A Cytological Study of the Effects of Self-Pollination on Pseudotsuga menziesii (Mirb.) Franco[1]

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Introduction

The mating systems of plants and animals have interested breeders for many years, particularly in respect of the relative merits of inbreeding and outbreeding, the one favouring reproduction by closely related parents and the other by parents not closely related. An even closer form of inbreeding is possible in hermaphroditic plants as self-pollination results in fusion of the male and female gametes from the same plant. This inevitably results in some reduction of the supply of hereditary variability and many of the angiosperms, for example, have developed various devices to prevent its occurrence, among which are the genic systems of incompatibility. BATEMAN (1952) has explained that such systems differ from the many devices that interpose a mechanical barrier to self-pollination in that they act at a later stage of interposling a physiological barrier between self-pollination and self-fertilization. There have, however,been no detailed studies on the effects of self-pollination on the gymnosperms although such past investigators as SYLVÉN (1910), DENGLER (1932) and others have shown that it usually resulted in greatly reduced yields of viable seed. WHITEHOUSE (1950) has speculated. that no mechanism based upon the incompatibility of pollen and style appearsd possible in the gymnosperms.. He added that the thickness of the nucellar tissue was hardly sufficient to be capable of interposing a physiological barrier to pollen tubes. BATEMAN (1952) pointed out that since a single layer of cells in the stigma of certain angiosperms was sufficient to prevent self-pollination, the massive nucellus of most gymnosperms should be quite capable of acting as a physiological sieve to pollen tubes. He added that the absence of a style in the gymnosperms, therefore, was not sufficient explanation for the absence of self-incompatibility from this phylum, if it was indeed absent. PLYM FORSHELL (1953), moreover, has claimed that a self-incompatibility system was operative in Pinus sylvestris and that her theory of incompatibility was not contrary to what was known about the genes of self-sterility in the angiosperms in which pollen tube growth was restrained after self-pollination. Both ALLEN (1942) and DUFFIELD (1950) had found that the self-pollination of Pseudotsuga menziesii resulted in little or no viable seed being produced and the species, therefore, was considered to be self-sterile. This species appeared to be a logical choice for a cytological study to determine whether a self-incompatibility system was responsible for the reported low yields of seed after self-pollination. The term self-incompatible has been used throughout this paper instead of that of self-sterile, as according to LEWIS (1954) it is the more correct.

Material and methods

The cytological study was divided into two parts as the first controlled self- and cross-pollinations were made on two trees near Vancouver in 1952 and the remainder on six trees on Vancouver Island in 1954. Extreme care was used in making these pollinations and the techniques employed have been described in detail (ORR-EWING, 1956). The general procedure was to remove sample cones at different dates after both self- and cross-pollination and to dislodge the ovules from ovuliferous scales. The ovules were then killed and fixed, dehydrated and embedded. In the 1954 study, the integuments of the ovules were also removed prior to killing and fixing. Sections were finally cut at twelve microns and stained with either HEIDENHAIN'S iron haematoxylin or with FEULGEN counterstained with fast green.

Results of the 1952 controlled pollinations

No cytological work was conducted until a germination test had been made on the seed from the remaining cones of the two trees. It was then found that the effects of self-pollination varied considerably (ORR-EWING, 1954) so that the cytological study had to be confined to the tree on which the effects had been the most drastic. This tree is henceforth referred to as tree 1.

The first sections of self-pollinated ovules were made to determine whether there was any inhibition of either pollen germination or of its subsequent development. Pollen was considered to have germinated when the outer exine layer had ruptured and tube growth had commenced; the actual stages of development after germination are recorded in Table 1. The results conclusively showed that

Table 1. — Pollen development after self and cross-pollination. Tree 1

Date of cone removal	Type of pollination	Days after pollination	Ovules sectioned	No. of pollen grains	No. of germinated pollen grains	Generative and tube cell stage	Body and stalk cell stage	Division of body cell stage
14/4	Self	12	15	37	36	17	19	0
"	Cross	9	10	63	46	19	27	0
19/4	Self	17	2	9	9	2	7	0
26/4	Self	26	15	84	76	4	72	0
"	Cross	21	13	30	28	8	20	0
1/5	Self	27	13	76	73	0	70	3

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no inhibition was present and that development was comparable with that observed within cross- pollinated ovules removed at approximately the same dates. Pollen tube development after self-pollination is illustrated in Figures 1 and 2.

