The Embryo-Sac and Embryo of certain Penaeaceae.

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With Plates XXV and XXVI.

THE Penaeaceae are a small family of shrubby Dicotyledons, of an ericoid habit, entirely confined to the south-west region of Cape Colony. The order has yet to be studied from a systematic standpoint, but five genera, with twenty-two species, are recognized by Gilg¹—*Sarcocolla*, *Penaea*, *Brachysiphon*, *Endonema*, *Glischrocolla*. Of these, I have investigated the following species, representing three of the genera—*Sarcocolla squamosa*, *S. fucata*, *S. formosa*, *Penaea mucronata*, *P. ovata*, *Brachysiphon imbricatus*. Of *Endonema* and *Glischrocolla* I have not yet been able to obtain material suitable for embryological work. All the species investigated show exactly the same life-history, and the account that follows may be taken as applying to them all. Material was collected for the most part on the slopes of the mountains of the Cape Peninsula during 1907, and again during a visit to S. Africa from July to September, 1908. Material of *Geissoloma marginata*, belonging to the allied order Geissolomaceae, was collected for comparison at Garcia's Pass in the Langeberge Mountains in September, 1908, during an excursion, kindly arranged by Prof. Pearson for that object; information as to the exact locality of this very rare plant was obtained through the kind agency of Dr. Marloth. The material examined was fixed in acetic-alcohol (two parts of absolute alcohol to one of acetic acid), mercuric chloride (solution in one per cent. acetic acid), various chromo-acetic mixtures, or Fleming's solution. The best results for nuclei and for mature embryo-sacs at about the fertilization period were given by the acetic-alcohol mixture; in the free nuclear stages, however, this fixative caused contraction (cf. Pl. XXV, Fig. 14); for these and for the developing embryo, the chromo-acetic and Fleming's solution

¹ Gilg, in Engler and Prantl, 1894, p. 208.

were more satisfactory. Combinations of Licht-Grün with Diamant Fuchsin (a rapid and clear stain for embryological work), and of the haematoxylins with eosin were the chief stains used.

OVULE AND EMBRYO-SAC.

Throughout the order a four-carpellary, four-locular ovary is found. In *Sarcocolla*, *Penaea*, and *Brachysiphon* each loculus contains two to four erect anatropous ovules inserted in axile placentation near the base; in *Endonema* and *Glischrocolla* the four ovules in each loculus are inserted on an axile placenta, two erect and two hanging. Each ovule is surrounded in its earlier stages by two free integuments, each two cells thick (Pl. XXV, Figs. 1, 2); these, however, soon coalesce with one another, and with the nucellus, and are then recognizable, below the level of the micropyle, only as four regular cell layers at the periphery of the ovule (Pl. XXVI, Fig. 24). In the later stages of their development the outer integument projects beyond the inner. Before they coalesce, the cells at the lip of each integument have begun to divide (Fig. 3), and their further divisions cause each lip to swell out into a cushion-like ring, which, meeting at the centre, blocks the micropyle (Fig. 24).

The archesporium consists of a single sporogenous cell, which at its earliest recognizable stage is found sunk one layer deep beneath the epidermis (Fig. 1). The arrangement of the cell or cells in the layer immediately above it indicates that they and the sporogenous cell were probably derived from a single hypodermal cell by periclinal division. In one case only was there any sign of another sporogenous cell; this is shown in Fig. 2, where the large cell above the embryo-sac is clearly differentiated from the rest of the nucellus by its size and staining properties, and may possibly represent a second potential megaspore mother-cell which has not functioned. The sporogenous cell of Fig. 1 enlarges considerably, becoming meanwhile sunk four to five layers deep by the periclinal divisions of the overlying cells, until it attains the size shown in Fig. 3, when it passes into the megaspore mother-cell condition; the nucleus of the cell there figured is in an early stage of synapsis. In a preliminary note,¹ it was stated that this cell 'appears to form a row of three (?) macrospores'. Further examination leaves no doubt that the reduction division takes place in the embryo-sac itself, and that a preparation formerly interpreted as a row of three megaspores, the upper two disintegrating, must really represent an enlarging mother-cell capped by crushed nucellar cells. Characteristic stages of the reduction division are shown in Figs. 4-8. These are apparently passed through with some rapidity, as ovules in the same ovary may show all stages from the mother-cell nucleus before synapsis to the four-nucleate sac of Figs. 9-11. Material was fixed at different times of day

¹ Stephens, 1908, p. 329.

with the object of securing these divisions, and all the stages here figured, and also the dividing endosperm nucleus of Fig. 25, were found in material fixed on several occasions within a few minutes of mid-day. Other nuclear divisions showed no such relation to any particular time of day. The metaphase of the heterotype division was only seen in one case (Fig. 6), the spindle being here arranged obliquely to the axis of the ovule. The gametophytic number of chromosomes is eleven or twelve. The metaphase of the homotype division is shown in Fig. 7, and the four daughter-nuclei derived from this division in Figs. 8-12. These nuclei usually lie tetrahedrally or crosswise, but they show some variation in this respect; thus in Fig. 10 three are grouped at the bottom of the sac, and the fourth lies towards the top.

