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ON SOME STAGES IN THE LIFE HISTORY OF GNETUM.*

BY H. H. W. PEARSON, Sc.D., F.R.S., AND MARY R. H. THOMSON, B.A.

(With Plates XLV-L.)

Since the time of Griffith,† the life history of *Gnetum* has been studied by many botanists, each of whom has done something to advance the knowledge of this remarkable genus, which, nevertheless, is still very incomplete. Material for the study is difficult to obtain, and when obtained is usually more or less disappointing in the results which it yields.‡

The present investigation resembles those that have preceded it in respect of its incompleteness. It carries the story a little farther, but it leaves considerable gaps to be filled by future work. It has extended over a period of seven years, in the course of which material from West Africa,§ Poona, Darjeeling, Ceylon, Penang, Singapore, and Buitenzorg, representing *G. africanum*, *G. Buchholzianum*, *G. Gnemon*, *G. scandens*, and two species of doubtful identity have been studied. Thousands of sections have been examined || and some results have been published.¶ This paper contains probably the last contribution to the life history which this material will furnish.

Our failure to elucidate certain stages in the life history, in spite of this large supply of material carefully collected and fixed by those who have been so good as to assist us, is due to causes which themselves suggest some interesting conclusions regarding the biology of some species of *Gnetum*. We find no indication of specific disease such as Lotsy describes

* Percy Sladen Memorial Expeditions in S.W. Africa, No. 78 (in part).

† Griffith, 1859.

‡ Cf. Lotsy, 1899, 1903.

§ As previously stated (Pearson, 1912, 1915 A & B), we owe this material to the kindness of the Director of the Buitenzorg Gardens, Major Gage, Mr. I. H. Burkill, Prof. Gammie, Mr. W. Talbot, the late Dr. R. H. Lock, Mr. C. C. Calder, Mr. G. H. Cave, Mr. J. Gossweiler, Herr G. Zenker, and the Percy Sladen Memorial Expedition in S.W. Africa, 1908-1909.

|| We are indebted to Miss F. Goldmann, B.A., for some assistance in preparing sections.

¶ Pearson, 1912, 1915 A, 1915 B; Caporn, 1916; Thomson, 1916.

for *G. Ula*.* Pollination in *G. africanum* appears very frequently to fail. Lotsy had the same experience with *G. Gnemon*,† due in this case to the local rarity of male trees of a species long cultivated in the east‡ for the sake of the edible leaves and seeds. *G. africanum*, however, is not cultivated, and the failure of pollination here must be due to some degree of lack of adaptation to its environment. The fact that in this species the whole contents of the embryo-sac have frequently disappeared entirely is probably due to the exhaustion of available nutriment by the few ovules which do succeed in attaining their full size, with or without proembryos. The failure of fertilisation does not appear to affect adversely the development of endosperm and associated seed structures other than the embryo. Lotsy§ records that in *G. Gnemon* the embryo-sac becomes filled with endosperm if no fertilisation occurs. In *G. africanum*, seeds whose external characters are indistinguishable from those in which many proembryos are found, frequently contain a normally developed endosperm in which no proembryos appear to be present.|| The proembryos are not conspicuous, but with a little experience they are readily recognised, and their absence in these cases may safely be affirmed.

Another difficulty of constant occurrence in all our material is the almost synchronous development of all the ovules of the spike. In the early stages of the male spike the successive nodes do not develop in acropetal order,¶ and presumably the female spike has the same character.** Later, the ovules at successive nodes are in practically the same stage of development at a given time; still later one or more of them outpace the rest, which then develop no further.†† It is further probable that all the ovules on a particular plant at a given time represent a remarkably close developmental series. Certain it is that many ovules representing a single collection and presumably obtained from the same plant have yielded very few stages, and these close together.

Finally, if we may judge from our material of *G. africanum*, fertilisation and the changes which immediately follow it are rapidly completed when once the pollen-tube has reached the sac. We have seen both pollen-tubes and free-nucleate proembryos in scores, if not in hundreds, but not a single case of fertilisation or of the first division of the oospore; oospores themselves are recognised in only two of our preparations.

* Lotsy, 1899, 1903.

† Lotsy, 1899.

‡ Karsten, 1893 A, p. 204.

§ Lotsy, 1899, p. 107.

|| Cf. Thoday, 1911, p. 1101.

¶ Pearson, 1915 B, fig. 2 A.

** Cf. Lotsy, 1899, fig. 10.

†† Cf. Lotsy, *loc. cit.*, figs. 5, 6, 7.

1. THE NUCELLUS AND POLLEN-CHAMBER.

The pollen-chamber of *G. Gnemon* has been described and figured by Lotsy.* In *G. africanum*† the chamber is formed in the same manner, but it becomes deeper and narrower than in *G. Gnemon* (Plate XLV, figs. 2–4). In *G. scandens* it is broad and shallow as in the latter species and in *Welwitschia*‡ (Plate XLV, fig. 1).

After pollination the micropyle becomes blocked by tissue in the manner already described§ ; we have frequently seen large papilla-like cells || projecting from the lower end of the blocking tissue into the cavity of the pollen-chamber (Plate XLV, fig. 2).

Soon after pollination, sometimes before this occurs, the tissue of the nucellar-cap below the level of the floor of the pollen-chamber, may undergo disintegration similar in character to that described by Coulter for *G. Gnemon*.¶ Occasionally, as in the latter species, the epidermis becomes infolded and a slit-like constriction appears in this region of the nucellus. More commonly, however, in *G. africanum* (Plate XLV, fig. 3) the epidermis remains unaffected and the disintegration begins in the underlying tissues. It gradually extends inwards and downwards producing a broad, irregularly funnel-shaped cavity which may extend to the top of the embryo-sac (Plate XLV, fig. 4). During a stage in the later growth of [the endosperm its micropylar apex may turn aside into part of the cavity thus formed (Plate XLV, fig. 5), and it may thus acquire a somewhat irregular form. At a still later stage, with the destruction of all but a thin layer of crushed nucellar-cap cells, the endosperm assumes the more regular form already described.**

There is no indication of the meaning of this curious deformation of the nucellar cap. It is clearly not incited by pollination nor by the growth of the pollen-tube, for it may occur in unpollinated ovules, and often there is no sign of it in ovules which have been fertilised. Its occurrence does not appear to be detrimental to the normal development of the seed, and it is therefore unlikely that it is a stage in the nucellar disease described by Lotsy. It imposes limitations on the possible directions of growth of the pollen-tubes, though in its absence their course is not materially different. The cells of the cap not affected by this degeneration are packed with starch grains.

* Lotsy, 1899, p. 94, figs. 18, 35.

† Thoday, 1911, fig. 14.

‡ Bertrand, 1878, fig. 10; Pearson, 1906, figs. 28, 29.

§ Thoday, *loc. cit.*, p. 1120.

|| *Cf.* Berridge, 1911, fig. 4.

¶ Coulter, 1908, fig. 5 A.

** Thoday, 1911, Text-fig. 9.

2. THE POLLEN-TUBE AND SPERM-CELL.

There is no conclusive evidence as to the pollinating agent in any species of *Gnetum**; the results of its action are very variable in *G. africanum*. Very frequently, as already stated, the ovule is unpollinated; otherwise one, two, or three pollen grains are commonly found in the pollen-chambers; frequently the number is larger. In one case, in *G. Gnemon* (Ceylon material), the large number present in the micropyle (Plate XLVI, fig. 7) recalls the condition often seen in *Welwitschia*.† There is every indication that pollination in the latter genus is much more efficient than in *Gnetum africanum*.

The adult pollen-grain in the microsporangium of *G. africanum* contains three free nuclei.‡ The same condition for *G. Gnemon* is described by Lotsy§ and for *G. funiculare*, *G. Rumphianum*, *G. latifolium*, and *G. Gnemon* by Karsten.|| In no species has a generative cell been seen in a pollen grain. In *G. funiculare* the pollen-grains on the nucellus contain only two free nuclei,¶ one having disappeared. In *G. Gnemon*, either two or three free nuclei are present in grains found in a micropyle (Plate XLVI, fig. 7). Presumably the same is true for *G. africanum*; in our material of this species pollinated ovules all show the pollen in the course of germination; in many cases a third nucleus is seen (Plate XLVI, fig. 8) in the proximal end of the tube, in others there is no trace of it. In all these species then, as in *Welwitschia*,** three free nuclei are characteristic of the mature pollen-grain; one of the three plays no part in its germination; it disappears either before or soon after pollination or, if it persists, never, as far as is known, enters the pollen-tube. Such a nucleus must be regarded as constituting the last vestige of the prothallial tissue. It is noteworthy that this very considerable reduction of the prothallial tissue, to a condition occasionally found in the Angiosperms,†† is characteristic both of *Gnetum* and *Welwitschia*; in *Ephedra* the prothallus is represented by one or two fully constituted cells with at least a partial formation of cell-walls.‡‡

The pollen-grains of *Welwitschia* and *Gnetum* do, however, differ in the organisation of the generative cell. In *Welwitschia* this cell is organised before pollination.§§ In *G. africanum* (Plate XLVI, figs. 9, 10) as in the other

* Cf. Karsten, 1892, p. 213; Lotsy, 1899, p. 94.

† Cf. Pearson, 1909, fig. 11.

‡ Pearson, 1912, p. 618, fig. 20.

§ Lotsy, 1899, p. 94.

|| Karsten, 1893 B, p. 359.

¶ *Loc. cit.*

** Pearson, 1906, p. 281, figs. 13-16.

†† Coulter and Chamberlain, 1903, p. 135.

‡‡ Strasburger, 1892, pp. 10, 11; Land, 1904, p. 6; Berridge, 1909, fig. 2.

§§ Pearson, 1906, p. 281, fig. 16.

species described,* it is first recognised after germination. The marked difference in the sizes of the tube and generative nuclei by Karsten† is not observed in *G. africanum*; here the tube nucleus, before it shows signs of deterioration, is usually somewhat smaller than the other (Plate XLVI, figs. 9, 10).

In the pollen chamber in *G. africanum* the grain swells, splitting and casting off the exine (Plate XLVI, fig. 8, *ex*); this is described also for other species of *Gnetum*,‡ for *Ephedra*,§ and for *Welwitschia*.|| The pollen-tubes usually follow a fairly direct line from the base of the pollen-chamber towards the summit of the sac. The diameter of the axial tissue of the nucellar cap is frequently so limited by the degeneration already referred to (p. 233), that the passage of three or four pollen-tubes almost completes its disorganisation, leaving a broad irregular cavity.

The pollen-tube, so far as we have seen, always enters the embryo sac at a point very near its apex. In *G. Gnemon*, according to Lotsy,¶ it sometimes penetrates the sac-wall at a lower point, but always in the upper half. When there are several pollen-tubes, the destruction of nucellar tissue is particularly great just above the summit of the sac (Plate XLVI, fig. 11, *c*). If the pollen-tube contains three nuclei at the time of its germination, one of these does not enter the tube (Plate XLVI, fig. 8). Of the other two, the second, soon after entering the tube, becomes incorporated in a cell—the generative cell (Plate XLVI, figs. 9, 10). The organisation of the protoplasm of this cell must be effected very rapidly; no stages intermediate between the free nucleus and the apparently fully-organised cell have been observed.

Soon after the organisation of the generative cell, two nuclei are seen in it (Plate XLVI, figs. 12, 13, 14; and Plate XLVII, fig. 15). The division which yields this 2-nucleate generative cell has not been seen. The corresponding division of the generative cell of *Welwitschia* has been described.** The two nuclei in *Gnetum africanum* are usually unequal in size or staining capacity, as they are in *Welwitschia*, and, as also in the latter, the advantage may lie either with the leading or the following nucleus.†† Their shape does not vary to the same extent as in the homologous nuclei of *Welwitschia*; ‡‡ they are usually spherical or nearly so; an exception is shown in Plate XLVI, fig. 14. Usually they are both retained in the same unit of protoplasm; in

* Karsten, 1893 B, p. 360, figs. 59–63; Lotsy, 1899, p. 94.

† *Loc. cit.*

‡ Karsten, *loc. cit.*, p. 359.

§ Land, 1907, p. 275; Berridge and Sanday, 1907, p. 132; Berridge, 1909, fig. 2.

|| Pearson, 1906, fig. 17; 1909, p. 358.

¶ Lotsy, *loc. cit.*, p. 94, fig. 40.

** Pearson, 1909, figs. 44, 48.

†† In *G. funiculare*, etc., the two sperm nuclei are said to be quite alike. Karsten, 1893b, p. 360.

‡‡ Pearson, 1909, p. 360.

some cases the cell becomes divided into two uninucleate portions, probably as a result of a simple mechanical strain as is suggested for *Welwitschia*.* Both these conditions are recorded by Karsten for *G. Rumphianum* and *G. ovalifolium*.† In *G. funiculare* the protoplasm of the binucleate cell remains undivided until it enters the embryo sac.

The nucleolus of the sperm nucleus is large and the surrounding nuclear substance contains numerous coarse chromatin granules. The male nuclei of *G. ovalifolium* and *G. Gnemon*, figured by Karsten‡ and Lotsy‡ respectively, possess no nucleoli but “more or less regular balls of chromatin accumulated in a mass which resembles a raspberry somewhat.”§ These characters have not been seen in our material. Lotsy’s figure represents these nuclei just before their entrance into the sac. In earlier stages figured by Karsten,|| they appear to be very similar to those seen by us in *G. africanum*. We have identified the sperm nuclei just before they enter the sac; they still retain the characters of the nucleolus and chromatin described above.

We have not succeeded in obtaining certain proof that the smaller of the two sperm nuclei is in process of disintegration, though its condition (*e. g.*, Plate XLVI, figs. 10, 13) frequently suggests this conclusion. In the lower part of the nucellar cap it is frequently not possible to find the second sperm nucleus (Plate XLVII, fig. 16). It is therefore not improbable that one of them may disappear as is sometimes the case in *Welwitschia*.¶ If this is so, there is little doubt that the disappearing nucleus is a sperm nucleus and not the homologue of the so-called “stalk-nucleus” of *Ephedra* and the lower gymnosperms. The history of the male gametophyte of *Gnetum* and *Welwitschia* is shortened, not only by the partial elimination of the prothallus, but also by the complete suppression of one of the later divisions, almost certainly the first division of the “body cell” of the lower forms. The tendency to reduce the series of events intervening between the spore-mother-cell and the functional gamete is seen in the history of the microspore as in that of the macrospore.

