosperm	Embryo
10 days	Growing slowly.	Small cubical cells with dense contents.	Cell contents of innermost layer shrinking.	Two cells thick in places.	Very few nuclei.	Not seen.
20 days	Much larger, cells rounded on outer walls.	Normal in appearance.	Outer layer intact, next only partially broken down.	Rather thicker than normal.	Often cellular, sometimes more than one cell thick.	One celled.
24 days	Growth slowing down.	As yet unthickened.	Outer layer still unchanged.	Not increasing in thickness.	Up to 3 or 4 layers of cells.	Two celled.
28 days.	Little increase in size.	Showing signs of thickening in some ovules.	No further breakdown.	Little change.	Occasionally half filling ovule, often much less.	Up to about 16 celled.
32 days	Virtually ceased growing.	Thickening in every case now.	Remaining layer has signs of degeneration.	Not as thick as in primrose x oxlip.	Little more than half filling ovule, cells irregular and variable in size and shape, some nuclei abnormal.	Small and spherical.
36 days	Slowly maturing, appears fairly normal.	About 1/3 thickened.	Contents of outer layer disappearing.	A well developed, uniformly thick layer.	Filling about 2/3 of ovule, more regular than at 32 days, food material appearing.	Still spherical.
40 days	As above.	Half thickened.	Last layer now almost broken down.	As above.	Variable, sometimes almost filling ovule.	Size depending on amount of endosperm, still spherical.
44 days	As above.	Almost 2/3 thickened.	Now completely degenerated.	As above.	Practically filling ovule in most cases.	Becoming heart-shaped.
48 days	As above.	Thickening increasing.	As above	As above.	Loosely filling whole ovule, packed with food material.	Up to twice as long as broad.

B. Primrose x Cowslip (P. vulgaris x P. veris) R = 1.69

integuments. Outer integument 1 barely becomes thickened at all, and the cell walls remain thin and flimsy, forming an insubstantial layer (Figs. 39 & 40, p. 76). The thickening of the outer integument 2 is very slow in developing, and irregular, the net result being that the outer integument is very flimsy. The inner integument breaks down rapidly, the process being complete by the time the seeds are 28 days old, with the exception of the chalazal region.

embryo sac. There is a good deal of variation in endosperm development, but even in the most strongly developed it is small in quantity and very abnormal in appearance. In many seeds (Fig. 38, p. 75) the endosperm rspidly degenerates, leaving a structureless, unidentifiable mass of tissue in the seed. Cell wall formation is generally delayed until about 40 days have passed. The embryo at 48 days is still a minute spherical structure in the best seeds, and probably does not go much further than this.

The result of this cross is to produce large seeds with both underdeveloped integuments and contents. Graphs of seed and embryo length are in Fig. 41, p. 77.

P. vulgaris x P. veris. Fig. 37 x 150; Figs. 38 & 39 x 80; Fig 40 x 56.

Fig. 37: 10 days. Integuments appear fairly normal at this stage. Endosperm tenuous with few nuclei. Note embryo near micropyle, and the vascular supply sweeping behind the chalazal region to the chalazal pocket.

Fig. 38: 40 days. Complete degeneration of embryo sac contents, and collapse of the thin and under-developed integuments. This type of seed is fairly frequent.

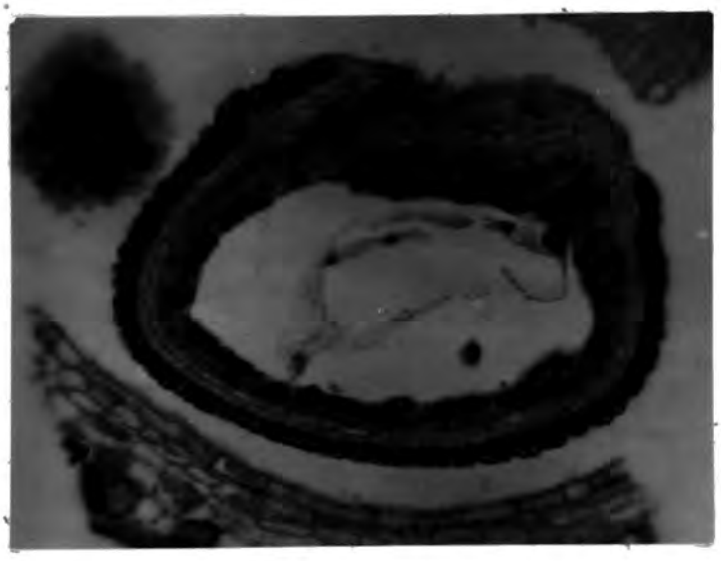


Fig. 39: 40 days. Even in the more fully developed seeds the endosperm is sparse and irregular, and the embryo is a minute sphere. Note the extreme flimsiness of the integuments of these seeds.

Fig. 40: 48 days. One of the better developed seeds. The endosperm is semi-degenerate, and the embryo, not seen here, is barely larger than that in fig. 39.

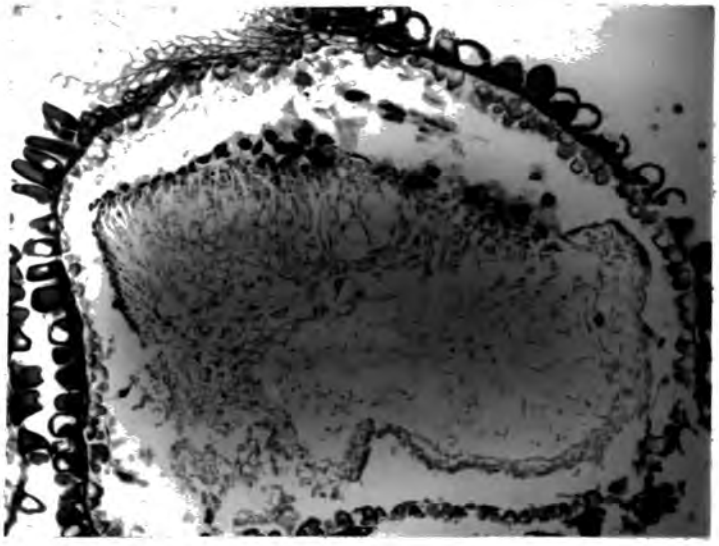
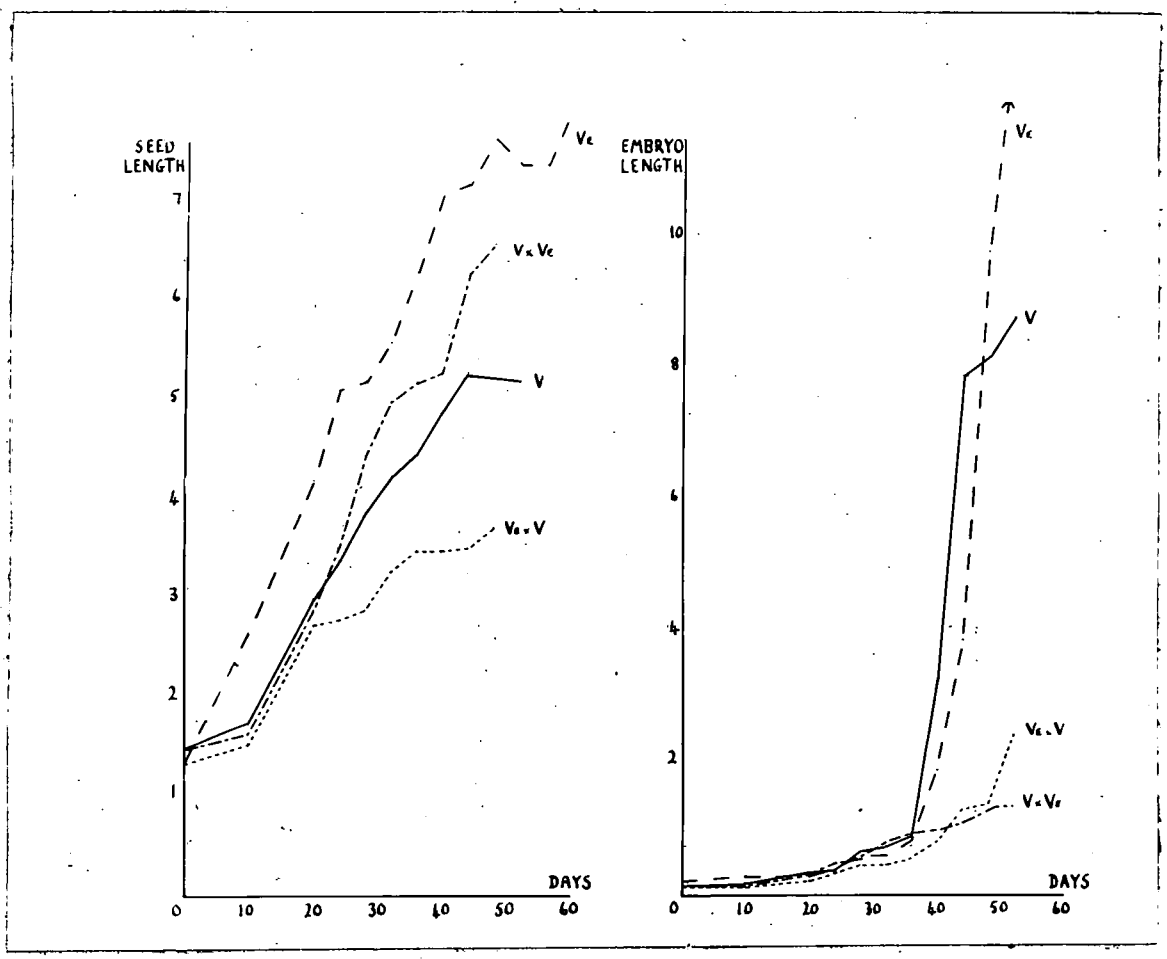


Fig. 41. Graphs of (a) seed length and (b) embryo length increase with time. P. veris (Ve); P. vulgaris (V); P. veris x P. vulgaris (Ve x V) and P. vulgaris x P. veris (V x Ve). Scales for seed and embryo lengths in arbitrary units.



PRIMROSE x COWSLIP.

Time	Outer layer of outer integument	Inner layer of outer integument	Outer layer of inner integument	Inner layer of inner integument	Endosperm	Embryo
10 days	Cells small and thin walled.	Small cubical cells.	Contents of inner layers beginning to shrink.	In some cases rather excessive growth is occurring.	A very tenuous layer with few scattered nuclei.	One cell.
20 days	Cells much larger, still with thin walls.	Cells becoming rather irregular.	Inner layers already degenerated, outer layers losing contents.	Degenerating, but rather overdeveloped in chalazal region.	Single layer, sometimes cellular, very few nuclei.	Not seen.
24 days	Walls of cells still flimsy.	Cells irregular.	Degenerated except at chalaza.	Degenerating rapidly, except at chalaza.	Very small in quantity, not always cellular yet.	Up to about 8 cells.
28 days	No thickening in the cells yet.	Not at all regular, no thickening yet.	A few remnants remain in chalazal region.	Still well defined in chalazal region.	Very rarely cellular, rather tenuous, few nuclei.	From 8 - 40 cells, very small.
32 days	Still unthickened.	A flimsy irregular layer of cells.	As above.	As above.	Slightly increased in quantity.	Up to about 40 - 60 cells.
36 days	As above.	Slight thickening on inner walls.	As above.	As above.	Little increase in quantity, still rarely cellular.	Slightly larger.
40 days	As above.	Thickening slowly increasing.	As above.	As above.	Rarely cellular, often many nuclei, sometimes completely structure-less, small in quantity.	Still small, up to 100 cells, sometimes degenerating.
44 days	A very flimsy layer, with brittle cell walls.	More thickened.	As above.	As above.	Increasing in quantity, more often cellular, about 3 cells thick.	Very little increase in size.
48 days	As above.	Thickening still increasing slowly.	As above.	As above.	Occasionally half filling ovule, more often much less, still frequently not cellular.	Slightly grown, largest seen in ovule which contained no endosperm.

P. veris and P. elatior.

These are the extreme species crosses, whose deviation of R from normal is greatest, and germination figures are lowest.

A. Cowslip x Oxlip (P. veris x P. elatior) R = 1.28.

integuments. The outer integument is slow in the laying down of thickening, but in the mature seed is a substantial layer, which is, however, surpassed by the inner integument. As in the other type A seeds, inner integument 1 is slow in degenerating, and inner integument 2 is very thick, the thickening being evenly distributed, and the cells resembling those in Primrose x Oxlip. (Figs. 44 & 45, p. 81). Collapse of the seeds usually takes place by about 44 days.

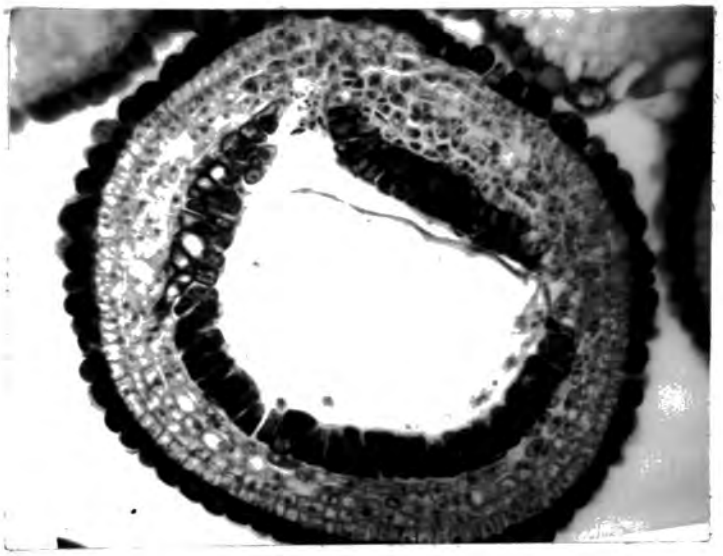
embryo sac. The endosperm is slow in growth, small in quantity, and contains few cells. It begins to degenerate after about 30 days, and the embryo, which by this time is a small sphere, persists for a long time afterwards (Fig. 45).

This cross produces a more extreme type A seed than previously, but with a similar pattern of growth, followed by early breakdown and collapse.

P. veris x P. elatior. Fig. 42 x 150; Figs 43 - 45 x 80.

Fig. 42: 10 days. Inner integument 1 barely starting to degenerate, inner integument 2 already showing signs of hypertrophy. Endosperm very tenuous with few nuclei.

Fig. 43: 20 days. Collapse has occurred very early in this seed, and the endosperm has already been reduced to a few degenerate remains.



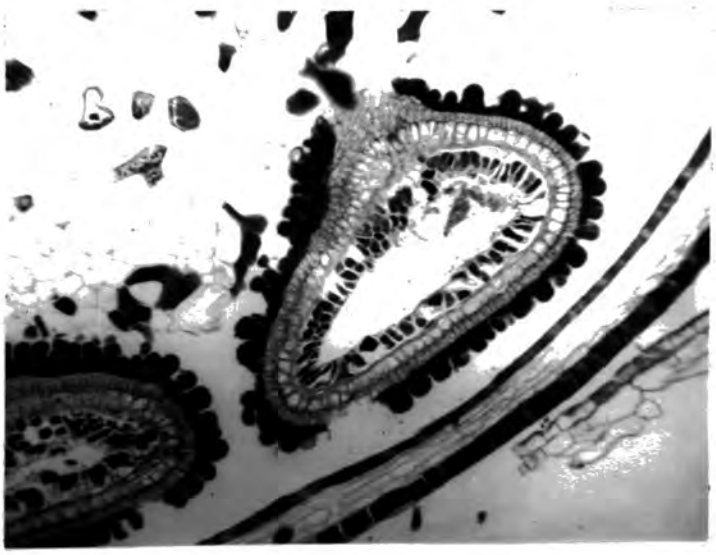
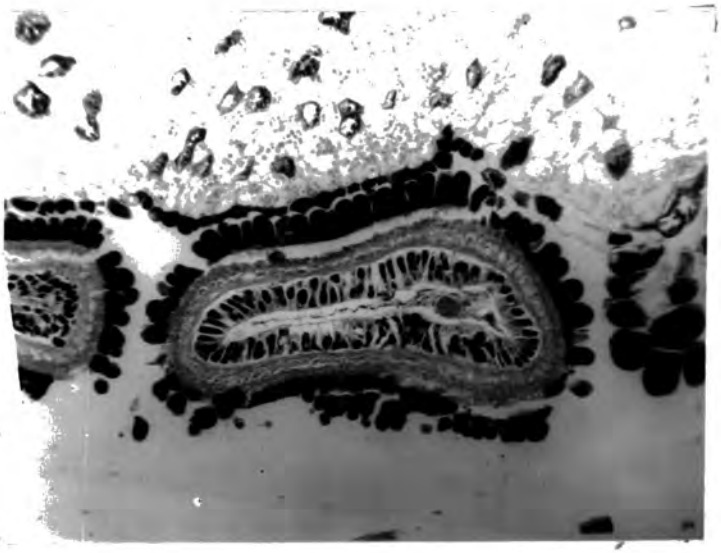


Fig. 44: 28 days. This was about the maximum endosperm development seen in this cross. It is composed of irregular cells, and it is difficult to distinguish the nuclei. Note that the embryo is relatively well grown. Cells of inner integument 2 are large and apparently active.