Sections were next made to determine whether actual fusion of the male and femal gametes at syngamy was in any way inhibited. The number of ovules in which fertilization was taking place and the stages of fertilization within the archegonia are shown in Table 2. It is evident that there was no inhibition of either syngamy or later development after self-pollination and this is also illustrated in Figures 3, 4. and 5.

Table 2. — *Development in archegonia after self and cross-pollination.* Tree 1

Date of cone removal	Type of pollination	Days after pollination	Ovules sectioned	Fertilized ovules	No. of archegonia with fertilization stages	Sperm nuclei present	Syngamy	First and second zygotic divisions	Proembryo development
26/5	Self	54	15	13	27	6	13	8	0
1/6	Self	58	4	3	6	0	1	0	5
„	Cross	55	11	3	8	5	1	1	1

These previous results indicated that the inhibition of normal seed development in tree 1 must occur after fertilization at some time during development of the embryo. Sections, therefore, were made from self-pollinated ovules which had been removed at regular intervals after fertilization. At each of these collections the number of embryos in each ovule and the number of nuclei in the terminal embryo were recorded. The number of nuclei used was that obtained by counting those visible in the embryo in one focal plane and not to the total number contained by the embryo. The terminal embryo was considered to be the dominant one in each ovule; it is usually the largest as it has the advantage of being at the apex of the corrosion cavity whereas the other embryos are forced to develop behind it. Buchholz (1918) has reported that in *Pinus*, the terminal embryo was the successful one in the struggle for supremacy among the embryos. At each collection date the condition of the terminal embryo was carefully checked for any signs of collapse. It was fully realized that killing and fixing could produce artifacts and that embedding and sectioning might damage the young embryos to the extent that the material might be misinterpreted. The procedure throughout the study, therefore, was to classify embryos as "collapsed" only when this condition was quite obvious. The results of the study, together with the range of nuclei number present in the sound terminal embryos, are summarized in Table 3 and the details are as follows.

The first sections were made from the ovules of a self-pollinated cone removed two weeks after syngamy had been first observed. All ten ovules had been fertilized and eight of these contained vigorous young embryos, one of which is illustrated in Figure 6. It is evident that considerable variation in development within the ovules from the same cone must occur in *Pseudotsuga menziesii* as syngamy was observed in the archegonia of four ovules in which embryos were also present.

