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PRELIMINARY ACCOUNT OF THE OVULE, GAMETOPHYTES, AND EMBRYO OF *WIDDRINGTONIA CUPRESSOIDES*

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(WITH PLATE XI AND THREE FIGURES)

INTRODUCTION

The genus Widdringtonia contains about six species of trees in equatorial and south Africa and Madagascar. A plant from tropical Africa figured by GOEBEL (**16**) and by EICHLER in ENGLER and PRANTL (**12**) as *Callitris quadrivalvis* is probably also very closely related to Widdringtonia, but differs in the much smaller number of ovules and also to some extent in the foliage, and was separated from that genus by MASTERS (**26**) as *Tetraclinis articulata*. I have not had an opportunity to examine this plant.

The genus Callitris has unfortunately been repeatedly confused with Widdringtonia, and the species of the latter are included under the former genus by BENTHAM and HOOKER (**2**), EICHLER (**12**), JACKSON (**17**), BOLUS and WOLLEY-DOD (**3**), and MARLOTH (**25**). Widdringtonia was clearly distinguished as a genus, however, by ENDLICHER (**13**), and is recognized by such authorities as MASTERS (**26, 27**) and RENDLE (**30**), as well as by SIM (**34**) and MAIDEN (**24**), the chief authorities on the forest floras of South Africa and Australia respectively. The two genera differ widely in cones and foliage, and while Widdringtonia (excluding fossil species) is restricted to south and central Africa and Madagascar, Callitris is as rigidly restricted to the Australian region. I have been fortunate in having a good opportunity to study several species of both genera growing in the Government Plantations at Tokai, the majority of the trees

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coning freely, and am confident that no one who took advantage of a similar opportunity would still wish to unite the two genera.

Only two species of conifers occur native in the Cape Peninsula, and both are confined to the tops and upper slopes of the mountains; these are *Podocarpus Thunbergii* Hook. and *Widdringtonia cupressoides* Endl. (3). The development of Podocarpus is fairly well known, having been studied in other species (COKER 6, BURLINGAME 4), but that of Widdringtonia (excluding Tetraclinis) is entirely unknown so far as I am aware.

In attempting to work out the life-history the chief difficulty has been the collection of the material. The plant occurs only at an altitude of about 2000 feet and upward, and it has always required at least four or five hours to obtain a single collection. Furthermore, a considerable portion of the cones contain only abortive ovules, especially in certain localities, and this has often made the collection of even a small number of ovules very tedious. As a consequence it has only been possible to make collections at rather long intervals, and these have each only included a small number of ovules, particularly in the later stages. As these difficulties will again be encountered in trying to fill in the gaps in the present account, it is thought best to publish the results now presented. Here and there comparisons are made with Callitris, the development of which is very similar. I hope later to be able to study this genus more in detail.

I am glad to take this opportunity of thanking the authorities at Tokai for permission to collect cones of all species of Widdringtonia and Callitris grown in the Government Plantations, and also my friend Mr. E. P. PHILLIPS, for kindly collecting and fixing material on three occasions.

METHODS

The material has in almost all cases been fixed in the field, and all figures are drawn from such material. Various fixing agents have been tried, including different strengths of chromacetic acid with and without osmic acid, but the following has been found the most generally useful (CHAMBERLAIN 5, p. 20): picric acid (saturated solution in 50 per cent. alcohol), 100^{cc}; glacial acetic acid, 5^{cc};

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corrosive sublimate, 5^{gm}.[1] Material was fixed for 24 hours and then washed in 50 per cent. alcohol until the alcohol was no longer colored yellow (a week or more as a rule), then 12–24 hours in 75 per cent., 85 per cent., and 94 per cent. alcohol, and at least 60 hours in absolute alcohol changed three or more times. Both xylol and cedar oil (in 25 per cent. grades with alcohol) were tried to precede infiltration with paraffin, but the former was entirely discarded after a few trials. Material was taken from cedar oil through 25 per cent., 50 per cent., and 75 per cent. solutions of soft paraffin in cedar oil, to pure paraffin, melting point 48° C., using tiny wire-gauze baskets, for the purpose, as described by FERGUSON (**14**), and allowing 48 hours in each solution. At least 48 hours longer was allowed in two fresh lots of soft paraffin (an indication of the time required here is given by the time taken to wash out the fixing agent), 12–24 hours in a mixture of soft and hard paraffin, and finally 24 hours in hard paraffin, melting point 55° C., in which it was imbedded. These very long periods in the oven were found to be absolutely necessary to insure proper infiltration of the paraffin.

In young stages the ovules were fixed whole, or when very young a small part of the tissue of the cone was cut off bearing the ovules. Later the integument becomes too hard to cut, and it was necessary to dissect out the nucellus, a process which can be carried out fairly easily in practice. In some stages after fertilization the prothallus was dissected out, and where nearly mature embryos were present these were fixed alone. The staminate cones were fixed whole, being very small, but required even longer periods in the oven than those mentioned above.