Fig. 45: 28 days. The endosperm is still persisting after the degeneration of the endosperm in this seed. Note the resemblance of inner integument 2 to that in P. vulgaris x P. elatior. (Figs. 24-27, pages 60-61).



COWSLIP x OXLIP.

Time	Outer layer of outer integument	Inner layer of outer integument	Outer layer of inner integument	Inner layer of inner integument	Endosperm	Embryo
10 days	Fairly normal in appearance.	Small densely packed cubical cells.	Barely any signs of degeneration.	Rather thick, two layers of cells at chalaza.	Thin tenuous layer with few nuclei.	Single cell.
20 days	Slight thickening of cell walls.	Normal in appearance.	All layers but the outermost degenerated.	Very thick, thickest at the chalaza.	A tenuous, incipiently cellular layer, with few nuclei, sometimes already beginning to break down.	About 4 - 6 celled.
24 days	Slight increase in cell size.	Occasionally proliferating into more than one layer.	Little change.	Still a very thick layer.	Sometimes slightly increased in quantity, sometimes more degenerate.	About 6-celled.
28 days	Very little growth from now on.	Thickening now commencing.	Outer layer intact, others forming a thin compacted band of tissue.	Hypertrophied to a varying degree, often occupying whole cavity, ovule often collapsed.	From none at all to a few highly abnormal cells. No noticeable nuclear irregularities.	Occasionally a small densely stained embryo present.
32 days	Probably finished growth.	Thickening increasing.	Outer layer showing signs of breakdown.	As above, chalazal pocket still open.	Degenerating rapidly.	Very few left.
36 days	As above.	About 1/3 thickened.	All layers now fully degenerated.	Often as thick as rest of integuments together; cells large, distinct, apparently active.	A few wisps of decaying tissue sometimes left.	Rarely still to be found, usually now degenerated.
40 days	As above.	Thickening still on the increase.	As above.	As above.	As above.	As above.
44 days	From here on any ovules not already collapsed do so; any endosperm or embryos left degenerate.					

B. Oxlip x Cowslip. (P. elatior x P. veris). R = 1.90

The seeds from this cross are variable, but the basic pattern of breakdown is similar in all of them.

integuments. Outer integuments 1 and 2, though flimsy, are more fully thickened that in Primrose x Cowslip. Inner integument 1 rapidly degenerates, and is followed more slowly by inner integument 2. The latter layer is occasionally more persistent, however. (Fig 49, p. 85; Fig 53, p. 86).

embryo sac. The endosperm shows all the abnormalities of the other type B crosses, only more severely. It never becomes more than a thin, vacuolated layer, rarely cellular, and with irregular nuclei. Breakdown is rapid, and the seeds often contain quantities of unidentified amorphous material; possibly unutilised food materials. The embryo is minute, and degenerates with the endosperm.

The seeds from this cross have integuments that are not markedly abnormal, but grossly aberrant endosperm, and a tiny embryo. They mark the extreme of type B seeds among the interspecific crosses.

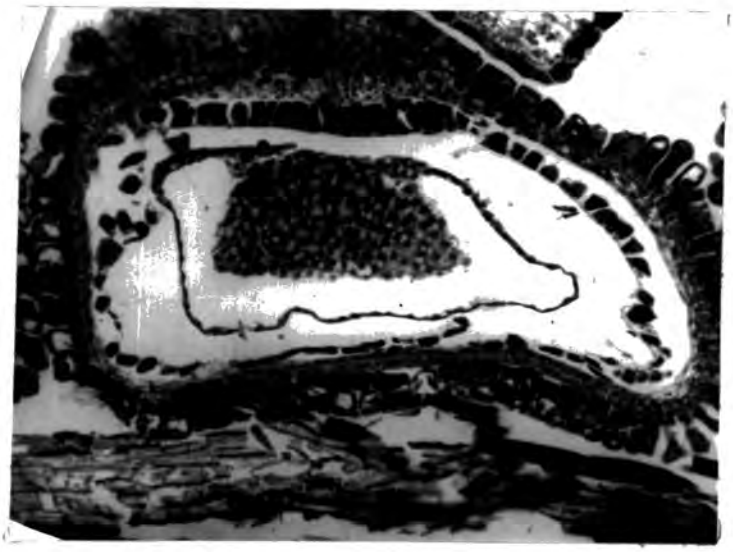
Graphs of seed and embryo length in this cross and its reciprocal are in Fig. 54, p. 87).

P. elatior x P. veris. Figs 46, 47 & 50 x 80;
Figs 48, 49, 51-53 x 56.

Fig. 46: 20 days. In this section part of the endosperm is seen in surface view. Note that there are many nuclei; development has been rapid initially. The nuclei are large and very densely staining.

Fig. 47: 28 days. Endosperm already showing signs of degeneration. The embryo is at about its maximum size here, and soon breaks down. The cells of outer integument 2 are commencing thickening.

Fig. 48: 36 days. The integuments here are rather flimsy, and the endosperm, though cellular, is small and shows large nuclei and vacuoles.



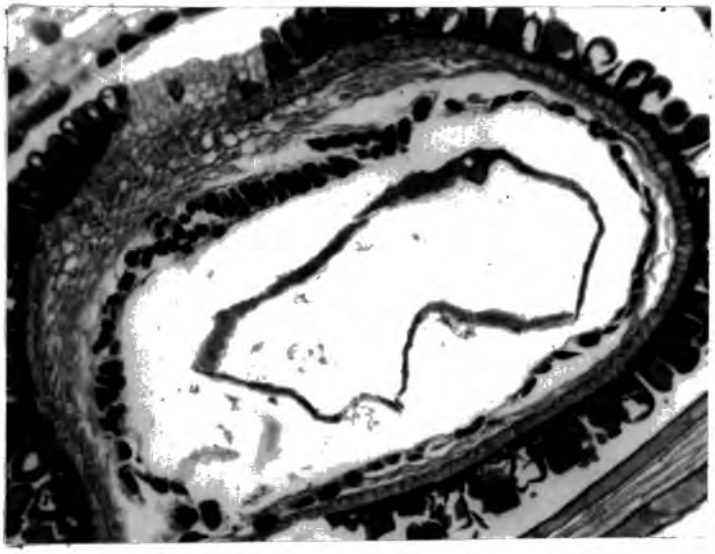
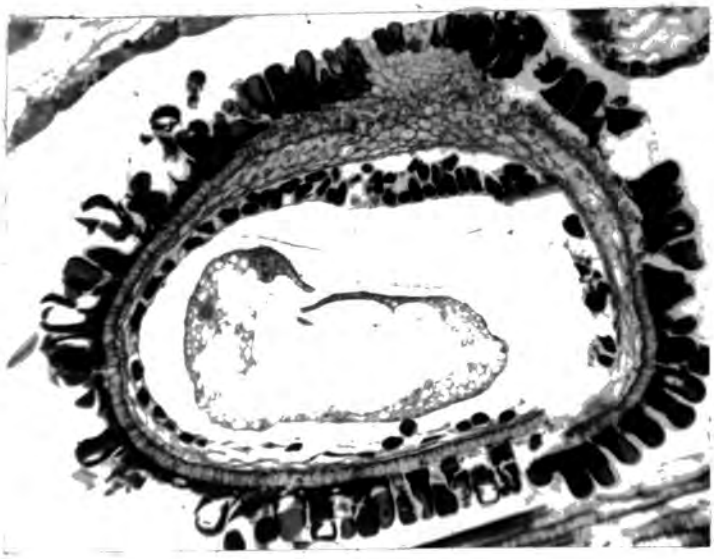


Fig. 49: 36 days. The integuments are more normal in this seed, and the endosperm has reached the greatest development seen in this cross, but it is vacuolate and unhealthy in appearance. The embryo is a minute sphere. Note the traces of amorphous substance near the chalazal region.

Fig. 50: 40 days. This seed, smaller than the majority, shows extreme abnormalities. The inner integuments form a large, tumour-like mass, and the embryo sac contents have completely disappeared. This is possibly an unpollinated ovule stimulated to growth by adjacent developing seeds.

Fig. 51: 44 days. The endosperm here is rapidly degenerating. Note the much large amounts of amorphous matter in the chalazal region. This is possibly unused food material.



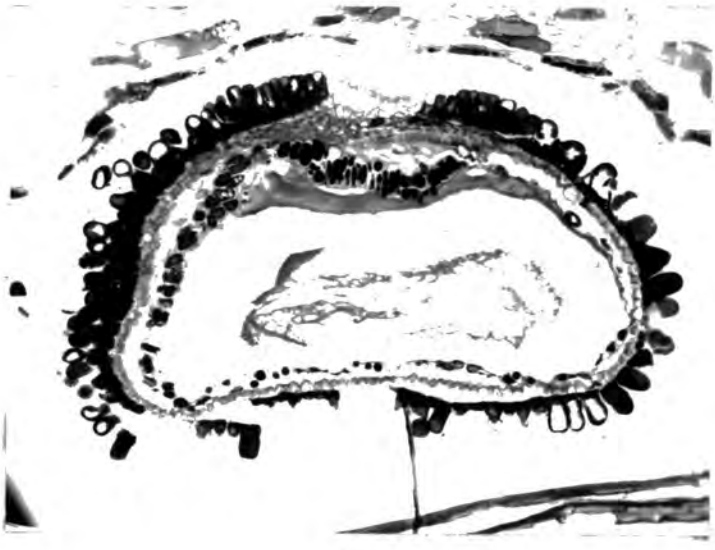
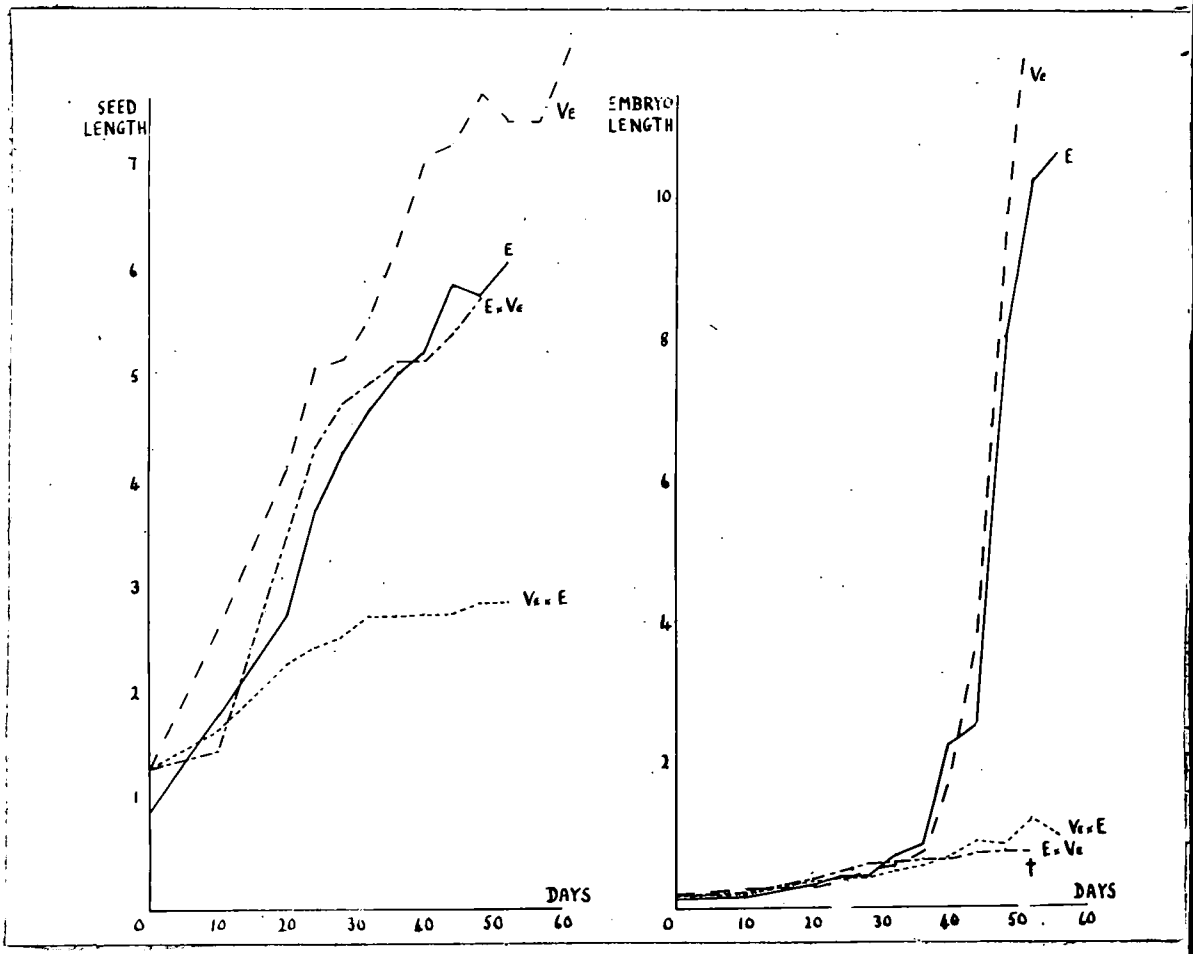


Fig. 52: 48 days. This seed is in an advanced state of degeneration, and is abnormal even for this cross.

Fig. 53: 48 days. A more typical seed at this stage. The outer integument 2 is well thickened, the inner integument is fully degenerate, and the endosperm has deteriorated to a few scrappy remains.

Fig. 54. Graphs of (a) seed length and (b) embryo length, increase with time. P. veris (Ve); P. elatior (E); P. veris x P. elatior (Ve x E) and P. elatior x P. veris (E x Ve). Scales for seed and embryo lengths in arbitrary units. († signifies death).



OXLIP x COWSLIP.

Time	Outer layer of outer integument	Inner layer of outer integument	Outer layer of inner integument	Inner layer of inner integument	Endosperm	Embryo
10 days	Cells still small and thin walled.	Small cubical cells.	Cell contents of inner layers shrinking.	Rather thick and irregular.	Very tenuous indeed, with few nuclei.	Not seen.
20 days	Great increase in size, very thin walls to cells.	Still small regular cubical cells.	Degenerated already except at chalaza.	Rapidly degenerating except at chalaza.	A thin irregular layer with abnormal nuclei.	4 - celled.
24 days	Still growing in size, flimsy.	Fairly normal in appearance.	As above.	Degeneration nearly complete.	Becoming vacuolated.	About 8 cells.
28 days	Cells very large and thin walled.	Slight thickening on inner walls.	As above.	Degenerated except at chalaza.	Small in quantity, highly vacuolated, with 'giant' nuclei.	Up to 16 cells, very small.
32 days	A layer of thin walled, brittle cells.	Thickening up to 1/3 of cells.	As above.	As above.	Much structureless debris, many large nuclei.	Degenerating.
36 days	As above.	About 1/2 thickened.	Degeneration now spreading to chalaza.	Breaking down even at chalaza.	Slowly degenerating.	Rapidly breaking down.
40 days	As above.	Nearly fully thickened.	Completely degenerated.	Usually fully degenerated, but sometimes actively growing rather irregularly.	Sometimes replaced by a structureless jelly.	Not often remaining.
44 days	As above.	Completely thickened.	As above.	As above.	Rapdily degenerating.	Usually fully degenerated.
48 days on	From now on the integuments change very little, endosperm and embryo finally break down if they have not already done so, and generally collapse of the ovules ensues.					

Autotetraploids.

Crosses involving autotetraploids are of considerable interest, and can throw a good deal of light on the problem of seed failure. The results briefly summarised here will be discussed later. The species used for the three crosses, 4n x 4n; 4n x 2n and 2n x 4n was P. veris.

Tetraploid x Tetraploid.

Development in the autotetraploid follows a similar course to that in the diploid, but the seeds reach maturity slowly.