The tube-nucleus usually maintains its spherical form to the end (Plate XLVII, fig. 16). Sometimes it becomes crescent-shaped, as occasionally in *Welwitschia*** Karsten doubtfully recognises the partially disorganised tube-nucleus in the embryo-sac†† in *G. Rumphianum* and *G. ovalifolium*; in

* *Loc. cit.*

† Karsten, *loc. cit.*, p. 361.

‡ Karsten, 1893b, figs. 68, 69; Lotsy, 1899, fig. 41.

§ Lotsy, *loc. cit.*, p. 95.

|| Karsten, *loc. cit.*, p. 360, fig. 64.

¶ Pearson, 1909, p. 360.

** *Loc. cit.*, fig. 43.

†† Karsten, 1893 b, p. 368, fig. 71.

G. Gnemon it "usually, if not always, remains inside of the pollen-tube."* We have never been able to distinguish the tube-nucleus in *G. africanum*, either in the sac or in a pollen-tube which has reached the end of its growth.

3. THE EMBRYO-SAC AND ENDOSPERM.

Several stages in the development of the embryo-sac and endosperm have been described.

It seems probable that more than one macrospore mother-cell is organised,† though perhaps only one usually gives rise to macrospores; but according to Karsten,‡ "aus den Embryosack-Mutterzellen geben nun durch weitere Quer-und Längstheilungen eine ganze Anzahl von Embryosackanlagen hervor." He further states § that he never saw more than three cells formed by the transverse division of a single mother-cell. According to Lotsy,|| the embryo-sac of *G. Gnemon* arises by the division of the mother-cell into two, each of which may become an embryo-sac. Our material gives no information regarding these early stages. Usually only one embryo-sac is organised in *G. africanum*; in *G. Gnemon*, as stated for this and other species by other observers, two or three are not infrequently present.¶

The earliest stage of the sac shown in our preparations contains four free nuclei. Later stages up to and including the septation of the lower part of the sac and the fusion of the free nuclei in each compartment have been described.** In *G. africanum*, as in *G. Gnemon*, the embryo-sac before septation is hardly more than four times as long as it is broad. In *G. scandens* it is much longer (Plate XLV, fig. 1) in proportion to its breadth, a character which appears to be constant in this species. The sac here outlined (Plate XLV, fig. 1) shows the aggregation of free nuclei at the chalazal end. There is yet no indication of septation. Later stages have not been seen in this species.

Neither Karsten †† nor Lotsy ‡‡ saw any stage of mitosis in the free nucleate sac. We have seen one only (Plate XLVII, fig. 17). The ovule in which it occurs is pollinated, and pollen-tubes have already penetrated about one-third of the length of the nucellar cap. In this sac the central vacuole is still present, and the nuclei are uniformly distributed throughout the thin

* Lotsy, 1899, p. 95.

† Strasburger, 1879, p. 116, Taf. xiii, figs. 51-55.

‡ Karsten, 1893 B, p. 354.

§ *Loc. cit.*

|| Lotsy, 1899, p. 102.

¶ Strasburger, 1879, Taf. xiv, figs. 59, 60; Karsten, 1892; p. 210; Lotsy, 1899, p. 90; Pearson, 1915 A, fig. 2.

** Pearson, *loc. cit.*, p. 318 (on p. 319 of this paper, "multinucleate," the last word in line 8 from the top, should read "uninucleate.")

†† Karsten, *loc. cit.*, p. 356; ‡‡ Lotsy, 1899, p. 92.

protoplasmic layer. All the nuclei are in precisely the same stage of division. It may be assumed that all the divisions which occur in the sac before septation are simultaneous, as they appear to be in *Welwitschia* * and in *Ephedra*.† The chromosomes cannot be counted with certainty, but they are probably twelve—the number determined by Coulter in the reduction divisions of the microspore mother-cell of *G. Gnemon*.‡

Lotsy § suggested the possibility that all the free nuclei of the “fertile” (micropylar) region of the sac were descended from one of the two daughter-nuclei resulting from the first mitosis in the macrospore; those of the chalazal end from the other. The absence of dividing nuclei from his material “made it impossible to follow this question up, yet it is a point of primary importance.” In view of what is now known of the origin of the endosperm in *Welwitschia* and in *Gnetum*, the point no longer possesses that degree of importance which in 1899 was very naturally attributed to it. In the early stages figured by Strasburger,|| as in those seen by us, the arrangement of the nuclei does not support Lotsy’s suggestion; and the later stages already known, or now to be described, indicate that the primary nuclei of the sac differ from one another only in the accident of position.

One of the many puzzling features of the life-history of *Gnetum* has been the occurrence of fertilisation before the formation of endosperm in some species ¶ (*G. Rumphianum*, *G. funiculare*) and after it in others (*G. Gnemon*).* This difference of character has resulted in the informal separation of the species of *Gnetum* already investigated into “Karsten’s *Gneta*” and *Gnetum Gnemon*,†† the latter being regarded as “an intermediate form between *Welwitschia* and the *Gneta* described by Karsten.”‡‡

Karsten’s statements, however, do not justify such a conclusion. While fertilisation does occur in Karsten’s *Gneta* before septation begins,§§ this is not always the case, for, having described and figured the free nuclear sac of *G. ovalifolium*,||| he proceeds, “In diesem Zustande nun erwartet der Embryosack den herannahenden Pollenschlauch. Eine weitere Differenzirung seines Inhaltes tritt nicht ein, höchstens spannen ein paar Plasmafäden im Scheitel von einer Seite des wandbelages zur andern, quer durch den Embryosack hindurch, oder es wird ganz in dem Chalazaende eine Endo-

* Pearson, 1909, p. 346, figs. 18, 19.

† Jaccard, 1894, p. 15, figs. 24, 25; Land, 1904, p. 10, figs. 33, 35.

‡ Coulter, 1908, p. 47.

§ Lotsy, 1899, p. 92.

|| Strasburger, 1879, figs. 59, 61, 62, 63.

¶ Karsten, 1893b, p. 356.

** Lotsy, 1899, p. 93.

†† Lotsy, *loc. cit.*, p. 101; 1911, pp. 353, 359; Pearson, 1909, p. 357.

‡‡ Lotsy, *loc. cit.*, p. 103.

§§ Karsten, 1892, p. 212, figs. 8–11 (*G. funiculare*).

||| Karsten, 1893 b, p. 356, Taf. x, figs. 56, 57.

spermzellbildung um die freien wandständigen Kerne begonnen." A similar formation of endosperm before fertilisation may also take place in *G. Rumphianum*, for in his earlier paper Karsten figures * a sac of this species "unmittelbar vor dem eintreffen des Pollenschlauches," in which the chalazal region shows the "anfang der Endospermbildung"—no less than 13 multinucleate cells appearing in the section. It is clear, therefore, that in these two of Karsten's species endosperm-formation does sometimes precede fertilisation.

In Lotsy's account of *G. Gnemon* he describes the sac awaiting fertilisation as "a sac constricted at some point below the middle, the lower end up to the constriction containing a prothallium, the part above it containing a large number of free nuclei. In this stage the embryo-sac can be fertilised, no further changes occur." †

The last sentence has frequently been interpreted as meaning that the formation of endosperm to the extent described ‡ is, in this species, constantly antecedent to fertilisation. This, however, was not Lotsy's meaning, for on the same page he says, "this prothallium, as I will call it, is undoubtedly present, at least in many cases, before fertilisation has taken place." And elsewhere § he states that "if no fertilisation occurs the embryo-sac becomes filled with endosperm." Lotsy therefore leaves it an open question whether or not fertilisation may occur in *G. Gnemon* before endosperm-formation has commenced. A comparison of his figures 34 and 36 makes it clear that the amount of endosperm at the time of fertilisation is not constant. According to Karsten, therefore, fertilisation may occur in *G. ovalifolium* and *G. Rumphianum* after the chalazal region of the sac is filled with endosperm, as, perhaps usually, is the case in *G. Gnemon*. Since in the latter species endosperm-formation proceeds towards the micropylar end of the sac if fertilisation is not effected, it is highly probable that this may take place also at a later period than those described by Lotsy, who does not definitely assert that it never precedes the formation of endosperm in the chalazal region. Coulter, || on the other hand, states that in this same species all the sac-nuclei are free when fertilisation occurs. We have abundant confirmation of the accuracy of Lotsy's account, but Karsten's statements and, more particularly some new facts now established for *G. africanum*, make it probable that the condition described by Coulter may also occur; in other words, that both accounts are correct for *G. Gnemon*. The evidence as it stands seems to indicate that in any species fertilisation does not necessarily take place at any particular stage in the formation of the endosperm, though

* Karsten, 1892, figs. 9, 9 A (here called *G. edule*, see Karsten, 1893 B, p. 338).

† Lotsy, 1899, p. 93.

‡ Cf. Lotsy, *loc. cit.*, figs. 34, 36, etc.

§ *Loc. cit.*, p. 107.

|| Coulter, 1908, p. 44.

there may be a tendency for it to take place before (as in *G. Rumphianum*) or after (as in *G. Gnemon*) this has commenced. It cannot occur before the end of the last free nuclear division in the sac nor after the completion of the endosperm, because it is only between these two limits that female gametes are available.* It is probable that in every species hitherto investigated the conditions of the sac itself are favourable to fertilisation at any time between these two limits.

Certainly this is the case in *G. africanum*, if we may assume that fertilisation immediately follows the introduction of the male gametes into the sac. In this species the pollen-tube reaches the sac before septation commences—*i.e.* while all the sac-nuclei are free—or at any stage in the formation of endosperm-tissue; and we have preparations in which the whole sac is filled with endosperm while the tips of the longest pollen-tubes are at various depths between the bottom of the pollen-chamber and the top of the sac, or, in some cases, the ovule is not pollinated.

When the life-histories of these and other species are more fully known it will probably be found that in all of them the formation of endosperm beginning at the chalazal end of the sac proceeds gradually and without any marked interruption upwards until all the available and accessible † free nuclei have been utilised in fusion, and that fertilisation may occur at any time so long as there are free gametes in the sac. Given this condition, the time at which fertilisation is effected is probably determined by the accidents of pollination and the growth of the pollen-tube. The relations of the gametes to the endosperm are not the same in *Gnetum* as in *Ephedra* and the lower *Gymnosperms*. In the latter the endosperm, a prothallus, produces the gametes, and therefore necessarily precedes them in ontogeny. The constitution of the gametes is the essential condition which must be realised before fertilisation can take place. This implies a pre-existent endosperm (prothallus) in the lower *Gymnosperms*, but not in *Gnetum*. In *G. africanum*, and probably in the other species as well, fertilisation appears to be quite independent of endosperm-formation except that when the latter process is once completed fertilisation is rendered impossible because the supply of female gametes is exhausted. These two processes are equally independent of one another in *Welwitschia*, but their independence is obscured by the extreme form of siphonogamy achieved in this genus; septation, the first step in the constitution of the endosperm, being also a necessary antecedent to the formation of the embryo-sac-tubes, and therefore to fertilisation.

The account already given ‡ of the septation of the sac and conversion of the multinucleate compartments into uninucleate cells by nuclear fusion is fully confirmed for *G. africanum*, and the same processes have now been

* But see p. 243.

† See p. 243.

‡ Pearson, 1915 A.

proved to occur in *G. Gnemon** (Plate XLVIII, fig. 21 A, B). We have found no indication that a primary uninucleate endosperm cell is ever formed except in this manner. These processes commence at the chalazal end of the sac and proceed gradually, though sometimes a little irregularly, upwards. Sometimes a compartment may be formed above a number of nuclei which are not yet enclosed (Plate XLVII, fig. 18), and frequently the nuclei in lower compartments may still remain free when fusion has occurred in those above them (Plate XLVII, fig. 19). If the enclosed nuclei do not fuse—as occurs occasionally when an unduly small number is enclosed—the compartment increases in size † and its protoplasm becomes highly vacuolated (Plate XLVII, fig. 19 E). This is also true of similar compartments in the endosperm of *Welwitschia*.‡ Nuclear fusion in an endosperm compartment is frequently followed immediately by cell-division (Plate XLVII, fig. 19 D); whether occurring immediately or not, the cells resulting from the division are smaller than those which gave rise to them.§

It should be emphasised that, with the exception of such cases as that shown (Plate XLVII, fig. 19), in which the enclosed nuclei are always few in number, every cell of the primary endosperm is uninucleate, the nucleus being formed by gamete-fusion. When this nucleus divides a persistent cell-plate appears and uninucleate cells result; so far as is known this also is always true for the primary cells. In later stages, however, the endosperm cells may become multinucleate (Plate XLVIII, fig. 20), and frequently in the seed the endosperm appears to be mainly composed of multinucleate cells. There are some indications that this condition results from nuclear fragmentation; the appearance and reactions of the free nuclei are always consonant with the view that they are in process of degeneration. This multinucleate condition of the old endosperm cells of *Gnetum* is probably to be ascribed to the causes which produce similar results in the prothalli of *Ephedra*,|| *Taxus*,¶ and other of the lower gymnosperms.

While the origin of the endosperm of the lower half of the sac is now known, that of the micropylar region is still in doubt. Lotsy** describes it (in *G. Gnemon*) in the following terms: "About the time of the formation of zygotes it is seen that a greater or smaller number of the

* *Cf.* Pearson, *loc. cit.*, p. 321.

† This is probably the explanation of the curious cell figured by Lotsy (1899 fig. 62), and described by him as an "Archeonium-like formation."

‡ Pearson, 1909, p. 353, fig. 38 B.

§ Pearson, *loc. cit.*, p. 352. *Cf.* also Pearson, 1915 A, fig. 23.

|| Strasburger, 1880, pp. 106, 109; Jaccard, 1894, p. 34.

¶ Jaeger, 1899.

