HYBRIDS IN CYCADS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 348

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(WITH THIRTEEN FIGURES)

It is doubtful whether any true hybrids in cycads have ever been described, although there is a note on hybrid cycads dating back as far as 1883. In Focke’s Pflanzen-Mischlinge (1881), all the principal reputed plant hybrids are listed in systematic order, but no cycads appear on the list. He makes the statement, however, that the pollen of cycads “has occasionally been employed to fertilise the female of other species; but I am unaware whether perfect seeds have been obtained or hybrids raised.”

In the note on hybrid cycads, Hemsley states as follows:

Mr. Katzer, chief of the Imperial Gardens at Paulilowsk, near St. Petersburg, had succeeded in raising a hybrid between Ceratozamia longifolia and C. mexicana. In this instance, a male of C. longifolia first developed an inflorescence, and Mr. Katzer collected the pollen and preserved it in a sealed bottle until a plant of C. mexicana produced a cone. When the flowers of the latter were in a receptive condition they were sprinkled with the pollen of C. longifolia, resulting in the development of perfect seeds, some of which germinated last year (1882).

These were claimed to be the first hybrid cycads; but as soon as this claim appeared, it was found that Weilbach, Curator of the Botanical Garden at Copenhagen, had fertilized Ceratozamia robusta Miq. with the pollen of C. brevifrons Miq., and obtained seeds which germinated. These hybrids were sent to various gardens. Miqel himself determined the species of the parents; but Warming later identified the so-called C. robusta as C. longifolia, and C. brevifrons as C. robusta.

Hemsley remarks:

There is only one thing that is certain in all this, and that is the uncertainty of the so-called species. It is quite possible, and indeed very probable, that we have here in both instances to do with the male and female of the same species.

1 Hemsley, Wm. B., Hybrid cycads. Gardeners Chronicle 19:466-467. 1883.
A study of *Ceratozamia* in the field and in the greenhouse for a period of twenty years enables me to state with confidence that the uncertainty in those determinations is even greater than Hemsley intimated. DeCandolle, in his *Prodromus*, lists *C. longifolia* and *C. robusta* under *species dubiae*. They are more than doubtful. It is safe to say that seeds of a single cone of *C. mexicana* could be made to yield plants of *C. mexicana*, *C. longifolia*, *C. robusta*, *C. Miqueliana*, and *C. brevifrons*; and this result could be brought about by raising the plants in containers of various sizes and by planting in the ground in various conditions. The different characters could probably be developed in less than twenty years.

My principal study of *Ceratozamia* in the field was made near Jalapa, Mexico, on the steep, well drained mountain side opposite the extinct crater of Naolinco. All the plants seemed to be typical *C. mexicana*. Plants raised from seeds collected here and planted at the University of Chicago were easily diagnosed as *C. mexicana*; but after fifteen years many of them had become *C. longifolia*. None appear to be *C. brevifrons*, but that may be due to the fact that none of them were kept in small containers. *C. Küsteriana* may be a good species, but it looks as if the rest could be raised from *C. mexicana*.

The pollen of cycads is short lived. It gives the greatest percentage of germination when first shed; after a few days there is a notable decrease in the number of spores which germinate; at the end of ten days less than half the spores germinate; at the end of three weeks very few spores germinate; and a month from the time the pollen has been shed I have not been able to germinate the pollen of any cycad. Mrs. Alice Bailey, who is particularly skillful with culture media, was able to germinate pollen a few days after my attempts failed, but a month can be regarded as the limit of life of *Ceratozamia* pollen.

This would mean that while Katzer went through the motions of pollination, it is practically certain that there was no fertilization. That embryos were developed and seeds obtained I do not doubt; but it is well known that in *Ceratozamia* and *Encephalartos* the female cones and their seeds develop to full size, even without any pollination. Sedgwick\(^2\) proved conclusively that *Encephalartos* sometimes

produces embryos without any pollination. There is, in this case, a fusion of the ventral canal nucleus with the egg nucleus, just as one finds it occasionally in Pinus, Picea, and Ginkgo. It is practically certain that Katzer's so-called hybrids were of this type, or at least were produced without fertilization by sperms. To summarize, it may be stated, as it was said in 1851 and 1882, that the plants here described are the first hybrid cycads.

Even in limited collections in greenhouses there are occasional opportunities for crossing cycads. The pollination should be done just as the scales of the female cone have opened enough to allow the pollen to enter easily. When the opening between the scales is at its maximum, the pollination drop at the end of the micropyle is in the best condition for receiving the pollen. If pollen is abundant, it may be placed on a sheet of paper and puffed against the female cone; but if the pollen is from a small cone, like that of Zamia latifoliolata, a method devised by our head gardener is better. He takes a long glass tube, with one end drawn out like the tip of a pipette, touches some pollen with this tip and blows it into the crevices between the scales.