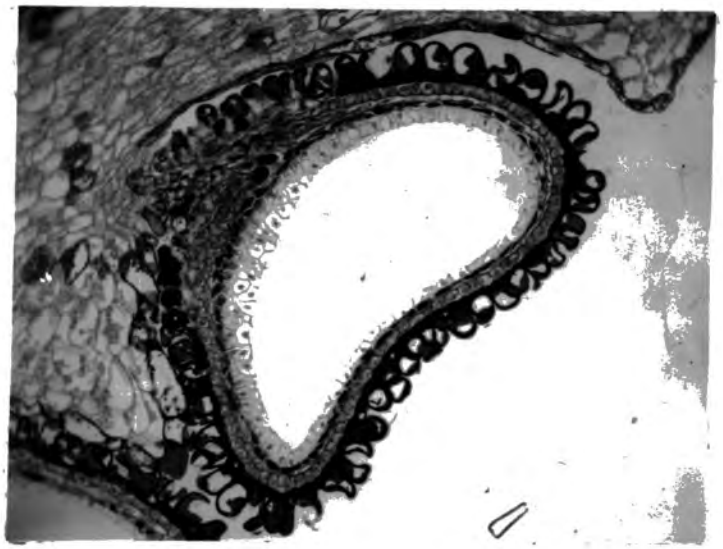
integuments. These differ little in development and appearance from those of the diploid. If anything the thickening of outer integument 2 is completed more rapidly. Degeneration of the inner integuments goes on at about the same rate as that in the diploid.(Figs. 55 - 58, pages 90 - 91).

embryo sac. The endosperm is slower in growth than in the diploid, and it has scattered through it a few large nuclei. Such minor irregularities might be expected in an autotetraploid. The embryo is correspondingly less rapid in its growth. The seeds at maturity are larger and more variable than those of the diploid, and there are not many in each capsule.

P. veris. Tetraploid x Tetraploid. All x 56.

Fig. 55: 32 days, endosperm still a single layer of cells except in the chalazal region. (compare the diploid at the same stage, Fig. 21, p. 56.)

Fig 56: 36 days. Endosperm now fills the seed, and the embryo is still fairly small. Note scattered large nuclei in the endosperm.



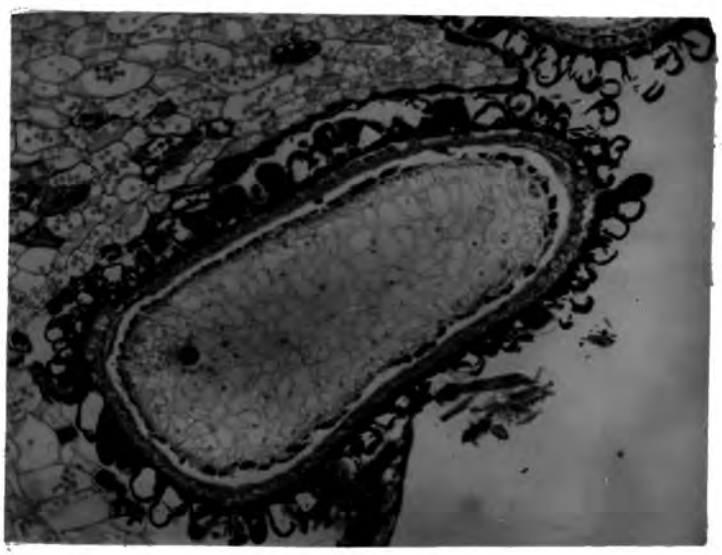
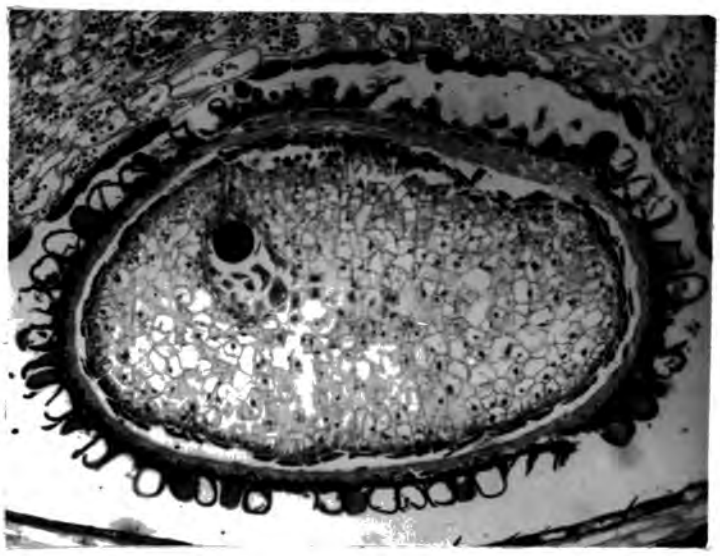
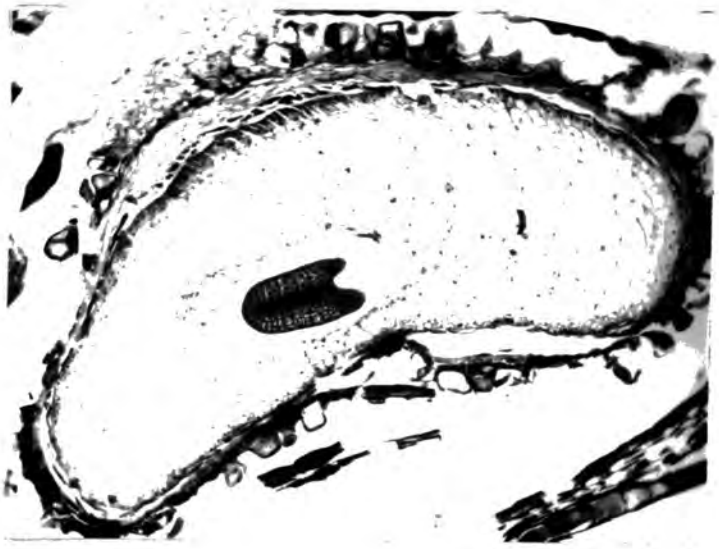


Fig 57: 40 days. The endosperm is a little irregular, and the embryo is at about the same stage as a 36 day embryo in the diploid. Outer integument 2 nearly completely thickened.

Fig. 58: 44 days. Embryo now rapidly lengthening. From this time on development continues as in the diploid.





COWSLIP TETRAPLOID x TETRAPLOID.

Time	Outer layer of outer integument	Inner layer of outer integument	Outer layer of inner integument	Inner layer of inner integument	Endosperm	Embryo
Unpollinated	Cells cubical and regular, dark brown.	Small cells with dense cytoplasmic contents.	Normal in appearance.	Rather irregular in arrangement of cells.	Wall of the embryo a thin membrane lining the ovule cavity.	
10 days	Cells much larger.	Some division has occurred.	Cell contents of inner layer slightly shrunk.	Normal in appearance.	Rather few nuclei in a thin cytoplasmic layer.	Not seen.
20 days	Slight thickening of cell walls.	Cells now cubical, contents less dense.	Only outermost layer not degenerated.	Beginning to break down except at chalaza.	Still very few nuclei.	Two cells.
24 days	Thickening greater.	Little change.	Fully broken down except at chalaza.	Degenerated except at chalaza, rarely rapidly proliferating.	Sometimes becoming cellular.	Two cells.
28 days	Little change.	Thickening now commencing.	As above.	As above.	Single layer of cells.	Very small.
32 days	As above.	Thickening increasing.	As above.	As above.	About 2 cells thick.	About 60 cells.
36 days	As above.	Up to 2/3 thickened.	Now breaking down at chalaza.	Degenerating in chalazal region.	Half filling ovule.	Growing very rapidly, still spherical.
40 days	As above.	Fully thickened.	Completely degenerated.	Completely degenerated.	Regular and normal in appearance, now filling ovule.	Elongated heart shaped.
44 days	As above.	As above.	As above.	As above.	Stored food material starts to accumulate.	Rapidly elongating.
48 days on	From now on the ovules develop to maturity much as do the normal cowslip diploid seeds. They are rather larger than in the diploid, however, and not a very large percentage develop in each capsule.					

A. Tetraploid x Diploid. (P. veris) R = 1.25.

The genetic ratio of the seeds from this cross is the lowest yet encountered, and would lead one to expect seeds with extreme type A characteristics.

integuments. Outer integument 2 does develop a certain amount of thickening, but it is not completed. Inner integument 1 breaks down, but the outermost layer tends to persist, the cells having shrunken contents, however. Inner integument 2 shows extreme hypertrophy at first, and then the cells begin to show undirected growth, forming tumour-like ingrowths in the embryo-sac cavity (Figs 59-64, pages 94-5).

These seeds are very small, but occasionally larger ones develop. These, however, resemble type A seeds, and have degenerate contents.

embryo sac. The endosperm can be seen in early stages as a tenuous cytoplasmic layer containing very few nuclei. By 20 days it has virtually disappeared, its place having apparently been taken by the tumour-like ingrowths from the inner integument.

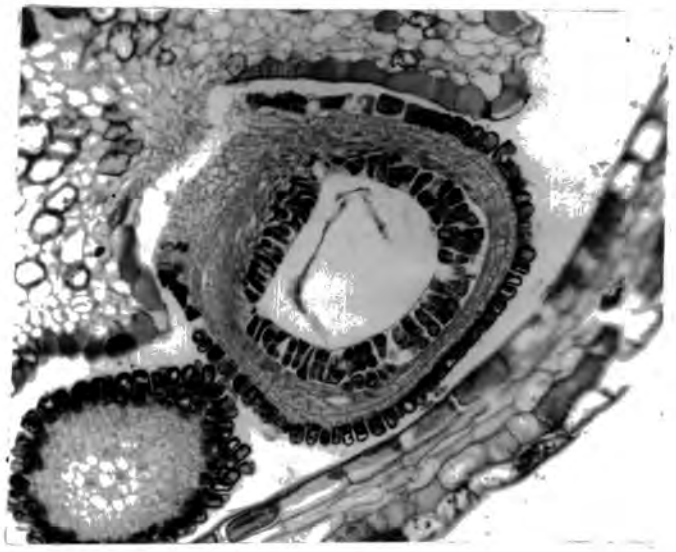
These seeds are thus of extreme type A developmental pattern, with the additional feature that the inner integument 2 becomes malignant in growth.

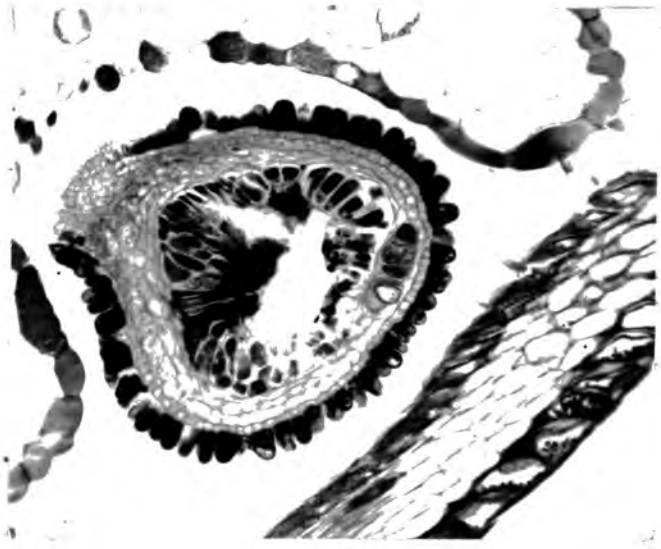
Tetraploid x Diploid. (P. veris). Fig. 62 x 56; Figs. 59-61, 63-64 x 80).

Fig 59: 10 days. Closely resembles seeds of P. veris x P. elatior at this stage (cf. Fig. 42, p. 80). Inner integument 2 showing signs of activity.

Fig. 60: 20 days. Inner integument 1 slowly breaking down. Some cells of inner integument 2 producing cancer-like ingrowths. Contents of embryo-sac have disappeared.

Fig. 61: 24 days. Thickening of outer integument 2 just commencing. In this seed a few scraps of endosperm residue remain.





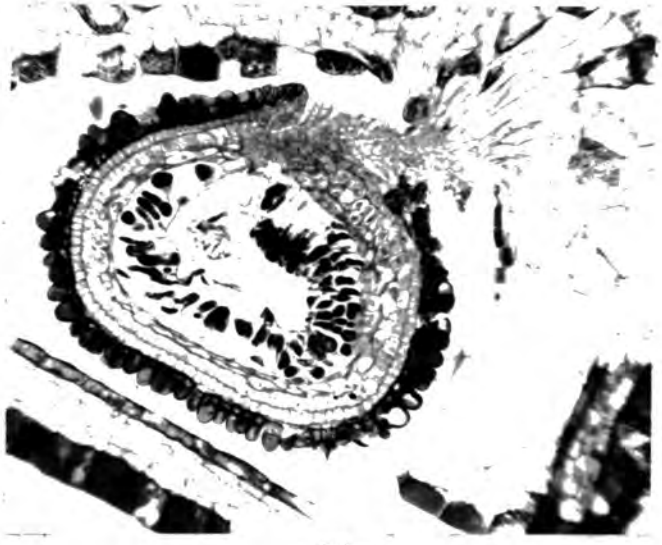
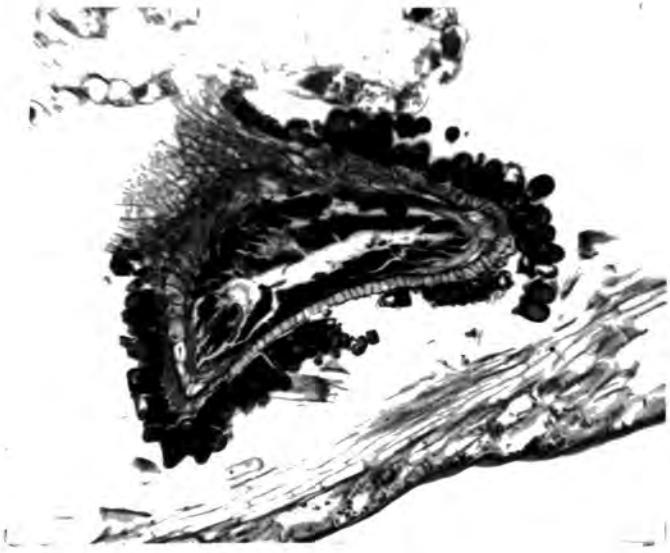


Fig. 62: 28 days. The majority of the cells of inner integument 2 have now produced structureless ingrowths. No contents are discernible in the seed cavity.

Fig. 63: 32 days. Collapse has occurred. Note that the thickening in outer integument 2 has barely progressed. Many of the inner integument 2 cells have broken down into an amorphous mass.

Fig. 64: 40 days. Occasionally seeds of this type are produced, much larger than the rest, but with a greatly hypertrophied inner integument 2, and degenerate contents.



COWSLIP TETRAPLOID x DIPLOID.

Time	Outer layer of outer integument	Inner layer of outer integument	Outer layer of inner integument	Inner layer of inner integument	Endosperm	Embryo
10 days	Cells small, cubical.	Normal in appearance.	Contents of cells shrunk, especially inner layers.	Very thick and overdeveloped.	Small in quantity, dense with few nuclei.	Not seen.
20 days	Somewhat thickened, outer walls rounded.	Small dense cubical cells.	Outer layer intact, others degenerating.	Greatly hypertrophied, especially at chalaza; in parts cells not distinguishable, merely amorphous growth.	Degenerating.	About 4 cells, degenerating.
24 days	Little change.	Little change.	Outer layer showing signs of breakdown.	Sometimes producing large amorphous tumour-like ingrowths.	Degenerated.	Degenerated.
28 days	As above.	Thickening now starting.	Outer layer now degenerating, except at chalaza.	Greatly hypertrophied.	As above.	As above.
32 days	As above.	Thickening barely increased.	Degenerated except at chalaza.	As above.	As above.	As above.
36 days	As above.	About 1/4 thickened.	As above.	Filling whole cavity due to collapse of ovules.	As above.	As above.
40 days on	Collapse has generally occurred by now, and if not, soon will. The ovules are rather variable in size and degree of degeneration, but all follow the general pattern.					

Diploid x Autotetraploid (P. veris) R = 2.0.

The genetic ratio of the seeds from this cross is higher than the highest of the interspecific crosses, and is at the extreme type B range.

integuments. The outer integument is more robust than the interspecific type B seeds, but still less so than normal. Outer integument 2 is fully thickened by 44 days. Inner integument 1 is slow in degenerating, and inner integument 2 persists as a rather thick layer in the chalazal region, while breaking down elsewhere.

embryo sac. The endosperm rapidly disappears, few traces being visible after 28 days. The embryo is minute, but it persists for a while after the disappearance of the endosperm. The seeds usually collapse by about the 40th day. Occasional seeds are found that are not so extremely type B (Fig. 67, p.98).