As the embryo-sac enlarges a central vacuole is formed, and these nuclei are relegated to the parietal layer of protoplasm (Figs. 11, 12). Each nucleus now divides (Fig. 12), so that four pairs of nuclei are formed (Fig. 13). In one case five groups of nuclei, consisting of four pairs and one single nucleus, were present at this stage (Fig. 14). Possibly this may mean that one of the four original nuclei has divided in advance of the others, and its daughter-nuclei have divided again after becoming separated as the sac enlarged. This preparation was fixed in acetic-alcohol, and is so much contracted that the wall of the embryo-sac has become drawn in, and the nuclei of the nucellar cells which have been crushed by the growth of the sac can be seen imbedded in it. This was the only case observed, at this or at any later stage, where more than the normal number of nuclear or cell groups were present. The four pairs of nuclei normally formed are found usually lying crosswise, one at each end of the sac, and the two at the sides; and as the embryo-sac shows much less variation in the arrangement of its nuclei at this than at the four-nucleate stage, it is probable that the nuclei usually take up this position as the sac elongates. This also is the arrangement most commonly found in the mature embryo-sac (cf. Figs. 17, 18, 25, 26), but it should be noted that both the free-nuclear groups, and the cells that are later formed from them, may take up any position around the periphery of the sac (cf. Figs. 15, 16, 19, 21).

Each of the nuclei composing these four pairs now divides again (Fig. 15), so that four groups, each consisting of four nuclei, are formed (Fig. 16), one group from each of the four original nuclei. The two successive divisions to form these tetrads are usually simultaneous, but occasionally one nucleus divides in advance of the others. Protoplasm now aggregates around three of the nuclei in each group, and a definite limiting membrane appears round each of the cells thus formed,¹ while the fourth nucleus remains free (Fig. 17). While these membranes are appearing, or even after they have been formed, the fourth free nucleus of each group migrates to the centre of the embryo-sac (Fig. 18). There these four meet (Fig. 19), and gradually

¹ Exceptionally, two nuclei may be enclosed in a single cell (cf. Fig. 25).

fuse to form the primary endosperm nucleus. A series of stages of this fusion is shown in Fig. 20. The process of fusion seems to occupy a comparatively long time. The nuclei can usually be seen in contact in the centre of the sac in the ripe flower bud, but the mature definitive nucleus (Fig. 20 *d*) has only been seen in a couple of cases where some of the other ovules in the same ovary had already been fertilized. While these nuclei are fusing, the three cells that remain in each of the four peripheral groups have taken on more or less of the appearance and arrangement of an egg-apparatus. In Fig. 19 *a*, the group at the bottom of the sac already shows this form, and the other groups are assuming it. It will be noted that the cells in the group indicated are rather more equal in size and form than is quite typical for an egg-apparatus, and this equality is often much more marked (cf. Pl. XXVI, Fig. 25). Another variation in form occasionally seen is that of the uppermost group of Fig. 17, where one of the cells is small and the two others are elongated, with their nuclei lying at their free ends—the whole group appearing to consist of two eggs and one synergid. This is exactly the appearance described by Modilewski¹ as typical for the two lateral cell-triads of the sac of *Euphorbia procera*, which is constructed similarly to that of the Penaeaceae. In the case of the Penaeaceae, however, this or any other deviation from the normal form of an egg-apparatus is shown indifferently by any of the groups, and examination of several hundred embryo-sacs about the fertilization period has made it clear that these groups are as alike in structure as they are in development. Indeed, when (as quite often occurs) two groups are equidistant from the apex of the sac (Fig. 21 *a*), or, as has been several times observed, three groups are thus placed (Fig. 21 *b*), it is impossible to say before fertilization which is to function as the egg-apparatus. For this reason I have preferred to use the non-committal term 'peripheral group' for all of these cell-triads.

In the usual arrangement of these groups (that of Figs. 17–19), naturally the one at the apex of the sac has the best chance of being fertilized, and from it the embryo therefore usually arises. In several cases, however, embryos have been observed developing at the sides of the sac in positions so far removed from its apex as to suggest that, if the sac possessed before pollination the structure shown in Figs. 17–19, fertilization must have taken place in one of the lateral groups instead of that nearest to the apex. The clearest case of this—one in which the remains of the apical group can still be seen—is shown in Fig. 29. This also illustrates the only certain case of polyembryony noted. As there is no indication of parthenogenesis or apogamy, but on the contrary pollen-tubes are freely formed and fertilization has been several times observed (though in the apical group only), this might perhaps be provisionally interpreted as a case where normal fertilization has occurred in two of the other peripheral groups instead of in the apical one. This suggestion, however, is only tentative.

¹ Modilewski, 1909, p. 22.