** Lotsy, 1899, p. 97.

nuclei of the fertile (*i.e.* micropylar) part of the embryo-sac surround themselves with a denser protoplasm and a membrane and thus form cells. I will call these retarded prothallium cells of the fertile part. They may form regularly in the neighbourhood of the young zygotes,* or they may be found rather irregularly distributed,† or they may be limited to a very small number, frequently but one or two.‡ After these changes have taken place the rôle of the fertile part of the embryo-sac has been played, nothing more occurs in it, it is gradually obliterated owing to the growth of the sterile part; its retarded prothallium cells play no rôle whatever." It has been clear for a long time that "this account may require some amendment,"§ but it is only lately that we have been able to obtain convincing evidence.

Lotsy's "retarded prothallium cells" are multinucleate endosperm cells of precisely the same kind and origin as those which are formed in the process of endosperm-formation in the chalazal region. The nuclei they contain are, as in the former case, unfertilised sac-nuclei. When there is little or no disturbance caused by the entrance of the pollen-tubes or the activity of the oospores, septation and nuclear fusion proceeds without any sign of interruption to the very summit of the sac. Two of a series yielded by our preparations are shown in Plate XLVIII, fig. 21 B (*G. Gnemon*), and Plate XLVIII, fig. 22 A, B (*G. africanum*). In the former (Plate XLVIII, fig. 21 B) a small part of the micropylar end is still unseptate; the endosperm below this is clearly of uniform origin (*cf.* Plate XLVIII, fig. 21 A). In the latter (Plate XLVIII, fig. 22) septation has proceeded to the summit of the sac; three of the uppermost compartments contain groups of free nuclei whose constituents are in process of fusion (Plate XLVIII, fig. 22 B). In Plate XLIX, fig. 23, which shows at least two oospores, numerous groups of free sac-nuclei are seen, though the preparation does not yield proof that cell-walls enclosing them have yet appeared. Plate XLIX, fig. 24, represents an interesting and not uncommon case in which the septation of the micropylar region is much less regular. This is almost certainly due to the influence of oospores, of which one (o) appears in the section. Septation at a particular point may be prevented, or multinucleate compartments may be destroyed, just as uninucleate endosperm cells are destroyed, by the oospores and proembryos. It is quite possible that a multinucleate compartment may sometimes suffer from the activities of a uninucleate endosperm cell in its neighbourhood. But there can be no doubt that compartments which do not succumb to the growth of the proembryo, or perhaps occasionally to the greater vigour of more advanced endosperm

* *Loc. cit.*, figs. 44, 45.

† *Loc. cit.*, fig. 60.

‡ *Loc. cit.*, figs. 53, 55, 58.

§ Pearson, 1915 A, p. 322.

cells, become a permanent part of the endosperm. In other words, the endosperm of the micropylar region is formed in exactly the same manner as that of the chalazal end. It is, therefore, a little misleading to call the micropylar region "fertile" and the chalazal "sterile."* Both regions alike produce sterile (endosperm) tissue and in the same manner; fertilisation is usually, though possibly not always, confined to the micropylar region merely because the pollen-tube enters it and the sperm-nuclei there meet with free female gametes.

Owing, no doubt, to the disturbances caused by the entrance of the pollen-tube and the formation and growth of the oöspores, it frequently happens that a few of the micropylar sac-nuclei, usually situated close to the wall of the sac, escape enclosure in an endosperm compartment (Plate XLIX, figs. 25 A, B). These do not fuse, and their appearance and reactions suggest that they play no further part in the economy of the sac; they occupy a position in the life-history which corresponds to that of the gametes in the embryo sac-tubes of *Welwitschia* which remain unfertilised. The "crowding out" of a few nuclei in this manner is not uncommon; a similar case is recorded by Lotsy.† The fact that this occurs at all is evidence for the view expressed later (p. 262) that the whole process of septation and nuclear fusion is due, in the main, if not entirely, to causes which are inherent in the physical and physiological conditions of the sac. Any of the sac-nuclei may be fertilised; any or all of them may fuse in groups after septation, producing endosperm; if, owing probably to simple mechanical causes, a number of them are excluded from both possibilities, neither the results of fertilisation nor of the nuclear fusion which produces primary endosperm cells are in any way interfered with, and the excluded nuclei have no future. These facts involve physiological questions of deep significance, but from a morphological point of view we are mainly concerned with the phylogeny of the nuclei; the fusion *inter se* is probably a new character, whose origin is to be sought in the conditions under which they are formed.‡

The endosperm later increases greatly in amount and becomes to some extent differentiated. Two massive growing points are organised, one at each end; that at the chalazal end is more active than the other; a result of its activity is the destruction of the pavement tissue § of the nucellus. The micropylar end advances into the nucellar cap; in the most advanced stages seen by us, this region of the nucellus is reduced to a thin plate of crushed and disorganised cells (Plate XLVIII, fig. 22 A, N). The peculiar form of the upper end of the advanced endosperm described by Coulter || for *G. Gnemon* has been seen in this species but not in *G. africanum*.

* Lotsy, *loc. cit.*

† Lotsy, 1899, fig. 44, W. o. E.

‡ *Cf.* Coulter, 1911; Pearson, 1915 A, p. 329.

§ Coulter, 1908, figs. 2-3 A; Pearson, 1915 A, fig. 19.

|| Coulter, *loc. cit.*, p. 45; fig. 6. (*Cf.* *Welwitschia*, Pearson, 1909, figs. 89 I.K.)

Soon after the completion of the primary endosperm there commences at a point not far from the centre of its axis the differentiation of a small-celled tissue, which later becomes a very marked feature.* Longitudinal and transverse divisions result in the formation of a tissue of cells (Plate L, fig. 26). These divisions extend upwards and downwards from the point at which they commence, and ultimately produce a columella-like cylinder, which extends without interruption from growing point to growing point, and is broad enough to be conspicuous to the naked eye in a bisected endosperm. Towards and into this cylinder the early growth of the proembryos is directed. In its early stages the cells contain no starch; later this region as well as the larger-celled peripheral portion of the endosperm are well provided with starch. The position, form and cell-characters of this "central cylinder" and its relation to the proembryos suggest that it is a simple form of absorbing, storage and conducting tissue. Whatever its precise functions, its presence and that of the two growing points indicate a considerable degree of differentiation.† There is no doubt that the endosperm of *Gnetum* is not only a "special formation," but "an organism."‡

4. THE OÖSPORE AND PROEMBRYO.

Cells believed to be oöspores are shown in Plate XLIX, figs. 23 and 24. But as fertilisation has not been seen, nor the relations between these cells and the proembryos determined, their identification requires confirmation. It is not clear from Lotsy's account that he saw the fusion of the gametes. He states that the oöspores are distinguishable by the large size of their nuclei § and that they occur in pairs, each pair resulting from the action of the two sperm-nuclei from one pollen-tube. These oöspores were later seen || to produce tubular proembryos. In *G. ovalifolium* and *G. Rumphianum* also each of the two sperm-nuclei of the pollen-tube fuses with a female gamete, ¶ but in *G. africanum* the number of oöspores is certainly not regularly twice the number of pollen-tubes, since in some cases the sperm-cell contains only one nucleus when it reaches the sac (p. 236).

Information regarding the details of fertilisation is greatly to be desired. In the case of *Welwitschia*, the protoplasm of the male cell plays an important part in the constitution of the oöspore.** Since in *Gnetum* the female gamete is a free nucleus it might be anticipated that the same would hold

* Coulter, *loc. cit.*

† *Cf.* Pearson, 1909, p. 371.

‡ Lotsy, 1899, p. 93.

§ Lotsy, 1899, p. 96.

|| Lotsy, *loc. cit.*, p. 97; fig. 55.

¶ Karsten, 1893, p. 372.

** Pearson, 1909, pp. 361, 362.

good for this genus also. Karsten figures a generative cell of an unnamed species with a female nucleus lying in its protoplasm,* but free male nuclei are shown in the embryo-sacs of *G. Rumphianum*,† and Lotsy describes the passage of the generative nuclei into the sac,‡ and states that the fusion nuclei surround “themselves with a rather dense mass of protoplasm, which can be plainly distinguished from the protoplasm of the embryo-sac.”§ There appears to be a tendency to reduce the protoplasm of the male cell in the Gymnosperms.|| If, therefore, the male gamete is a free nucleus in *Gnetum* while in *Welwitschia* the whole cell is concerned in fertilisation, the difference is of interest. It has been suggested that an ancient character has reappeared in *Welwitschia*,¶ probably as an adaptation to a unique method of fertilisation, itself perhaps part of a general adaptation to extremely xerophytic conditions.** It must nevertheless be regarded as not unlikely that, as is suggested by Karsten, the same character may be found in some species of *Gnetum*.

In *G. Gnemon* the oöspore produces a single proembryo.†† In other cases (*G. ovalifolium*, *G. Rumphianum*) the oöspore immediately undergoes a series of successive free nuclear divisions from which there results a mass of proembryos.‡‡ This appears to be the case in *G. africanum*, in which a mass of cells, believed to be proembryos, are frequently seen near the summit of the endosperm in ovules with a single pollen-tube. In view of the behaviour of the oöspore in *Ephedra* and *Welwitschia* respectively, it will be of some interest should the occurrence of both these cases in *Gnetum* be confirmed.

The proembryo becomes a long tubular cell, whose direction of growth is very irregular, but in general by way of the axial cylinder of the endosperm towards the chalaza. The single nucleus gives rise to a small number of free nuclei (Plate L, figs. 27, 28), which is probably a general character in *Gnetum* as it is in *Ephedra* and *Welwitschia*. Karsten figures a proembryonal tube of an unnamed species of *Gnetum* with two free nuclei §§ and implies that a similar stage is found in *G. Gnemon* and *G. funiculare*. Lotsy shows free nuclear stages in *G. Gnemon*,||| and in *G. ula*,¶¶ but with regard

* Karsten, 1892, fig. 12.

† Karsten, 1893, fig. 71.

‡ Lotsy, 1899, p. 95.

§ Lotsy, *loc. cit.*, p. 96.

|| Nawaschin and Finn, 1912.

¶ Nawaschin and Finn, *loc. cit.*, p. 50.

** Pearson, 1909, p. 387.

†† Lotsy, 1899, p. 97, fig. 55.

‡‡ Karsten, 1892, p. 239; 1893, p. 372.

§§ Karsten, 1892, p. 230, fig. 29.

||| Lotsy, 1899, fig. 50.

¶¶ Lotsy, 1903, p. 399, figs. 8-12.

to the latter there is some doubt as to whether the multinucleate structures described and figured are proembryos.* Coulter describes the proembryos of *G. Gnemon*; † as many as four free nuclei were observed. We find in *G. africanum* from two (Plate L, fig. 27) to five (Plate L, fig. 28); our material has not furnished any later stages. Coulter gives some further information regarding these, and Bower ‡ has described the development of the embryo; there is, however, much yet to be learned about both.

SUMMARY.

1. *Nucellus and Pollen-chamber.*

A disintegration of the nucellar cap tissue, in general similar to that described for *G. Gnemon*, frequently occurs also in *G. africanum*. It does not appear to interfere in any way with the functions of the ovule.

The form of the pollen-chamber appears to be a specific character.

The pollen-chamber of *G. africanum* often becomes partially filled by papilla-like cells growing down from the tissue blocking the micropyle.

2. *The Pollen-tube and Sperm-cell.*

In *G. Gnemon* and *G. africanum* the pollen-grains in the micropyle contain two or three free nuclei. If three are present, one does not enter the pollen-tube. The last is identified as the prothallial nucleus which, in the case of the 2-nucleate grains, has disappeared before pollination.

In *G. africanum* the generative cell is organised soon after the beginning of germination. Later, this cell becomes binucleate; the two nuclei are unequal; it is probable that sometimes one of them disappears before the pollen-tube reaches the embryo-sac. Rarely the protoplasm of the cell breaks into two uninucleate portions. The tube nucleus, usually smaller than the generative nucleus, usually disappears before the growth of the pollen-tube is completed. The first division of the generative cell into "body" cell and "stalk" cell is eliminated.

3. *Embryo-sac and Endosperm.*

The Mitoses in a free nuclear stage in *G. africanum* are simultaneous.

The pollen-tube reaches the top of the embryo-sac before or at any time after the beginning and before the completion of endosperm formation. There is reason to believe that this is also true for other species.

Endosperm is formed in the micropyle region of the sac in precisely the same manner as at the chalazal end, except that the processes of septation and nuclear fusion at any point may be interfered with by the activities of a pollen-tube or oöspore. Under such circumstances a few of the sac-

* Cf. Lotsy, 1911, p. 352.

† Coulter, 1908, p. 46, figs. 6, 7.

‡ Bower, 1882.

nuclei may escape enclosure in a compartment and so play no further part in the history of the sac. If fertilisation is not effected, endosperm-formation proceeds without interruption until it fills the sac.

Occasionally, as in *Welwitschia*, an endosperm compartment encloses an unusually small number of free nuclei. In such a case fusion is certainly delayed and probably does not occur at all; the cell undergoes a great increase in size and probably in the end collapses under the pressure of the surrounding tissue. Apart from such cases, which in *Gnetum africanum* appear to be rare, the primary endosperm compartments always enclose many free sac-nuclei, so far as is known, the primary endosperm-cell being later constituted by nuclear fusion. In the seed the endosperm-cells are very frequently multinucleate, the nuclei showing signs of deterioration. This condition is possibly attained by nuclear fragmentation. The endosperm organises two growing points, one at each end, and an axial column of small cells among which the growth of the proembryos mainly takes place.

4. *The Oöspore and Proembryo.*

In *G. africanum* the oöspore almost certainly gives rise to a small mass of proembryos. Each proembryo becomes a long tortuous tubular cell containing a number of free nuclei, as indicated by Karsten, Lotsy, and Coulter for other species.

When the outstanding facts of the life-history of *Welwitschia* were presented certain theoretical questions, arising from the peculiar characters of endosperm found in that genus, were discussed.* More particularly, attention was directed to the possibility of the endosperm of the Angiosperm being derived from a more primitive form whose main characters are preserved in *Welwitschia*. The use of the characters of the sac of *Welwitschia* in attempting to establish a hypothesis of this nature was always open to the criticism that their value as evidence of phylogeny is problematic, owing to the aberrant characters of this highly-specialised genus. But the value of the evidence furnished by *Welwitschia* can no longer be doubted now that it is established that the same type of endosperm, in a less specialised form, occurs also in *Gnetum*. While the case from the side of the *Gnetales* has been thus strengthened, later researches have not justified certain conclusions then adopted regarding the Angiosperm-sac. Also there seems now to be a possibility of relating the *Gnetum* and *Welwitschia* type of endosperm to the prothallus of the lower gymnosperm—a possibility which was not then clear. Consequently it seems desirable to restate the case in the light of such new facts as appear to be relevant.†

* Pearson, 1909.