The seeds produced from this cross are, then, what would be expected from the high value of R. They are of an extreme type B developmental pattern, though they have slight variations from the interspecific type B seeds.

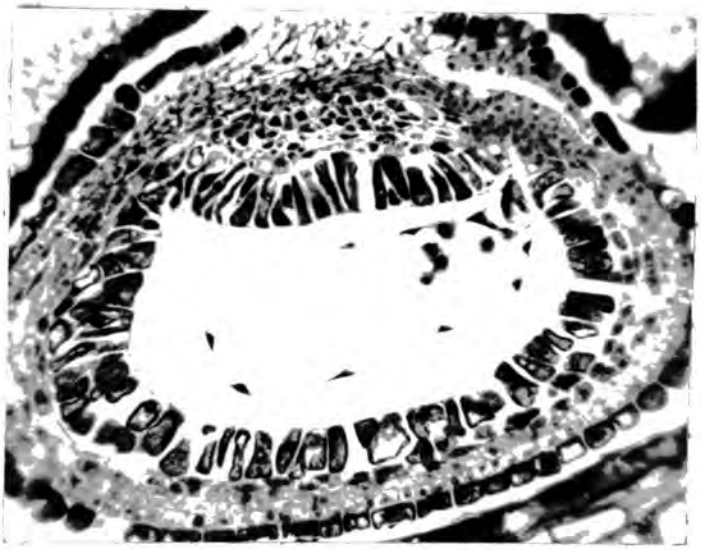
Fig. 68, p. 99, shows graphs of seed and embryo length in P. veris, its autotetraploid and the crosses between them.

Diploid x Tetraploid (*P. veris*) Fig 65 x 150, Figs 66 & 67 x 80.

Fig. 65: 10 days. Here inner integument 2 is thick. The endosperm has more nuclei than that of the reciprocal at this stage.

Fig. 66: 32 days. The majority of seeds have partially or completely collapsed. This is a typical seed at this stage.

Fig. 67: 36 days. Some seeds have a less degenerate inner integument 2 (cf *P. elatior* x *P. veris*).



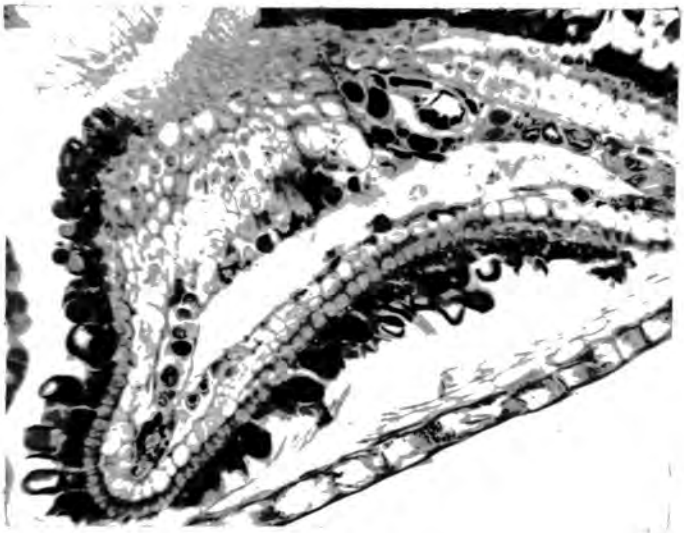
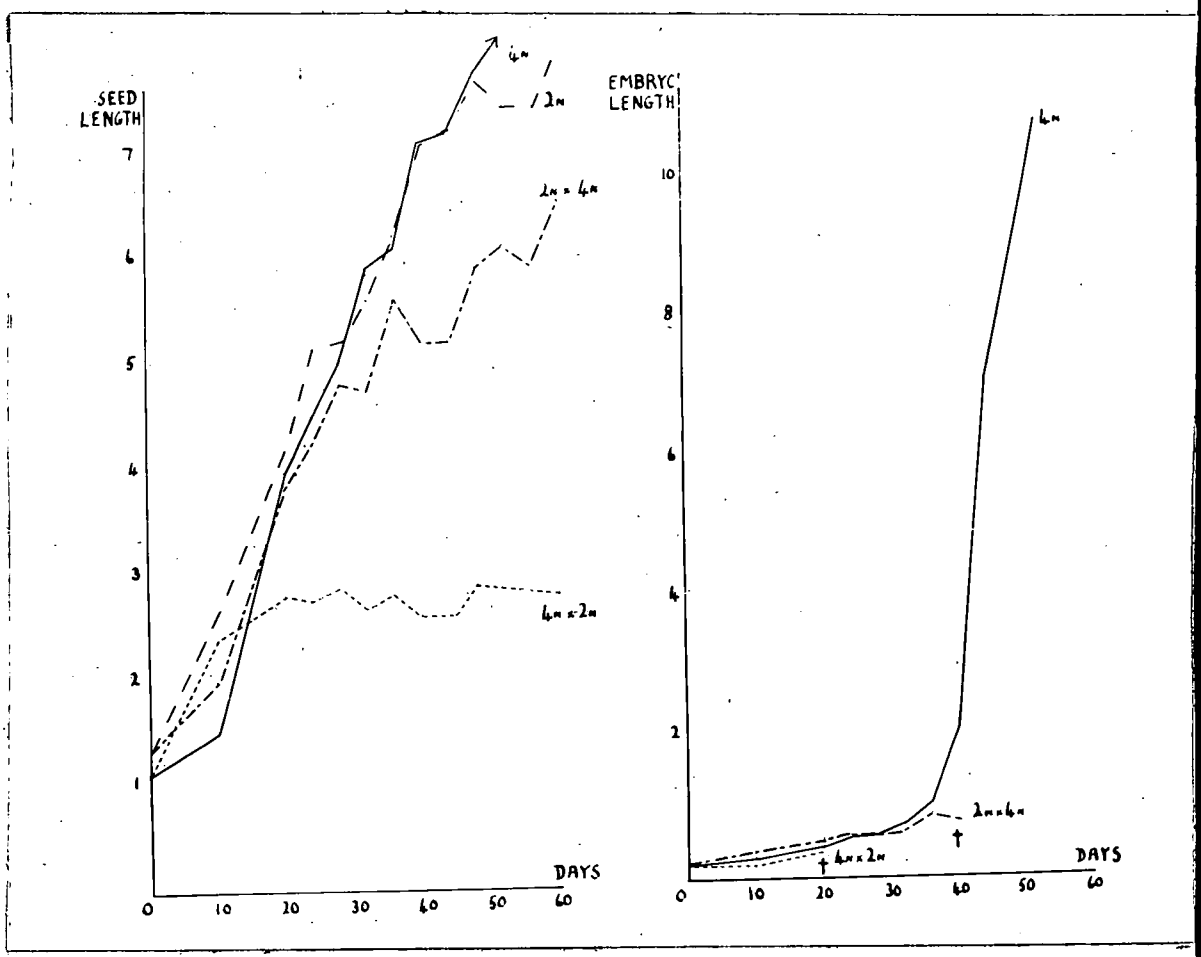


Fig. 68. Graphs of (a) seed length and (b) embryo length increase with time. P. veris diploid (2n); P. veris tetraploid (4n); tetraploid x diploid (4n x 2n) and diploid x tetraploid (2n x 4n). Diploid omitted from embryo length graph for sake of clarity (see Fig. 54, p. 87). Scales for seed and embryo lengths in arbitrary units. († signifies death).



COWSLIP DIPLOID x TETRAPLOID.

Time	Outer layer of outer integument	Inner layer of outer integument	Outer layer of inner integument	Inner layer of inner integument	Endosperm	Embryo
10 days	Cells small and thin walled.	Small cubical cells with dense contents.	Inner layers degenerating.	Thick layer with thin walled cells.	Very tenuous, with scattered nuclei.	One cell?
20 days	Cells large, very thin walled.	Dense cubical cells.	Nearly degenerated, even in chalazal region.	Rapidly breaking down.	Small in quantity, vacuolated, with large nuclei.	About 4 cells, degenerating.
24 days	Becoming slightly thickened.	Inner walls starting to thicken.	Nearly fully broken down.	Somewhat overgrown at chalaza, elsewhere degenerated.	Few wisps of tissue remain.	Degenerating.
28 days	Little change.	Thickening slowly increasing.	Not quite fully degenerated.	Little change.	Few scraps of dense granular tissue.	Up to 16 cells, very unhealthy.
32 days	Cells with very flimsy outer walls, rather large.	About 1/4 thickened.	Empty cells of outer layer still persist.	Still overgrown at chalaza.	As above.	Small and spherical, individual cells indistinguishable.
36 days	As above.	Half thickened.	Empty cells still persisting.	As above.	Structureless and degenerate.	Rapidly degenerating.
40 days	As above.	3/4 thickened.	Last layer now going.	As above.	As above.	Degenerated.
44 days	As above.	Fully thickened.	Completely collapsed.	As above	As above.	As above.
48 days on.	Final collapse of those ovules that have not already become flattened now ensues, and degeneration of the tissues goes on.					

P. veris diploid and P. elatior tetraploid.

We have seen that seeds from the crosses between the tetraploid and diploid of a single species show the extreme manifestations of type A and type B crosses. The bearing this has on the genetic value hypothesis will be discussed later. Further manipulations of the genetic ratio can be made by combining polyploids of one species with diploids of another. The autotetraploid of P. elatior was crossed as male and female with the diploid P. veris. The genetic value of P. elatior is 1.0, and that of its tetraploid, if it is twice the diploid, will be 2.0. P. veris has a genetic value of 1.8. This is quite close to the 2.0 of the autotetraploid P. elatior, and the genetic ratio R of either of the crosses will not be far from the normal value of 1.5. Its significance will be discussed later.

Valentine has also made these crosses and tested germination of the seeds. He found that none of the seeds from the cross with tetraploid oxlip germinated, but that a moderate percentage of those from the reciprocal germinated to produce triploid plants.

P. elatior tetraploid x P. veris diploid. Fig 73 x 56; Figs 69-72 & 74 x 80.

Fig. 69: 20 days. Inner integument 2 already thick, endosperm very tenuous, with small embryo. Note that here (as in all other type A seeds) the chalazal pocket is not occluded.

Fig. 70: 24 days. Inner integument 1 has degenerated. The endosperm is degenerating, but the embryo is growing.

Fig. 71: 32 days. One of the few seeds of second type. Thickening of inner integument 2 not so marked, embryo well developed, in a poor endosperm.

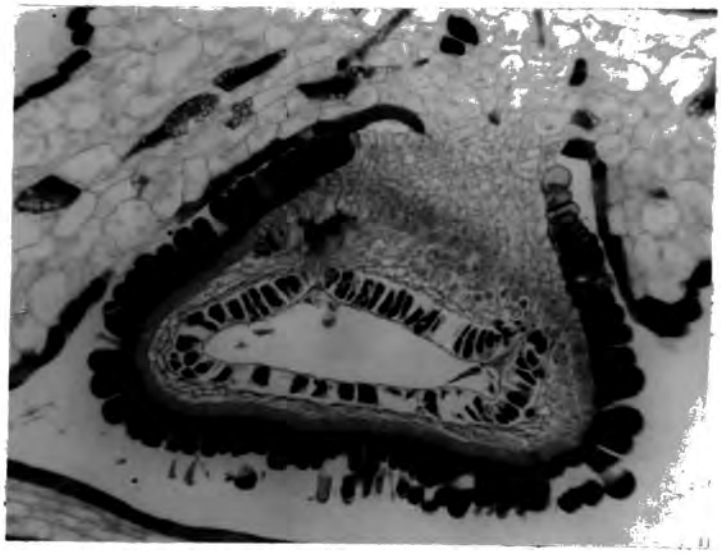
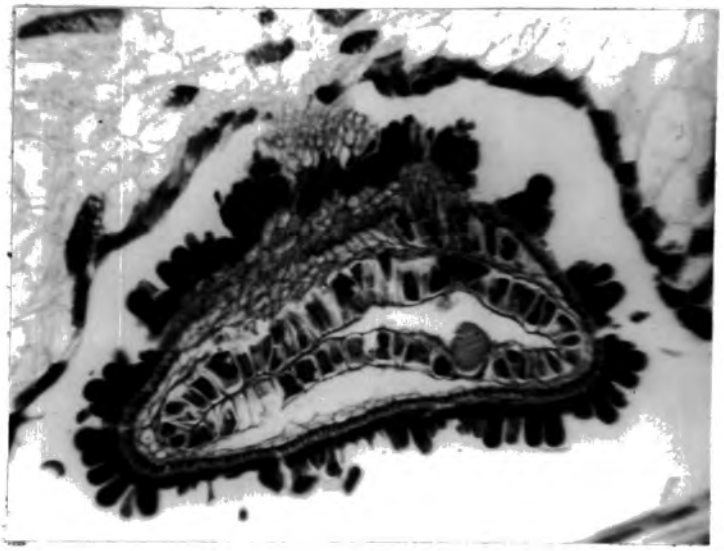
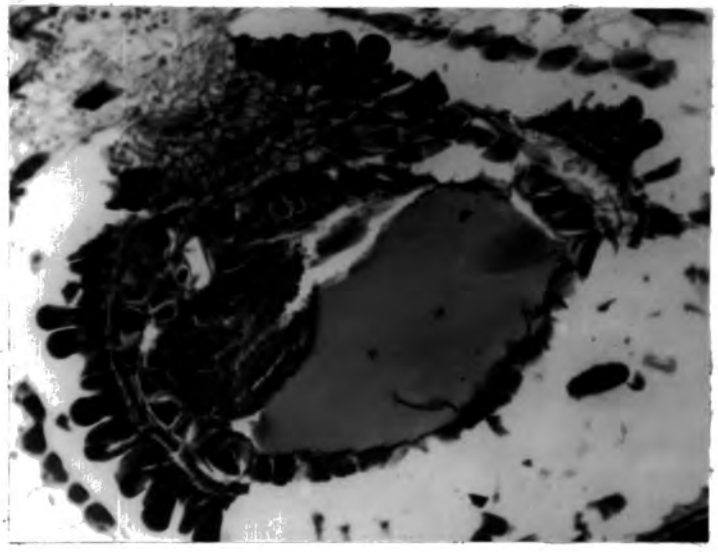


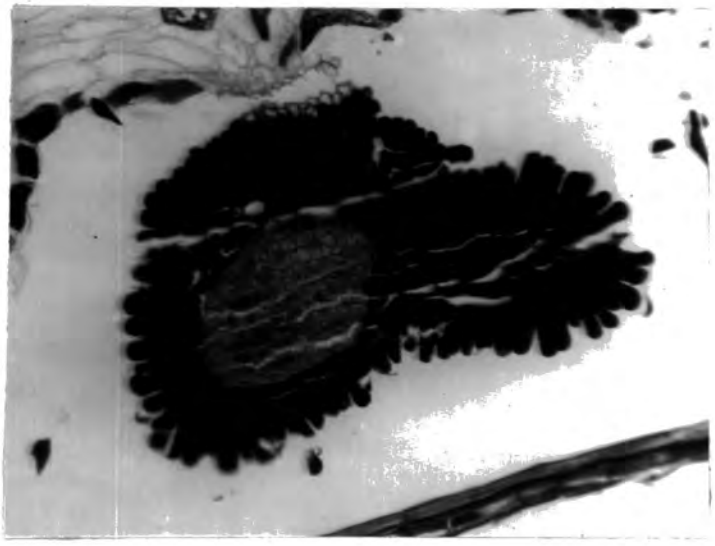
Fig. 72: 32 days. Endosperm has completely gone, but the embryo is still growing, apparently at the expense of the integuments.

Fig. 73: 44 days. An anomalous seed, which contains a large embryo surrounded by structureless jelly-like substance. (cf. Blakeslee et al).

Fig. 74: 52 days. The embryo is now quite large, but is enveloped by a seed coat so thick and hard that it has split in sectioning.







integument, until it appears at a late stage to be completely surrounded by integument (Fig. 74, p. 104.)

The less common type of seed does not follow a course of development that is so extreme. The seeds grow rapidly, and inner integument 2 is less hypertrophied. Many of these seeds show signs of collapse by about 28 days, but some persist, and the oldest seen, 32 days old, is shown in Fig. 71, p. 103. Its integument is similar to that of type B seeds, and its endosperm is of moderate quantity and rather irregular. The embryo, though quite large, is not healthy in appearance.

One or two seeds are of a still more unusual type, and are of interest in that they resemble some noted by Blakeslee et al (1950) in certain crosses of Datura. They have a well developed outer integument, a thick inner integument 2, and a large embryo surrounded by a mass of structureless jelly-like material that fills the seed completely. The seeds of this type are much larger than the others from the cross. (Fig. 73, p. 104).

