A case of apogamy with Dasylirion acrotrichum *Zucc.*

BY

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with Plate V.

In the summer of 1904 a specimen of Dasylirion acrotrichum Zucc. was in bloom in the Utrecht Botanical Garden. The home of this tree-like Liliacea is in Mexico; on a short stem it bears a bundle of flat leaves wit thorny margins. Although the plant is pretty often cultivated in European botanical gardens it is very seldom seen in bloom. Hence constant attention was paid to the here mentioned specimen. The inflorescence was two metres long; the principal axis was ramified and had a great number of steeply erected lateral axes in the axils of bracts; each of these carried some 50 to 150 unstalked female flowers. Dasylirion is dioecious so that male flowers were entirely absent.

Each flower had a perianth consisting of six green leaflets and a pistil; this latter consisted of a triangular ovary with a short style and three stigmas. The ovary was unilocular and had on its bottom three ovules.

After the flowers had finished blooming it seemed as if some ovaries began to swell. As there could be no question of fertilisation in the absence of male sexual organs it was thought that perhaps a new case of apogamy or parthenogenesis was present here. The ovaries were now regularly examined; they more and more assumed the appearance of little fruits, looked like small nuts provided with three wings and strongly reminded one of the fruitlets of Rheum. It appeared that many ovules swelled, but never more than one in each ovary. Not nearly in all flowers this phenomenon was observed, in no more than 10 to 40 percent it was at all visible.

For a detailed investigation these ovules were now fixed in Flemming's fixing solution (the weak solution) and then washed in the usual manner and gradually placed in strong alcohol. This was done for the first time on August 15; from 158 ovaries 49 ovules were obtained, i.e. 31 percent. This was a maximum however, for when later material was collected in the same way on August 22, September 3, 10, 13, 19 and 25, October 8 and 22, November 12, Decembeı 15 and 24 and on January 19, 1905, each time more and more ovules appeared to be unfit for use, as they began to wrinkle. Such as looked more or less swollen were fixed; among these some had grown thicker and finally the impression was that some seeds had ripened. But ultimately not a single germinable seed appeared to be on the plant and after January 19 no material fit for investigation could be got. Nothwithstanding this, the preserved material was examined, since it was possible that only the unfavourable conditions under which Dasylirion lived in the Botanical Garden at Utrecht, were the reason why no ripe seed was formed.

On microscopical examination phenomena were indeed observed which seemed to point to apogamy or parthenogenesis, but the material proved insufficient to obtain a consistent result. Leaving apart even the already mentioned fact that not a single ripe seed was produced, the number of ovules in which ultimately anything particular could be observed, was extremely small. For microscopic examination revealed that most ovules which outwardly showed nothing abnormal, yet were already in all stages of disorganisation.

Although we are unable to offer a finished investigation, yet it seemed desirable to us to publish what we have seen. For Dasylirion blooms so seldom in Europe that for us the chance of finishing our investigation is practically zero, while now at least attention has been drawn to it, so that perhaps in the mother country of the plant some one may feel inclined to re-examine it.

Moreover the number of known cases of apogamy or parthenogenesis is so small that there is every reason to publish each new case. And finally the material examined by us presents some points which deserve attention for special reasons.

The fixed material was embedded in paraffin, cut with the microtome and then stained, as a rule with safranin only, sometimes with safranin, gentian violet and orange G.

The ovules of Dasylirion are anatropous and furnished with two integuments (fig. 7 and 8); the outer one consists, besides of an exterior and interior epiderm, of cells, situated rather irregularly in 2 to 4 rows; towards the chalaza it is much more strongly developed. The inner integument consists of two layers of closely adjacent cells. The micropyle (fig. 1) is formed by the inner integument only, the edges of which are strongly swollen — the cells are larger and the thickness is here about four cells — and are closely adjacent so that they only leave a narrow slit between them.

The tissue of the nucellus is small-celled near the chalaza, but for the rest it consists of large cells with very little protoplasm and apparently very much cell-sap. The more peripheral cells (fig. 1—4) are smaller, their cell-walls are perpendicular to the integument, especially near the micropyle, but the others are greatly lengthened in the direction of the chalaza so that they have become tube-shaped. These tubes are often more or less bent, so that longitudinal sections present an appearance which is rather difficult to disentangle. The swelling of the ovules was in many cases to be ascribed to the strong turgescence of these nucellus-cells only; in older stages also the cells of the outer integument began to increase their volume, evidently also by the increase of the cell-sap only.