† The endosperm-hypothesis as stated in 1909 has been reviewed by Lotsy (1911) and by Samuels (1912). It is rejected by the former (see Pearson, 1915 A, pp. 324-327), and favourably considered by the latter.

The nuclei which, in *Gnetum* and *Welwitschia*, fuse to form the nuclei of the primary endosperm-cells are potential gametes which lose this potentiality by fusing. This statement rests upon the following evidence:

1. All the nuclei present in the sac at the end of the free nuclear condition are alike in all visible characters and reactions and in origin, from which they are equally distant, *i. e.* they are all of the same nuclear-generation.

2. Each sac-nucleus follows one of three courses, viz. (1) it becomes fertilised, (2) it fuses with other sac-nuclei to form a primary endosperm nucleus, (3) it does neither, in which case it participates no further in the life-history.*

3. In some species of *Gnetum* (*G. africanum*, *G. ovalifolium*, *G. Rumphianum*), fertilisation may take place while all the sac-nuclei are still free. In this case, according to Karsten,† “jeder einzelne der sämtlichen kerne des Embryosackes gleich geeignet als Eikern zu fungiren.” In *Welwitschia*, under some circumstances, all the sac-nuclei may remain free‡ and in their appearance and reactions resemble those which are fertilised.

4. All the sac-nuclei may participate in the endosperm fusions, in which case fertilisation is of course impossible. This occurs not uncommonly in *Gnetum* and occasionally in *Welwitschia*. In *Gnetum* it is due to the late arrival of the pollen-tube (p. 240), in *Welwitschia* to causes which interfere with the normal growth of the sac in its early stages.§

5. In *Welwitschia* the gametes in the embryo-sac tube may fuse to form a fusion-nucleus in all respects like that of a uninucleate endosperm-cell.|| A small tissue of seven cells seen in an embryo-sac tube¶ is interpreted as an endosperm formed by the division of such a nucleus.

If, then, both in *Gnetum* and *Welwitschia*, *all* the sac-nuclei may participate in the endosperm-fusions while under other circumstances *some* of them become fertilised; if, further, all the sac-nuclei are potentially sexual, as is asserted by Karsten for *Gnetum*, and as seems to be indicated for *Welwitschia* by the abnormal case referred to in § 3 above; while in both cases they are all equal in origin, descent, and visible characters, the state-

* Viz. The unfertilised gametes in the embryo-sac-tubes of *Welwitschia* and those micropylar nuclei of *Gnetum* which are not fertilised and are “crowded out” from the endosperm-fusions (fig. 25, A, B). Cf. Lotsy, 1899, fig. 44, W.o.E.

† Karsten, 1893, p. 357.

‡ Pearson, 1909, p. 353.

§ Pearson, 1909, p. 354.

|| Pearson, *loc. cit.*, figs. 30 A, B.

¶ Pearson, *loc. cit.*, fig. 37 A, cf. fig. 37 B.

ment that "the nuclei which, in *Gnetum* and *Welwitschia*, fuse to form the nuclei of the primary endosperm-cells are potential gametes which lose this potentiality by fusing," would appear to be fully justified.

A more detailed examination of the facts established for *Gnetum* and *Welwitschia* shows that of the three courses which a sac-nucleus may follow, (1) its exclusion from further participation in the life-history is due entirely to the accident of position in both genera; (2) in *Gnetum* its fertilisation or inclusion in the endosperm-fusions is determined (*a*) by the time of arrival of the male gamete, (*b*) by its position with reference to the point in the sac to which the male gamete is carried by the pollen-tube. In *Welwitschia* this alternative is decided mainly by its position in the sac—the greater elongation of the micropylar region making for the retention of sexual characters, the lesser growth of the chalazal and middle regions for endosperm-fusion.*

Lotsy,† in criticising this conclusion (that the free nuclei of the sac are all potential gamete-nuclei), considers that all nuclei of an *x* generation are of equal value, and one can, if one wishes, consider all to be potential gamete-nuclei. This seems to depend upon the exact meaning of the term "potential gamete." This term was formerly used,‡ and is now used, of nuclei which, given the opportunity, will fuse with a male gamete to produce a new sporophyte. There is, as we have tried to show, good reason to believe that all the sac-nuclei after the last free-nuclear mitosis in *Gnetum* and *Welwitschia* are potential gamete-nuclei in this sense. This is not the case, for example, in *Pinus*: the gamete is not present among the free sac-nuclei; it appears at the end of a series of divisions which are undergone by a few of these nuclei only. The rest form prothallial cells, which, like all their descendants, are entirely vegetative in function; they are not potential gametes, nor do they produce potential gametes. It is true that there seems to be a tendency among the Coniferae towards the condition in which all the sac-nuclei are potential gametes, as they are in *Gnetum* and *Welwitschia* (see p. 248). But no Conifer so far described has yet reached this condition. Whether the physiological difference between a gamete-nucleus and a vegetative nucleus, as these appear in the Conifer sac, is great or small, the difference exists. Our position is that this difference no longer exists in *Gnetum* and *Welwitschia*; all the sac-nuclei present in these genera before septation begins are believed to be potential gametes in the sense defined above. Lotsy's comparison of the nuclear fusion which occurs in the prothallial cells of *Taxus*, *Ephedra*, and other of the lower gymnosperms with the nuclear fusion in the endosperm compartments of *Welwitschia* is not valid because (1) the fusing nuclei in the former cases are not gametes, and

* Pearson, 1909.

† 1911, p. 340

‡ Pearson, 1909.

(2) the results of the fusion are entirely different. This question has already been dealt with.*

Systematic writers frequently use as a distinguishing character between the Angiosperms and the Gymnosperms, the formation of endosperm before fertilisation in the latter, after in the former. In certain species of *Gnetum*, possibly in all, either condition may occur. Here, then, the character is of little importance. But in all species of *Gnetum*, as in *Welwitschia* (and in the Angiosperms), *the gametes are always constituted before the formation of endosperm commences*. One most outstanding character of the prothallus is that it produces the gametes if these are not eliminated from the cycle. If both prothallus and gametes are present, the former precedes the latter in ontogeny. The characters of the fusion-nuclei also are not those of a gametophyte. In a life-history which includes reduction phenomena of a normal character, a tissue whose nuclei contain a number of chromosomes which is not constant, but always greater than that characteristic of the sporophyte, can hardly be regarded as a gametophyte. Therefore to apply the term "prothallus" to the endosperm of *Gnetum* or *Welwitschia* is, in our view, a quite unjustifiable "Spiel mit Worten." †

On the other hand, there appears to be no reasonable ground for regarding this tissue as a sporophyte. The sperm-nucleus takes no part in its formation—it is formed even in ovules which are not pollinated—and there is no evidence whatever that it is a monstrous embryo of apogametic origin. The number of the fusing nuclei, the number and character of the fusion-nuclei and all the circumstances of the fusion are opposed to this view.

It appears to be more in harmony with the facts to regard the endosperm of *Gnetum* and *Welwitschia* as a tissue, probably rather an organism, ‡ which has been "side-tracked" from the direct line of the life-cycle—a "by-product resulting from the fusion of potentially sexual nuclei, and functioning in the same manner as the prothallus of the lower seed-plants which it replaces in the nutrition of the embryo. § In order to emphasise the fact that it is neither a gametophyte nor a sporophyte, it was proposed to designate it "trophophyte." § In view of the recent additions to our knowledge of the life-history of *Gnetum*, this proposal seems unobjectionable so long as there is no implication that the trophophyte is a new "generation" intercalated into the life-cycle. Diagrammatically its place in the life-cycle may be represented as on the following page.

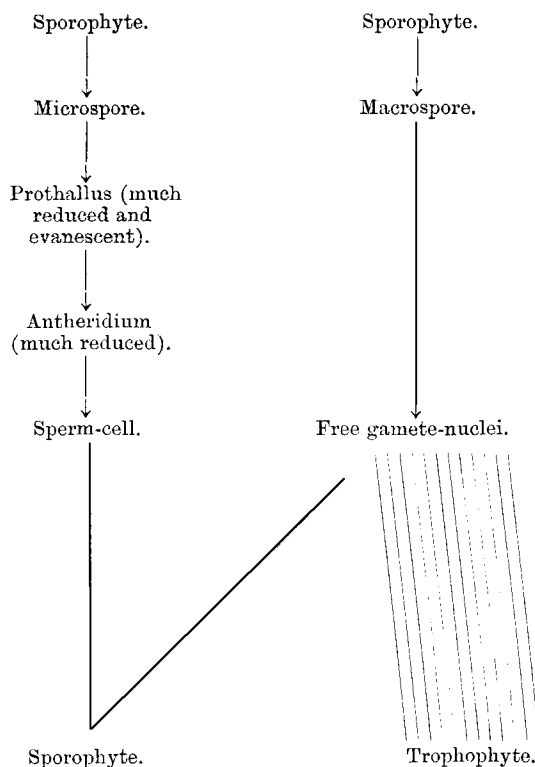
There are two marked differences between the endosperms of *Gnetum* and *Welwitschia*, viz. (1) the septation in *Gnetum* occurs progressively from the base upwards; in *Welwitschia* it is practically simultaneous throughout

* Pearson, 1915 A, pp. 324-327.

† Lotsy, 1911, p. 340.

‡ Lotsy, 1899, p. 93.

§ Pearson, 1909, p. 355.



the length of the sac* ; (2) in *Gnetum* fertilisation occurs within the sac before septation is completed or even begun ; in *Welwitschia* fertilisation is effected after the completion of septation and not in the sac, but in the nucellar cap into which the female gametes are conducted by tubular prolongations of the compartments in which they are enclosed—embryo-sac tubes.† A reasonable view of the relations between these forms, as indicated by the differences disclosed in the development of their sacs, is that *Gnetum* is the more primitive.‡ The formation of primary endosperm-cells by septation and nuclear fusion occurs throughout the sac in both cases. But these processes are completed more rapidly in *Welwitschia* than in *Gnetum*, and to this is due the fact that the functional gametes are enclosed in a compartment in the former case, free in the sac in the latter. The speeding up of these occurrences in *Welwitschia* may perhaps be correlated with the extreme form of siphonogamy which prevails in this genus, and this may quite well be of the nature of an adaptation to the necessity for shortening

* Pearson, 1909, p. 351.

† *Loc. cit.*, p. 349.

‡ See p. 240.

the period separating the beginning of spore-germination and the consummation of fertilisation—an adaptation to zerophytic conditions.* On this view the embryo-sac of *Welwitschia* in its development up to the stage of fertilisation is highly specialised. Strasburger thought that no great weight was to be attached to the occurrence of embryo-sac-tubes in *Welwitschia*. But the relationships between *Gnetum* and *Welwitschia* would be established on a sounder basis if any indications of a tendency to form such structures were recognised in *Gnetum*. So far there is no reliable evidence of such a tendency. On the analogy of the pollen-tube, it might be anticipated that the embryo-sac-tube in its original form was haustorial in function. Tubular prolongations, presumably haustorial, from the micropylar end of the sac are described for *Gnetum funiculare* and *G. verrucosum*,† but since these are formed very early in the development of the sac and are destroyed ‡ long before its septation, there is little or no justification for regarding these as in any way related to the embryo-sac-tubes of *Welwitschia*. Lotsy § has recently adopted the view that structures in the embryo-sac of *G. Ula*, which he first regarded as apogametic embryos,|| are more probably the homologues of the embryo-sac-tubes of *Welwitschia*. But, owing to the imperfections of his material, the life-history of this species is only very partially known, and the value of the suggestion is consequently doubtful. But at least it directs attention to the possibility of gaining further knowledge of the origin of these remarkable structures and, incidentally, of the relationships between *Gnetum* and *Welwitschia*, from species of *Gnetum* of which at present little or nothing is known save the fact of their existence.

The phylogeny of the *Gnetum* type of embryo-sac is at present obscure. Prominent among the facts which justify the conclusion that it was immediately derived from that of the lower seed-bearing plants are the existence of *Ephedra*, which retains the intrasporic prothallus, and the characters of the nucellus, of the micropyle and of the pollen-grain in *Gnetum* and *Welwitschia*. It has been shown (p. 236) that the pollen-grain, both in the course of its development from the microspore and in its later germination, exhibits unmistakable signs of affinity with the gymnosperm pollen-grain. It is probable that the products of the germination of the macrospore are equally related to those which are typical of the lower gymnosperm. There are no grounds for seeking elsewhere for the origin of the *Gnetum* sac.

The fundamental differences between the sac of the lower Gymnosperm and that of *Gnetum* are: (1) The presence of vegetative tissue of the gametophyte in the former and its complete absence from the latter;

* Strasburger, 1872, p. 295; Pearson, 1909, p. 387.

† Karsten, 1893, pp. 354, 355, Taf. 9, figs. 45, 46.

‡ *Loc. cit.*, p. 354.

§ Lotsy, 1911, pp. 350-352.

|| Lotsy, 1903.

(2) the presence of archegonia in the former and their complete absence from the latter; (3) the reduction of the oosphere of the former to a free nucleus in the latter. These differences are physiologically very considerable. Nevertheless, if the one sac is directly derived from the other, there is every reason to suppose that it is the consequence of a comparatively simple series of reductions, a series, perhaps, not less simple than those which have resulted in the reduced type of pollen-grain. *Primâ facie* it is not improbable that parallel series of reductions affecting the development of the free microspore, and the microspore retained permanently within the sporangium, should produce very divergent results. In the case of the pollen-grain the autophytic prothallus of the pteridophyte has lost its assimilating function and undergoes its shortened development under new physiological conditions. But the physiological changes which are bound up with the evolution of the prothallus of the seed from the free-living type are more considerable. While the development of the microspore is much less shortened than that of the microspore, it has not only lost the power of assimilation, but, for a prolonged period, it leads the life of a parasite, becoming in due course the host of a new parasitic sporophyte. It is not unlikely, therefore, that its stability is considerably impaired, and that a further series of reductions should produce results apparently out of proportion to the causes to which they are immediately due.