The stains were (1) Delafield's hematoxylin diluted 8–10 times, allowed to stain 4–12 hours, extracted with very dilute acid (aqueous), and followed by a thorough washing in water; and (2) Flemming's triple stain. The best results were obtained with the former, especially after the fixing agent mentioned above. It appears to be always the case that the very best staining effects of Delafield's hematoxylin are obtained after a fixing agent containing corrosive sublimate. The value of this hematoxylin as a nuclear stain has not at present been realized.

[1] I am indebted to Mr. A. J. BALLANTINE for suggesting the use of this reagent.

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DESCRIPTION

The youngest ovulate cones seen were about 3 or 4^{mm} across, when the two decussate pairs of scales were widely spreading. The ovules are generally 20–30 in number and appear to be evenly distributed over the broadened end of the axis. The subsequent development proves this position to be only apparent, and that they are actually situated on the fused bases of the scales. By considerable growth of the basal part of the scales the ovules are carried farther apart, and after the former have grown together the latter are found on the sides of the ridges where the lower and upper scales meet.

The youngest ovule found is shown in *fig. 1*. The comparatively long tubular micropyle is very noticeable even at this early stage; the upper cells are already dead and empty and growth of the integument proceeds only at its base. The layer of small cells with dense contents lining the basal part of the micropyle grow actively some time after pollination and narrow considerably the micropylar opening at this part. Apparently, however, the micropyle is never completely closed by this means, but only by the accumulation of dust, etc., at its apex. The integument so soon becomes too hard to section satisfactorily that it is impossible to speak with confidence on this point.

The staminate cones are mature at about the same time that the ovules are found in this condition. They are very small and so inconspicuous until they change color at or near maturity that I have never succeeded in collecting immature cones with a view to following the development of the microspores. The sporophylls are peltate and somewhat pointed toward the apex of the cone and bear five pollen sacs abaxially placed on the stalk, the wall of the microsporangia being only one cell thick (*fig. 2*). The pollen grains have an exceptionally thick cell wall, and only a single nucleus can be distinguished in the mature grain, and in early stages of germination (*figs. 3, 4*). It seems usual in the Cupressineae for no prothallial cell to be formed in the male gametophyte, COKER (**8**) having noted their absence in eight or nine genera, including Callitris. It is of course impossible to assert their absence in Widdringtonia without obtaining preparations of microspores of various ages, but it may be assumed that none is present. As a large number of pollen grains have been

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examined in the stages here figured, it seems scarcely possible that a second nucleus can be present, but it is unusual for the division giving rise to the generative and tube nuclei to be delayed so long. COKER (**8**) reports that this division occurs after shedding in certain cases, but says that it occurs before shedding in *Callitris* sp. As will be seen, however, the whole history of the male gametophyte shows a minimum of nuclear activity.

The pollen grain has a fairly thin cellulose endospore and a very thick and rather hard exospore (*fig. 4*), and on germination (as shown in the figure) the exospore alone begins to grow, without any immediate growth of the endospore or any trace of tube formation. The number of pollen grains which begin to germinate in each ovule varies from one to four, three being the most usual number (*fig. 3*). The ovule here figured closely resembles that of *fig. 1*, and therefore only a few of the cells have been drawn.

No trace of a megaspore mother cell or cells can be seen in ovules of this age, and stages to illustrate megasporogenesis are unfortunately wanting at present. GOEBEL (**16**) figures an ovule of Tetraclinis (*Callitris quadrivalvis*) in which a considerable number of what are probably megaspore mother cells are figured. Several ovules considerably older than that just mentioned show the nucellus clearly differentiated into peripheral and central regions. This central region and the innermost cells of the peripheral region are represented in *fig. 5*. The cells of the central part are somewhat larger and are characterized by having only a very scanty supply of cytoplasm. Probably these cells are a large group of megaspores, one of which later grows to form the prothallus. In this case GOEBEL'S figure of Tetraclinis would doubtless also serve to illustrate the corresponding structure in Widdringtonia.

It is certain in any case that normally only one megaspore develops, since no "secondary prothalli" have been found, as described by LAWSON (**20**) in *Sequoia sempervirens*. An ovule of *Callitris verrucosa*, however, has been sectioned, which contained two secondary prothalli (*text fig. 1*). In the next stage figured the very large prothallus is already formed. The embryo sac is lined with cytoplasm containing a single layer of free nuclei and bounding one large central vacuole. *Fig. 6* is a sketch of a whole ovule in

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optical section at this stage, showing the winged integument (the wings are only one cell thick), nucellus, and prothallus. The long tubular micropyle is now very noticeable. The ovules are nearly all curved like this one, so that there is only one plane of symmetry in the ovule. Occasionally a second curvature is found at right angles to this one, in which case longitudinal sections are apt to be puzzling unless a whole series is carefully studied. Cones containing ovules of about the age shown in *fig. 6* are growing on the middle branch in the accompanying photograph (*text fig. 2*). Those on the right have just been pollinated and those on the left have young embryos, all being gathered February 28, 1909.