The seeds that are typically produced in this cross, though of type A as expected, are much more extreme than the genetic ratio would suggest.

TETRAPLOID OXLIP x DIPLOID COWSLIP

Time	Outer layer of outer integument	Inner layer of outer integument	Outer layer of inner integument	Inner layer of inner integument	Endosperm	Embryo
10 days	Very thin walled cells.	Rather irregular.	Cell contents shrinking slightly.	Contents shrinking.	Very small in quantity, with few nuclei.	1 - 2 celled.
20 days	Walls thickening.	Regular, rather dense contents.	Contents nearly gone, but cells not wholly collapsed.	A very thick overgrown layer.	Incipiently cellular, very densely cyto-plasmic, large irregular nuclei, degenerating.	About 4-celled.
24 days	Little change.	Thickening of inner walls starting.	Cells now collapsing.	Very thick.	Almost completely degenerated.	Increasing in size rapidly, spherical.
28 days	As above.	Cells half thickened.	Degenerated.	Greatly hypertrophied. Ovules collapsed.	None remaining.	Growing rapidly, completely surrounded by integument.
32 days	As above.	Heavily thickened, rather irregular.	As above.	As above.	As above.	Much larger.
36 days	As above.	Completely thickened.	As above.	As above.	As above, one or two ovules filled with structureless jelly.	Still rapidly growing.
40 days on	From now on the only apparent changes are the hardening of the extremely thick integument, and the continued increase in size of the embryo, apparently growing at the expense of the integumentary tissue.					

P. veris diploid x P. elatior tetraploid. R = 1.56.

The genetic ratio R suggests that seeds from this cross will be of type B. There is less variation among the ovules than in the reciprocal.

integuments. The outer integument is less thick than normal; in fact is similar to that of most type B seeds, having a flimsy outer layer and a slowly thickened inner layer. Thickening of outer integument 2 is completed by about 52 days.

Inner integuments 1 and 2 both degenerate rapidly, and by the 36th day both layers have been completely broken down.

embryo sac. The endosperm develops very slowly, and does not become cellular until about 40 days (Fig 76, p. 108). At 44 days it is about 2 cells thick, and then it grows very rapidly, filling the seeds by 56 days. Some seeds collapse partially, but continue to develop. The endosperm resembles that of a moderate type B seed, being large celled and irregular with scattered large nuclei. The embryo grows rapidly when the endosperm starts its main period of growth. The product of this cross is a type B seed, very much of the quality that the genetic ratio would lead one to expect.

P. veris diploid x P. elatior tetraploid.

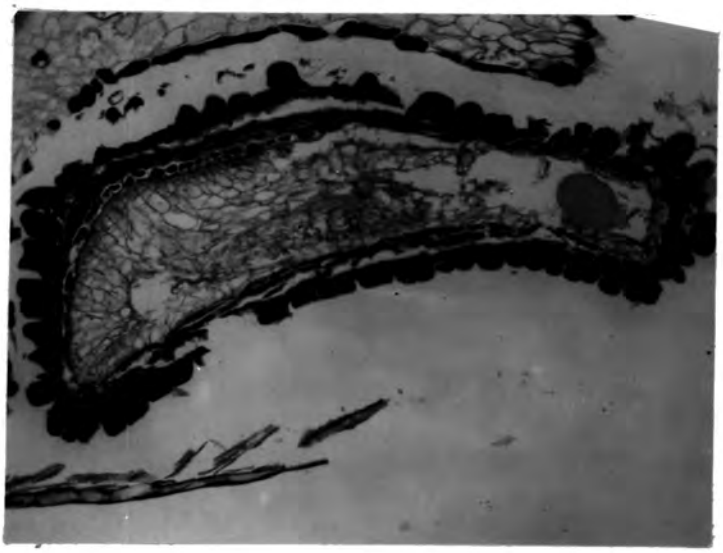
Fig. 75 x 80; Figs 76-78 x 56.

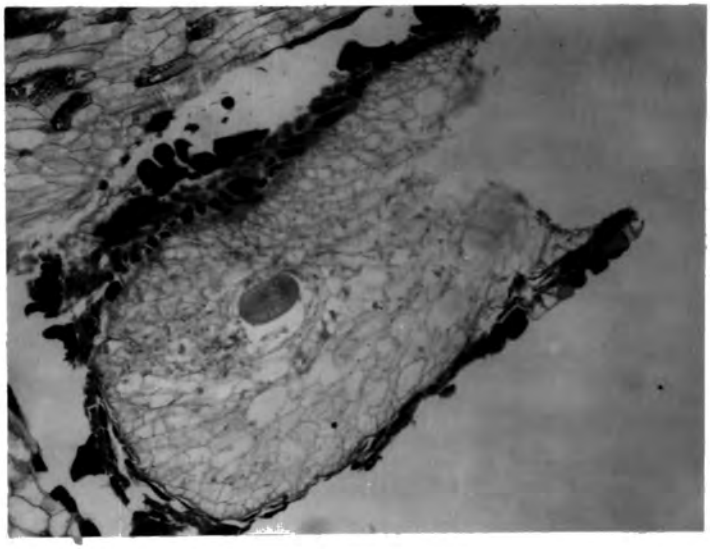
Fig. 75: 20 days. Not a great deal has happened, apart from an increase in size. The inner integuments are still nearly intact. The endosperm is very tenuous and the embryo contains about four cells.

Fig. 76: 40 days. The inner integuments are now a compacted layer of cell debris. The endosperm has just become cellular, still only consisting of a single layer, and the embryo is a small sphere.

Fig. 77: 56 days. Partial collapse has occurred in this seed, but the integument is relatively strong, and the embryo is the largest found at this stage.

Fig. 78: 56 days. Here there is more endosperm; note the scattered large nuclei, and the irregular nature of the tissue. The integument is flimsier in this seed. Elongation of the embryo has commenced.





DIPLOID COWSLIP x TETRAPLOID OXLIP.

Time	Outer layer of outer integument	Inner layer of outer integument	Outer layer of inner integument	Inner layer of inner integument	Endosperm	Embryo
10 days	Regular and normal in appearance.	Cubical cells with dense contents.	Little shrinkage of contents.	Slightly hypertrophied.	Very tenuous indeed, with very few nuclei.	Not seen.
20 days	Slightly thickened.	Cytoplasm becoming rather sparse.	Inner layers breaking down, outer intact.	Slight degeneration.	Very tenuous and rather dense.	About 4-celled.
24 days	Little change.	Very regular.	Degeneration slowly continuing.	Still thicker than normal.	Little change.	4-6 celled.
28 days	As above.	As above.	Outer layer still not breaking down.	Little change.	Very thin and dense.	4-6 celled.
32 days	As above.	Sometimes commencing thickening.	Outer layer now degenerating.	Degenerating.	Little change.	About 8-celled.
36 days	As above.	About 1/3 thickened.	Nearly degenerated.	Degenerated.	Still very tenuous.	About 16 cells.
40 days	As above.	1/2 thickened.	Degenerated.	As above.	Becoming cellular, one cell thick.	About 40 cells.
44 days	As above.	3/4 thickened.	As above.	As above.	Two cells thick.	About 60 cells.
48 days on	The integuments harden, the inner layer of the outer integument becoming fully thickened. The endosperm increases to a moderate quantity, with very irregular large cells, and little food material. The embryo grows slowly and is rather dense.					

Seed development: The major features.

Type A seeds.

As the genetic ratio gets smaller, the main trends apparent in type A seeds become more marked. They are:

1. A slowing down of growth in the outer integument, and a lessening of the rate of deposition of thickening.
2. Excessive growth of the inner integument 2, (endothelium), resulting in a layer that in the more extreme cases is as thick as the rest of the integuments together. There is a tendency for this layer to be extra thick in the chalazal region, but occlusion of the chalazal pocket has not been observed.
3. A reduction in the endosperm, progressively greater as the genetic ratio becomes less.
4. A corresponding reduction in embryo size and growth rate

Type B seeds.

These show a similar progressive abnormality with increase in the genetic ratio, the type of breakdown being, however, very dissimilar to that of type A seeds. The main trends are:

1. A lack of thickening and consequent fragility of the outer integument.

2. A less rapid than normal breakdown of the inner integument,

3. A lessening of the quantity, and a great deterioration in quality of the endosperm, even in the least extreme type B seed. The endosperm contains vacuoles and large scattered nuclei.

4. An embryo which in all but the least extreme cross is rarely found to have progressed beyond the small sphere stage.

V. THE PROBLEMS.

Many problems are raised by this study, and not all can be adequately dealt with here. Those which are of most importance and interest are:

1. Genetic value. Valentine made measurements of seed size and weight, and estimates of seed contents, the interspecific crosses, and later the crosses involving hybrids, being considered. From his results he was able to arrange the crosses in order, and he derived his hypothesis of genetic values, by means of which he was able to explain the results. Do the histological results confirm his, and do they support the hypothesis of genetic values?

2. The histological investigation has shown that the abnormalities that occur in the hybrid seeds are totally different in the reciprocal crosses; the whole pattern of seed development in type A and type B seeds differs. What is the explanation for this?

3. The differences between two species are presumably qualitative. The differences between a diploid and its autotetraploid are quantitative. Yet the crosses between

the diploid and its autotetraploid produce seeds whose developmental pattern is strikingly similar to that of seeds from interspecific crosses. What is the correlation between these two types of cross?

4. P. veris and P. elatior are almost completely incompatible; germination of the seed rarely succeeds, and very few wild hybrids have been found. If, however, the chromosome number of P. elatior is doubled, it can be used as male parent in the cross with diploid P. veris and produce a good percentage of viable seed. The development of this seed is not far removed from normal. What bearing does this fact have on the problem of seed incompatibility?

5. There is a good deal of variation between seeds in the same capsule, and between those in different capsules, both on the same and different plants. In the majority of crosses, there is a wide range of variation, but in P. elatior x P. vulgaris two distinct types of seed are found. These two types are present in the reciprocal cross also, but here they are less easily discerned. Both the questions of seed variation and the two types in this cross need some comment.

6. Finally, the cause of developmental abnormalities needs elucidation. Do our results allow an explanation for the breakdown of these seeds, either along the lines suggested by previous workers, or by the proposal of a new hypothesis? Related to this is the whole problem of seed incompatibility, its occurrence in different groups and its evolutionary importance.

VI DISCUSSION.

A. The hypothesis of genetic value.

Valentine's classification, on a genetic basis, of the seeds obtained from hybridization in Primula has been described in an earlier section (pages 35 - 38). He allotted each of the three species genetic "values" which were based on his results, and then showed that the genetic "ratio" (ratio of genetic values of the endosperm and maternal tissue) of a hybrid could be a guide to the sort of seed one could expect. The further the genetic ratio deviates from the normal value of 1.5, the less successful is the cross. He also showed that they type of seed produced depends upon the direction of the cross. When the genetic ratio is less than 1.5, seeds are produced that he termed tye A, and when it is higher, the seeds formed are, by Valentine, termed type B.

From these beginnings, Valentine predicted that crosses which involved hybrids as parents would produce seed that could by its characteristics be fitted into his classification in positions indicated by genetic ratios. This prediction was confirmed.

As a study of the results summarised in the previous section will show, the division into two types observed by Valentine is reflected in every stage of development of the seeds. The histological characteristics of type A and type B seed have been described. Of particular relevance is the fact that the greater the deviation of the genetic ratio from normal, the more severe are the abnormalities in the developing seed. Without any of Valentine's data, it would have been possible to arrange the hybrids in order of increasing abnormality, and the order would have been identical with his.

Valentine had not carried out any crosses between diploids and their autotetraploids, so there was no indication of what sort of abnormalities might be found in seed from such crosses, except that afforded by the genetic ratios. If it is assumed that doubling the chromosome number of a species doubles its genetic value as well, the genetic ratio of the diploid x autotetraploid cross will be 2.0, and that of the reciprocal will be 1.25. If these ratios are related to the type of seed produced, the diploid x tetraploid should produce seeds

that are extreme type B, and the reciprocal should give extreme type A seeds.

On pages 89 - 100 (Figs. 55 - 67) these two crosses and the control, tetraploid x tetraploid, are described. It will be seen that the seeds from the tetraploid x tetraploid cross are almost perfectly normal in their development. There are small abnormalities, but no more than would be expected. On the other hand, the tetraploid x diploid seeds show the characteristics of type A seeds in a more extreme form than is displayed in any of the interspecific crosses, and the reciprocal cross produces extreme type B seeds.

This cross involves parents whose differences are purely quantitative, yet the course of development of their offspring is similar in almost every respect to that of seeds from wide interspecific crosses. This fact gives strong support to the genetic value hypothesis.

The autotetraploid has been used here to produce genetic ratios as far removed from normal as possible. The next step is to use it to produce ratios as near to normal as possible, thus reducing quantitative differences to a minimum, while at the same time using different

species in order to retain qualitative differences. If this can be done, then if qualitative and quantitative differences are of the same nature in Primula, they should cancel out and produce relatively good seed.

The genetic value of P. elatior is 1.0. An autotetraploid will have a ratio of 2.0, assuming, as we did before, that it is doubled together with the chromosome number. This value is not very different from that of diploid P. veris, which is 1.8. Crosses between diploid P. veris and tetraploid P. elatior will produce genetic ratios as follows:

Tetraploid P. elatior x diploid P. veris 1.45 type A.

diploid P. veris x tetraploid P. elatior 1.56 type B

Neither of these ratios is far from normal. If we put these two crosses in their place in the table of genetic ratio, we can forecast that in both cases the seeds should be nearly normal. This is on quantitative grounds. Any deviation from the expected path of development should be due to the qualitative differences between the two species. It can be argued that unbalance between parents of different chromosome number will also affect the result.

The development of the seeds from these two crosses is described in pages 101 - 110, (Figs. 69 - 78). The results of these crosses indicate that it is not only genetic value that is determining the course of seed development in Primula. However, it is important to note that the attempt to balance the genetic values has resulted in a partial success in overcoming the barriers present between these two species. When the diploid P. veris is female parent seed development is variable, but a certain number of good seeds are produced, (and germination tests made by Valentine have shown that about 50% of the seeds from this cross germinate). The result in the reciprocal cross is not so successful, and for a possible explanation of this it is necessary to recall the interspecific crosses.

In the type A seeds from interspecific crosses, inner integument 2 is hypertrophied. In P. vulgaris x P. elatior this is very marked, and it is as prominent in P. veris x P. elatior. In the third type A seed, however, P. veris x P. vulgaris, the hypertrophy is less severe. The two crosses in which it is most

prominent, then, are those involving P. elatior. It is possible that the genome of P. elatior is exerting an influence that is greater than that suggested by its genetic value. If this is so, then when it is present in double quantity as in the cross between tetraploid P. elatior and diploid P. veris, the effect it exerts will be far greater. This may account for the unexpected abnormality of this cross.

The crosses involving the tetraploid P. elatior and diploid P. veris, though not very successful, do lend a certain amount of support to the genetic value hypothesis, since despite the qualitative differences, and the possible chromosome unbalance, the balancing of the genetic values does allow the production of a certain amount of viable seed.

Our results indicate that Valentine's genetic value hypothesis is not without foundation. It must be emphasized here, though, that none of the three protagonists of this hypothesis, Stephens, Howard and Valentine, have claimed that it explains the phenomenon of seed incompatibility. It merely provides the genetical basis for an explanation.

B. Seed variation.

Variation in most of the crosses appears to be continuous. In the crosses between P. elatior and P. vulgaris, however, two types of seed can be distinguished. Valentine could only distinguish them when P. elatior was the female, and in fact they are much more clearly seen here than in the reciprocal, but the present investigation has shown that they do occur in that cross. Unfortunately it was not possible to estimate the proportions of the two types of seed.

Since Valentine found about 25% sound seeds in the cross P. elatior x P. vulgaris, he could put forward a simple genetic explanation for the phenomenon. In each of the three species of Primula, uniformly sound seed is produced in intraspecific crosses. This indicates that each species is homozygous for genes controlling genetic value, and hence that a single type of seed should predominate. Now if both P. elatior and P. vulgaris are heterozygous for one gene controlling genetic value, a 3:1 ratio would be obtained in the F1, giving approximately 3 empty seeds to 1 sound seed. He had to then assume that this gene was effective only when

R was 1.65, and not effective when it was 1.5 as in the intraspecific cross, or 1.36 as in the reciprocal. This assumption may be unnecessary in the light of the segregation into two types of the seed from the reciprocal cross. There is still no evidence to indicate what the real basis of this segregation is.

It is doubtful whether there is a satisfactory explanation of the variability between seeds, especially that within a single capsule. However, the point is worth a little of our attention.