These strongly lengthened nucellus-cells at first caused us to believe that more than one embryosac is formed, but an accurate examination of the preparations finally gave us the conviction that only one embryosac is found. Certainty on this point will be obtained only by investigating the development and for this purpose the collected material was unsuitable, for also in the youngest ovules the embryosac was already completely formed. It is long-drawn, somewhat in the shape of a dumb-bell, at the base extending near the chalaza, at the top near the micropyle surrounded by a single layer of nucellus-cells (fig. 1).

Now it appeared that in the great majority of these embryosacs nothing particular could be observed; sometimes a little protoplasm or more or less disorganised and swollen masses, but no egg-apparatus, no polar nuclei and no antipodal cells; so that presumably in nearly all the ovules a disorganisation had already taken place before they were fixed.

Only a few ovules presented more particularities and these we shall describe here, in the first place those where a young embryo was found.

In an ovule, collected on August 22, there is found at the top of the embryosac and there filling this latter entirely, a cellular body with eight normal looking nuclei, making the impression of an embryo (fig. 3). The rest of the embryosac is empty and only some disorganised masses lie in it; of an endosperm nothing can be seen, no more than of antipodals or embryosac-nucleus; concerning this latter, however, the possibility must be granted that it has fallen from the preparation during the staining, although we do not think this probable.

In an ovule, collected on September 10, tbe top of the embryosac is filled by a cell-mass of some 20 to 30 cells, the walls of which are strongly swollen (fig. 4); the nuclei are small and are in a state of disorganisation as well as the rest of the protoplast. The whole makes the impression of a more or less disorganised embryo. Further there is in the embrosac a pretty large quantity of protoplasm in which we could find no nuclei.

Finally we found in an ovule, collected on August 22, a still larger cellular body, reminding us of an embryo (fig. 5). It consists of about 40 cells, the contents of which are still more disorganised, with swollen cell-walls which strongly absorb staining substances. Having regard to the former two preparations we are of opinion that this also must be looked upon as an embryo, the development of which has already for some time been stopped and which now is in progress of becoming disorganised. Also here nothing peculiar was further found in the embryosac.

Of course we looked also for the presence of an egg-apparatus, especially in the younger stages, but there is only one preparation in which anything of this kind can be detected (fig. 2). It is an ovule, collected on August 22, where in the top of the embryosac three cells are found, two shorter ones with distinct nuclei and a third which is larger with disorganised cell-contents in which the nucleus can still be discovered, however. We believe this to be the egg, the others synergids. Here also nothing else is found in the embryosac besides protoplasm which stains strongly.

In 10 other ovules an endosperm was observed in various stages of development. It must be stated at once that in none of these anything of the nature of an embryo is seen. Although it may be objected that for some ovules the series of sections is not complete, yet this is certainly not the case with the majority. Especially where the micropyle is seen in the section, the embryo would be sure to be observed if it were there, but also in this case no trace of it can be found. So we arrive at the conclusion that here an endosperm has been formed without the embryo having developed.

An ovule, collected on August 15, shows the smallest quantity of endosperm (fig. 6). The upper part ($\frac{2}{3}$ to $\frac{3}{4}$) of the embryosac is filled up with it. The shape of the embryosac has been changed; it is swollen, has become cylindrical or somewhat broader towards the bottom, has a thickness of 0,4 mm., while the nucellus has a maximum diameter of 1,0 mm. The lower part of the embryosac in which no endosperm is found, has entirely collapsed and has evidently been squeezed by the surrounding cells. This same shape of the embryosac was met with only once without an endosperm having been formed in it, namely in an ovule, collected on the same day. In the lining protoplasmatic layer no nuclei could be seen, but still we oelieve that this was a first beginning of the formation of an endosperm. Now the endosperm of the just-mentioned ovule consists of thin-walled cells of varying size; normal nuclear divisions occur but also nuclei of abnormal size with a number of nucleoli, indicating fragmentation. At one of the sides of the embryosac the formation of the endosperm has not yet been completed.