If we may assume the existence of certain tendencies and their complete realisation in the final stages of the evolution of the Gymnosperm prothallus, the conditions now obtaining in the embryo-sac in certain species of *Gnetum* could be realised. These tendencies are: (1) The extension of the potentialities of an Archegonium-initial nucleus to every nucleus in the sac; (2) the reduction of the Archegonium to one (or more) gametes; (3) the organisation of these at a period antecedent to the formation of cell-walls.

During recent years a number of Gymnosperms have been investigated in which the Archegonia (or archegonial initials) are very numerous and irregularly distributed upon or within the prothallus. These occur both in the Cycads (*Microcycas*)* and in the Conifers (*Araucaria*,† *Agathis*,‡ *Sequoia*,§ *Widdringtonia*,|| *Callitris*,¶ *Actinostrobus***). In *Sequoia* the pollen-tube appears to exercise at least a directive influence upon the development of the archegonial initial.†† In *Widdringtonia*, *Callitris*, and

* Caldwell, 1907.

† Seward and Ford, 1906, pp. 366, 367.

‡ Eames, 1913, p. 5.

§ Lawson, 1904, pp. 14 and 599, fig. 17.

|| Saxton, 1910 A, text-fig. 1.

¶ Saxton, 1910 B, p. 560.

** Saxton, 1913, p. 327.

†† Lawson, 1904, p. 17; *i. e.* not confined to a group at the micropylar end of the prothallus.

Actinostrobis the position of the pollen-tube "determines which of the very numerous archegonial initials become functional archegonia."* It is, therefore, clear that nuclei in any part of the prothallus in some Conifers may acquire the potentialities of those of archegonium-initials, though these potentialities sometimes owe their realisation to conditions not inherent in the prothallus. If this is the expression of a tendency which develops as the evolution of higher forms proceeds in the Conifers or other archegoniate seed-bearing groups, its complete development will convert the prothallus into a mass of archegonia, with the elimination of all vegetative tissue. That there is adequate evidence of the existence of such a tendency in the Coniferae is the opinion of some who have made a study of the group.†

Coulter and Chamberlain recognise a further tendency "among Pinaceae, and notably among Coniferales in general . . . to develop archegonia earlier and earlier in the ontogeny of the gametophyte, a tendency that expresses itself somewhat irregularly but none the less clearly, and that reaches its extreme expression in the appearance of archegonial initials immediately after the free nuclear stage of the gametophyte."‡ This condition is attained, for example, in *Torreya*, in which "any earlier differentiation would occur in the stage of the free nuclei and would result in the selection of eggs and in the elimination of archegonia."§ *Cupressus Goveniana* is stated by Land,|| on evidence furnished by Juel,¶ to have taken "a step beyond the archegonium and formed free eggs." Such a case occurring among the Conifers would be of particular interest, but Land's statement goes beyond Juel's facts. The latter found that cell-formation in the embryo-sac failed in nearly all cases studied, but "einmal fand ich ein zelliges endosperm mit jungen Archegonienlagen."** Fertilisation was not observed; failing this, it is impossible to regard the free nuclei of the sac as gametes. The one case in which the formation of vegetative cells and archegonial initials was observed rather suggests that the majority of the ovules were immature or abnormally developed in the artificial conditions|| under which the tree was growing.

The typical form of archegonium in the gymnosperms is already greatly reduced; reduction carried very little further would eliminate all but the essential part of it, the gamete. The neck-canal cell has disappeared and a general tendency to eliminate the ventral canal cell is recognised.††

There is, therefore, some evidence that these tendencies do exist among

* Saxton, 1913, p. 327.

† Coulter, 1909; Saxton, 1913; Land, 1907.

‡ Coulter and Chamberlain, 1909, p. 263.

§ *Idem, loc. cit.*, p. 330.

|| Land, 1909, p. 285.

¶ Juel, 1904.

** Juel, 1904, p. 57.

†† Coulter and Chamberlain, 1910, p. 420.

the Coniferae and that they are not confined to this group. An embryo-sac, in which all three have reached their utmost limits, would contain free nuclei, each nucleus being capable of functioning as a gamete—the condition believed to be realised in *Gnetum* and *Welwitschia* before septation.

In the primitive condition of such a sac it is probable that fertilisation would occur while all the nuclei were still free—as in species of *Gnetum*. The free gamete-nuclei which remained unfertilised would constitute the only material from which a new nutritive tissue for the needs of the embryo could be organised. Of the factors which led to the septation of the sac-protoplasm and the fusion of the free nuclei enclosed in each compartment so formed, we have no knowledge. As a result of such a series of reductions as has been assumed, new physical and physiological conditions would prevail in the sac. It is possible that the causes inducing the new behaviour both of the protoplasm and of the free nuclei were inherent in the physical and physiological conditions prevailing in a large cell under uniform pressure with vacuolated protoplasm and many nuclei, none of which possessed a full complement of vegetative characters.

There is not sufficient evidence at present available to justify the direct derivation of the embryo-sac of *Gnetum* and *Welwitschia* from that of the Coniferales. The seed appeared independently in more than one group. The tendencies which seem to be present in the evolution of the prothallus in the Conifers and less certainly in the Cycads, probably existed in all primitive seed-bearing groups, and may have prevailed in more than one. It is true that *Ephedra* “is most reasonably connected with the Coniferales,”* and that striking comparisons can be instituted between *Gnetum* and certain Conifers.† But the relationships of *Gnetum* and *Welwitschia* to *Ephedra* are by no means clear, and *Ephedra* possesses very marked characters which do not occur in the Coniferales. Land’s conclusion that “there is no proof that the Gnetales have been derived from or are directly related to any living group”‡ is still fully justified.

A morphological comparison of the embryo-sac and endosperm of *Gnetum* with those of the Angiosperm is inevitable, because (1) the primary endosperm-nucleus or nuclei are in both cases constituted by the fusion of nuclei related in the same manner and degree to the functional gamete; (2) the physiological results of the fusion are closely similar; and (3) these, with *Welwitschia*, are the only known cases in which endosperm is formed in this manner.§

* Coulter and Chamberlain, 1910, p. 402.

† Cf. Saxton, 1913, p. 340.

‡ Land, 1907, p. 288.

§ Land (1907, pp. 278, 279) calls attention to the occurrence in *Ephedra* of an intra-archegonial “ephemeral nutritive mass” whose origin and structure are obscure. He sees in this “a suggestion of the origin of endosperm . . . in the

We may first consider some characters of the Angiosperm-sac which appear to possess importance in this connection. In the form most commonly met with, the 8-nucleate sac, the nuclei show a considerable degree of physiological differentiation; six of them are incorporated in units of protoplasm, two remain free; of these, only one normally functions as a gamete; three, frequently increased by division (antipodals) seem either to be of no physiological account or to be concerned in the nutrition of the sac in its early and middle stages; two (synergids) are very definitely associated with the oosphere in the egg-apparatus; the two free nuclei fuse to form the primary endosperm-nucleus. There are, however, many cases in which a much lower degree of differentiation obtains. The function of the oosphere may be performed by a synergid; * embryos are said also to arise from antipodal cells and from polar nuclei; † in *Alchemilla* ‡ all the antipodals, as well as the polar-nuclei, are included in the fusion which constitutes the primary endosperm-nucleus.

TABLE I.

	Total sac-nuclei.	Synergids.	Fusing sac nuclei.	Antipodals.
<i>Euphorbia virgata</i> §	16	1	14	0
<i>E. procera</i> 	16	2 ?	4	3 ?
<i>E. palustris</i> ¶	16	2 ?	4	3 ?
<i>Peperomia hispidula</i> §	16	1	14	0
<i>P. pellucida</i> §	16	1	8	6
Penaeaceae §	16	2 ?	4, 5 or 6	3 ?
Gunnera §	16	2	7	6
Some Podostemaceae §	4	2	0	0
Cypripedium	4	1	2	0

The sac-nuclei are frequently less or more than eight in number. In these we find varying degrees of specialisation and varying numbers of fusing-nuclei. The numbers of synergids, fusing-nuclei, and antipodals found in some of these are contrasted in Table I.

These differences in the number and behaviour of the sac-nuclei are very

strict Angiosperm sense." The appearance in *Ephedra* of an endosperm of the same character as that of *Gnetum* is not improbable, and its recognition would be of great interest. The facts at present known regarding this "nutritive mass" are not sufficient to justify a comparison.

* Coulter and Chamberlain, 1903, figs. 102, 103.

† *Loc. cit.*, Guignard, *ex* 221; Opperman, 1904.

‡ Murbeck *ex* Coulter and Chamberlain, 1903, p. 93. Apparently the number of fusing-nuclei is very inconstant.

§ See Table II.

|| Modilewski, 1908.

¶ Modilewski, 1911.

striking, and any explanation of the origin of the Angiosperm-sac and of the significance of the endosperm-fusion must take them into account. At least the following conclusions are clearly indicated, viz.: (1) The number of the sac-nuclei is not constant; the functions of the sac may be adequately performed by 4, 8, or 16 sac-nuclei; (2) the degree of differentiation exhibited by the sac-nuclei varies within wide limits; (3) the gamete characters are not always resident in the oösphere alone; (4) the production of the primary endosperm-nucleus by the fusion of two polar-nuclei is not in any way dependent upon any specific characters present in the latter and absent from other sac-nuclei.

The first three of these conclusions, which are completely established by the facts, constitute evidence in favour of the view that the Angiosperm-sac is descended from one in which the nuclei were not differentiated at all. Such a sac is known in *Gnetum* and perhaps also in *Juglans**—a sac in which all the free nuclei are believed to be potential gametes, *i. e.* actually capable of being fertilised. It is therefore suggested that in exactly the same sense as the oösphere-nucleus of the Angiosperm is homologous with the female gamete of *Gnetum*, so the other sac-nuclei of the Angiosperm are homologous with those of *Gnetum*, and therefore the endosperm of the Angiosperm is homologous with that of *Gnetum*. With regard to the last, Coulter† lays stress on the fact that the endosperm of the Angiosperm is undifferentiated—"growth, not organisation." Its undifferentiated character compared with *Gnetum* (p. 243) is explicable if it be a reduced form. The fact that five of the Angiosperm-nuclei have usually acquired new functions which prevent them from being fertilised and from participating in the endosperm-fusion does not affect this homology, though it places difficulties in the way of its recognition.

A marked feature of the sac is probably the majority of the Angiosperms is its polarity, which has usually exercised a considerable influence upon the theories advanced to explain the origin of the sac in this group. It is conceivable that this character has favoured the development of that degree of specialisation which we find among the sac-nuclei. The egg-apparatus, in which the retention of sexual characters is most marked, generally occupies the position which is most favourable to fertilisation; the antipodal group, in which vegetative characters are most strongly developed, is placed where it can most efficiently perform a nutritive function. With regard to the polarity itself, it may be noted that it could not be strongly marked in a sac crowded with nuclei like that of *Gnetum*; the fact that there are few nuclei in the sacs of most Angiosperms may of course be quite unconnected with their strong polarity, but when there are many (*Euphorbia virgata*, *Peperomia hispidula*, *Juglans*, etc.) they are not usually segregated into groups at the

* Nawaschin, 1895; Karsten, 1902.

† Coulter, 1911.

poles of the sac, and they usually show a lower degree of specialisation than in a sac in which polarity is strongly developed.

The fact that most of the sac-nuclei (sometimes only a small minority) are incorporated in units of protoplasm is a character of the Angiosperm as compared with Gnetum. The transition from the state of a free nucleus to that of a uninucleate-cell is very frequently effected in the ontogeny of the individual. In Gnetum and Welwitschia we have examples in the constitution of the generative-cell in the pollen-grain or pollen-tube, and, if Karsten and Lotsy are correct in stating that the male gamete functions as a free nucleus, in the cell-formation which must immediately precede or follow fertilisation. The nuclei of the Angiosperm-sac are free in their earlier stages; the later incorporation of some of them in uninucleate-cells does not invalidate a comparison with the free nuclei of Gnetum.

We have already seen that the fusion which produces the primary endosperm-nucleus does not depend for its success upon any particular morphological or physiological characters inherent in the polar-nuclei as such. For the further consideration of this question reference may be made to Table II wherein are diagrammatically indicated certain characters of ten selected embryo-sacs.* In each case the descent of the sac from the mother-cell is traced, the nuclei present in each generation being indicated by dots. The nuclei which fuse to form the primary endosperm-nucleus are enclosed in an inner figure.† The participation of the second sperm-nucleus in the fusion is disregarded (see p. 264). The embryo-sac in the forms represented may reach the condition in which it is ready for fertilisation immediately after the 6th, 5th, 4th, or 3rd simultaneous nuclear division, commencing with and including the first division of the mother-cell. For the sake of convenience these are called the 7th, 6th, 5th, or 4th nuclear generation, respectively. In *Euphorbia virgata* (Col. i) the embryo-sac is matured in the 7th nuclear generation, in most Angiosperms (Col. ii) in the 6th, in six other cases (Col. iii-viii) in the 5th; in *Lilium* and *Cypripedium* (Cols. ix, x) in the 4th.

* Little of the very extensive literature on this subject is available here. The information contained in the table is derived from—

Dessiatoff, 1911 ex Samuels, 1912 (*Euphorbia virgata*).

Johnson, 1914 (*Peperomia hispidula*).

Campbell, 1908; Brown, 1908 (*Peperomia pellucida*).

Stephens, 1909 (Penaeaceae).

Samuels, 1912 (Gunnera).

Modilewski, 1909 ex Samuels, 1912 (Onagraceae).

Went, 1908 (Podostemaceae).

Pace, 1907 (*Cypripedium*).

The macrospore mother-cell is indicated in the table by the symbol \oplus .

† Except in Column vii. We have not access to Modilewski's original paper, and the details of the endosperm-fusion are unknown to us.