During the past five years several cross pollinations have been made in the University of Chicago greenhouse, most of them between different species of the same genus, but some of them between different genera. With the parents and the F₁ generation well labeled, it may be possible, within the next ten years to pollinate intelligently for an F₂ generation.
Zamia latifoliolata × Z. pumila

On December 20, 1921, I pollinated the female cones of two plants of Zamia latifoliolata with pollen of Z. floridana. The Z. latifoliolata was brought from Porto Rico, and the Z. floridana was secured from Miami, Florida. The scales of the female cone were tightly closed, and it is doubtful whether any of the pollen not washed off by spraying survived until the scales opened. A week afterward the scales of the female cones opened and both cones were pollinated with pollen from a plant of Z. pumila which had been secured at Hawks Park, Florida. One of the cones produced three good seeds and the other produced four, all of which were
planted and four of which germinated. The condition of one of these cones when the seeds were ripe is shown in fig. 1. The cone at the left in this figure is a year younger. Its scales are just beginning to open. Two or three days after this negative was made, several of the scales were open and the cone was pollinated (December 15, 1922) with Z. floridana. This also seemed to be a successful pollination, for the cone grew, and on November 21, 1923, the seeds were ready to fall out.

The four seedlings resulting from pollination by Z. pumila grew vigorously, and on February 26, 1925, a little more than three years after the pollination (December 30, 1921), and two years after planting the seeds (January 9, 1923), they presented the appearance
shown in fig. 2. The size relations are shown somewhat diagrammatically in fig. 3.

It is evident that, in general habit and the character of the leaves, *Z. latifoliolata* is dominant, and that *Z. pumila*, which has comparatively small narrow leaflets, is recessive. The F₁ generation also resembles the dominant parent in the general contour of the leaflets, their serration, and in the number and branching of the veins.

The contour of the typical leaflets of *Z. latifoliolata, Z. pumila*, and their hybrid is shown in fig. 4. The veins are very numerous in *Z. latifoliolata*, often more than sixty; while in *Z. pumila* there are about twenty. In the F₁ generation the average number is about forty. We do not have seedlings of *Z. latifoliolata*, but four leaflets on a leaf developing from a bud scale had 33–40 veins. In the cycads such leaves resemble those of seedlings, or at least are juvenile in character. So it is probable that, so far as the number of veins is concerned, the F₁ generation closely resembles that of *Z. latifoliolata*. The histological character of the leaflet is also more like that of *Z. latifoliolata*, especially in the loose texture of the parenchyma between bundles.

If these four F₁ plants should reach the coning stage, as they may within the next ten years, and both sexes should be represented, and male and female cones should be produced at the same time, it would be interesting to see what would appear in the F₂ generation. Since the cones of the two parents are as distinct as their leaves, another pair of characters could be contrasted even before the F₂ generation is secured. Although *Z. latifoliolata* has a larger leaf, which is dominant in the hybrid, *Z. pumila* has a larger stem and larger cones.
Zamia latifoliolata × Z. floridana

The cone of *Z. latifoliolata*, shown at the left in fig. 1, was pollinated December 15, 1922, with pollen from *Z. floridana*. The seeds were planted November 25, 1923, and four seedlings survived. Their appearance on February 23, 1925, is shown in fig. 5 and in the somewhat diagrammatic fig. 6, which records details of size relations.

Here again it is evident that *Z. latifoliolata* is dominant and that *Z. floridana* is recessive. The seedlings look very much like those of *Z. latifoliolata × Z. pumila*; but it must be remembered that the female in this case is not only of the same species as that of the first hybrid described, but that it is the same plant. Besides, *Z. pumila* and *Z. floridana* are very nearly related species. The leaflets have the contour, serration, and venation of *Z. latifoliolata*. Fortunately in this case there are seedlings of *Z. floridana* for comparison (fig. 7). Its long narrow leaflet, with comparatively little serration, contrasts
sharply with the broad leaflet of *Z. latifoliolata*. Even the F, seedling shows double the number of veins found in the adult leaflet of *Z. floridana*.

In foliage *Z. floridana* differs more widely from *Z. latifoliolata* than does *Z. pumila*, and in the histological structure of the leaf the two species can be distinguished easily. In transverse section, the leaflet of *Z. latifoliolata* shows 6–8 suberized tracheids around the vascular bundle, while *Z. floridana* shows two or three times as many. The hypodermal layer of the abaxial side of the leaflet of *Z. floridana* is usually more or less sclerotic, while such a thickening is confined to the epidermis in *Z. latifoliolata*, except of course at the bundles, where all the cycads have sclerotic tissue.

The hybrid shows no sclerotic hypodermis on either side of the leaflet. The number of the suberized tracheids surrounding the bundle, as seen in the transverse section, varies from five to seven, while in the seedling leaflet of *Z. floridana* the number varies from eleven to fourteen. The mesophyll of the hybrid has large air spaces
like those of *Z. latifoliolata* and not at all like those of *Z. floridana*, in which this tissue is even more compact than in *Z. pumila*. In short, the foliage of the hybrid resembles that of *Z. latifoliolata*, both in topography and histological structure. Consequently, the dominant parent is the one with the larger leaves and broader leaflets.