The mature embryo-sac, therefore, in all the species studied contains four peripheral groups of cells, each group more or less resembling an egg-apparatus, and four nuclei fusing to form the primary endosperm nucleus. Some interesting exceptions to this normal type may now be noted. In six cases it was found that more than four nuclei were fusing to form the primary endosperm nucleus. In two of these cases, the total number of nuclei in the sac could not be accurately computed; one of the two showed either seven or eight nuclei thus fusing, and only three peripheral groups present, two of which were normal, while the composition of the third was uncertain. In the remaining four, sixteen nuclei altogether were present in each case, and it would seem probable that the sixteen-nucleate stage of Fig. 16 had been reached as usual, but that then more than the customary four nuclei had been contributed to form the primary endosperm nucleus. Two of these cases showed five nuclei thus fusing (cf. Fig. 20 *b*), one of the peripheral groups being correspondingly two-celled; in the remaining two there was seen a fusion of six nuclei, an extra nucleus having been contributed from two of the groups, each of which consisted of only two cells (cf. Fig. 23). Two cases should also be noted in which it seemed as if less than sixteen nuclei had been formed in the embryo-sac. One of these is shown in Fig. 22. Here the large nucleus at the apex of the sac may perhaps be interpreted as one of the four original daughter-nuclei which has not undergone division (becoming directly converted into an egg) or has only divided once. The latter is the more probable interpretation, as while the fusion nucleus seems here to be made up of only three nuclei, the other peripheral group figured is not connected with it by the usual protoplasmic strands, and does not seem to have contributed to it. If this interpretation be correct, the second mitosis has been omitted in this group also, as only two cells are present in it. In the other similar case, one peripheral group is again represented by a single cell, and probably here also the original nucleus of this group has only undergone one division, for only the normal four nuclei are fusing to form the definitive nucleus. The remaining groups in all these cases are quite normal.

ENDOSPERM.

With the few exceptions above recorded, the primary endosperm nucleus is always formed by a fusion of four nuclei. Whether a male nucleus usually enters into its composition is still uncertain, owing to the difficulty of interpreting the fertilization processes in these plants—the embryo-sac being small, and the male nuclei showing no differentiation in size, form, or staining properties to distinguish them from the other nuclei present in it. Fertilization, apparently normal, has been observed in the apical group of cells; figures of it have been reserved till the point mentioned has been settled. In *Euphorbia procera*, where the male nuclei are easily

distinguishable, such a fusion of the second male nucleus with the primary endosperm nucleus is seen,¹ and there is no reason why it should not also take place in the Penaeaceae.

If fertilization does not take place, the endosperm nucleus ordinarily disorganizes and disappears with the other contents of the sac. In one case, however, it was seen to divide before fertilization had occurred (Fig. 25). A similar formation of endosperm before fertilization (apparently the only clearly proven case among plants where fertilization normally occurs²) has been reported as being occasionally seen in *Ranunculus*.³ Coulter suggests that the endosperm nucleus might here have been stimulated to divide through the influence exercised by the presence of the pollen-tube in the style being felt in the embryo-sac. The same explanation is possible in the case here figured, as pollen-tubes had already entered several other ovules in the same ovary. A like stimulus of the endosperm nucleus to division is seen in the *Caprifig*,⁴ the exciting cause here being the presence of the egg of the *Blastophagus* wasp. Such cases of division of the primary endosperm nucleus before fertilization furnish a suggestive comparison with the behaviour of the same nuclei in *Welwitschia*.⁵

Immediately after fertilization, the embryo-sac begins to enlarge rapidly at the expense of the surrounding nucellar tissue, until by the time the seed is ripe the nucellus has entirely disappeared. Even before fertilization the cells in the mid-line of the nucellus seem to part with some of their contents, losing in consequence their rounded outlines, and fitting together more compactly than the surrounding tissue (Fig. 24). If the ovule is not fertilized these axial cells simply collapse together under the pressure caused by fertilized ovules developing in the same loculus; but if fertilization does take place, they rapidly disorganize (Fig. 26), and the embryo-sac, which is remarkably long and narrow, grows down into the cavity thus formed (cf. Figs. 27, 28 a, 30 a, 35). The unfertilized cell-groups usually break down and disappear as soon as the embryo-sac begins to enlarge, but sometimes (very rarely) one or more may persist through the first few divisions of the embryo. As a rule, the endosperm nucleus begins to divide immediately after fertilization, or while fertilization is proceeding, and before the fertilized egg, 8–12 nuclei being present by the time the embryo is two-celled (cf. Fig. 27). (Fig. 29, however, if correctly interpreted, shows a case in which fusion of the primary endosperm nucleus is not yet complete, though fertilization has apparently taken place in the apical group and the other peripheral groups are already disorganizing.) I have not observed the stages of its first divisions, but later the endosperm nuclei have often been seen actively, and for the most part simultaneously, dividing (cf. Fig. 32). As might be expected from their multinuclear origin, they have a large number of chro-

¹ Modilewski, 1909, p. 23.

² Coulter and Chamberlain, 1904, pp. 167, 170.