Curiously enough the next stage in the development of the endosperm was observed with an ovule, fixed on December 15 (fig. 7). Here the greater part of the tissue of the nucellus has been displaced, so that it forms only a narrow layer round the endosperm, somewhat thicker near the chalaza (greatest thickness of the embryosac 1, 2 mm., of the nucellus 1,5 mm.). Here also the lower part of the embryosac is not filled, but is entirely abortive. The endosperm-cells are of rather unequal size, most nuclei do not look normal, but still divisional stages occur; in the more peripheral cells small grains which strongly absorb staining substances appear outside the nucleus. As in some other cases, the impression is got here that the formation of the endosperm takes place rather irregularly, as if in various spots within the embryosac pieces of endosperm-tissue would form which grow towards each other so that seemingly more than one endosperm lies in the embryosac. At any rate this seems to be so when one limits his attention to one preparation; by comparing, however, the different successive sections of one ovule there finally appears to be only one mass of endosperm. The formation of the endosperm begins in the lining of the wall of the embryosac and from there proceeds inwardly; in this process the cavity is gradually filled up, the endosperm now meets itself from various sides and it is these divisional lines that remain visible.

That the formation of an endosperm starts indeed at the periphery of the embryosac, appears e.g. from an ovule, collected on September 19 (fig. 8). Here the size of the whole endosperm is greater than in the already mentioned ovules (diameter 1,35 mm.), so that only a very narrow layer of nucellus tissue is visible all round, mostly at the chalaza (greatest diameter of the nucellus 1,4 mm.); but the whole endosperm is hollow and in this cavity remnants of the protoplasm of the embryosac are visible. The endosperm-cells are here of very different sizes and so also the nuclei vary much. Some of them look normal, show karyokinesis, others are enlarged, have assumed all sorts of capricious shapes, the number of nucleoli has greatly increased and a number of fragmentation stages can be observed (fig. 10, 11, 12 and 13).

Two ovules, collected on September 10, show a still further developed endosperm. The nucellus tissue has been more displaced, the shape of the endosperm-cells is pretty regular, their cell-wall is somewhat thickened, the nuclei are almost normal; in any case there is much less indication of fragmentation than with the just mentioned ovule.

In an ovule, collected on September 19, the endosperm is so strongly developed that of the nucellus-tissue hardly anything remains visible. This also applies to the cases which will be described presently. The endosperm-cells have strongly thickened but still fairly gelatinous walls; the contents of the cells consist of a number of small grains which stained very strongly and which somehow make the impression of nucleoli; of a nucleus nothing is found any longer, unless we apply the name to some thick, coloured masses.

Three ovules, fixed on December 15, all showed the same picture (fig. 9). A strongly developed endosperm is present with very thick cell-walls, absorbing safranin more or less, and protoplasts which are entirely foamy and in which nothing of a finer structure is found. This endosperm must evidently be reckoned among the horny ones; it was extremely difficult to cut. Sections of the ovules could only be made after treament with hydrofluoric acid. It is not impossible, of course, that the foamy appearance of the protoplasts must be ascribed to this treatment, although we do not think this probable on account of other experience with this method. In the endosperm some fissures are visible, the last remnants of the cavity of the embryosac.

Finally an ovule with an endosperm was found among the material collected on January 19. Here also cutting was only possible after treatment with hydrofluoric acid. The endosperm is entirely disorganised, borders of cells can scarcely be recognised. No more than in the preceding cases we think this must be ascribed to the manner of treatment.

We have now described all cases of formation of an endosperm, observed by us. It will have been noticed that the order is not chronological, the arrangement was such that we gradually proceeded from the least developed to the complete endosperm. From this it follows already that the formation of an endosperm takes place very irregularly with these ovules, now sets in sooner, then later, and that the endosperm may pass into disorganisation at various stages of development.

Summarising, it appears that with Dasylirion acrotrichum an endosperm is formed without fertilisation. This endosperm finally disorganises; it may do so already at a pretty early stage of development, but it may also first attain its complete development. But an embryo could never be found together with such an endosperm. From this it does not follow, however, that it could never be formed together with an endosperm, especially since in three ovules — in which, to be sure, no endosperm was formed — in the top of the embryosac a cell-body was found which we take to be an embryo, which however very soon passes into a state of disorganisation.