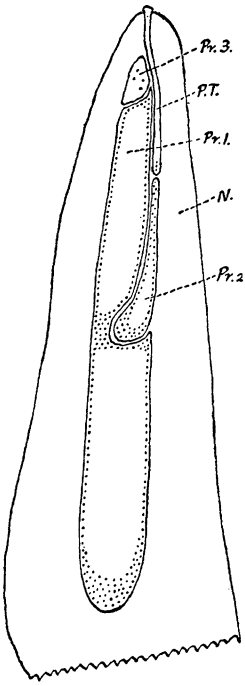


FIG. 1.—Median longitudinal section of nucellus, *n*, of *Callitris verrucosa: pt*, pollen tube; *pr* , primary prothallus; *pr*², *pr*3, secondary prothalli. ×40.

Fig. 7 shows the apex of a nucellus about the same age as that in *fig. 6*. Both preparations indicate that only a single pollen tube is present and a case is only rarely met with in which more than one pollen tube develops; though, as seen above, three usually begin to germinate. Only two very small nuclei are found in the pollen tube of this age, no doubt the tube and generative nuclei.

Fig. 8 represents a very small part of the prothallus with its lining layer of cytoplasm and two of the free nuclei. In some preparations, such as the one figured, the nuclei can be seen to be paired, probably indicating that a simultaneous division has recently taken place. None of these divisions have been seen, but if, as is probable, they occur always very rapidly and simultaneously there might be only about ten separate periods of an hour or two each, extending over three months or more, during which the requisite stages could be obtained. It may also be that the time of day or night at which these divisions occur is always about the same, though there is no evidence that this is so with other divisions; at any rate

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in the sporophyte, collections at 9 A. M., 12 noon, and 6 or 7 P. M. show an approximately similar number of nuclear figures.

It is noticeable in *fig. 8* that the nuclei project slightly into the vacuole. Later on the layer of cytoplasm thickens considerably and the nuclei are then completely sunk in it. *Figs. 9* and *10* indicate the principal changes met with after the last stage figured. *Fig. 9* shows clearly the process of wall formation in the prothallus,



FIG. 2.—*Widdringtonia cupressoides* (see text). ×½.

alveoli being organized as described by SOKOLOWA (**35**) and other writers for various gymnosperms. The cells at first formed are invariably uninucleate, and the original cell walls persist. The binucleate and multinucleate condition met with later (see below) does not arise, therefore, in the same way as the binucleate prothallus cells of Cryptomeria described by LAWSON (**21**). The pollen tube has meanwhile reached the tip of the prothallus, and even before wall formation begins, it penetrates the megaspore membrane and grows down just inside of it, on or near the surface of the prothallus, to about one-third or one-half way down.

Fig. 10 is drawn from the same series of sections as *fig. 9* and represents the tip of the pollen tube which has in this way pene-

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trated nearly half-way down the prothallus. Three nuclei are now met with in the pollen tube, all imbedded in a rather dense mass of cytoplasm. One nucleus is considerably larger than the other and is clearly differentiated from the cytoplasm. The two smaller (stalk and the tube nuclei), on the other hand, are only with difficulty distinguished either in structure or staining capacity from the surrounding cytoplasm. The larger nucleus with its cytoplasm is evidently the body cell, and its structure is shown in detail in *fig. 11*.

Fig. 12 is a sketch of a longitudinal section of the upper half of a nucellus at a slightly later stage, when cell division in the prothallus is just completed. The tube with its conspicuous body cell can be seen in the position described above. The end of this pollen tube is shown in detail in *fig. 13;* and no nucleus except that of the body cell can now be found. A careful and repeated search in this and adjoining sections of the series (which is quite complete) failed to reveal any trace of the tube and stalk nuclei, even with an oil-immersion objective. Unfortunately the tube figured was the only one showing this stage (all others were before cell division in the prothallus was complete), and the figure will probably therefore be regarded as abnormal; but the whole structure of the ovule and pollen tube seemed perfectly normal in other respects, the fixation was entirely satisfactory, as was the staining, and there was not the least indication that any part of the tube or its contents had washed off the slide during staining, etc. The staining reactions of the tube and stalk nuclei of *fig. 10*, together with their absence in *fig. 13*, indicate that they break down completely and that their substance becomes blended with the cytoplasm.

The cytological characters of the body cell nucleus are indicated in *fig. 14*, and a comparison with the nucleus in *fig. 11* suggests that division will shortly take place, giving rise to two sperm nuclei. Up to this point no trace of archegonia or archegonium initials can be seen.