³ Coulter, 1898, p. 83.

⁴ Du Sablon, 1908.

⁵ Pearson, 1909.

mosomes, and usually several nucleoli (cf. Figs. 31-3). The endosperm exists at first only as a number of nuclei, scattered through the parietal layer of cytoplasm which surrounds the large central vacuole that is formed as a result of the growth of the embryo-sac (cf. Figs. 27, 28 *a*, 30 *a*). As may be seen in Figs. 28, 31-3, at the micropylar end of the sac, this layer closely invests the embryo. In the thicker protoplasm found at the lower end of the sac as it extends downwards into the nucellus, from three to eight of the endosperm nuclei become associated in a deeply-staining group, their nucleoli at the same time fusing to form one large and deep-staining nucleolus in each. This group is doubtless haustorial in function, passing on to the rest of the embryo-sac food obtained from the disorganization of the axial cells of the nucellus. After these cells have been absorbed the group is no longer recognizable. It is not until the embryo has attained to the size shown in Fig. 33 that any cell-walls are formed in the endosperm. Cell-wall-formation continues until by the time the cotyledons have appeared (Fig. 35), the greater part of the embryo-sac is filled with a delicate endosperm tissue. This is all used up by the developing embryo, which grows down into it.

EMBRYO.

The most striking fact in connexion with the development of the embryo is the absence of any form of suspensor in even the youngest stages. The first wall formed is transverse (Fig. 27); a longitudinal wall is then formed in each cell (Figs. 28, 29 *b*), and the whole of the spherical proembryo thus formed enters into the formation of the embryo, which preserves this spherical outline until the formation of the plerome (cf. Fig. 32). The dermatogen early becomes differentiated (Figs. 30, 31), and the plerome, is marked out at a slightly later stage (Figs. 32, 33). The embryo begins to elongate (Figs. 33, 34), and the cotyledons are formed (Fig. 35). They are very slightly developed (Fig. 36), the storage function, which is their normal one in an exalbuminous seed, being here performed by the unusually massive hypocotyl, whose cells are closely packed with starch. This great development of the hypocotyl, and its adaptation thus early in life to the function of food storage, may very probably be considered as an adaptation to a xerophilous habit. The seedlings of geophilous xerophytes have been shown to exhibit two marked characteristics¹—a tendency towards reduction in the size of the cotyledons (usually by a partial fusion, or by the abortion of one), and an adaptation of the hypocotyl for the storage of food after the seed has germinated. In this strongly xerophilous and probably ancient order it is then not surprising to find similar characteristics so marked even in the seed.

The root-tip of the embryo in the mature seed (Fig. 37) has some peculiar features. It is of unusual breadth, and the growing point is situated at the bottom of a slight depression. There is no root-cap, and the

¹ Sargent, 1903, pp. 78-81.

dermatogen is a single external layer. It may be anticipated that a root-cap is formed, when germination begins, by the periclinal division of the dermatogen overlying the tip, but as uniform failure has attended all attempts to germinate the seeds, this point is still in doubt.

The chief peculiarities of the embryo are thus :—

- (1) Absence of a suspensor.
- (2) The great development of the hypocotyl and its adaptation thus early in life to the function of food storage.
- (3) The reduction in size of the cotyledons.
- (4) The absence of a root-cap at the stage found in the mature seed.

DISCUSSION.

Before discussing the nature of this type of embryo-sac it may be worth while to note a few facts regarding the affinities and distribution of the little-known order in which it occurs. The Penaeaceae show no very close relationships to other orders, except to the monotypic Geissolomaceae, which is united to them by Bentham and Hooker. Both Engler and Bentham and Hooker have placed the order near the Thymeleaceae (Engler among the Thymeleales, Bentham and Hooker among the Daphnales), and this seems to be its natural position; Van Tieghem, however, mainly on the strength of its anatomical characters, places it next to the Melastomaceae.¹ It was hoped that this investigation might throw some light upon its true systematic position, but the whole life-history here disclosed is so abnormal that it merely serves to isolate the order still further. It is true that Modilewski has recently described in *Euphorbia procera*² an embryo-sac which, so far as his investigations have been carried (from the four-nucleate stage to the first divisions of the embryo), shows a structure almost exactly the same as that here described. But as the embryo-sac in other species of *Euphorbia* is of the normal type, and as the two orders otherwise show no points of resemblance sufficient to warrant a close relationship, there appears to be no doubt that this is a case of parallel development. As regards the other orders with which the Penaeaceae are grouped, the Thymeleaceae is the only one of whose life-history any account has been published, Strasburger³ having recently investigated species of *Wikstroemia*, *Daphne*, and *Gnidia*, none of which show a similar departure from the normal. The same may be said for *Cryptadenia uniflora*,⁴ of which I have made a preliminary examination. *Geissoloma marginata*, the sole representative of the Geissolomaceae, appears to show other peculiarities, which will be described later.

The peculiarly limited distribution of this order may be of interest in connexion with its rather isolated systematic position. It is entirely

¹ Van Tieghem, 1893, p. 291.