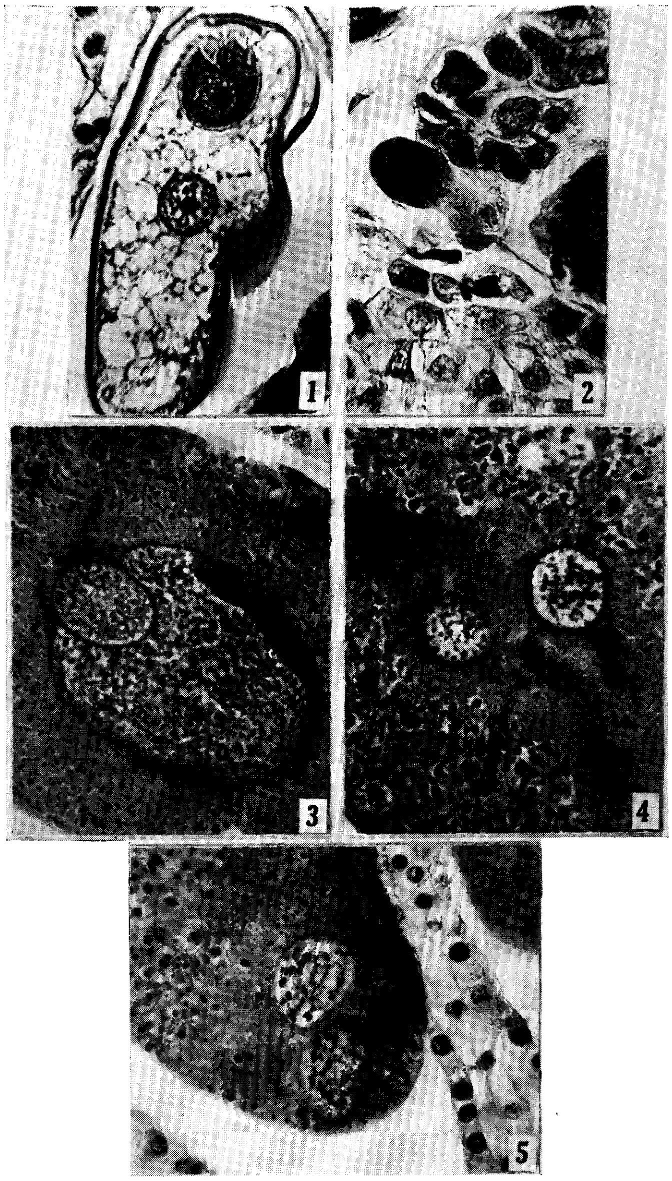

Fig. 1—5. — (1) Pollen tube development 17 days after self-pollination. Note the tube, generative, stalk and prothallial cells. × 336. — (2) Pollen tube penetrating the nucellus 54 days after self-pollination. × 336. — (3) Fusion of the sperm and egg nuclei 54 days after self-pollination. × 244. — (4) First division of the zygote 54 days after self-pollination. × 244. — (5) Early proembryo development 50 days after self-pollination. × 244.

The first sign of embryo collapse was noticed in the next sample which was removed on June 16th. Embryo collapse in its early stages is illustrated in Figure 7 and was characterized by cell shrinkage, excessive vacuolization and very dense staining with haematoxylin, the entire cell contents being commonly stained. The terminal embryos in the remaining ovules were small and cell division was observed in only one of them.

Collapse of the terminal embryos became more pronounced with each successive sample and careful examination of the ovules showed that this collapse was general, there being no cases of a second embryo taking the place of the collapsed terminal. In the sample removed on June 21st, seven of the ovules contained collapsed embryos while only limited cell division was observed in the terminal embryos of the remainder. The cells of the gametophyte at this date showed evidence of becoming filled with densely staining material. These cells were perfectly normal in appearance regardless of the condition of the embryo.

A direct comparison of embryo development after both self- and cross-pollination was made from the samples removed on June 28th. Only three of the self-pollinated ovules contained normal embryos whereas no embryo collapse was observed in the cross-pollinated samples. The striking difference in the relative vigour of these embryos is illustrated in Figures 8 and 9 and it can be seen from Table 3 that the range of nuclei number of the embryos from cross-pollination was considerably higher. Some of the terminal embryos resulting from self-pollination were already in the late stage of collapse at this date; this was characterized by the complete collapse of the cell walls and erratic nuclear staining as is illustrated in Figure 10.

The completeness of embryo collapse after self-pollination in tree 1 was demonstrated by the sample removed on July 7 th, when collapsed embryos were observed in all of the twenty-four ovules. Figure 11 illustrates a typical ovule with three collapsed embryos which, however, are surrounded by healthy gametophyte tissue, the cells of which are filled with dark staining material.

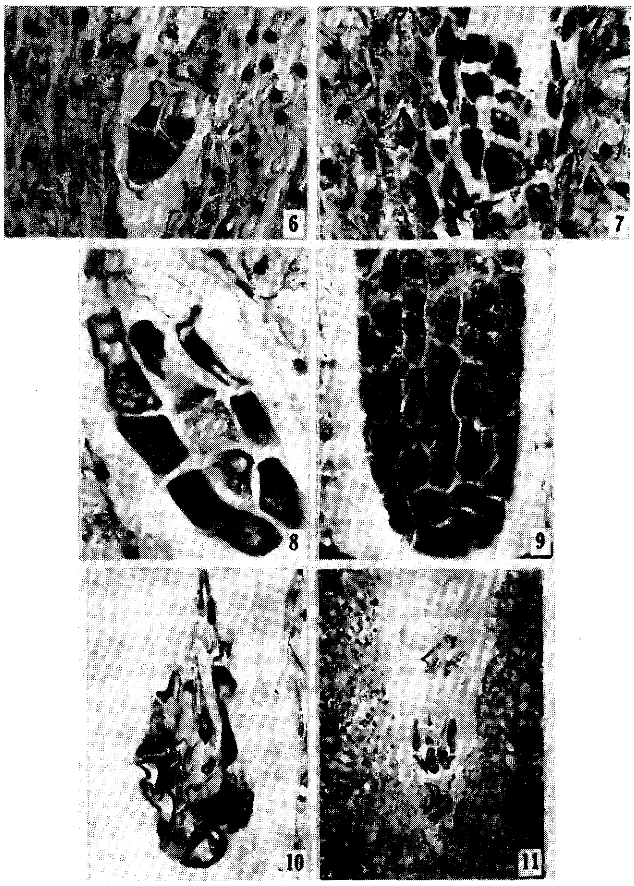

Fig. 6—11. — (6) Terminal embryo 68 days after self-pollination. × 187. — (7) Terminal embryo collapsing 76 days after self-pollination. × 187. — (8) Terminal embryo collapsing 87 days after self-pollination. × 187. — (9) Terminal embryo 84 days after cross-pollination. × 187. — (10) Collapsed terminal embryo 87 days after self-pollination. × 187. — (11) Collapsed embryos 96 days after self-pollination. × 49.