Fig. 15 represents the upper half of the prothallus (and part of the nucellus) at a considerably later stage, after the archegonia are mature and fertilization has been effected.[2] The distribution of the archegonia is very remarkable and recalls that described for *Sequoia*

[2] I was unfortunately unable to make any collections for a number of weeks at this time.

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sempervirens by ARNOLDI (**1**) and LAWSON (**20**). Their distribution is evidently determined largely by the position of the pollen tube. Thirty-eight archegonia are shown in this figure, but only those are indicated parts of which at least could be seen in a single section; the total number is slightly over fifty. Of several prothalli collected on the same date, one other showed an almost identical structure, but the rest were somewhat older and the archegonia were disorganized. There was an indication, however, that the number of archegonia in these other prothalli was considerably less, though their distribution must have been essentially similar.

The lowest group of archegonia from *fig. 15* has been drawn on a larger scale in *fig. 16*. Of these archegonia the lowest but one and the lowest but three have evidently been fertilized, as each contains a proembryo. This fact, together with the presence of a single pollen tube, indicates that, as is usual in Cupressineae (LAWSON **23**), two sperm cells are organized and both are functional. However, only indirect evidence is available in the present case. Unfortunately the unfertilized archegonia of the group are not in very good condition, having doubtless been organized some time previously and being about to disintegrate; hence it is quite possible that the details exhibited are to some extent different from those of a recently formed archegonium.

Only the lowest archegonium of the group has any trace of what might be interpreted as neck cells, and even this cell (shaded in the figure) is probably only a prothallus cell which happens to lie immediately over the archegonium. It is quite clear that the archegonia arise from cells deep in the prothallus, and possibly this may be the reason why no neck cells are formed, though LAWSON (**20**) records them in Sequoia; but in that genus the archegonia grow in such a way as to push their necks to the surface, whereas in Widdringtonia they remain deep-seated in the prothallus. It may be that the neck cells disintegrate entirely and leave no trace, and this would be the natural explanation in the case of fertilized archegonia, as noted by KILDAHL (**18**) in connection with a similar absence of neck cells in *Phyllocladus alpinus;* there is no apparent reason, however, to suppose that the neck cells have completely disappeared in all the unfertilized archegonia, and I believe that none are ever formed.

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Scarcely any trace of jacket cells can be seen in the group of archegonia of *fig. 16*, and they are never more than very feebly organized. In this respect the condition in Cephalotaxus as described by COKER (**9**) may be compared, where jacket cells are sometimes replaced by ordinary prothallial cells; and in Torreya where COULTER and LAND (**11**) note the absence of jacket cells until after fertilization. Their absence in this genus and in Widdringtonia is probably to be correlated with the small size of the archegonia.

If the nuclear phenomena in these partially abortive archegonia are to be taken as representing normal conditions, the central nucleus divides (*fig. 16*, sixth archegonium from bottom) to form the egg and ventral nuclei (see also the top archegonium). The ventral nucleus seems usually to disappear completely, leaving only a centrally placed egg nucleus, as shown in four of the archegonia in the figure.

The lower of the two proembryos referred to above (*fig. 16*) closely resembles the proembryo of Sequoia (LAWSON **20**), but the upper shows that more cells are formed than in that genus. It is noticeable that the proembryo practically fills the archegonium in each case. The only conifers previously described in which this is the case are Torreya (COULTER and LAND **11**) and Sequoia (LAWSON **20**). This fact is likely in all three cases to be correlated with the small size of the archegonium, and is probably of no phylogenetic importance. Only one of the several cells of the proembryo forms the suspensor and one forms the embryo. The others must disintegrate rapidly, as in the next stage they are no longer recognizable (*fig. 17*).

Figs. 16, *17*, and *21* show stages in the development of the multinucleate endosperm mentioned above. Although in early stages numerous uninucleate cells may be seen, as well as a large number of binucleate cells, yet the actual origin of the binucleate condition has only been indicated by two karyokinetic figures and the remains of a spindle between the two nuclei of one cell. Sometimes two nuclei come to lie almost in contact in a cell and have thus often suggested that direct division of the nucleus has occu red, but a careful search has failed to confirm this suggestion. It seems perfectly clear, therefore, that the binucleate (and in some cells multinucleate) condition arises by karyokinetic division of the original single nucleus.

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Although in *Widdringtonia cupressoides* the evidence upon which this conclusion rests is perhaps slender, yet the same phenomenon has been seen in *W. Whytei* and in two species of Callitris. In the latter abundant evidence of the origin of the binucleate condition has been obtained in both *C. cupressiforme* and *C. Muelleri*.[3] Here a considerable number of nuclei have been found in every stage of karyokinetic division from earliest prophase to fully formed binucleate cells. The four-nucleate condition is very much less common in both Widdringtonia and Callitris, and it is therefore not surprising that a second mitotic division has not been met with, but doubtless it also occurs. A very limited number of cases is found in which five nuclei are present in a single cell.