² Modilewski, 1909.

³ Strasburger, 1909.

⁴ Mr. A. J. Ballantine kindly preserved material of this for me.

confined to the south-western region of South Africa, a region in which 'an extraordinary number of species, many of them belonging to a few genera and orders elsewhere rare, are massed . . . between the sea and a desert interior'.¹ Hooker has pointed out² the striking affinities that this flora shows with that of Australia, especially South-West Australia, and the less marked relationships of these two floras to that of the Antarctic regions. This connexion between these three southern floras, combined with their separation from the tropical floras which succeed them to the north, seems to him to indicate that they may have been 'members of one great vegetation, which may once have covered as large a southern area as the European now does a northern'.³ Schönland, recently re-examining the evidence derived from the distribution of the plants composing these floras,⁴ comes to the conclusion that there was a direct land-connexion between Australia and South Africa at the time when the types common to the two developed, and a later one between Australia and South America. The isolated groups of this now dismembered flora are being encroached upon everywhere by northern forms, through whose usurping tendencies the small local genera are beginning to disappear.⁵ Bolus says:—'Few botanists who, like the present writer, have spent many years in South Africa, and especially in the south-western districts, have not been penetrated by a gloomy impression that the south-western flora is dying out and is doomed to extinction. . . . In general, species of the *Bruniaceae*, *Penaeaceae*, and *Proteaceae*, so peculiar to this region, seem to have become much more rare.'⁶ He adds:—'No weight can be attached to this, for it is wanting in adequate evidence'; but as regards the *Proteaceae*, Phillips's recent investigations⁷ show that the power of setting seed is in many species on the wane, and in the case of the *Penaeaceae*, it may be significant in this connexion to note that although they set seed freely, all attempts to germinate these seeds have failed, and in the genera examined careful search on many occasions has failed to discover any seedling plants. Moreover, even young plants less than about a dozen years old seem extremely rare; I have not found above five such in all the species examined.

This order then may probably be regarded as belonging to an ancient flora, and its restriction to the one region within that flora would seem to indicate either that it is a 'survival' which has lingered on in this south-western region, while dying out in other regions where the flora to which it belonged still flourishes; or else that it is a new type developed since the isolation of this region. Two considerations would seem to point to its being an original member of the flora rather than a lately-evolved

¹ Hooker, 1859, p. xcii.

² loc. cit.

³ Hooker, 1859, p. civ.

⁴ Schönland, 1909.

⁵ Hooker, 1859, p. cv.

⁶ Bolus, 1905, p. 235.

⁷ Ballantine, 1909, p. 161.

type, namely, the absence of close affinities to other types in the same region, and the pronounced adaptation it shows to xerophilous conditions—an adaptation which extends even to details of the embryonic structure, and must have been the result of a prolonged exposure to such conditions. Additional weight is lent to this view by the fact that in all three genera investigated the embryo-sac shows the peculiar structure that has been described; these genera then would appear to have been evolved since the establishment of this type of sac. In considering the morphological bearing of any deviation from the normal angiosperm life-history shown by such a group there are two possibilities to be kept in view; they may be relatively primitive features retained by it, or recent developments of no phylogenetic significance. The former view would seem more probable on the preceding facts, and it would be quite legitimate to regard this sixteen-nucleate embryo-sac as more primitive than the usual eight-nucleate type if it were formed, as is usually the case in the latter, from one of four megaspores. Indeed, although this is not the case, it is still quite possible to consider that the reduction division by which these megaspores are formed has here been shifted, and takes place at the germination of the spore—that is, the megaspore mother-cell functions directly as the embryo-sac, and all the nuclei formed in it belong to the gametophyte. This view is held by Ernst, who has discussed¹ this point for the sixteen-nucleate embryo-sacs of *Gunnera*, *Peperomia*, and the Penaeaceae. In all of these the formation of walled megaspores is omitted, and this is a strong argument in favour of regarding them as derived rather than primitive forms. If they were really primitive in respect of their embryo-sac structure, the normal row of four megaspores might be expected. Ernst, however, arguing from such cases as that of the Liliaceae, where tetrad-formation may be partly or entirely suppressed within the limits of a single order without influencing the development of the embryo-sac, considers that these processes of development that go on within the embryo-sac are quite independent of its origin, and can be considered apart from it. He points out that the divisions within the macrospore mother-cell are the last divisions within a macrosporangium, while those within the embryo-sac represent the germination of one of its derived spores, and concludes that these two processes can undergo various grades of reduction quite independently of one another; thus if the two divisions forming the spore-tetrad are omitted, chromosome reduction will be shifted so as to take place within the spore at its germination.² This he considers has happened in the cases under discussion.

But on the other hand, sporogenesis is a normal phase in the life-history—the culminating point of the sporophyte generation—and some

¹ Ernst, 1908 (B), pp. 26–9. As regards the Penaeaceae, Ernst was misled by my former statement (see ante, p. 364) that three megaspores appeared to be formed.