No two seeds in any capsule are likely to be genetically identical. In intraspecific crosses this is of little importance, since the vast majority of seeds are vigorous and reach maturity, but it may have some effect in interspecific crosses, where some of the seeds are going to fail.

Minor environmental differences, both between and within capsules, may have some effect on seed development. One of Blakeslee's co-workers, Sanders (1948) was of the opinion that environmental differences; temperature, light, moisture etc., both at the time of pollination and afterwards might be responsible for

many seed failures in Datura, and that variation between reciprocal crosses is related to conditions in the immediate vicinity of the embryo in the seed. It is unlikely that her first suggestion merits serious consideration, but it does serve to point out the possible importance of environmental variation.

One environmental factor that I think may be of some importance is competition for nutrients between seeds within a single capsule. After pollination of a flower has taken place, pollen grains will probably not all germinate at the same time, nor will they grow at the same rate, and this means that not all the ovules will be fertilized simultaneously. It may be that in some cases an interval of a day or two may elapse between the fertilization of the first and last seeds. The same results will ensue if there are two successive pollinations, the first with insufficient pollen to fertilize all the ovules. The first seeds to begin development will have a start on the later ones that will probably always be maintained, and will claim a more than average share of the available nutrients. This will be unimportant in an intraspecific cross, apart from seed size variation, but in

interspecific crosses it may have a much more profound effect.

It seems likely that a seed which potentially has the capacity to develop to a viable maturity has to reach a certain stage in development beyond which it will successfully reach maturity. If it fails to pass this point, it will fail. It is conceivable that two seeds of similar potentialities may have different histories; one failing and one succeeding, because one of them gets off to a better start than the other. In crosses where the number of seeds that develop to maturity is very low, this may be a critical factor in survival. P. veris x P. elatior is a cross which rarely produces viable seed. In such a cross very minor environmental factors may make all the difference between success and failure.

C. The main lines of thought on seed incompatibility.

Before considering the possible causes of seed failure in Primula, the hypotheses put forward by previous workers to explain seed breakdown, and their validity both to the case under discussion, and to their own results, should be examined critically. I have already pointed out that none of them have claimed to be complete explanations, and that they are linked inmmany ways, but they are conveniently separated into five main lines of thought.

The genetic value hypothesis has already been discussed, and it, least of all, attempts to explain the mechanism of seed breakdown. It can be used as a basis for such an explanation, and will be so used later in this discussion. The vast majority of work in this field has centred on histological investigations, and so it is not surprising that three of the remaining hypotheses are directly based on such investigations, and the fourth developed from a follow-up of early histological work.

The suggestion first put forward by Kihara and Nishiyama (1932); the "pollen stimulation" hypothesis, has been severely criticised, notably by Muntzing (1934), and I think it is because some workers have failed to discriminate between rapid development and good seed set on the one hand, and viability of seed on the other. It is perhaps unfortunate that the way in which they presented their results gives the impression that the most successful cross is that in which the male parent has a higher chromosome number than the female. It is more successful as far as rate of development is concerned, but the reciprocal is more successful where viability is concerned, and this is the important factor in the production of a hybrid plant. What they did in fact establish was that in the cases they studied, more viable seeds were produced when the female had the higher chromosome number, and this meant that their cases fell into line with the general rule proposed by Watkins (1927) that crosses of this type would be more successful.

Their basic premise was that the pollen entering the ovule would exert a certain stimulus to growth and development. Pollen of high chromosome number would

impart a greater stimulus than that of low chromosome number. Their own results supported this claim, and indicated that the stimulus could be too high; so high that it initiated an excessively rapid rate of growth and eventually caused failure. The work of Katayama (1933) and Ledingham (1940) supports this hypothesis, but in other cases of crosses involving parents of different chromosome number, there is no evidence for such stimulation. (In some cases the lack of such evidence may be due to failure to look for it). The nature of the "stimulation" that the pollen imparts is not clear, and Sachet (1948) is one of the few workers in this field who have speculated upon it. She suggested that the pollen brings some substance, either directly, or as a precursor, into the ovule at fertilization. Increased or reduced quantities of this substance could have different effects on the young seed, as could alien substances. Such alterations of quantity or nature of the substance might retard, accelerate or unbalance development of the young seed. In the absence of further evidence it is impossible to be any more precise than this.

The nutrient competition hypothesis was first put forward by Brink and Cooper, as an explanation of the phenomena they observed when making self- and cross-pollinations in Medicago sativa. When this species is selfed most of the ovules abort, and a histological investigation revealed that there is gross hypertrophy of the nucellus, with a consequent occlusion of the chalazal pocket. The endosperm appears to be starved and collapse ensues. They termed this process "somatoplastic sterility", and then sought to apply the hypothesis to interspecific crosses. They observed similar phenomena in several different groups; in some cases it is the nucellus, in some the inner integument that is hypertrophied. In most of these cases occlusion of the chalazal pocket does not occur, and Brink and Cooper suggested that they were manifestations of "incomplete somatoplastic sterility."

Though the concept of somatoplastic sterility has not gained much acceptance, and indeed Brink and Cooper soon modified their hypothesis in the light of further evidence, it is a pity that some subsequent workers have failed to distinguish between somatoplastic

sterility as Brink and Cooper originally defined it, and competition for nutrients. Somatoplastic sterility is the phenomenon of blockage of the nutrient supply to the endosperm by hyperplasia of maternal tissue, with subsequent failure of the endosperm. Endosperm and maternal tissue may compete for nutrients without such a blockage occurring, and this appears to be much more frequently the state of affairs.

Brink and Cooper almost certainly realised this, and they preferred later to explain seed failure in terms of success or failure of the endosperm to compete, and corresponding abnormal development of the maternal tissue. This is not somatoplastic sterility.

The picture that comes out of their ideas is one of endosperm and maternal tissue vigorously competing for the available nutrients. The endosperm in a normal seed maintains a slight advantage over the maternal tissue, but in crosses it loses that advantage through genomic unbalance, and fails to compete for the available food materials. The non-utilized food is diverted to the integuments, producing the hypertrophy observed in so many cases.

This is a plausible hypothesis, but it leaves many gaps, and it cannot be applied to all groups. Primula is one of the groups that does not fit it too well. In the hybrid seeds, hyperplasia of maternal tissue occurs in Type A seeds only, but not in type B. If this is due to failure of the endosperm to compete, one would expect that the endosperm would be sparse in type A seeds, and well developed in type B. In fact the opposite is true. Seeds from a cross such as P. vulgaris x P. elatior, which have a very much overdeveloped inner integument, have a well developed endosperm, whereas those from the reciprocal cross have integuments that are less well developed than normal, and very sparse endosperm.

The third hypothesis of histological origin is that of Weaver (1955), who places the responsibility for seed failure on the embryo, at least in certain crosses. The general consensus of opinion has been that the embryo plays little part in seed failure. This would certainly seem to be the case in Primula, where the success of the embryo appears to be completely dependent

on the endosperm, at least until it reaches a size at which it is capable of supporting itself. By that time the development of the seed has reached a stage where its fate has already been decided. Embryo culture work has shown that an embryo must be at a certain stage before it can successfully be grown in culture after excision. It is possible that within the developing seed, if the embryo reaches a certain level of development, it can continue to grow even if the endosperm fails. This is suggested by the occurrence of large embryos in seeds which are empty of endosperm, in the cross P. elatior tetraploid x P. veris diploid.

Weaver based his hypothesis on the fact that when he crossed different species of Gossypium, seeds were occasionally produced that contained a full endosperm but no embryo. Failure was general in this cross, and so the indications were that the embryo was responsible.

The main argument against the suggestion that the embryo is responsible for seed failure is that embryos can be excised from seeds that normally fail, and grown to maturity on culture media. Once removed from the

surrounding tissues they are able to develop. This would suggest that in the failing seed the embryo is being retarded by the other tissue in the seed, and not that it is causing seed failure.

Histological investigations are necessarily limited in the amount of information they can give us. They are essential to enable us to determine what changes are going on in the tissues of the seed, but they cannot tell us the mechanism behind these changes. More fundamental investigations should follow, and unfortunately only one group has been studied in detail from the biochemical point of view.

Blakeslee and his associates obtained a great deal of information from their histological work, but there were attempts fairly soon to explain the results in terms of the biochemistry of the seed. (Sachet, 1948). The discovery of so-called "ovular tumours" was the decisive factor that launched them on the hunt for the toxic factors in the abnormal seed. Extraction from these tumours of various substances was carried out, and after one or two

false starts it was established that the substance that appears to be most active in seed failure is indole 3-acetic acid. Further investigation has indicated that amino-acid regulation may be important (J. Rietsema personal communication).

It is not possible to say at this stage whether the findings of this group of workers are applicable to other cases, such as Primula, and a great deal more work along these lines is needed before we can say anything decisive about the processes going on within the developing seed.

It becomes increasingly obvious that without a great deal more information than we have at our disposal, it is impossible to find a satisfactory explanation for seed incompatibility. All that can be done at this stage is to suggest a possible mechanism behind the failure of hybrid seed in Primula, and to hope that new lines of attack will be opened up in the near future.

D. Seed incompatibility in Primula

i. The nature of "genetic values."

In attempting to formulate an hypothesis to explain seed failure in Primula, I shall make the assumption that the existence of "genetic values" or "genetic strengths" is, if not proven, at least likely. It would seem the place to make some speculations as to the nature of genetic values. What is the difference between two species of different genetic value? In the answer to this question may lie the key to the whole problem of the relationship of qualitative and quantitative differences between species.

A possible clue to the answer is the similarity of seed failure in diploid - autotetraploid crosses to that in interspecific crosses. It is not unreasonable to assume that the production of similar types of breakdown is caused by similar parental differences.

Howard (1947), discussing the crosses he made with Nasturtium, suggested that the allopolyploid N. uniseriatum had a "physiology of seed production" intermediate between those of diploid and autotetraploid N. officinale

and that it had reached this state by evolving part of the way toward the diploid condition. This does not seem unlikely. Nor does the opposite process, that is, the evolution of a diploid toward the polyploid condition.

If we assume, that of the three species with which we are concerned, the nearest to the diploid state is P. elatior, then on this hypothesis the primrose, (P. vulgaris) has evolved some way toward the polyploid condition, and the cowslip (P. veris)has gone further. Alternatively, if P. veris is nearest to the original condition, P. vulgaris and P. elatior have evolved toward the haploid state.

A possible explanation of the method of such evolution in one direction or another would be in terms of loss or duplication of genetic material. The plants of high genetic value may have large amounts of genetic material, in their nuclei, larger than those of low value. This situation could be brought about in two ways. If the evolution has been from the diploid toward the polyploid state, the increase in genetic material could be due to the accretion of a large number of minute duplications, each of minor effect, but together bringing the genetic complement near the polyploid state without

an actual increase in chromosome number. The alternative possibility, that of evolution toward the haploid state by gradual loss of genetic material seems less likely. There must be a limit to such loss. The true answer may lie between these two alternatives. P. vulgaris could be nearest the original diploid state, and P. veris and P. elatior would then have evolved in different directions, one by the addition and the other by the loss of genetic material.

If this is the sort of process that has occurred, the similarity in seed type between offspring of interspecific crosses and diploid-autotetraploid crosses is more easily explained. Interspecific differences can be considered in quantitative terms. The purely quantitative differences will be modified by the qualitative differences between species, but will largely influence the course of development of hybrid seed.

If this explanation of interspecific differences in quantitative terms is accepted, the problems of seed incompatibility in Primula are more easily encompassed.

ii. A possible explanation.

The main problem confronting us in our attempt to explain the Primula results is the difference between type A and type B seeds. The presence of such great differences in the seeds from reciprocal crosses is uncommon. If we can assume, however, that differences in genetic value are equivalent to differences in ploidy, the explanation is made less difficult. In almost every case that has been investigated, reciprocal crosses between parents of different chromosome numbers produced dissimilar seed. But as we have seen, no really satisfactory explanation has yet been put forward to account for these differences. Instead of concentrating on one feature of breakdown, as has often been done in the past, I shall attempt to relate the different features in one hypothesis.

If the introduction of foreign pollen into the ovule initiates seed development, it follows that the type of pollen introduced will affect the ultimate result. This fact was used as the basis of the pollen stimulation hypothesis. There is some evidence that in

Primula the introduction of pollen of either higher chromosome number or higher genetic value than that of the female genome does initiate a more rapid rate of development than when the reverse is the case. This means that type B seeds are stimulated to more rapid development; type A seeds getting a slower start. It seems probable that type B seeds, especially the more extreme ones, are given a stimulus that is too strong, and they "overgrow their strength." The less extreme crosses produce seeds that can sometimes recover from this initial overstimulation, whereas the wider crosses do not recover. However, type B seeds grow to a large size, and weigh more than their type A counterparts. I would suggest that this is a result of the dissipation of the available nutrients in the production of large empty seeds.

Type A seeds. on the other hand, get a stimulus lower than normal. This appeara to affect the contents of the embryo sac more than the integuments, and although both develop, the endosperm is put at somewhat of a disadvantage. The integuments are therefore able to

utilise some of the material that normally goes to the embryo sac, and are more strongly developed than normal. In less extreme crosses the effect is small, and a fair proportion of the seeds reach maturity, but in wider crosses the endosperm is less well balanced with the maternal tissue, and its increased weakness allows the integuments to take over a large part of the nutrient supply. In the most extreme cases it seems possible that the inner integument can assume an aggressive role and expand at the expense of the embryo sac contents.

In both types of seed it seems likely that the key tissue is the endosperm. The stimulus it receives at fertilization is the important factor in deciding the fate of the seed. The relationship that can determine the success or failure of the seed is that between the endosperm and the maternal tissues. Genetic ratio is a means of expressing this relationship.

However, though this is a step in the direction of explaining seed failure, the relationship is a much more complex one than a simple quantitative endosperm : maternal tissue balance. One must not forget the fact that since embryos can be excised from otherwise inviable

seeds and grown to maturity, the endosperm here must be acting as a buffer protecting a viable embryo from the toxic influences of the mother plant. The physiological and genetical differences between species will loom large in the determination of seed development. The endosperm stands out as the tissue that, in its peculiar position, is the sole connection between mother plant and embryo. Much more research into the nature of the endosperm is needed, and here the histological apporach is limited.

Briefly, the present evidence suggests that there are two key relationships in seed development:

1. The genome relationship, which can result in a greater or lesser stimulus to seed growth, and especially to the growth of the embryo sac contents.
2. The endosperm : maternal relationship, the unbalance of which can result in overgrowth of the integument, and/or failure of normal endosperm development.

These basic relationships must be considered in the light of physiological and genetical differences between species.

E. The problems that remain.

A hypothesis of seed incompatibility has been put forward that is necessarily limited by the lack of more evidence. It would not be out of place for me to suggest here some of the remaining problems, and some possible approaches to them, that might help to a better understanding of the nature of seed breakdown.

1. If genetic values are of the nature that has been suggested, then it follows that a species of high genetic value will have a larger amount of gemetic material in its genomes than will one of low value. If some estimation of the actual amount of chromatin in the cells of the different species could be made, the suggestion made earlier as to the nature of genetic values could be tested.

2. A detailed microchemical investigation of the tissues of normal and abnormal seeds at different stages, developing the approach of Blakeslee and his associates, would probably bring us nearer to the solution of the problems than any other single approach.

3. The use of embryo and seed culture techniques,

combined with that of various growth substances, would be of value. If whole ovaries could be cultured, then ovaries containing hybrid seed could be removed from the parent plant and raised to maturity. This would tell us whether the immediate maternal environment is the sole factor concerned in relation to the endosperm, or whether the plant as a whole has an effect on the seeds borne upon it. The use of various substances, both *in vitro* and *in vivo* can help to elucidate the chemical relations within and around the seed.

4. Chromosome unbalance within hybrid seed can be investigated by a study of endosperm cytology. Some preliminary attempts at this have been made, but a satisfactory technique remains to be worked out. (see Appendix B, page 192).

VII. SUMMARY.

1. The importance of post fertilization breakdown as a barrier to interspecific hybridization has been emphasized, and the use of the term "seed incompatibility" for such a phenomenon has been suggested by Valentine.

2. Early workers came to the conclusion that as a general rule, when two species of different chromosome number were crossed, the cross would be more successful when the plant with the higher number was used as female.

3. There have been several lines of thought on the problem during the last thirty years, and of these the most important are:

   a. The pollen stimulation hypothesis.
   b. The nutrient competition hypothesis.
   c. The view that the embryo causes seed failure.
   d. The hypothesis that some sort of chemical unbalance is responsible for seed failure.
   e. The genetic "value" or "strength" hypothesis.

   None of these hypotheses sets out to be a complete explanation, and each is related to the others.