In the remaining sample removed on July 13th, the cells of the gametophyte had begun to break down and the collapsed embryos barely stained as illustrated in Figure 12. One ovule, however, contained a well developed embryo which was definitely past the stage where embryo collapse usually ocurred; it is illustrated in Figure 13. There can be little doubt, nevertheless, that very few embryos developed to this stage as only two viable seeds were later obtained from thirty-five self-pollinated cones of this tree. There was no inhibition of fertilization or early embryo development, however, as no less than two hundred and thirty-five embryos were counted in the ninety-two fertilized ovules which were sectioned.

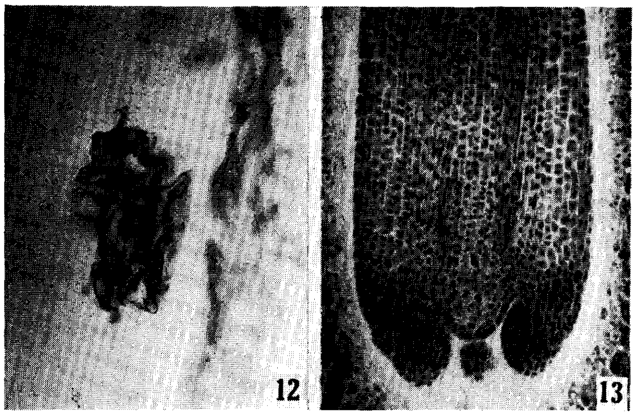

Fig. 12—13. — (12) Collapsed terminal embryo 100 days after self-pollination. × 244. — (13) Terminal embryo 100 days after self-pollination. × 66.

Table 3. — *Summary of ovule examination after self and cross-pollination. Tree 1*

Date of cone removal	Type of pollination	Days after pollination	Ovules sectioned	Unfertilized ovules	Ovules with sound terminal embryos	Range of nuclei no. of sound terminal embryos	Ovules with collapsed terminal embryos	Total number of embryos counted
9/6	Self	68	10	0	8	2—4	0	15
16/6	Self	76	8	2	4	7—9	2	18
21/6	Self	80	31	6	18	4—34	7	67
28/6	Self	87	21	0	3	11—29	18	58
„	Cross	84	14	8	6	13—82	0	14
7/7	Self	96	26	2	0	0	24	63
13/7	Self	100	6	0	1	1)	5	14

1) Nuclei too numerous to count.

Results of the 1954 controlled pollinations

The controlled pollinations conducted in 1952 had shown that the effects of self-pollination on the two trees varied considerably so that it was considered desirable to investigate this variation further. Accordingly in 1954, self- and cross-pollinations were made on three trees near Victoria and on another three farther north at Lake Cowichan, both localities being on Vancouver Island. The effects of self-pollination on these six trees would normally not have been known until germination tests had been conducted on the seed from the remaining cones. It was found, however, that, with removal of the integuments prior to killing and fixing, the condition of the ovules in the central productive region of the cone was a very useful guide to the effects of self-pollination on the six trees.

There are two unproductive regions near the tip and base of the cones of *Pseudotsuga* and the ovules in this region do not develop past the rudimentary stage. Such ovules were discounted as their condition was not related to the effects of pollination. The condition of the ovules in the central productive region, however, received particular attention. The ovule is normally firm and white in colour; but towards the end of June and beginning of July, some of the ovules became limp and discoloured, finally shrivelling up completely. Lyons (1956) has used the term "aborted" for such ovules in *Pinus resinosa* and this term will be used to distinguish between ovule and embryo collapse. Figure 14 illustates a normal ovule and aborted ovules. Ovule abortion can be caused either by lack of fertilization or by the prior collapse of the embryo, as it was found that an ovule will not persist to maturity if no embryo is present. The numbers of normal and aborted ovules were, therefore, recorded for each tree as soon as the first signs of abortion were observed and it was soon apparent that there was considerable variation among the six trees. Ovule abortion after the self-pollination of three of these trees was much more pronounced than in the remainder and there was little doubt that this must have been caused by the prior collapse of the embryo. Self-pollination resulted in complete ovule abortion for one of the three trees and it was, therefore, selected for the cytological study. The course of ovule abortion for this tree, henceforth referred to as tree 4, is shown in Table 4. It is also evident from this table that abortion after self-pollination was more gradual than that occurring in the ovules from cones which had not been pollinated.