² See also Farmer, 1907, pp. 192, 196.

writers even consider that the four nuclei formed by the reduction division should be always regarded as the nuclei of four spores, whatever may be their subsequent history.¹ The gametophyte of plants, normally a post-meiotic phase,² is to be regarded on this view as always post-meiotic, the number of cell-generations in it corresponding to the number of post-meiotic divisions of these four nuclei.

This view has lately been elaborated for the embryo-sac of the angiosperms by Coulter,³ who applies it to all cases in which the mother-cell divides only once, or else functions directly as the embryo-sac; and therefore in which, according to him, two spores in the first case, and four in the second have been involved in forming the embryo-sac. That this is probably the case in *Peperomia* has been shown by Brown,⁴ who describes the formation of evanescent walls or cell-plates between the first four nuclei of the embryo-sac in the species he has studied. Though careful search has discovered no signs of such walls or cell-plates in the first divisions of the macrospore mother-cell in the *Penaeaceae*, the absence of a row of megaspores, combined with the tetrahedral arrangement of the first four nuclei formed, makes it seem possible to the present writer that the peculiarities of the embryo-sac may here also be best explained by regarding it as a specialized type, in the development of which all four megaspores have become included, the germination of each ceasing at the four-nucleate stage. Some similar cases of four potential megaspore nuclei being included in a single cell (only one, however, germinating) are cited by Brown.⁵ Evidence on this point is unfortunately wanting in *Euphorbia procera*, since Modilewski's material showed nothing earlier than the four-nucleate stage. If the early stages of development in this form should prove to be the same as in the *Penaeaceae*, it is probable that the two may be regarded as cases where specialization has proceeded on parallel lines.

Whether the embryo-sac of these *Penaeaceae* is to be regarded as a relatively primitive one, or as one which has been reduced and specialized in the manner suggested, two explanations of its structure will still both be applicable. One of these has been advanced by Ernst, who applies to the *Penaeaceae* ⁶ the homologies suggested by Porsch ⁷ as an explanation of the embryo-sac and double fertilization in the angiosperms. Porsch interprets the egg-apparatus as being homologous with a Gymnosperm archegonium, the synergids representing the neck cells,⁸ and the antipodal cells as being equivalent to another 'sexually-degenerate' archegonium.

¹ Davis, 1905, pp. 471-2.

² Farmer and Moore, 1905, pp. 548-9.

³ Coulter, 1908.

⁴ Brown, 1908.

⁵ *ibid.*, pp. 453-4.

⁶ Ernst, 1908 (A), p. 434; 1908 (B), p. 33.

⁷ Porsch, 1907, pp. 19, 20.

⁸ Chamberlain (Bot. Gaz., xlv, p. 155, 1908) and Went (1909, p. 13) interpret Porsch as holding the synergids homologous with neck *canal* cells, but this seems a misinterpretation of Porsch's view.

The polar nuclei are homologized with ventral canal cells, and as the ventral canal cell has been shown capable of fertilization, the fusion of the second male nucleus with them can be regarded as a kind of fertilization, and the endosperm as of the nature of a modified embryo. Ernst, applying this 'Archegontheorie' to the Penaeaceae, considers then that each of the four peripheral groups represents an archegonium, and that the four nuclei fusing to form the primary endosperm nucleus are the four ventral canal cells of these archegonia. This would hold equally well if each group were regarded as formed from a macrospore, only in this case the sac would be reduced rather than primitive, as Ernst interprets it. But it is difficult to imagine that a gametophyte so greatly reduced as that of the Angiosperms could continue to form the rudiments of such specialized structures—structures which in the Gymnosperms appear only at a much later stage in the life-cycle, and moreover have disappeared in the highest group of Gymnosperms, so that in *Welwitschia* and *Gnetum* the naked cells of the prothallus function as eggs. Moreover, a view that regards the synergids as homologous with neck-cells affords no explanation of such a case as that described for *Naias major*,¹ where a synergid is often fertilized and gives rise to an embryo. Porsch, it is true, regards such 'Synergidenbefruchtung' as of no more significance than the fact that embryos can arise from nucellus or even integuments,² but the cases are surely very different, the latter being merely sporophytic budding. For these reasons, the present writer is inclined to look for a more probable explanation for the peculiarities of this embryo-sac.