In these cases the number of sac-nuclei taking part in the endosperm-fusion varies from 0 to 14 (see Table I). Further, while the fusing-nuclei are always of the same generation* as the oosphere-nucleus, their number is in no way dependent upon the generation to which they belong, *e.g.* there are

14 in the 7th and 5th generations (Cols. i and iii).

2 in the 6th and 4th generations (Cols. ii, ix, x).

14, 8, 7, 6, † 5, † 4, or 0 in the 5th generation (Cols. iii, iv, v, vi, viii).

These facts establish at least a probability that the essential character which qualifies a sac-nucleus to participate in this fusion is its freedom. In all cases included in the table this is the only obvious character possessed by these nuclei which is not shown by others which do not join in the fusion. The inconstancy of the number of fusing-nuclei in certain cases—4, 5, or 6 in *Sarcocolla squamosa*‡—shows that, in these at least, the number is determined by circumstances, not by fixed characters inherent in the nuclei themselves. In *Cypripedium* a synergid§ which becomes free from the egg-apparatus and wanders down the sac, fuses with the single antipodal nucleus. It appears from the description that the synergid-cell as a whole undergoes this change of position “as if it were being pushed from its actual position by the inrush of the contents of the (pollen) tube.” It therefore enjoys the same freedom as a free nucleus. The opportunist character of its participation is suggested by the possibility that it sometimes fails.|| In Went’s *Podostemaceae*, the primary antipodal nucleus develops no further, and there is therefore only one polar nucleus; no fusion occurs and no endosperm is formed. In *Peperomia hispidula*, the sac contains only two cells, viz. one synergid and the oosphere; all the other nuclei are free and these all fuse.¶ Similar conditions and the same results are seen in *Euphorbia virgata*** *Peperomia pellucida* with a two-celled egg-apparatus and six peripheral-cells in the antipodal region leaves only seven nuclei free for the endosperm fusion.†† The difference between this species and

* In *Pandanus*, in which the antipodal-nuclei are of a later generation than the oosphere and the upper polar, a varying number of antipodal-nuclei—one to six or more, usually two or three, fuse with the single upper polar-nucleus to form one, sometimes two primary endosperm-nuclei. Campbell, 1911.

† Occasionally five or six sac-nuclei take part in the fusion in the *Penaeaceae* (Stephens, 1909, p. 367, figs. 20 B, 23).

‡ Stephens, *loc. cit.*

§ Pace, 1907, p. 359.

|| *Loc. cit.*, p. 360.

¶ Johnson, 1914, fig. 84. (The details of certain abnormal cases were not fully interpreted, *loc. cit.*, p. 369, fig. 87).

** *Vide* Samuels, 1912, p. 104.

†† Campbell.

P. hispidula is of particular interest; in each case the fusing-nuclei are all those which are free. In *Gunnera macrophylla* there are seven free nuclei which all fuse.* In the 8-nucleate sac of *Alchemilla sericata*, the antipodal nuclei remain free and all four fuse with the upper polar.† These facts certainly afford strong support to the opinion already expressed by Coulter, that the "conditions in the embryo-sac favour fusion of any free nuclei, in any number and of any origin."‡ But if the whole explanation of the act of fusion is found in the fact that the participants are free to respond to conditions favouring fusion, these conditions being inherent in the structure of the sac, the results of the fusion itself may nevertheless be of profound importance. By hypothesis, the fusing-nuclei in their primitive form were potential gametes; in any particular case they may or may not have undergone a measure of reduction. If they have so far reverted to the conditions of vegetative nuclei that they can directly produce a nutritive tissue, then an endosperm may be formed without fusion. Otherwise the formation of an endosperm appears to be dependent upon nuclear fusion which is comparable with the act of normal fertilisation in so far as it produces a stimulus to vegetative growth.§ We should, therefore, expect to meet cases in which the endosperm is eliminated if no fusion occurs, as in Went's Podostemaceae;|| and others in which the vegetative characters of some of the sac-nuclei are sufficiently developed to enable them to produce a vegetative tissue without fusion, as in *Helosis* and a few other forms.¶ A vegetative tissue so formed by the polar or any other of the sac-nuclei is, on the view adopted here, the morphological equivalent of that which frequently results from the division of the antipodal-cells. The sac-nuclei from which it arises have reverted to the vegetative condition from which they have emerged in *Gnetum*. The tissue is gametophytic though it lacks one of the essential characters of the prothallus in that its organisation is necessarily later than that of the gamete, which therefore it does not bear.

In the Angiosperm usually only one fusion-nucleus is constituted, but there may be two**; in *Gnetum* there are many, and the sac becomes septate before fusion. In the Angiosperm no more than 14 sac-nuclei are known to enter into this fusion; in *Gnetum* and *Welwitschia* some hundreds are concerned. In view of the later history of the fusion-nucleus there is probably a limit to the number of gamete-nuclei which can enter into

* Samuels, *loc. cit.*, fig. 18.

† *Vide* Coulter and Chamberlain, 1903, fig. 42.

‡ Coulter, 1911.

§ *Cf.* Sargent, 1900, p. 705; Pearson, 1909, p. 384.

|| Went, 1908.

¶ *Vide* Coulter and Chamberlain, 1903, pp. 166, 167.

** Campbell, 1911, p. 786.

its composition. The septation of the sac in *Gnetum* and *Welwitschia* and the formation of many fusion-nuclei may be consequences of this limitation.

In the Angiosperm the fusion-nucleus usually undergoes a series of simultaneous divisions and the protoplasm in which the resulting free nuclei lie becomes completely septate to uninucleate cells or incompletely to multinucleate compartments in which ultimately all the nuclei fuse.* But in some cases, as in *Peperomia*, cell-walls are formed in all the divisions of the fusion-nucleus and its descendants,† and the endosperm is cellular from the beginning and throughout its development. This is also characteristic both of *Gnetum* and of *Welwitschia*. When, as is usual in the Angiosperms, “endosperm begins with free nuclear division, a rudimentary plate often appears suggesting derivation from an endosperm in which nuclear division was followed by cell-formation,” ‡ as in *Peperomia*.

Attention has frequently been called to the curious resemblance between the common behaviour of the fusion-nucleus and its products in the Angiosperms and the process by which the prothallus is formed in the macrospore of the Gymnosperm and the massive proembryo in the archegonium of the Cycads and Ginkgo. The endosperm of the Angiosperm is clearly not phylogenetically related to both these latter structures, and this fact in itself makes it the more probable that the resemblance is no evidence for a phylogenetic relationship with either. It is much more likely that the whole explanation is to be sought in the similar conditions under which a single nucleus produces a multicellular tissue in a large cell.

The “triple fusion,” now recorded for an increasing number of Angiosperms, is peculiar to this group.§ Its occurrence is not confined to the 8-nucleate sac; it is observed in *Cypripedium*;|| a sperm-nucleus is one of the five constituents of the primary endosperm-nucleus in *Euphorbia procera*,¶ and it probably fuses with the seven sac-nuclei in *Gunnera*** The participation of the second sperm-nucleus is therefore independent of the number of fusing-nuclei contributed by the sac itself. It is also clear that it is not of universal occurrence among the Angiosperms. It sometimes fails in species in which it normally occurs (as *e.g.* *Naias major*),†† and

* Strasburger, 1880, figs. 46–64; Tischler, 1900.

† Johnson, 1914.

‡ Coulter and Chamberlain, 1903, p. 172.

§ The “double fertilisation” described for *Juniperus virginiana* (Ottley, 1909, p. 40) and for *Thuja* (Land, 1902) is probably not in the same category as the triple fusion of the Angiosperms (*cf.* Samuels, 1912, p. 90). It appears rather to be a case of the true fertilisation of a gamete which in most Archegoniatae is arrested, correctly described as “double fertilisation.” *Cf.* Blackman, 1898.

|| Pace, 1907.

¶ Modilewski, 1908, fig. 7.

** Samuels, 1912.

†† Guignard, *vide* Coulter and Chamberlain, 1903, p. 216.

apparently the development of the endosperm is not affected. But even now, the number of Angiosperm-sacs investigated is comparatively small, and the facts are insufficient to establish any general conclusions regarding the phylogenetic or physiological significance of this phenomenon. But since it does not occur in some Angiosperms, its absence from Gnetum does not weaken the comparison between the two sacs which it is now sought to establish.

It has been suggested above that the free nuclei fuse because they are free—in other words, that the physical and physiological conditions of the sac are favourable to the fusion of nuclei whose situation imposes no obstacle. This is the view suggested by Coulter * for the Angiosperms as by the writers for Gnetum and Welwitschia. If it is true for the Angiosperm, the sperm-nucleus which has escaped the influence of the egg-apparatus † may be drawn into fusion with the free sac-nuclei by the influences which cause these to fuse.‡ In such a case the participation of the male nucleus is, in its origin, of no more significance than that of any of the sac-nuclei.‡ It may nevertheless exercise an important influence in increasing the vigour of the fusion-nucleus as it does in introducing foreign characters into the endosperm. Since the endosperm can be formed without it, the view that the endosperm, which results from the triple fusion or its equivalent (as in *Euphorbia procera*), is a monstrous embryo appears to be without justification.

It seems, then, that some of the characters of the Angiosperm-sac which have not yet been satisfactorily explained, may be more or less accounted for on the hypothesis that this sac has had its origin in a less specialised form not necessarily very different from that now found in Gnetum. Such a derivation implies a very considerable shortening of that portion of the life-cycle which connects the macrospore-mother-cell with the functional gamete. It remains to inquire whether there is any evidence of a tendency to accomplish such a shortening of this series of events. The Gnetales at present furnish no certain evidence on this point. It can therefore only be looked for among the Angiosperms themselves; if it has had an effect in producing the Angiosperm type from a form essentially similar to that of Gnetum, it is not improbable that it continues to act among the Angiosperms.

Recent writers on the life-history of the Angiosperm have recognised the existence of a tendency to include in the embryo-sac § the results of the reduction divisions of the mother-cell (see Table II). The results of this tendency are seen in Columns iii–vi, ix, and to a less extent in viii and x, if, as is probable, these are correctly regarded as derived from a more ancient

* Coulter, 1911.

† In *Naias major*, e. g. the second sperm-nucleus may fuse either with a synergid or with the polars (Coulter and Chamberlain, *loc. cit.*).

‡ Coulter, *loc. cit.*

§ Johnson, 1914, pp. 384 *et seq.*

type in which the spore-mother-cell formed a tetrad or row of four spores, one of which became the embryo-sac, as in the forms represented in Columns i, ii, and vii. This tendency prevails regardless of the final structure of the sac resulting from its action. A similar tendency is perhaps present in the Gnetales, for, according to Lotsy, the macrospore-mother-cell of *Gnetum* undergoes only one complete division, the nuclei resulting from the homotypic division being included in the embryo-sac.*

But there seems to be evidence in the facts presented in the table that this tendency may become operative in the later as well as, or instead of, in the earlier stages.

In Modilewski's Onagraceae (Col. vii), in which the reduction divisions are normal, the oosphere appears in the 5th instead of in the 6th generation; in *Cypripedium* only the homotypic division takes place in the sac, nevertheless the oosphere belongs to the same generation as in *Lilium*—the 4th. If these cases are due to the prevalence of a tendency to shorten this part of the life-cycle, then *Euphorbia virgata* (Col. i) must be regarded as a more primitive form or a reversion to a more primitive form than that which is established in the majority of Angiosperms.

While the evidence is admittedly very meagre, there is still some reason to believe that the tendency to shorten the series of events between the mother-cell stage and the organisation of the gamete has been operative not only at the beginning of the series but also at later stages in the outogeny. If so, the gamete which in *Welwitschia* appears in the 12th or 13th nuclear generation, in *Gnetum* probably somewhat earlier, would in later forms be organised in earlier stages, as in the 7th, 6th, 5th, or 4th, as in existing Angiosperms. It is at least to the credit of the hypothesis that the tendency which, it is suggested, has yielded the Angiosperm-sac, is also believed to exist among the lower Gymnosperms,† and may have led to the evolution of the free gametes of *Gnetum* from the intrasporic vegetative prothallus of the lower forms.

In brief, the hypothesis now outlined derives the Angiosperm-sac from a primitive form whose essential characters are preserved in *Gnetum*. The evolutionary tendencies believed to have led to this result are (1) a tendency to shorten the series of simultaneous divisions of the sac-nuclei, with the results that (a) the nuclear generation in which the gamete or gametes appear is brought nearer to the macrospore-mother-cell and (b) the number of nuclei belonging to this generation is reduced in geometrical progression—involving perhaps the grouping of the reduced number of sac-nuclei at the poles of the sac; (2) a tendency to the functional differentiation of the nuclei so grouped, which involved the constitution of a number of cells within the sac and consequently a still further reduction in

* Lotsy, 1899, p. 102.

† See p. 254.

Table II.

I	II	III	IV	V	VI	VII	VIII	IX	X
<i>Euphorbia virgata.</i>	Many <i>Angiosperms. hispidula.</i>	<i>Peperomia hispidula.</i>	<i>Peperomia pellucida.</i>	<i>Penaeaceae. Gunnera.</i>	<i>Some Onagraceae. Podostemaceae.</i>	<i>Some Onagraceae. Podostemaceae.</i>	<i>Some Lilium.</i>	<i>Lilium.</i>	<i>Cypripedium.</i>
1	2	3	4	5	6	7	8	9	10

the number of free sac-nuclei available for the endosperm-fusion. There is some evidence for the existence of the first tendency within the Angiosperm group as at present constituted, and the fact that different degrees of polarity and of functional differentiation of the sac-nuclei are found among the Angiosperms may perhaps be regarded as evidence for the second.

It is further suggested that the conditions preserved in the *Gnetum*-sac were similarly derived from those of an intrasporic prothallus whose fundamental characters are found in the Cycads, Ginkgo, the Conifers, and Ephedra, by the operation of a similar tendency to shorten that portion of the life-cycle which lies between the macrospore-mother-cell and the functional gamete.

It is, however, not suggested that the *Gnetum*-sac is directly derived from that of the Conifers, nor the Angiosperm-sac from that of *Gnetum*.