The number of chromosomes in the division just mentioned is of course the reduced one. In Callitris both the haploid and diploid chromosomes have been approximately counted, the latter being about 24 and the former almost certainly 12 in both species mentioned. In *Widdringtonia cupressoides*, in one of the two nuclear figures mentioned above, the chromosomes were just starting to separate from the equatorial plane, and in the other they had advanced about half-way to the poles. It was possible to count the group of daughter chromosomes approximately in three out of the possible four cases, and the number was about 6 in each case. The sporophytic number has been found to be about 12 (certainly not more than 14). One dividing nucleus is figured (*fig. 22*) in which 12 chromosomes seem clearly indicated; this is from a very young embryo. It is curious in two genera so closely allied as Widdringtonia and Callitris that the number of chromosomes in the one should be approximately double that found in the other, but similar differences have been noted in even more closely related plants (GATES **15**, ROSENBERG **32, 33**). So far as I am aware, no case has previously been recorded in which a multinucleate prothallus persists in a conifer, but this is certainly the case in Widdringtonia, as *fig. 21* (from quite an old prothallus) clearly shows. An entirely binucleate prothallus is present in Cryptomeria at one stage (LAWSON **21**), but subsequent cell divisions

[3] I am not quite certain that the second species is correctly named, but both cones and foliage seem to agree very closely with description and figures given by MAIDEN (**24**).

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reestablish the uninucleate condition. Multinucleate jacket cells are reported by LAWSON (**23**) in various Cupressineae, also by COULTER and LAND (**11**) in Torreya, and by KILDAHL (**18**) in Phyllocladus. Apparently no case has previously been reported, however, in which a multinucleate prothallus persists in the conifers, but the record may easily have been overlooked by the writer, owing to a large proportion of the literature not being available to him. LAWSON (**23**), however, makes no mention of such a case. Parallel cases are reported among the Gnetales (LAND **19**, PEARSON **28**), but this in itself is probably of little importance, especially as the origin of such cells is different in these cases.

The development of the embryo shows no peculiarities and closely resembles the general sequence of events as described for other conifers, an apical cell being organized for a very short time (*fig. 18*); the presence of embryonal tubes is to be noted in *fig. 19*. In the mature embryo the cotyledons are two (very rarely three) in number and usually of the same length; sometimes, however, one cotyledon is conspicuously shorter than the other.

COMPARISON WITH WIDDRINGTONIA WHYTEI AND CALLITRIS

One collection of *W. Whytei*, growing in the Tokai plantations, was made on January 11, 1909, but did not yield results of much importance. It clearly agrees with *W. cupressoides* in the presence of laterally placed archegonia and in the multinucleate endosperm.

Single collections were also made of three species of Callitris (*C. verrucosa*, *C. cupressiforme*, and *C. Muelleri*)[4] during the second week in January. Of four ovules of the first-named species sectioned, one showed secondary prothalli (*text fig. 1*), and in each the prothalli were in the free nuclear condition and one pollen tube only was found, which had penetrated a short way down the side of the prothallus and contained three nearly equal nuclei (*text fig. 3*). The upper of the three, however, stains more sharply than the other two and suggests the same sequence of events as described above for Widdringtonia. The ovules of the other two species contained young embryos in different stages of development (similar to *figs. 17–19*). The unfertilized archegonia are much too disorganized to make out any-

[4] See footnote, p. 171.

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thing beyond the fact that they occur in a lateral group (or possibly more than one group). The formation of the multinucleate prothallial cells has already been described. Sufficient evidence has been obtained to indicate that the development in Callitris is essentially similar to that of Widdringtonia.

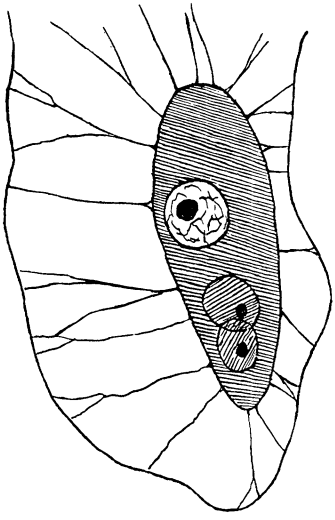


FIG. 3.—Tip of pollen tube of *Callitris verrucosa*. ×778.

DISCUSSION

With the exception of GOEBEL'S (**16**) figure of Tetraclinis (*Callitris quadrivalvis*) and COKER'S (**8**) statement about the pollen grain of Callitris, I am aware of no contribution to the knowledge of the gametophytes in the Actinostrobeae. As shown by MASTERS (**26**), there is a very close agreement between the four genera of this section of the Cupressineae in sporophyte characters, and the present investigation shows that the section is much more clearly differentiated from other Cupressineae in the gametophyte than in the sporophyte characters. Especially is this the case in the female gametophyte, where the peculiar position of the archegonia and the persistently multinucleate prothallus constitute a sharp distinction from typical Cupressineae. On the other hand, the position of the archegonia is similar to that found in Sequoia, and I hesitate to emphasize the differences in structure of the archegonia, as described above, until the development has been more closely followed. It has already been suggested that the Cupressineae have been derived from the Sequoiaceae, and it is quite possible that the Actinostrobeae constitute the connecting link between the two tribes.