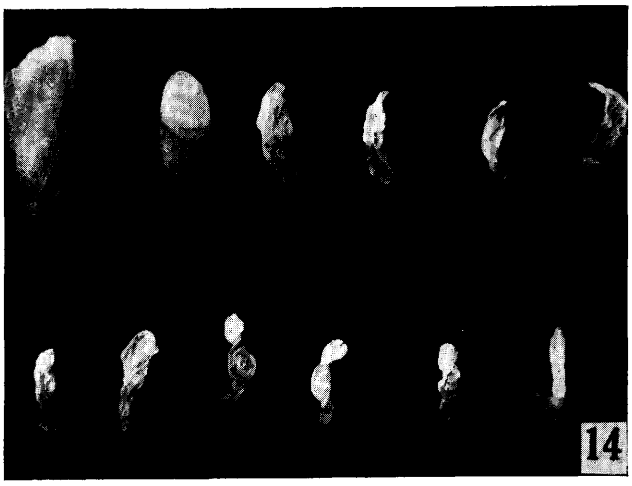

Fig. 14. — Normal ovule compared with aborted ovules. The seed on the left is approximately three times natural size.

The cytological study with tree 4 followed the same course as with tree 1 and it will be summarized briefly to avoid repetition. It was again found that there was no inhibition of either syngamy or early development of the embryos after self-pollination. The embryos, however, almost all collapsed before they had reached any size. The complete results of this study are shown in Table 5 and provide a significant comparison between the respective development of embryos from self- and cross-pollination. This is strikingly illustrated by Figures 15 and 16. It was again apparent that there was no inhibition of the early development of the embryos after self-pollination as the seventy-five fertilized ovules which were sectioned contained two hundred and thirty-one embryos. A *test of Chi square*, moreover, showed that there was no significant difference between this number and the number of embryos produced after cross-pollination.

Table 4. — *Comparative ovule abortion. Tree 4*

Date of cone removal	Type of pollination	Days after pollination	Normal ovules	Aborted ovules
23/6	Self	68	No abortion[2]	
"	Cross	"	"	
"	None[1]		"	
28/6	Self	71	37	5
"	Cross	"	No abortion	
"	None		"	
2/7	Self	75	35	8
"	Cross	"	46	3
"	None		No abortion	
7/7	Self	78	3	19
"	Cross	"	18	16
"	None		0	39
14/7	Self	85	0	37
"	Cross	"	24	17
"	None		0	41
21/7	Self	92	0	51
"	Cross	"	31	14
"	None		0	29

[1] Cones isolated and not pollinated.

[2] Number of ovules not counted.

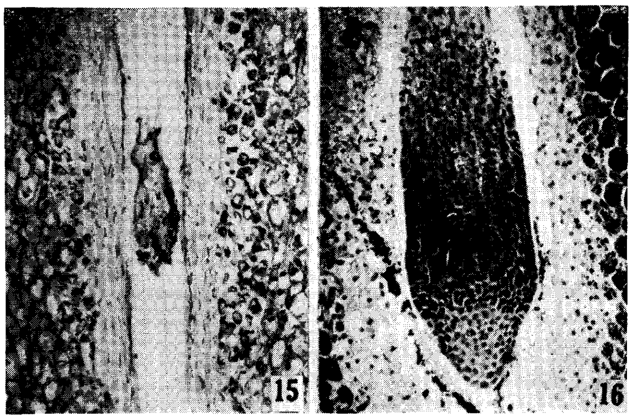

Fig. 15—16. — (15) Collapsed terminal embryo 81 days after self-pollination. Tree 4. × 66. — (16) Terminal embryo 82 days after cross-pollination. Tree 4. × 66.

Table 5. — *Summary of ovule examination after self and cross-pollination. Tree 4*

Date of cone removal	Type of pollination	Days after pollination	Ovules sectioned	Unfertilized ovules	Ovules with sound terminal embryos	Range of nuclei no. of sound terminal embryos	Ovules with collapsed terminal embryos	Total no. of embryos counted
16/6	Self	59	15	2	10	2—4	0	30
20/6	Self	63	10	7	3	7—11	0	6
„	Cross	65	15	1	14	2—6	0	46
23/6	Self	68	14	3	8	6—11	3	32
„	Cross	68	15	2	12	5—17	1	47
28/6	Self	71	15	5	4	8—16	6	28
„	Cross	71	21	2	13	15—46	6	67
2/7	Self	75	34	1	28	7—64	5	122
„	Cross	75	31	3	28	20—138	0	116
8/7	Self	80	3	0	0	0	3	7
„	Cross	75	16	1	15	30—128	0	45
14/7	Cross	82	5	0	5	—1)	0	20
21/7	Self	105	2	0	0	0	2	6
	Cross	89	3	0	3	—1)	0	7

1) Nuclei to numerous to count.