4. The three British species, Primula vulgaris,

P. veris and P. elatior are all diploids with n = 11. Their crosses partly or completely fail. Normal seed development follows a similar pattern in each species. The young seed enlarges rapidly after fertilization, the outer integument, which consists of two layers, becomes thickened, the inner integument degenerates rapidly. The endosperm remains non-cellular for about three weeks, and then cell formation takes place. After this the endosperm increases in quantity very quickly and soon fills the seed. The embryo remains very small until the endosperm has nearly filled the seed, and it then undergoes a period of rapid growth, elongation and differentiation.

5. In each pair of reciprocal crosses, a different type of seed is produced depending on which direction the cross is made. Valentine has called these two types of seed type A and type B. He has allotted each species a "genetic value" these values being based on the results of his crosses. From this genetic value the genetic ratio; the ratio between endosperm and mother plant, can be calculated. It is 1.5 in intraspecific crosses. When the ratio R is less than 1.5, the seeds are of type A,

if R is greater than 1.5, type B seeds are produced. The embryological investigation has clearly distinguished these two types.

6. Type A seeds gave an abnormally thick inner integument, and a reduced endosperm and embryo. The seeds are small. Type B seeds are large, and have thin integuments. The endosperm is very poor, and the embryo is correspondingly underdeveloped.

7. The seeds from the reciprocal crosses between an autotetraploid and diploid P. veris can be classed into the same two types, being more extreme in their abnormalities.

8. When a diploid P. veris (genetic value 1.8) is crossed with autotetraploid P. elatior (genetic value 2.0), R is nearly equal to the normal value of 1.5. Despite the specific difference, the cross shows a certain amount of success.

9. These facts are held to support the hypothesis that different diploid species may have different "genetic values". It is suggested that genetic value is in some way allied to ploidy, and that a diploid species may evolve toward the polyploid state as well as in the

reverse direction. One way in which such a process can occur, it is suggested, is by the accumulation of a number of minute duplications. By this means, genetic value and polyploidy can be compared. In Primula, they are similar in their effects, and this to some extent removes the distinction between quantitative and qualitative barriers between species.

10. It is suggested that the initial stimulus imparted by the pollen determines the rate of early seed growth. Type A seeds get a low stimulus, and the endosperm in particular is retarded, enabling the integuments to utilise some of the resulting surplus nutrients. Type B seeds get a strong stimulus, and the endosperm never recovers from the initial period of too rapid growth. In both cases the endosperm : maternal tissue relations are held to be important.

11. It is pointed out that further physiological investigations, cytological work, and tissue culture experiments are needed before the solution of the problem of seed incompatibility is much clearer.

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Note. Although the review of previous work in this field followed the main lines of thought only, this Bibliography includes many other important references. Those which have been quoted in earlier sections have been indicated with an asterisk.

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APPENDICES.

Appendix A.

The course of development of seeds in the intra- and interspecific crosses was presented in summarised and tabular form in Section IV of this thesis. There are, however, some details that could not be discussed there, as they were not necessary to the main argument. They have, therefore, been included in the more detailed stage by stage descriptions that are appended here.

The intraspecific crosses.

Though the cross described here is that between P. veris and P. veris, the details of development lof the other two species, P. elatior and P. vulgaris, are so similar as not to warrant repeating, the only differences being in timing and seed size.

The unpollinated ovule has two integuments. The outer integument consists of two cell layers. Outer integument 1 consists of roughly cubical cells, brown in colour, due to the presence of tannins. Outer integument 2 has cells which are elongated circumferentially, and which have very dense cytoplasmic contents. They are rather smaller than those of outer integument 1. Outer integument 1 is from three to six cells in thickness, the outer layer is regular, but the other layers are progressively less regular. All have dense contents. Inner integument 2 consists of a single layer of large cells, radially elongated. They, too contain tannins. (Dahlgren, 1916). These cells are irregularly shaped, especially in the chalazal region. This layer is interrupted in two places; at the micropyle, toward the lower end of the chalazal region, and at what may be called

the chalazal pocket, at the upper end. The nutrient supply sweeps upwards behind this region and is probably mainly delivered through this gap in the inner integument.

The embryo sac is a thin membrane lining the ovule cavity, and containing the egg cell, synergids and polar nuclei. The antipodals could not be distinguished at this stage (immediately prior to pollination).

10 days. The seeds have increased considerably in size. The cells of outer integument 1 are larger and have some thickening on their inner walls. Outer integument 2 has now many more cells, though still only a single layer. Inner integument 1 is changing in appearance, the cells showing shrinkage of their contents. The cells of inner integument 2 have elongated radially, especially in the chalazal region. The endosperm is a thin layer of cytoplasm lining the seed cavity, with a few nuclei scattered throughout. It is slightly thicker, and has a granular appearance in the chalazal region. The embryo appears to be marking time, and in fact the zygote does not seem to have yet divided.

20 days. Thickening is increasing on the inner walls of the cells of outer integument 1. All but the outermost cells of inner integument 1 are now degenerating, losing their contents and shape. This process is less advanced in the chalazal region. Inner integument 2 is also starting to degenerate. The endosperm contains more nuclei, but the embryo is still marking time.

24 days. The cells of outer integument 1 show little change, but a marked change is taking place in those of outer integument 2. The inner wall is becoming thickened, this thickening being extended for a little way up the lateral walls of the cells. This thickening thus forms a cup-shaped layer within each cell. The cell is still filled with dense cytoplasm.

The process of degeneration of the inner integument goes on apace. Only the outermost layer of the inner integument 1 remains relatively intact. Inner integument 2 is somewhat flattened. The remaining layers of inner integument 1 are empty of contents, and are becoming compressed.

The emphasis of development appears to be switching toward the endosperm now. It is now a single cell layer,

sometimes double in the chalazal region, where the cell contents are also more dense. The embryo is often at about a four-celled stage by this time.

28 days. The thickening in the cells of outer integument 2 has increased slightly, but the degeneration of inner integuments is now nearly complete. The endosperm is 2 - 3 cells thick by this stage, thicker in the chalazal region. The embryo has started a period of rapid growth, and in some seeds contains about 64 cells.

32 days. The cells of outer integument 2 are now filled to about $\frac{1}{4}$ of their height by the thickening of the inner wall. Degeneration of the inner integument is complete except in the chalazal region. The endosperm is several cells thick, and the cells adjoining the chalazal region are well filled with cytoplasm, those of the rest of the endosperm being scantily supplied with contents. The embryo is increasing rapidly, and is a small sphere, surrounded by endosperm, in the micropylar region.

36 days. The thickening on the inner walls of the cells of outer integument 2 now fills about half the cell cavity. The inner integument is now a thin, compressed layer of cell debris. The seed is generally completely filled by

endosperm. The endosperm cells have developed in rows upon the original cells that lined the cavity. The embryo, though much larger, is still spherical.

40 days. The cells of outer integument 2 are now about two-thirds filled by thickening. Even in the chalazal region the inner integument cells are now showing signs of breakdown. The endosperm cells are gradually being filled with cytoplasm, as their increase in number is slowing down. Around the embryo the endosperm is apparently being absorbed by the developing egg-shaped embryo.

44 days. The cells of outer integument 2 are now almost completely thickened, and this layer is better developed in the chalazal region, forming a thick wad of tissue. All but the cells of inner integument 2 have now degenerated in the chalazal region. The endosperm cells are beginning to show signs of the accumulation of food materials, mainly oil. The embryo is rapidly elongating, and is beginning to differentiate, the cotyledon initials being evident.

Throughout development there have been deposits of starch in the placenta, and they have increased as the seeds matured, the greatest concentrations being near the attachments of the seeds.

From this time on the integuments show little change. Their thickening becomes complete, and the outer integument forms a thick, hard layer around the seed. The remains of the inner integuments can be seen as a very thin, dark, compressed layer of broken down tissue.

The endosperm continues to accumulate food materials, until at maturity it is packed with stored nutrients. These are said by Dahlgren to be mainly oils and proteins. The embryo continues to elongate, until at maturity it is a long, straight structure, with two equal cotyledons, packed with food materials.

Illustrations of developing seeds of this and other crosses are to be found in Section IV (pages 45 - 112).

P. vulgaris x P. elatior.

10 days. The two layers of the outer integument are normal in appearance. The two outermost layers of inner integument 1 are still intact, the remainder having begun to degenerate. Inner integument 2 is rather thicker than normal. The endosperm, a thin cytoplasmic layer, has less nuclei scattered through it than in the intraspecific seeds. No division of the zygote appears to have taken place.

20 days. The outer integument is still normal in appearance. Inner integument 1 still has the outermost layer intact, the others having degenerated. Inner integument 2 is abnormally thick. The endosperm is still non-cellular, and the embryo is apparently still undivided.

24 days. The seeds are considerably smaller than normal. The outer integument is apparently normal, and there is still one layer in inner integument 1 remaining. Inner integument 2 is notably thicker than normal, this thickness being especially apparent at the chalazal region. The endosperm is variable, sometimes becoming cellular, sometimes not, and always appearing rather dense. The embryo is apparently still undivided.

28 days. The cells of outer integument 2 are showing signs of the commencement of thickening. The outermost layer of inner integument 1 is still persisting, longer than in normal seeds. Inner integument 2 cells are very large, espceially in the chalazal region. The chalazal pocket remains open, however, despite the thickness of inner integument 2.

The seeds can be divided into two groups at this stage, those in which the inner integument is very thick, and the endosperm is not too well developed, and the remainder, in which the inner integument is not so thick, and the endosperm is cellular, usually consisting of a single layer of cells. The embryo is often 2-celled.

32 days. The two types of seed, though easily distinguishable, differ little in their outer integuments. In both types, too, the outermost layer of inner integument 1 is still present, though the cell contents are becoming shrunken. It is in the inner integument 2 and the endosperm that the two types are most distinct. In some seeds several layers of cells are present in the endosperm, and in these seeds the inner integument 2 is

not very greatly overdeveloped. In the other seeds the endosperm is less well developed, but the inner integument 2 is correspondingly overdeveloped. In the seeds with well developed endosperms embryos at about the 64 celled stage are present.

In addition to the two types of seed described, occasional aberrant seeds are found, which are, by this time, already degenerating. They are about one-third of the size of the rest of the seeds. The outer integuments show little sign of thickening, the inner integument 1 has more than one layer intact, and the inner integument 2 is slightly overgrown. At this stage a small amount of very dense cellular endosperm can be seen, and occasional very small embryos can be discerned.

40 days. The outer integument is about one third thickened by this time. Inner integument 1 is still not completely broken down, while inner integument 2 is rather more thick, especially in the chalazal region. In the best developed seeds, the endosperm fills about half the available space. The cells are small and contain dense cytoplasm, and are granular in appearance, especially in the chalazal region. The embryo is growing more rapidly.

44 days. The two types of seed are still distinguishable. In both the outer integument 2 is about half thickened. Inner integument 1 is now fully broken down, but inner integument 2 fills about one third of the seed cavity in the most abnormal seeds, rather less in the remainder. In both types the endosperm now fills the remainder of the cavity, and has some food material in it. The embryo is lengthening, especially in the more normal seeds.

48 - 52 days. During this period the thickening in the cells of outer integument 2 fills about two thirds of the cell cavity. There is very little difference between the two types of seed as far as their outer integuments are concerned. The real difference lies in the inner integument 2. In one group of seeds, this consists of large, active-looking, elongated cells, forming a layer that fills one third to one half the space normally occupied by endosperm. In these seeds the endosperm is naturally restricted in quantity by the available space, but the cells do not fully fill that space, and the interstices between them are filled with unidentified matter. There is not a great deal of food material in

the endosperm, compared with the amount in the remaining seeds. In these, the inner integument 2 is not so greatly hypertrophied, especially in the chalazal region. The endosperm cells are in more or less regular rows, and are packed with food materials. In these seeds the embryo is well developed, and appears to be quite healthy. In the others, it is sometimes distorted, and is in any case smaller.

The possible reasons for the presence of two types of seed are discussed in another part of this thesis.

P. elatior x P. vulgaris.

10 days. The seeds are apparently almost normal in most respects. The endosperm has rather fewer nuclei scattered through it than that in a normal seed. The embryo is still a single cell.

20 days. The outer integument is developing normally. Inner integument 1 is degenerating rapidly, and the cells of inner integument 2 are shrinking, except in the chalazal region. The endosperm is variable, being always non-cellular, but in some cases being very dense in appearance. The nuclei are irregular in size, and there are signs of vacuolation in the endosperm of some seeds. The zygote has not yet divided.

24 days. In some seeds there is a commencement of thickening on the inner walls of the outer integument 2 cells, but the majority have not yet started. The inner integument has undergone a very rapid breakdown and now remains only in the chalazal region. The endosperm is increasing in quantity, and two types of endosperm are becoming apparent, one much more rapid in growth than the other, and more normal in appearance. The embryo in these seeds may be four celled.

28 days. The inner walls of the cells of outer integument 2 are now becoming thickened in all seeds. Degeneration of the inner integuments has now spread to the chalazal region. The endosperm is still non-cellular in all seeds, but some seeds contain a much smaller quantity than the rest. Both types show irregularities in the endosperm.

32 days. Outer integument 1 is rather less well thickened than normal, but outer integument 2 shows an increase in thickening. The inner integument has completely broken down, leaving only a compacted layer of cell debris. The endosperm is increasing slowly, and the embryo in the best developed seeds contains about 32 cells. It is generally about 8-celled, however.

36 days. From this time on the integuments show little change, except for the thickening of outer integument 2 which is completed by about 48 days. Some seeds have a cellular endosperm at this stage, but the remainder have a smaller quantity, which is non-cellular. The better developed endosperms have a spherical embryo of fair size, the rest have very small embryos, which from this time cease to increase in size.

40 days. The two classes of seed are now very clearly distinguishable, with very few intermediates. One type has a fair quantity of cellular endosperm, with scattered giant nuclei, and rather irregular cells. The other has a smaller quantity of non-cellular endosperm, which is dense, vacuolate, and contains many large and abnormal nuclei. The integuments are similar in both types. In the first type the embryo is now heart shaped, whereas that in the second type is small and unhealthy in appearance.

44 days on. The seeds of the first type, with good endosperm, continue to develop steadily. Food materials accumulate in the endosperm and in the rapidly elongating embryo. The contents of the remaining seeds undergo a slow but steady process of degeneration, until the endosperm appears completely structureless.

P. veris x P. vulgaris.

10 days. Outer integuments 1 and 2 are normal in appearance. Inner integument 1 shows some shrinkage of cell contents of the inner layers. Inner integument 2 is thick, and occasionally has two layers of cells. There are few nuclei in the endosperm, and the embryo is one celled.

20 days. The seeds are rather variable, but the majority of them follow the same course of development. The cells of outer integument 1 are much larger, and their outer walls are rounded off. One layer of inner integument 1 remains intact, and the next one is only partly degenerated. Inner integument 2 is thicker than normal, but not excessively so. The endosperm is often cellular, and is even occasionally more than one cell thick. The zygote has not yet divided.

Among the remainder of the seeds there are some in which the endosperm has not yet become cellular. A few small seeds are found in which the inner integument 2 is very thick and the embryo sac contents have already completely degenerated, leaving only a few wisps of unidentifiable tissue.

24 days. One layer of the inner integument 1 remains intact. Inner integument 2 has changed little. The endosperm may be three or four cells thick, and the embryo now is often two-celled.

28 days. Outer integument 1 changes very little from now on, and is fairly normal in appearance. Thickening of the outer integument 2 is commencing. The remaining layer of inner integument 1 shows some shrinkage of cell contents. Inner integument 2 is thick, but not as thick as in P. vulgaris x P. elatior. It is much more uniform in thickness than in that cross, and is not much more thickened in the chalazal region than elsewhere. The endosperm about half fills the seed, and the embryo is up to about 16 cells. The early degenerating types of seed seen earlier are now in the later stages of complete breakdown.

32 days. Outer integument 2 is being rapidly thickened. The remaining layer of inner integument 1 is slowly breaking down. Inner integument 2 has changed little, and from now on remains as a thick, uniform layer, with somewhat compressed cells. The endosperm often fills about two-thirds of the seed, and is not as regular

as in normal seeds. The embryo is a small sphere.

36 days. The contents of the cells of one remaining layer of inner integument 1 are now disappearing. The endosperm is increasing in quantity, and food material is starting to accumulate. The embryo is still spherical.

40 days. Outer integument 2 is about half thickened. Inner integument 1 is at last almost completely broken down. The endosperm, even at best, does not yet completely fill the seed, and it is variable in quality. The embryo is still spherical, its size being correlated with the amount of endosperm present.