It is just possible, however, that the developmental details may have a wider significance than this in indicating an approach to the conditions met with in the Gnetales. If the apparent absence of neck cells in the deep-seated archegonia is confirmed, a comparison is at once suggested with the multinucleate prothallial tubes of Tumboa

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(Welwitschia).[5] A consideration of this question and also of the possible relation between the multinucleate prothallial cells of the two genera is better postponed, however, until PEARSON'S later researches on Tumboa have been published, and a closer series has been obtained in Widdringtonia.[6]

One interesting point, suggested by the fact that karyokinetic divisions occur in the prothallus at about the same time as the ventral nucleus is cut off, is that all the cells of the prothallus are potential archegonia. If the neck cells are really absent, the only essential difference between an archegonium with its egg and ventral nuclei and the other binucleate cells of the prothallus is one of size.

SUMMARY

1. The genus Widdringtonia is quite distinct from Callitris and should be kept as a distinct genus, excluding Tetraclinis.

2. A fixing agent not generally employed in cytological work has been found to give better results than chromacetic mixtures with and without osmic acid.

3. The male gametophyte is of the most reduced type yet recorded in the gymnosperms, no division of the microspore occurring until some time after pollination, and the tube and stalk nuclei disappearing before the body cell divides and before the archegonium initials can be recognized.

4. A very large number of megaspores are probably formed, but only one of these ever forms a prothallus.

5. The early development of the prothallus is perfectly normal.

6. A very large number of archegonia (over 50) are formed, and these are arranged in a number of groups near the margin of the prothallus on the side down which the pollen tube grows. They are confined to the upper half of the prothallus (but absent from the apex).

7. The archegonia arise from deep-seated cells of the prothallus and never grow through to the margin.

[5] See RENDLE'S remarks (**30**) on the correct generic name of this remarkable plant.

[6] Since the above was written, an abstract of PEARSON'S paper (**29**) has been published. He now finds the origin of the endosperm in Tumboa to be quite different from what was expected (cf. PEARSON **28**).

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8. Jacket cells are either absent or sometimes very feebly developed, and apparently neck cells are entirely absent from the archegonia.

9. The central nucleus of the archegonium probably divides to form the egg nucleus and a ventral nucleus.

10. By karyokinetic divisions in the prothallus cells, about and after the time of fertilization, they become binucleate and in some cases four- or even five-nucleate.

11. The multinucleate condition persists, differing in this respect from Cryptomeria, as well as in the origin of the binucleate cells.

12. The haploid and diploid numbers of chromosomes are respectively 6 and 12 (approximately). In two species of Callitris the numbers are approximately 12 and 24.

13. From the occurrence of proembryos and embryos in pairs it is concluded that fertilization of two archegonia is effected by two sperms from a single pollen tube.

14. The proembryo contains eight or more cells, one of which forms a suspensor and the other the embryo. Early stages of the proembryo are wanting.

15. Embryo development is quite normal, and embryonal tubes are formed.

16. A resemblance is noted in certain points with the development of *Sequoia sempervirens*.

17. A comparison is provisionally suggested with the Gnetales, especially with the genus Tumboa.

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An asterisk indicates that the original paper has not been consulted.

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EXPLANATION OF PLATE XI

All figures were drawn with the aid of a Zeiss microscope, lenses, and camera lucida; *figs. 4, 8, 11, 14,* and *22* with a 2^mm oil-immersion objective, and the other figures with various dry objectives. Magnifications were measured directly by comparison with a stage micrometer in each case. Each figure is oriented with the long axis of cone or ovule vertical except *figs. 8* and *22*. The sections were cut out with a Cambridge rocking microtome to a thickness of 4 to 10 μ.

FIG. 1.—Median longitudinal section of very young ovule, not yet pollinated; January 7, 1909. ×180.

FIG. 2.—Median longitudinal section of staminate cone, slightly diagrammatic; only the apical three-fourths of the cone are shown; January 7, 1909. ×36.

FIG. 3.—Median longitudinal section of a pollinated ovule (drawn from two sections); January 7, 1909. ×147.

FIG. 4.—Germinating pollen grain from a similar ovule to that shown in *fig. 3;* January 7, 1909. ×725.

FIG. 5.—Median longitudinal section of central part of ovule, showing very large number of megaspores (?); May 3, 1908. ×180.

FIG. 6.—Sketch of whole ovule in optical section (cleared in cedar); oil at the apex of the nucellus is a pollen tube; June 30, 1908. ×10.