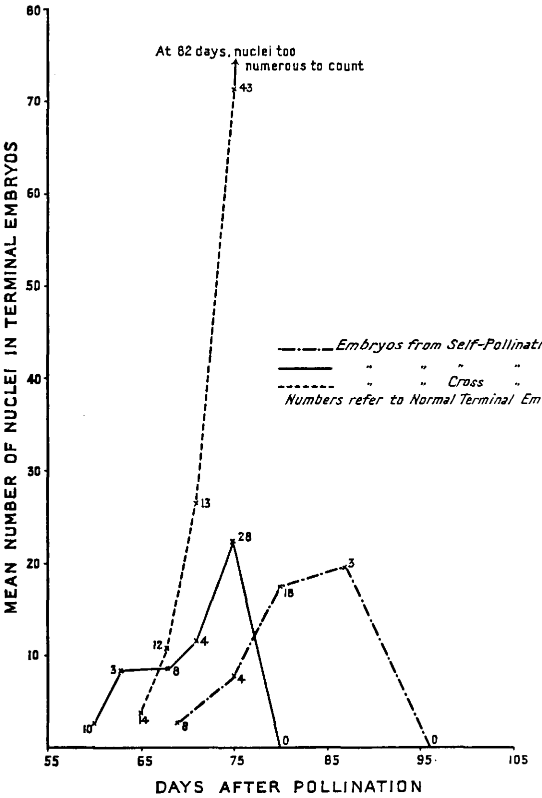

Fig. 17. — Comparative embryo growth following the self and cross-pollination of trees 1 and 4.

Conclusions from the cytological studies

There is little doubt that embryo collapse after self-pollination was common to both trees 1 and 4. It can be concluded that it was also responsible for the low yields of viable seed of two of the other trees pollinated in 1954 as was apparent from a few sections of their ovules. The difference in vigour between embryos from self- and cross-pollination was apparent at an early stage and was very striking. This is illustrated in Figure 17, the points on this graph being based on the mean number of nuclei of the terminal embryos which were visible in one focal plane.

It will be noted from both Tables 3 and 5 that not all the sectioned ovules contained embryos and it was somewhat surprising to find that such unfertilized ovules did not abort until some weeks after the date that fertilization would normally have occurred. One unfertilized ovule, which was removed more than two weeks after syngamy was first observed in other ovules, is shown in Figure 18. The archegonium had finally broken down and its former position was marked by a dark staining and shrunken mass but the nuclei of the gametophyte still stained sharply and considerable cell division was observed. Figure 19 shows another unfertilized ovule which was removed nearly six weeks after syngamy was first observed in other ovules. The corrosion cavity is clearly visible and Buchholz (1918) has reported similar findings in *Pinus* where the gametophytes had well developed corrosion cavities without any trace of embryos. Buchholz considered that the archegonia must secrete the digestive enzyme even though the eggs had not been fertilized. Schopf (1943) has also reported the occurrence of such corrosion cavities in *Larix* and has noted that the gametophyte tissue seemed to break down at the same time whether or not embryos were present. Schopf added that the breakdown of tissue left a residue in the corrosion cavities of these unfertilized ovules. A similar residue was observed in the unfertilized ovules of *Pseudotsuga.* It is clearly visible in the corrosion cavities in Figures 18 and 19 and was one means of distinguishing between fertilized and unfertilized ovules. The more significant difference between such ovules was that in the former, stainable material began to accumulate in the cells of the gametophyte a few weeks after syngamy had occurred. No such accumulation, however, was present in the gametophyte cells of unfertilized ovules and this is quite evident in a comparison of Figures 11 and 19. There seems

Fig. 18—19. — (18) Unfertilized ovule with remains of archegonium. × 244. — (19) Unfertilized ovule with residue in the corrosion cavity. × 244.

little doubt that fertilization must stimulate the gametophyte in some way in order that an adequate supply of food material is available for the embryo. It is possible that the embryo itself is the centre of this stimulus as the gametophyte cells were only filled with this dark staining material when embryos were present. PLYM FORSHELL (1953), on the other hand, has reported that in *Pinus sylvestris* neither the integument nor the female gametophyte can develop without the stimulus of pollen tubes. Such is definitely not the case with *Pseudotsuga* and it is strange that these two species should differ so widely in this respect.