Reviewing that portion of this hypothesis which touches the relations of the Gnetales and the Angiosperms, Coulter remarks, "while the argument . . . is not convincing, it shows that the case is still open."* This is a fair summing up of the situation. The endosperm of the Angiosperm presents one of the outstanding problems of morphology. We suggest that its solution is to be sought along the lines now indicated. But neither this nor any other hypothesis yet formulated can be accepted until it can muster in its support more convincing evidence than is yet forthcoming.

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* Coulter, 1916, p. 85.

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EXPLANATION OF PLATES XLV—L.

PLATE XLV.

FIG.

1. *G. scandens*. Diagram of longitudinal section of nucellus to show pollen-chamber and embryo-sac. $\times 30$.
2. *G. africanum*. Pollen-chamber partially blocked by papilla-like cells from micropylar tissue. $\times 180$.
3. *G. africanum*. *p.c.* Pollen-chamber. *p.t.* Pollen-tube. *c.* Degenerating tissue of nucellus. $\times 40$.
4. *G. africanum*. An unpollinated ovule. *c.* Cavities formed by degeneration of nucellar tissue. *end.* Embryo-sac filled with endosperm. $\times 32$.
5. *G. africanum*. A fertilised ovule containing many proembryos. Endosperm growing upwards into cavities (*c.*) formed by degeneration of nucellar tissue. Dotted area (figs. 1-6), active nucellar tissue. $\times 30$.
6. *G. Gnemon*. An ovule with several pollen-tubes (*p.t.*). σ . Sperm-cell. *end.* Endosperm. *pv.* Pavement tissue. *s.n.* Free sac-nuclei (*cf.* fig. 21B). $\times 30$.

PLATE XLVI.

7. *G. Gnemon*. Lower portion of a micropyle in oblique longitudinal section showing many pollen-grains. $\times 400$.
8. *G. africanum*. Portion of a pollen-chamber in longitudinal section showing three germinating pollen-grains, of which two retain the prothallial nucleus. *ea.* Split exine. $\times 700$.
9. *G. africanum*. A pollen-grain and young tube showing generative cell and tube-nucleus. $\times 1200$.
10. *G. africanum*. A generative cell and tube-nucleus from a tube which has penetrated to one-third of the depth of the nucellar cap. $\times 1200$.
11. *G. Gnemon*. A fertilised ovule showing large cavity (*c.*) above embryo-sac formed by destruction of nucellar tissue by pollen-tubes (*p.t.*). $\times 30$.
- 12-15. *G. africanum*. Binucleate sperm-cells (anterior ends directed downwards). Fig. 12 $\times 1720$; the rest $\times 1200$. Fig. 15. Plate xlvii.

PLATE XLVII.

16. *G. Gnemon*. Pollen-tube with uninucleate sperm-cell and tube-nucleus. Tip of tube is almost in contact with top of embryo-sac. $\times 1200$.
17. *G. africanum*. Portion of embryo-sac showing simultaneous division of free nuclei $\times 700$.
18. *G. africanum*. Lower part of embryo-sac showing endosperm-cells and free gamete-nuclei. $\times 400$.
19. *G. africanum*. Middle portion of an unfertilised sac showing the upper part of the septate endosperm and the lower portion of the free gametes (a little contracted). A mass of endosperm-cells (*d.*) formed by cell-division. A large compartment (*e.*) containing two free nuclei. $\times 420$.

PLATE XLVIII.

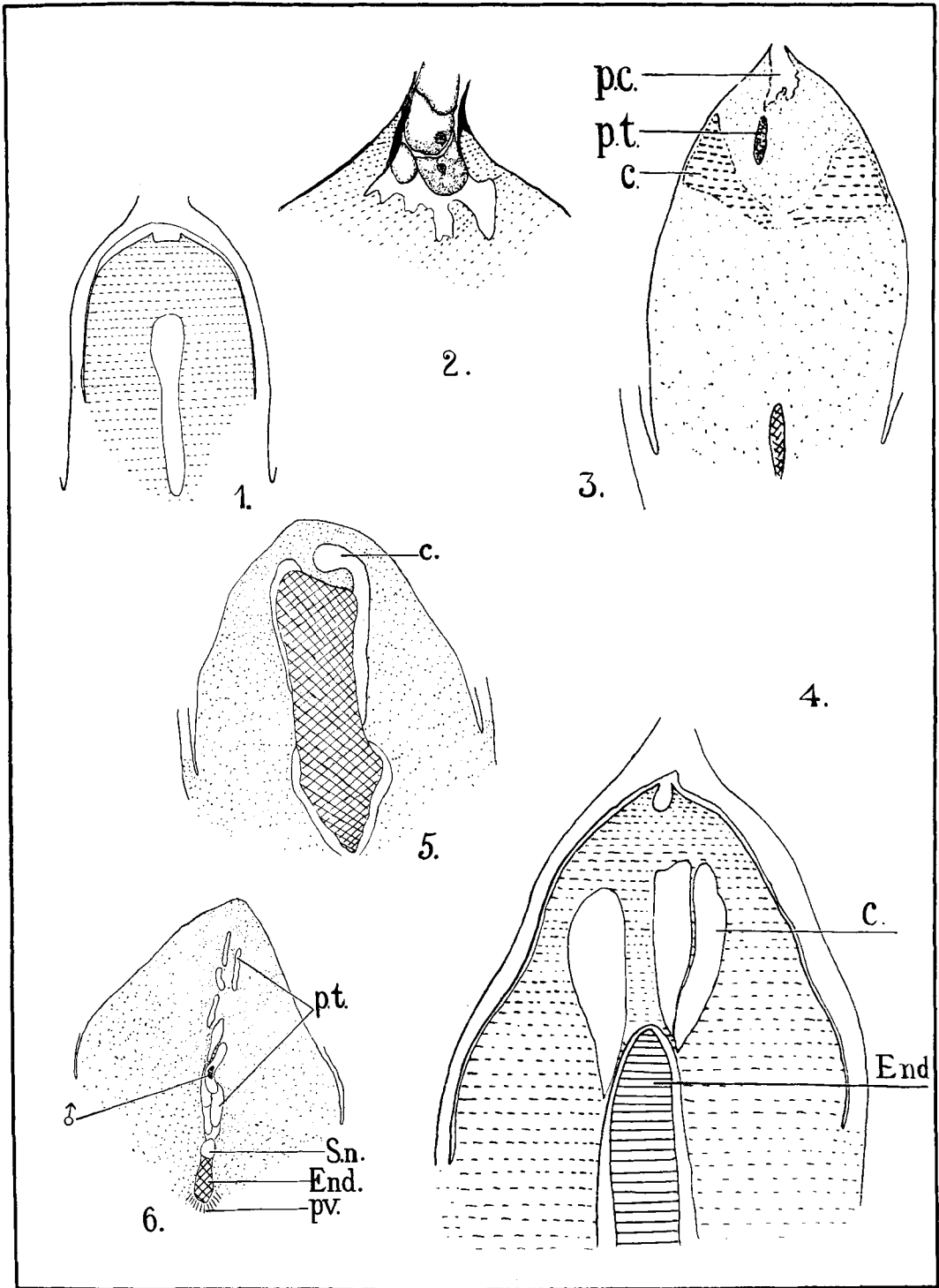
20. *G. africanum*. Two cells from the endosperm of an advanced ovule. The one to the right is multinucleate, the nuclei showing signs of deterioration. $\times 720$.
- 21A. *G. Gnemon*. The lower part of a sac showing multinucleate endosperm compartments. $\times 720$.
- 21B. The sac from the ovule of fig. 6 more advanced than 21A. Nuclear fusion has occurred in all the endosperm compartments shown. The apparent delay in the completion of the endosperm in the micropylar region is probably caused by the presence of oöspores. $\times 300$.
- 22A. *G. africanum*. The micropylar end of the sac showing three multinucleate endosperm compartments (*f.*) in which nuclear fusion is in progress. *N.* Remains of nucellar tissue. *d.* Endosperm-cells produced by division of primary cells constituted by nuclear fusion. $\times 300$.
- 22B. A group of fusing nuclei from one of the compartments of 24A. $\times 1200$.

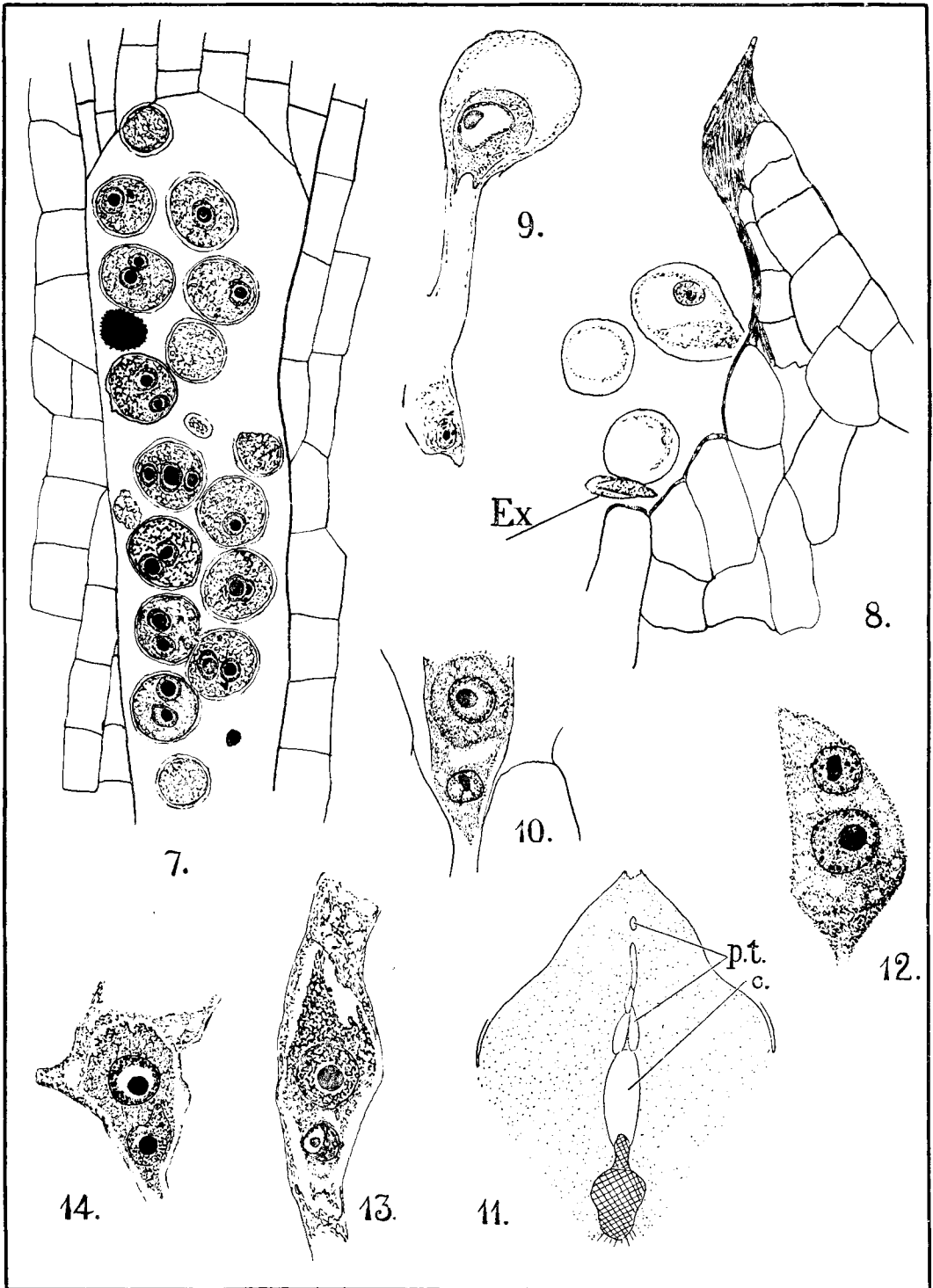
PLATE XLIX.

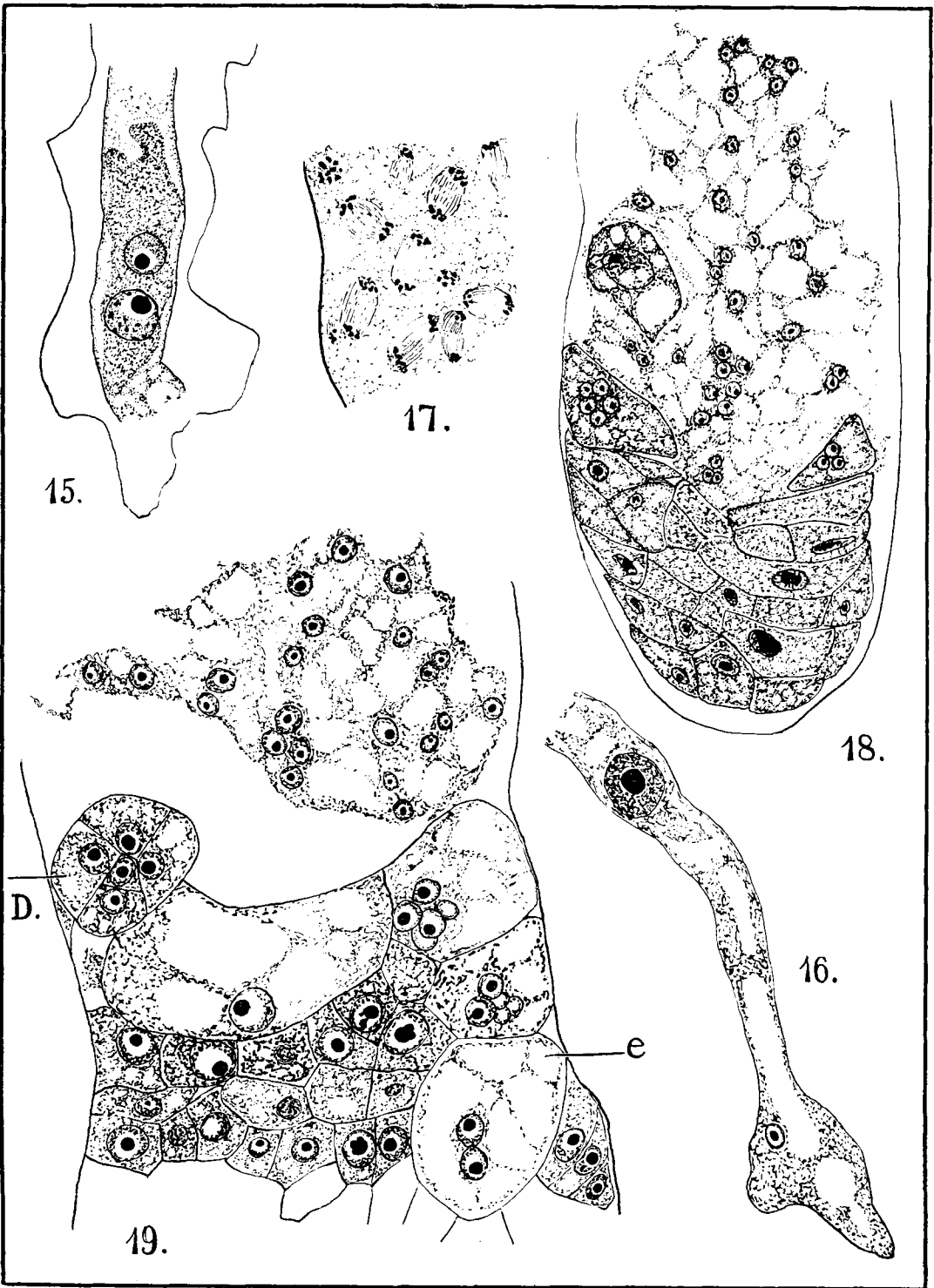
23. *G. africanum*. Portion of the micropylar end of a sac. Two cells (*O.*) are provisionally identified as oöspores. Two others above and to the right are more doubtful. Groups of sac-nuclei (*f.*) fairly certainly represent a state preparatory to that shown by the compartments (*f.*) in fig. 22A. $\times 1200$.
24. *G. africanum*. Micropylar end of sac. *O.* Oöspore. Endosperm-cells are continuous along the sac-wall; in the centre separation is very irregular, a condition considered to be due to the presence of this and other oöspores, the latter not seen in this section. $\times 500$.
- 25A. *G. africanum*. Micropylar end of a sac showing summit of the endosperm. *N.* Crushed nucellus. *s.n.* Sac-nuclei excluded from fertilisation and from incorporation in the endosperm by fusion (*cf.* Lotsy, 1899, fig. 44, W.o.E.). $\times 160$.
- 25B. Four of the nuclei (*s.n.* of fig. 25A). $\times 1700$.