One may now ask to what cause this disorganisation must be ascribed. It might be suspected that the circumstances of this Dasylirion were abnormal. Although we grant that these were different from the conditions in the mother country of the plant, yet we must remark that the plant was in the open air for a long time before and after it had bloomed during the very hot summer of 1904 and that there was no question of this specimen being sickly. We venture another supposition: to us it seems that this plant makes, so to say, an attempt to apogamous development, but that these endeavours do not succeed. For this would plead that the endosperm develops here independently of an eventual formation of an embryo and that the embryo is sometimes planned, but never grows to any considerable size. If this be the case, in the mother country of the plant similar phenomena should be observed, but at the same time normal fertilisation and seed-formation. We ought to know the development of the embryosac, in order to know why the apogamy is unsuccessful here, even though the plant makes an attempt in this direction. If in the embryosac mother-cell a reduction division has taken place, this would be very easy to understand and it would also explain the greater facility with which the endosperm is formed. For, after fusion of the two polar nuclei the normal number of chromosomes of the $2x$-generation (not, of course, of the endosperm) would be re-established again; we have tried to determine this number and it seemed to us to be 20 to 24. But as long as we do not know how the endosperm is formed this determination is of little value; for we owe to Treub [1]) the knowledge of a case of endosperm formation, with Balanophora elongata, where the endosperm nuclei are formed by division of one of the two polar nuclei. It is, to be sure, the only case on record where an embryosac fills with endosperm, without a normal embryo being formed. In this respect the ovules of Dasylirion, described by us, could be compared with Balanophora. On the other hand there is this great difference, that with Balanophora an embryo is later formed from part of the endosperm and of this there is no question with Dasylirion.

We put the word apogamy at the head of this communication because it leaves unsettled whether here phenomena of parthenogenesis were indeed observed. It is an open question to what extent the development of an endosperm without previous fusion of the polar nuclei with one of the generative nuclei of the pollen tube can be brought under one of these conceptions. Those who will not use the word fertilisation in the case of endosperm formation, like Strasburger, will object to it; those

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1) M. Treub. L'organe femelle et l'Apogamie du Balanophora elongata Bl. Ann. du Jardin botan. de Buitenzorg XV. 1898 p. 1. See also J. P. Lotsy, Balanophora globosa Jungh. Ann. du Jardin botan. de Buitenzorg 2me Série I. 1899, p. 174.

who embrace the opposite view, like Guignard and Bonnier, will think the use of these terms admissible. Although we incline towards this latter opinion, we shall not dwell on this point here.

But we think it desirable to point out that a closer study of unfertilised ovules, especially of dioecious plants will perhaps yield surprising results. Since we know through Loeb that chemical stimuli may cause the development of an egg, the possibility must be granted that this may also be the case with higher plants. When a normal fertilisation does not take place, such chemical stimuli would at any rate render a beginning of development possible. Looked at from this point of view the case of Dasylirion is perhaps important, but, as we stated already at the beginning of this communication, only an investigation in the natural place of occurrence of the plant can give an answer to this and allied questions.

EXPLANATION OF THE FIGURES IN PLATE V.

All the figures represent ovules or parts of ovules from Dasylirion acrotrichum; the dates are given, on which the material was gathered.

Fig. 1. × 100. Top of an ovule with 2 integuments, micropyle, top of the nucellus and embryosac (22nd August).

„ 2. × 240. Top of an ovule; only the inner integument is sketched. In the top of the embryosac two cells are seen, which perhaps belong to an egg-apparatus; in the next section, which is not figured, a third cell was visible (22nd August).

Fig. 3. × 240. Top of an ovule as in fig. 2. In the top of the embryosac a young embryo is visible with 6 nuclei; 2 other nuclei in the next section which is not figured (22nd August).

„ 4. × 240. The same as in fig. 2. In the top of the embryosac a small group of cells is visible, perhaps representing an embryo. The cell-walls are somewhat swollen, the cell contents disorganising, the nucleus only being visible in some of them (10th September).

„ 5. × 240. Almost the same as in fig. 4, only the number of cells in the top of the embryosac is greater and the disorganisation has gone further (22nd August).