A suggestion which seems of service here is that offered by Pearson regarding possible homologies in the embryo-sac of the primitive angiosperm. In an abstract of some recent observations on *Welwitschia*,³ he describes all the nuclei found in the embryo-sac at the end of free-nuclear division as being potential gametes. Some of these remain free and can function as gametes; the remainder fuse to form the primary endosperm nuclei. He suggests that the endosperm of the primitive angiosperms was homologous with that of *Welwitschia*. If the sixteen nuclei formed in the free-nuclear stage in the Penaeaceae can be similarly regarded as potential or reduced gametes, and the endosperm as physiologically homologous with that formed by the fusion-nuclei in *Welwitschia*, the chief peculiarities noted—the apparent formation of an egg-apparatus in each peripheral group; the equality in development and structure of these groups and their component cells; the formation of a normal endosperm by a fusion of four (exceptionally more) nuclei; the exceptional case of division of the primary endosperm nucleus before fertilization—all find an explanation. This suggestion thus seems to the writer to meet the facts of the present case. If this embryo-sac can be regarded as more primitive than the normal type,

¹ Guignard, 1901.² Porsch, 1907, p. 25.³ Pearson, 1909.

it forms an interesting transition stage between that type and a possible ancestor possessing a larger number of free nuclei, sufficient to form several primary endosperm nuclei where here only one is present. On the other hand, if, as seems more probable, it is a derived form, the same hypothesis is equally helpful, especially in explaining how it is possible for a normal endosperm to arise from a four-nucleate instead of the usual bi-nucleate fusion.

SUMMARY.

Species of *Sarcocolla*, *Penaea*, and *Brachysiphon*, three of the five genera of the rather isolated order Penaeaceae, have been examined. The development and structure of the embryo-sac and embryo in all three genera is the same. The megaspore mother-cell forms four nuclei by a reduction division. These are usually tetrahedrally arranged. They migrate to the periphery of the embryo-sac, and there as a rule lie cross-wise, one at each end and the other two at the sides; their position, however, often varies. Each gives rise to a group of four nuclei. Cells are formed round three of these nuclei in each group, and these cells in each case assume more or less of the appearance of an egg-apparatus; whether they can all function thus is uncertain. The four remaining free nuclei fuse in the centre of the sac to form the primary endosperm nucleus. After fertilization this forms a parietal layer of endosperm nuclei, cell-walls appearing only at a late stage.

The embryo has no suspensor. It divides to form a sphere of cells, which elongates as theplerome is formed. It has a broad root-tip, no root-cap, a massive hypocotyl, and rudimentary cotyledons.

This embryo-sac is probably to be regarded as a derived form, with all four megaspores included in its development, rather than as a primitive one; but in our present state of knowledge of the embryo-sac of the Angiosperms, this cannot be considered as definitely proved. It is suggested that in either case its endosperm is formed, like that of *Welwitschia*, by a fusion of potential gametes, all the nuclei in the sac being looked upon as potential or reduced gametes.

This investigation was begun in the Botanical Laboratory of the South African College, at the suggestion of Professor Pearson, to whom I am indebted for much help and advice in its early stages, and for assistance in procuring material. I wish also to thank Professor Seward for kindly allowing me to continue it in the Cambridge Botany School.

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EXPLANATION OF FIGURES IN PLATES XXV AND XXVI.

Illustrating Miss Stephens's paper on the embryo-sac and embryo of certain *Penaeaceae*.

Abbreviations used: *end.* endosperm; *em.* embryo; *e.s.* embryo-sac; *e.s.w.* embryo-sac wall; *int.* integument; *m.m.c.* megaspore mother-cell; *nuc.* nucellus; *o.* oospore; *peri.* periblem; *pl.* plerome; *b.g.* peripheral group; *p.n.* polar nucleus; *p.t.* pollen-tube.

PLATE XXV.

Fig. 1. Longitudinal section through young ovule, showing megaspore mother-cell, a periclinal division in the cell-layer above it, and developing integuments. $\times 390$. *Sarcocolla squamosa*.

Fig. 2. Longitudinal section through upper part of nucellus and embryo-sac (at stage of Fig. 13), showing a second undivided megaspore mother-cell (?) above the embryo-sac. $\times 390$. *Sarcocolla squamosa*.

Fig. 3. Longitudinal section through ovule just prior to division of the megaspore mother-cell, in the nucleus of which synapsis is beginning. $\times 390$. *Sarcocolla minor*.

Fig. 4. Nucleus of megaspore mother-cell, showing synapsis. $\times 1300$. *Penaea mucronata*.

Fig. 5. The same, showing chromosomes. $\times 1300$. *Penaea mucronata*.

Fig. 6. Longitudinal section of metaphase of first (heterotype) division of megaspore mother-cell. $\times 1300$. *Sarcocolla minor*.

Fig. 7. Optical section of embryo-sac, made up of two successive transverse sections, showing metaphase of second (homotype) division. $\times 1300$. *Penaea mucronata*.

Fig. 8. Optical section, made up of two successive transverse sections, showing telophase of the same. $\times 1300$. *Sarcocolla minor*.

Fig. 9. Four-nucleate embryo-sac, slightly older than that of Fig. 8, showing tetrahedral arrangement of nuclei. The nucleus marked *a* really lies at the same distance from the one that overlies it in the figure as it does from the two others. $\times 630$. *Sarcocolla minor*.

Fig. 10. The same, showing different arrangement of nuclei. $\times 600$. *Sarcocolla formosa*.

Fig. 11. Later stage, showing nuclei separating. The nuclei marked *a* and *b* occur at the beginning and end respectively of the series of longitudinal sections composing this embryo-sac; the other two lie about its middle. $\times 630$. *Sarcocolla minor*.