44 days on. The thickening of outer integument 2 is nearly complete by about 52 days. The breakdown of inner integument 1 is completed. The endosperm comes to fill the seed cavity in a few seeds, but is loosely packed. In many seeds the quantity is less. The embryo starts to elongate at about 44 days, and thereafter continues steady growth. The total number of mature seeds is not very great.

P. vulgaris x P. veris.

10 days. The integuments are not noticeably abnormal at this stage. The endosperm has very few nuclei, and the zygote has not yet divided.

20 days. The cells of outer integument 1 are very large with thin walls, and those of outer integument 2 are becoming irregular in shape and arrangement. The inner layers of inner integument 1 are degenerated, and the rest are losing their contents. Inner integument 2 is degenerating rapidly, except in the chalazal region, where it is rather thicker than normal.

24 days. Inner integument 1 is completely broken down except in the chalazal region. Inner integument 2 is rapidly degenerating, except in the same region. The endosperm is sometimes cellular, but is very small in quantity, and the embryo is about 8-celled.

28 days. The cells of outer integument 1 are unthickened, and those of outer integument 2 are irregular and also unthickened. Inner integument 1 has almost completely disappeared in all parts of the seed, but inner integument 2 persists in the chalazal region as a well defined layer, and continues to do so from now on. The endosperm is

tenuous, with few nuclei, and is very rarely cellular. The embryo has from 8 - 40 cells, but the cells are very small.

32 days. There is very slight thickening on the inner walls of the cells of outer integument 1, and this does not increase any more, the layer remaining flimsy. Outer integument 2 is a flimsy irregular layer. The endosperm has increased very little, and the embryo has up to about 60 cells.

36 days. There is a sign of slight thickening on the inner walls of the cells of outer integument 2. The endosperm and embryo are still slowly growing.

44 days. The thickening of outer integument 2 is slowly increasing. The endosperm is more often cellular than not, and may be up to 3 cells thick. The embryo remains very small.

48 days on. The outer integument 2 does not become much thickened and remains irregular. The endosperm may, in the best developed cases, half fill the seed, and it frequently remains non-cellular and begins to degenerate. The embryo, even in the best developed endosperms, never reaches a very great size, and remains spherical.

P. veris x P. elatior.

10 days. The outer integument appears normal, and there are barely any signs of degeneration in inner integument 2. Inner integument 2 is very thick, and often has two layers of cells in the chalazal region. The tenuous endosperm contains very few nuclei, and the zygote has not yet divided.

20 days. The seeds are growing very slowly. Outer integument 1 shows signs of thickening, but outer integument 2 is unchanged. Only the outer layer of inner integument 1 is intact. Inner integument 2 is very thick, especially in the chalazal region.

The endosperm is very tenuous, but occasionally shows signs of becoming cellular. Occasionally degeneration has already set in. The embryo is about 4 - 6 celled.

24 days. The outer integument 1 changes very little from this time on. Outer integument 2 shows occasional proliferation to form two layers, but this is rare. Inner integument 2 is very thick. The endosperm is often more degenerate, but in many seeds is growing very slowly, while the embryo is about 6 celled.

As in the cross P. veris x P. vulgaris, there are some more extreme aberrant forms, which are small, with very thick inner integuments and only a few remains of endosperm. These degenerate rapidly from now on.

28 days. Outer integument 2 has started to become thickened. The outermost layer of inner integument 1 is still intact, but the rest have degenerated. Inner integument 2 is hypertrophied to a varying degree. The cells are large and active, and the layer is fairly uniformly thick. Many of the seeds have collapsed by this time. The endosperm has sometimes disappeared completely, and is often very degenerate. A few seeds occur which contain a small quantity of irregular cellular endosperm, and in these there are small, dense embryos.

32 days. The outermost layer of inner integument 1 is now showing signs of breakdown. The endosperm has begun to degenerate in almost all the seeds, and the embryo is often following the same course.

36 days. Complete collapse of the seeds has always occurred by now. Outer integument 2 is thickened to

about one third the way up the cells. Inner integument 1 has finally completely degenerated, and forms a thin compacted layer. Inner integument 2 is often as thick as the rest of the integument together. The cells are large and distinct.

There are occasionally remains of endosperm present. but never cellular tissue. The embryo often persists in spite of the absence of endosperm. As far as can be seen the chalazal pocket remains open.

40 days on. Thickening of outer integument 2 continues slowly, even in the collapsed seeds. In one case an embryo was seen that had begun to elongate at 44 days, although there was no endosperm present. This seed had not collapsed at this stage. In general, there is little change from now on.

P. elatior x P. veris.

10 days. The cell contents of the inner integument 1 have begun to shrink. Inner integument 2 is rather thicker than normal. The endosperm is very sparse indeed, with only a few nuclei.

20 days. The seeds have rapidly increased in size. The cells of outer integument 1 have very thin walls. The inner integument 1 has already degenerated except in the chalazal region, and rapid degeneration is taking place in inner integument 2. The endosperm has increased very little in quantity, and is very dense in appearance, with scattered nuclei of various sizes, and occasional vacuoles. The few embryos seen at this stage were all 4 celled.

24 days. Inner integument 2 is now fully broken down except in the chalazal region. The vacuolation of the endosperm is more evident, and the embryos are up to 8 cells in size.

28 days. The outer integument 1 now consists of large, thin-walled cells, that are so flimsy that they have often been damaged in sectioning. Outer integument 2

is becoming thickened in most seeds. The endosperm is variable, sometimes being completely structureless, but occasionally contsining a few cells. Rarely, small embryos are to be seen, consisting of about 16 small cells. They do not appear to be healthy.

32 days. The outer integument 1 does not develop any thickening in these seeds, but remains in its present state; a layer of thin-walled rounded cells. However, the thickening on the cells of outer integument 2 is increasing rapidly, and about one-third fills the cells now. The endosperm is apparently slowly degenerating.

36 days. The inner integument is breaking down even in the chalazal region by this time. In a few seeds the inner integument 2 persists as a rather active looking layer, resembling in the appearance of the cells, (but not in thickness) the same layer in the seeds of the reciprocal cross. In these seeds the endosperm is possibly degenerating more rapidly than in the rest.

40 days. The thickening in outer integument 2 has proceeded extremely rapidly, and is now nearly complete. The inner integument is completely broken down, except in the few exceptional seeds mentioned above. The

endosperm is slowly degenerating, but in some seeds can still be seen to be cellular. One seed was examined in which the integuments were relatively normal, and this seed had an endosperm that was better developed than any other seen in this cross. Even so it was unhealthy in appearance, and the embryo was minute.

40 days on. The thickening of outer integument 2 is completed by about 44 days. This results in an outer integument that has a very flimsy outer layer and a normally thickened inner layer. The endosperm continues to degenerate, and eventually it almost or completely disappears. In many seeds, as the endosperm slowly degenerates, large quantities of structureless matter become evident, especially in the chalazal region. This does not consist of endospermic debris, but is clearly distinct from the remains of that tissue. It may well be food material that has passed into the seed, where it has remained unused owing to the inability of the endosperm to absorb it.

P. veris tetraploid x diploid.

10 days. The outer integument is apparently normal. The cell contents of inner integument 1 are shrunken, but the cells of inner integument 2 are large and active; variable in size, and they form a thick layer, resembling that of less extreme type A seeds. The endosperm is very tenuous, and rather dense. No embryos could be found at this stage.

20 days. The seeds have grown somewhat in size, and the outer integument 1 has a little thickening on its inner cell walls. Degeneration of the inner integument 1 is speeding up. Hypertrophy of inner integument 2 has already reached an extreme stage. The cells are very large and active, and give the appearance, especially in the chalazal region, of actively encroaching on the embryo sac, in the form of tumour-like ingrowths. In many seeds the embryo sac contents have disappeared, but occasionally fragments of endosperm, and a four-celled embryo, already degenerating, are seen.

24 days. Outer integument 2 has just started thickening in some seeds. The active ingrowth of inner integument 2 continues, and only a few fragments of endosperm can be

found. The embryo has disappeared.

28 days. The thickening in the cells of outer integument 1 has barely increased. Inner integument 1 is well broken down, except in the chalazal region. Inner integument 2 is apparently becoming structureless. The cells have broken down to form a mass of irregular cancer-like tissue.

32 days on. From now on the outer integument 2 becomes a little more thickened. Inner integument 1 finally degenerates completely. Inner integument 2 becomes more and more amorphous in nature, forming a mass of tissue in which individual cells cannot be distinguished. The seeds generally collapse at about 36 days.

Occasional very large seeds are found in this cross. Their integuments are fairly normal, except inner integument 2, which is very thick. However unlike that of most of the seeds in this cross, the individual cells in it can be distinguished, and there are no "tumours." The nature of such seeds is obscure.

P. veris diploid x tetraploid.

10 days. The outer integument is normal in appearance, though the cells of outer integument 1 have thin walls. The inner layers of inner integument 1 are breaking down. Inner integument 2 is thick, with large, thin-walled cells. The endosperm has very few nuclei. The zygote does not appear to have divided.

20 days. The cells of outer integument 1 have grown considerably, and are very thin walled. Inner integument 1 is nearly completely disintegrated, even in the chalazal region. Inner integument 2 has not maintained its initial thickness, and is rapidly degenerating. The small quantity of endosperm is vacuolated, and shows the typical large nuclei of the other type B seeds. The embryo is four celled at most.

24 days. The outer integument is little changed. Inner integument 2 persists in the chalazal region, as a layer rather thicker than normal. The endosperm is unchanged, and the embryo is up to about eight cells.

28 days on. Outer integument 2 becomes fully thickened by about 44 days. Inner integument 2 persists in the

chalazal region. The embryo sac contents completely degenerate, and complete or partial collapse ensues in all the seeds.

In all the crosses, intra- and interspecific, there occur small, degenerate seeds, usually towards the base of the placenta. They do not appear to contain any embryo sac contents; they are very small and their inner integuments, especially inner integument 2, are always to some extent hypertrophied. They cannot be easily explained, but it is likely that they are unpollinated ovules, which have been stimulated to a certain amount of growth by the diffusion of substances from the adjacent developing seeds.

Appendix B.

Endosperm cytology.

It was thought that a study of endosperm cytology would prove useful as a future line of attack on the problem of seed incompatibility, and with this in mind, some preliminary investigations were made. Although the method used needs some adjustment, and very few of the hybrid endosperms yielded any results, some of the preparations obtained suggest that further research along these lines would be fruitful. One or two examples of moderately successful endosperm squashes are therefore appended here.

The technique devised by Rutishauser & Hunziker (1950), using mass staining in Feulgen, was not successful with this material. The preparations illustrated in Figs. 79 - 81 were obtained as follows:

The seeds were fixed at 40 days after pollination, this having been found by trial and error to be the time when the endosperm is in the best condition. They were fixed in acetic alcohol, with the addition of a little iron as ferric acetate. They were kept in the deep freeze at about -10°C until required. The endosperm was teased out in a drop of aceto-carmine on a slide, covered, heated, and gently squashed under a cover slip.

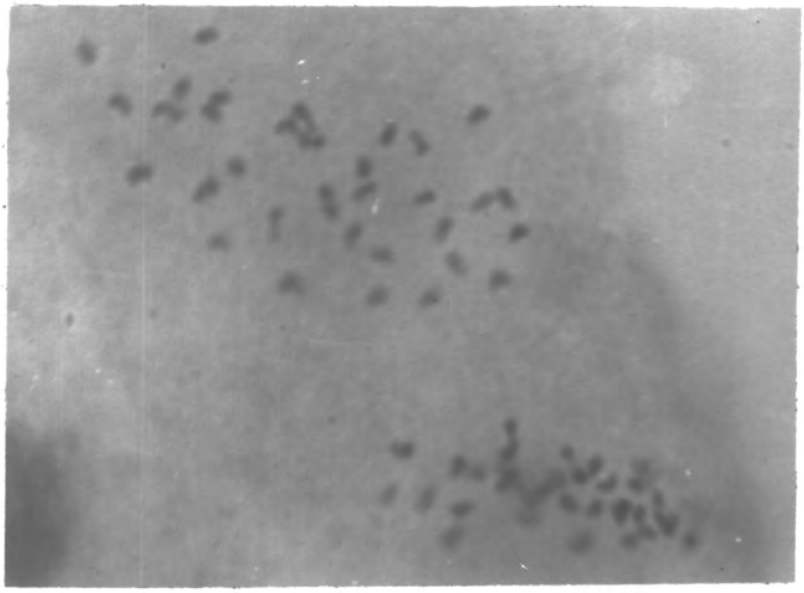
Sufficient material has not yet been examined, and accurate counts of chromosome number cannot therefore be made. However, the three photographs do illustrate one point that was obvious from the material examined. Although the normal endosperm, in the three species, has a chromosome number which is approximately that which would be expected, ie. the 3n number of 33, that of at least one of the hybrids, P. elatior x P. vulgaris

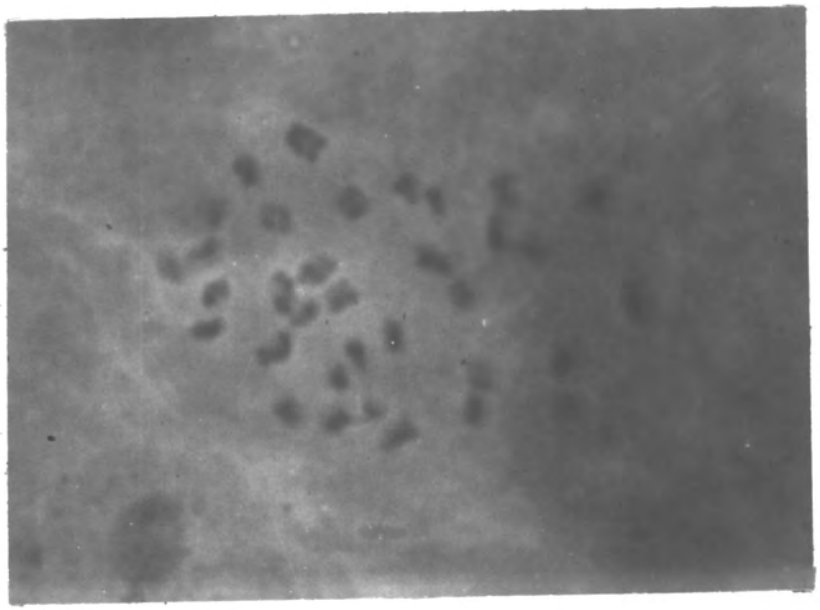
Figs 79 - 81. Aceto-carmine squashes of endosperm. All x approximately 3,500.

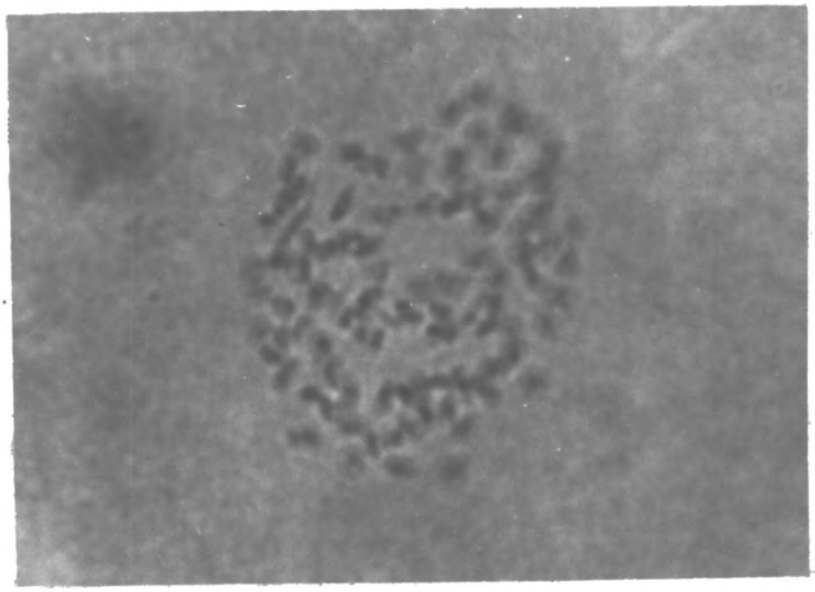
Fig. 79. Endosperm of P. veris

Fig. 80. Endosperm of P. vulgaris.

Fig. 81. Endosperm of the cross P. elatior x P. vulgaris.







has a number much larger than this. The number cannot be accurately counted, but has been estimated at being between 85 and 95.

The endosperm of the cross P. elatior x P. vulgaris does show large and irregular nuclei, so such a result was not altogether unexpected. A much more extensive series of observations and experiments is needed to determine how widespread this phenomenon is, and what are its causes.