FIG. 7.—Pollen tube in apex of nucellus of similar age to that shown in *fig. 6;* May 25, 1908. ×180.

FIG. 8.—Very small part of prothallus of same ovule as *fig. 7;* vacuole to the right, membrane to the left. ×1720.

FIG. 9.—Small part of a prothallus in the process of forming cell walls; August 25, 1908. ×310.

FIG. 10.—The tip of a pollen tube which has penetrated nearly half-way down the prothallus of *fig. 9* (cut obliquely and drawn from two sections). ×310.

FIG. 11.—The body cell of *fig. 10* more highly magnified. ×725.

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FIG. 12.—Upper half of median longitudinal section of nucellus after wall formation is complete in the prothallus, showing the characteristic position of the pollen tube; August 25, 1908. ×24.

FIG. 13.—Tip of pollen tube of *fig. 12*. ×310.

FIG. 14.—Body cell nucleus of *fig. 13*. ×1240.

FIG. 15.—Upper half of median longitudinal section of nucellus, *n*, and prothallus, *p*, showing position of pollen tube and of archegonia; drawn from several sections; *t*, pollen tube; January 7, 1909. ×28.

FIG. 16.—The lowest group of archegonia of *fig. 15*, two containing proembryos. ×130.

FIG. 17.—Suspensor bearing a single embryo cell at its apex; note multinucleate prothallus cells in this and previous figure; *s*, suspensor; *e*, embryo cell; January 7, 1909. ×310.

FIG. 18.—Very young embryo in median longitudinal section; March 8, 1908. ×505.

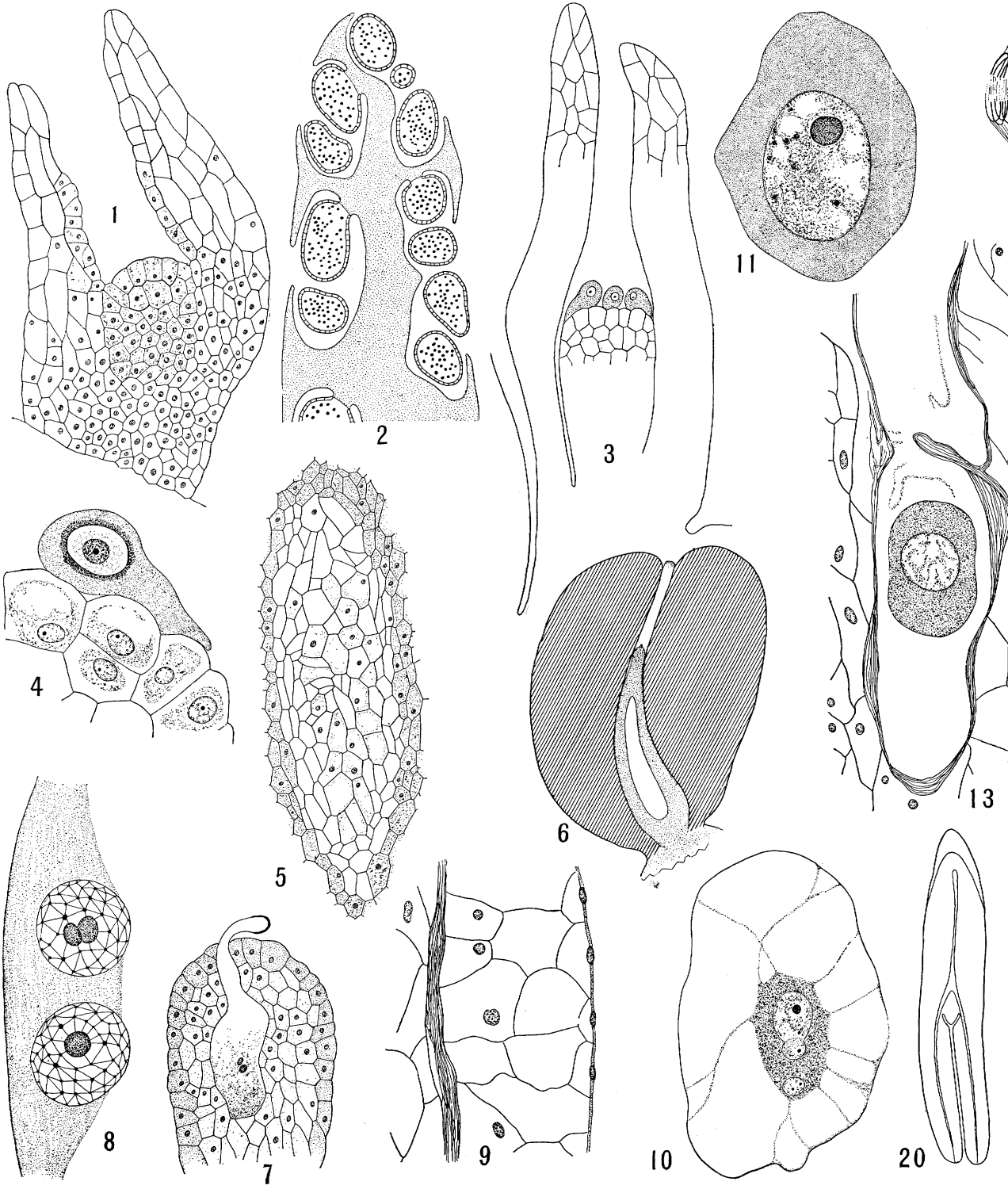
FIG. 19.—Older embryo, showing dermatogen differentiated and embryonal tubes; January 7, 1909. ×490.