PLATE L.

26. *G. africanum*. Beginning of the formation of the column of small-celled tissue near the middle point of the axial line of the endosperm. $\times 300$.
27. *G. africanum*. Portions of three proembryonal tubes shown in longitudinal section of the endosperm; one of them is binucleate. $\times 700$.
28. *G. africanum*. Distal end of a proembryonal tube containing five free nuclei, of which three are seen in the section. $\times 1200$.







15.

17.

18.

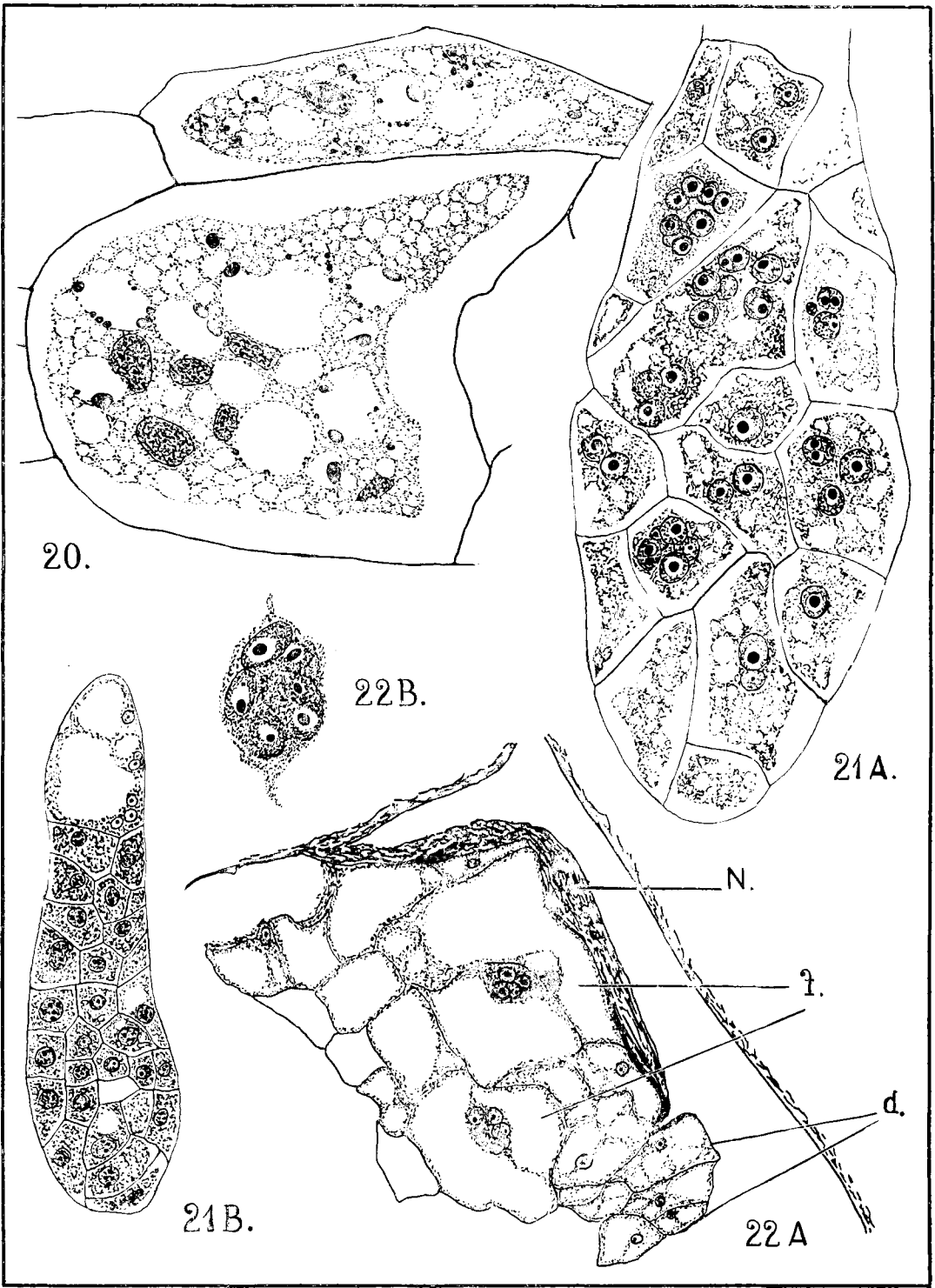
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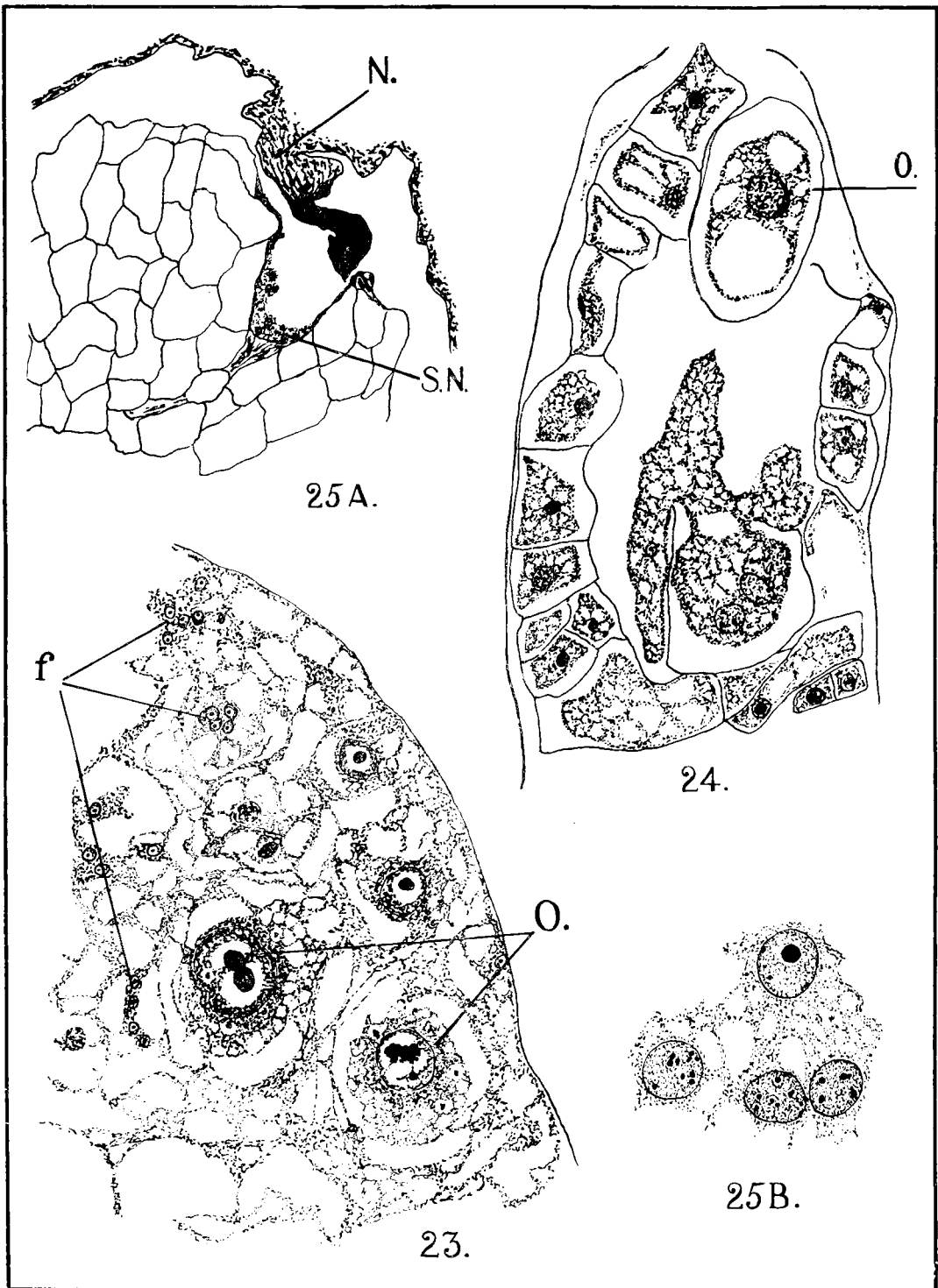
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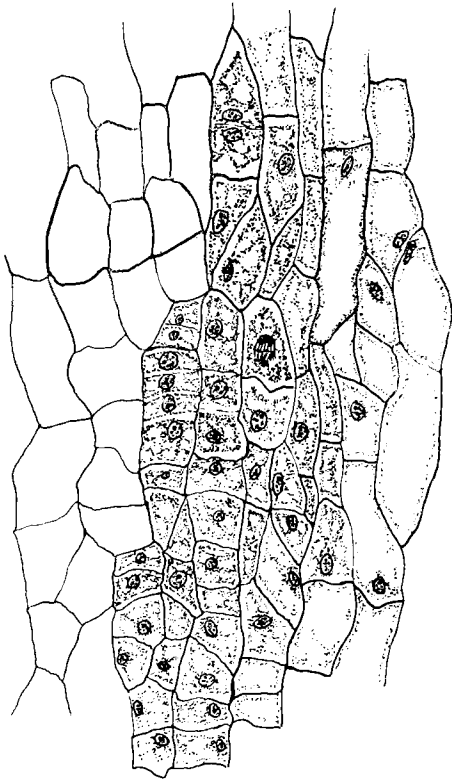
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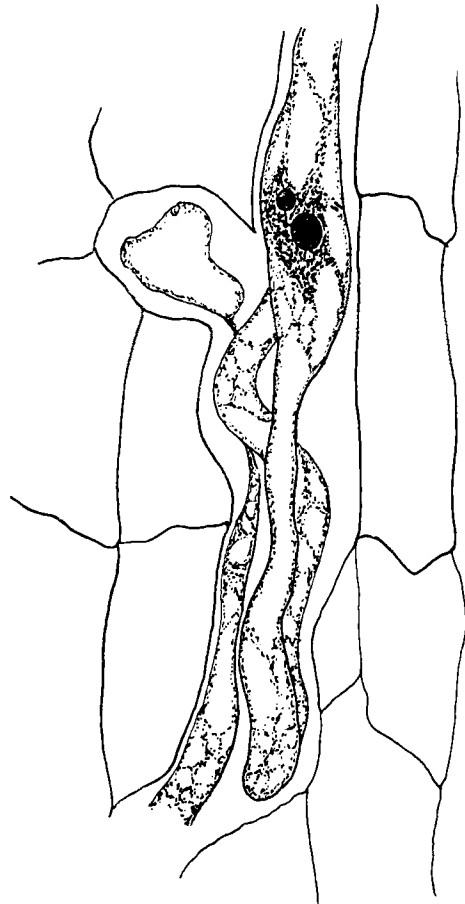


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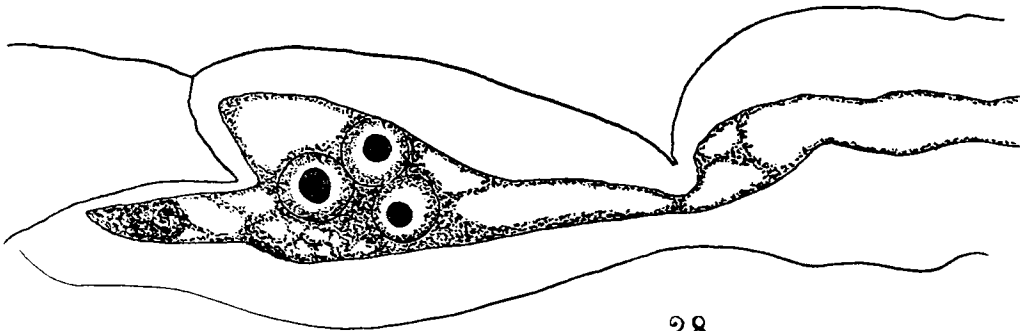




26.



27.



28.