Fig. 12. Later stage; nuclei in prophase of division. $\times 420$. *Sarcocolla minor*.

Fig. 13. Eight-nucleate embryo-sac. $\times 420$. *Sarcocolla formosa*.

Fig. 14, *a* and *b*. Same stage but with five groups of nuclei; the fifth group is shown in 14 *b*. (Fixed in acetic alcohol and much contracted, so that the embryo-sac wall can be seen; imbedded in it are the nuclei of disorganized nucellar cells). $\times 420$. *Sarcocolla squamosa*.

Fig. 15. Later stage; nuclei in prophase of division. $\times 333$. *Sarcocolla squamosa*.

Fig. 16. Sixteen-nucleate embryo-sac. $\times 420$. *Sarcocolla squamosa*.

Fig. 17. Later stage; cell-walls are being formed in the peripheral groups; one nucleus in each remains free (*p.n.*). $\times 630$. *Penaea mucronata*.

Fig. 18. Later stage; the free nuclei are moving to the centre of the sac. $\times 420$. *Penaea mucronata*.

Fig. 19, *a, b*. Later stage; the free nuclei are meeting in the centre of the sac. The fourth peripheral group lies in a plane perpendicular to the other three, and is seen in 19 *b* in transverse section. $\times 420$. *Sarcocolla squamosa*.

Fig. 20, *a-d*. Successive stages of nuclear fusion; in *b*, a fusion of five nuclei. All $\times 420$. *a, b, c*, *Sarcocolla squamosa*; *d*, *Penaea mucronata*.

Fig. 21, *a-e*. Diagrammatic outline of five embryo-sacs, showing varying positions of the peripheral groups. The upper half of *d* is shown in Fig. 22, and of *e* in Fig. 23.

Fig. 22. Upper half of embryo-sac of Fig. 21 *d*. with three nuclei fusing to form the primary endosperm nucleus. The two peripheral groups shown contain only one and two cells respectively, the remaining two groups are normal. $\times 630$. *Sarcocolla squamosa*.

Fig. 23. Upper half of embryo-sac of Fig. 21 *e*, with six nuclei fusing to form the primary endosperm nucleus. The two peripheral groups shown contain only two cells; the remaining two groups are normal. $\times 420$. *Sarcocolla squamosa*.

PLATE XXVI.

Fig. 24. Outline of longitudinal section through ovule at the time of fertilization, showing axial row of cells in nucellus. $\times 28$.

Fig. 25. Mature embryo-sac, showing exceptional case of division of the primary endosperm nucleus before fertilization. $\times 630$. *Penaea mucronata*.

Fig. 26. Embryo-sac after fertilization; the unfertilized peripheral groups are disappearing and the cells of the axial row of the nucellus are breaking down. $\times 630$. *Penaea mucronata*.

Fig. 27. Embryo-sac with two-celled embryo. $\times 420$. *Penaea mucronata*.

Fig. 28. Upper part of embryo-sac with four-celled embryo, seen in optical section. The wall dividing the upper two cells is in the plane of the drawing: that dividing the lower two is cut obliquely. An empty pollen-tube is seen in the nucellus. $\times 390$. *Sarcocolla squamosa*.

Fig. 28 *a*. Outline of embryo-sac of Fig. 28, showing endosperm. $\times 28$.

Fig. 29 *a, b*. Sections through the same embryo-sac showing two embryos of eight cells (29 *a*) and four cells (29 *b*) respectively. The latter is seen in obliquely transverse optical section, being in a plane almost perpendicular to that of the drawing. $\times 333$. *Penaea mucronata*.

Fig. 30. Longitudinal section through embryo, showing (probably) the beginning of the dermatogen. $\times 390$. *Sarcocolla squamosa*.

Fig. 30 *a*. Outline of ovule containing embryo of Fig. 30. $\times 15$.

Fig. 31. Longitudinal section of embryo older than Fig. 30, with investing layer of cytoplasm containing endosperm nuclei. $\times 300$. *Sarcocolla squamosa*.

Fig. 32. Longitudinal section through top of embryo-sac at a later stage, showing embryo in median section, and dividing endosperm nuclei. The shaded cell in the centre of the embryo probably represents the beginning of the plerome. $\times 300$. *Sarcocolla squamosa*.

Fig. 33. Longitudinal section through top of embryo-sac at a later stage, showing embryo in median section (with longitudinal division of two cells in the plerome region) and formation of cell-walls in the endosperm. $\times 196$. *Sarcocolla formosa*.

Fig. 34. Longitudinal median section through embryo at a later stage. $\times 300$. *Sarcocolla squamosa*.

Fig. 35. Longitudinal section through ovule at a later stage, showing endosperm tissue and embryo with developing cotyledons. $\times 15$. *Sarcocolla squamosa*.

Fig. 36. Mature embryo. $\times 15$.

Fig. 37. Root-tip of mature embryo. $\times 125$. *Sarcocolla squamosa*.

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