FIG. 20.—Outline sketch of median longitudinal section of nearly mature embryo; March 8, 1908. ×9.

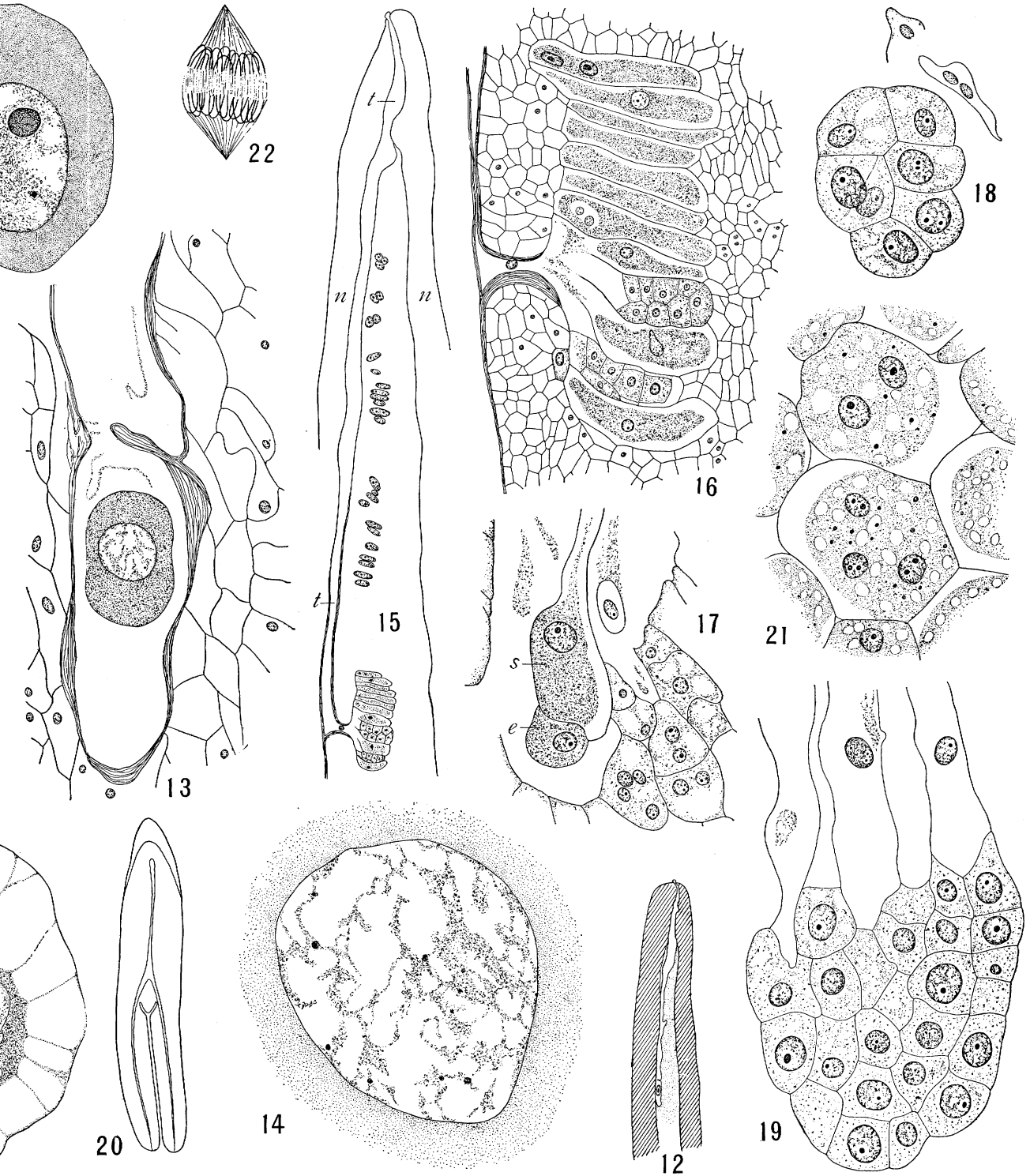
FIG. 21.—Two multinucleate cells from an old prothallus; March 8, 1908. ×540.

FIG. 22.—Dividing nucleus from an embryo between the ages of those shown in *figs. 17* and *18* respectively. ×1500.

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