Discussion

The results of the cytological studies with *Pseudotsuga menziesii* raise the question as to whether embryo collapse is part of a genetic self-incompatibility system or from some other factor. The conclusions of such investigators as EAST (1929), MATHER (1943), LEWIS (1944) and BATEMAN (1952) definitely suggest that embryo collapse is not part of a genic incompatibility system since fertilization is allowed to occur. It will be recalled, however, that BATEMAN (1952) had pointed out that there was no reason why a true self-incompatibility system should not be present in the gymnosperms. PLYM FORSHELL (1953), moreover, has claimed that fusion of the sex cells in certain ovules was inhibited after the self-pollination of *Pinus sylvestris*. Her conclusions have been carefully considered together with those of BRANSCHEIDT (1939) to whose previous work with *Taxus baccata* she has made frequent reference. Neither of these studies, however, are supported by any cytological evidence of the occurrence of an inhibiting substance. A more recent study with *Pinus sylvestris* by EHRENBERG et al. (1956), moreover, indicates that embryo collapse is the probable cause for the reduced seed yields after self-pollination.

It can be concluded at the present time, therefore, that embryo collapse is the most likely explanation for the reduced yields of viable seed reported after the self-pollination of many of the gymnosperms. Embryo collapse is probably an inbreeding effect caused by the increased homozygosity of recessive deleterious genes. DOBZHANSKY (1952) has effectively demonstrated that in time any normal breeding population will build up a mass of deleterious recessive genes by mutation. Many of these genes are lethal or semi-lethal but they are sheltered from selection pressure by the normal dominant alleles in a heterozygous organism. It is logical to assume that there would be such genes in a normally outbreeding species as *Pseudotsuga menziesii* and that they would be fully capable of destroying the embryo when homozygous. Their number, however, would certainly vary from tree to tree which could explain why the effects of self-pollination in terms of seed yield were found to be so variable in the eight trees. One of these trees, for example, produced more viable seed after self-pollination than was obtained after cross-pollination and it is significant that PLYM FORSHELL (1953) has also reported a similar finding with *Pinus sylvestris*.

The precise factors which cause embryo collapse in *Pseudotsuga* require much further investigation but it is evident that any drastic change such as the inbreeding of a normally outbreeding species could very readily upset the orderly sequence of development in the young embryo. BRINK (1952), for example, has pointed out that the early embryo in the angiosperms was dependent upon the endosperm for certain metabolites which, initially, it was quite incapable of synthesizing. The embryo later became progressively less dependent by acquiring for itself the synthetic qualities previously limited to the nurse tissue. Embryo collapse in *Pseudotsuga* would appear to be from some failure in this vital early relationship with the gametophyte tissue. The embryos regularly collapsed at an early stage of development when they were presumably still dependent upon the gametophyte. Collapse, moreover, invariably occurred in ovules whose gametophyte cells were full of dark staining material which was apparently of little value to the embryo. Embryos from cross-pollination, however, were able to develop vigorously under what appeared to be comparable conditions in the gametophyte.

It is evident that the low yields of viable seed frequently obtained after the self-pollination of *Pseudotsuga menziesii* result from the effects of inbreeding upon the genetic constitution of the embryos. This explanation can probably be extended to similar examples in other gymnosperms. It is, therefore, considered that the term self-incompatible is not applicable until definite proof of the existence of an incompatibility system in the gymnosperms has been presented.

Summary

Extensive investigations have shown that self-fertilization in many of the angiosperms is prevented by genic incompatibility systems. Past investigations have also shown that some of the gymnosperms produced little or no viable seed after self-pollination but the causes of this seed failure have not been investigated. A cytological study of *Pseudotsuga menziesii* was, therefore, made to determine whether or not a self-incompatibility system was responsible for the low yields of viable seed which had been reported after the self-pollination of this species. Controlled self- and cross-pollinations were made on eight trees growing in three separate localities. Cone samples were removed from each tree at regular intervals and the ovules embedded for later sectioning. The remaining cones from the eight trees were collected at maturity and the results of germination tests on the seed showed that the effects of self-pollination varied greatly from tree to tree. A detailed cytological study was made of the ovules of two of the trees on which the effects of self-pollination had been the most drastic. It was found that neither pollen germination nor its subsequent development were in any way inhibited after self-pollination and that syngamy and proembryo formation proceeded normally. The embryos, however, almost all collapsed at an early stage of development; this appeared to be caused by some failure in the vital relationship between young embryos and their surrounding gametophytes. It is concluded that embryo collapse is an inbreeding effect caused by the increased homozygosity of recessive deleterious genes. This explanation could account for the variable effects of self-pollination on different trees as the number of such genes would not be constant.