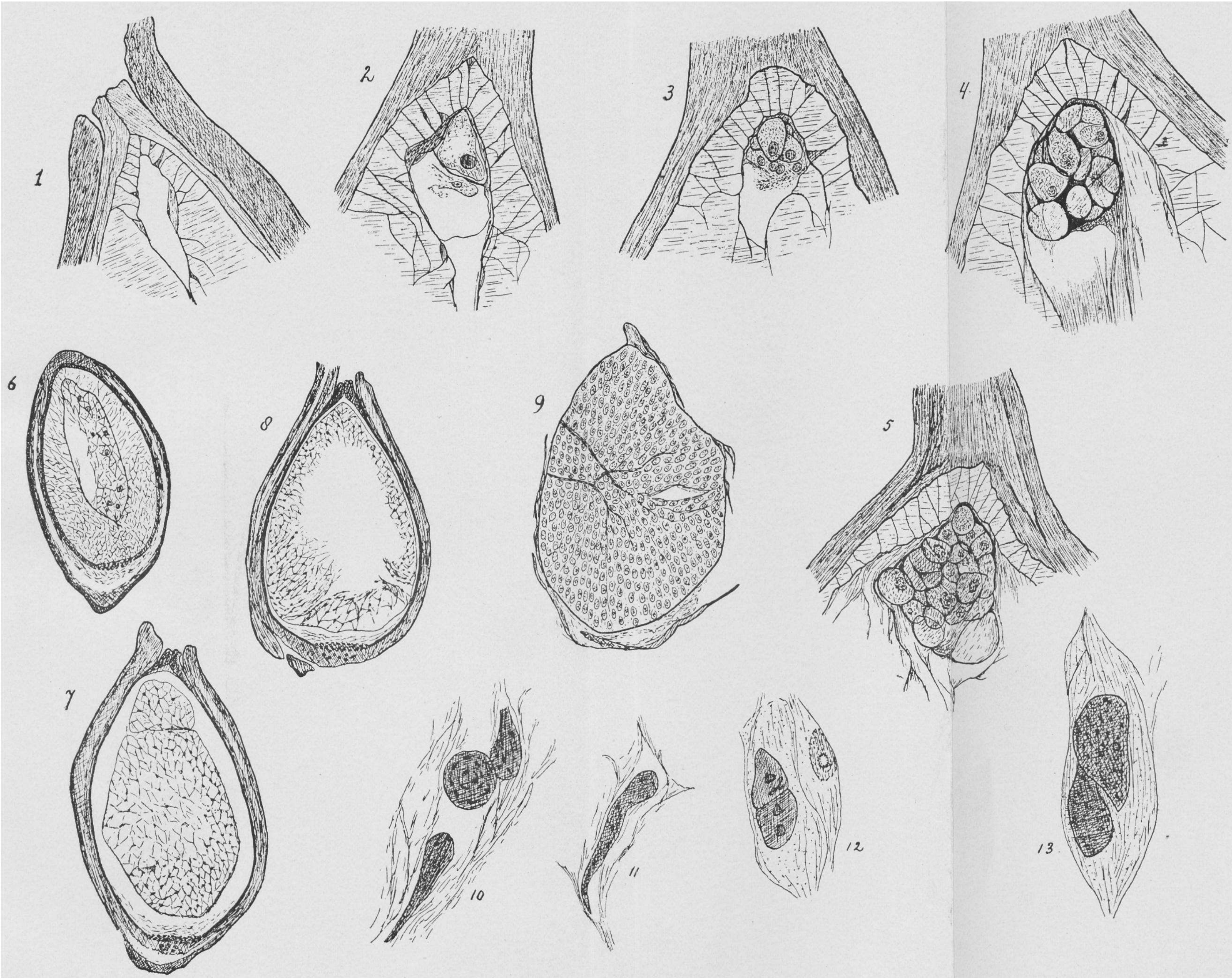
„ 6. × 20. Ovule with an embryosac, of which the upper part is almost filled up with endosperm (15th August).

„ 7. × 20. Ovule with an embryosac, filled with endosperm. The nucellus forms only a thin layer (15th December).

„ 8. × 24. Ovule with a much enlarged embryosac with a peripheral layer of endosperm. The nucellus has almost disappeared (19th September).

„ 9. × 24. Ovule with ripe endosperm with thickened cellwalls (15th December).

„ 10, 11, 12. 13. × 920. Fragmentation of nuclei in the endospermcells figured in fig. 8.



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