Zusammenfassung

Titel: *Eine zytologische Untersuchung über die Wirkung der Selbstbestäubung bei Pseudotsuga menziesii* (MIRB.) FRANCO. —

Bei vielen Angiospermen konnte festgestellt werden, daß eine genetisch bedingte Selbstunverträglichkeit die

Selbstbefruchtung verhindert. Auch bei den Gymnospermen ist schon bekannt, daß nach Selbstbestäubung nur wenige oder gar keine lebensfähigen Samen gebildet werden. Hierfür wurden aber die Gründe noch keiner näheren Untersuchung unterzogen. Bei *Pseudotsuga menziesii* wurden deshalb zytologische Untersuchungen durchgeführt mit dem Ziel, festzustellen, ob ein System der Selbstunverträglichkeit für den geringen Ertrag keimfähiger Samen nach Selbstbestäubung verantwortlich zu machen ist oder nicht. Hierzu wurden bei 8 Bäumen an 3 verschiedenen Orten kontrollierte Selbst- und Kreuzbestäubungen durchgeführt. Danach wurden in regelmäßigen Abständen von jedem Baum Zapfen entnommen und die Samenanlagen für spätere Schnitte eingebettet. Die restlichen Zapfen der 8 Bäume wurden reif geerntet. Wie die Keimproben der Samen ergaben, ist die Wirkung der Selbstbestäubung verschieden. An 2 Bäumen, an denen sie am ausgeprägtesten war, wurden ins einzelne gehende zytologische Untersuchungen vorgenommen, die ergaben, daß nach Selbstbestäubung weder die Pollenkeimung noch die anschließende Entwicklung beeinflußt wurden. Sowohl die Kernverschmelzung wie die Entwicklung des Proembryos waren normal. Die Embryonen jedoch kollabierten in einem sehr frühen Stadium. Dies wird auf eine Unstimmigkeit zwischen der Lebensfähigkeit des Embryos und des ihn umgebenden Gametophyten zurückgeführt. Es wird daraus geschlossen, daß das Kollabieren des Embryos ein Inzuchteffekt ist, der durch die verstärkte Homozygotie rezessiver ungünstiger Gene verursacht wird. Da die Anzahl solcher Gene bei den einzelnen Bäumen verschieden ist, würde diese Annahme auch die unterschiedliche Wirkung der Selbstbestäubung bei verschiedenen Bäumen erklären.

Résumé

Titre de l'article: *Etude cytologique des effets de l'autofécondation chez Pseudotsuga menzies*ii (Mirb.) Franco. —

De nombreuses expériences ont montré que chez plusieurs espèces d'angiospermes, une incompatibilité d'ordre génétique s'oppose à l'autofécondation. Les recherches antérieures ont également montré que certains gymnospermes ne produisent, par autofécondation, que très peu, ou même pas du tout de graines vivantes, mais les causes de cet échec n'ont pas été mises en évidence. L'étude cytologique sur *Pseudotsuga menziesii* fut entreprise dans le but de déterminer si un système d'auto-incompatibilité est ou non responsable de la faible quantité de graines viables obtenues après autofécondation de cette espèce. Des croisements et des autofécondations contrôlées furent faites sur 8 arbres poussant dans 3 stations différentes. Des cones furent prélevés à intervalles réguliers, et les ovules mises en inclusion en vue de faire des coupes. Les cones restants furent récoltés à maturité, et les résultats des essais de germination des graines ont montré que les effets de l'autofécondation varient beaucoup d'un arbre à l'autre. Une étude cytologique détaillée fut faite sur les ovules de deux des arbres pour lesquels l'autofécondation avait eu les effets les plus marqués; cette étude permit de voir que ni la germination du pollen, ni le développement du tube pollinique n'étaient affectés par l'autofécondation, et que la fécondation et la formation des préembryons étaient normales. Mais les embryons mouraient presque tous dès les premiers stades de leur croissance, ce qui semble dû à une perturbation dans les relations entre les jeunes embryons et le gamétophyte qui les entoure. On peut conclure que la mort des embryons est un effet de consanguinité (inbreeding) dû à l'augmentation des gènes létaux récessifs sous forme homozygote. Cette explication peut rendre compte de la variation des effets de l'autofécondation suivant les individus, puisque ces gènes ne sont pas en nombre constant.

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