**FIG. 8.—** *Z. pumila* × *Z. latifoliolata*, nearly mature cone

The cones and stems of *Z. floridana* are larger than those of *Z. latifoliolata*, but it will be about ten years before it will be known which parent is dominant in this respect.

**Zamia pumila** × **Z. latifoliolata**

On January 25, 1924, a female cone of *Zamia pumila* from Hawks Park, Florida, was pollinated by *Z. latifoliolata*. Only the upper
scales were open, but the cone grew to the usual size of cones of *Z. pumila*, and looked like a normal cone of this species (fig. 8). The first week in January 1925, the peduncle was accidentally broken, but the seeds were nearly ripe and the seed coats had an orange red color tinged with the darker red of *Z. latifoliolata*, rather than the light red with prominent orange which characterizes *Z. pumila*. The seeds were planted February 19, 1925, and several embryos broke the seed coat, but only one survived (fig. 9).

The color of the seed coat and the general appearance of the seedling are those of *Z. latifoliolata*; so that in this case, as in the reciprocal cross *Z. latifoliolata* × *Z. pumila*, the *Z. latifoliolata* is dominant.

**Zamia latifoliolata** × **Zamia monticola**

On March 15, 1924, a cone of *Zamia latifoliolata* was pollinated with *Z. monticola*, and four days later another cone was pollinated from the same male cone. The female cones grew to the usual size of cones of *Z. latifoliolata* and the seeds seemed to be normal. The color of the seed coat in the first cone was somewhat lighter and with more orange than in the typical cone of *Z. latifoliolata*; but in the second cone the seeds had the dark red color characteristic of *Z. latifoliolata*, with scarcely any tinge of orange.

The seeds were planted late in December 1924, but as yet no seedlings have appeared. Although the seeds have not decayed, it is doubtful whether any seedlings will be secured.

Ceratozamia mexicana × Zamia monticola

The most striking and successful cross made is one between Ceratozamia mexicana and Zamia monticola. One cone was pollinated March 23, 1924, with pollen from the same plant of Z. monticola which had produced the cones used in all the previous pollinations in which this species was one of the parents. Another cone of Ceratozamia on another plant was pollinated on April 25, 1924, with pollen from another cone of that same plant of Z. monticola. This new species is very favorable for hybridizing, because, during the period described in this paper, it has coned every year, producing from four to six cones at a time. The cones appear in succession, and shed their pollen in succession. Of the six cones which appeared in 1920, the first began to shed pollen December 14, 1920, and the last pollen from the sixth cone was shed February 9, 1921. Since each cone continues to shed pollen for about a week, there was a period of nearly two months in which any female cone, which might open its scales, could be pollinated. I have not had under continuous observation any other cycad in which the period of shedding pollen was so prolonged, but there can be no doubt that in Macrozamia Moorei, with its 20–100 males cones developing in spiral succession, the shedding period is much longer.

From these two cones nearly a hundred seeds appeared to be well developed. They began to fall out naturally November 27, 1924, and during the next few days all were planted, except a few which were reserved for dissection.

Ceratozamia is unique among the cycads in having only one cotyledon, while all the rest have two. This fact was noted first by VAN TIEGHEM, and later confirmed by WARMING, MATTE, Sister HELEN ANGELA, and others. MATTE, however, found two cases in

6 MATTE, HENRI, Note préliminaire sur les germination des Cycadées. Rennes. 1907.
7 ———, Memoires société Linnéene de Normandie. 23: 35–44. 1908.
which there were two cotyledons. Van Tieghem's observations were made upon four "hybrid" seedlings secured by pollinating C. longifolia with pollen from C. mexicana which had been shed three years before the female cone was pollinated. In three of the seedlings there was only one cotyledon, but the fourth seemed to have two very unequal cotyledons. In germinating a large number of seeds I never happened to notice more than one cotyledon. Sister Helen Angela examined more than a hundred embryos and every one had only one cotyledon. In microtome sections for an anatomical study, however, she noticed occasionally a few tracheids opposite the cotyledon. Ceratozamia is unique among cycads in another feature, the seeds drop out of the cone soon after fertilization and before the various regions of the embryo have differentiated. Cones were secured from Mexico shortly before the time for the seeds to drop out, and Sister Helen Angela fastened a great number of them to clinostats which were rotated until the embryos were mature. All of the embryos on the clinostats had two cotyledons; but checks, planted normally, had only one. This was conclusive evidence that Ceratozamia, phylogenetically, had two cotyledons, one of which had become suppressed.

If the seeds planted by Matte (some of which were of my own collection from the same locality which produced the seeds studied by Sister Helen Angela) were planted in any other position than on the side, I should expect them to develop two cotyledons. We always plant the seeds of cycads with the long axis parallel with the soil and nearly but not quite covered by the soil. More than sixty seeds planted in December 1924 germinated. Without disturbing the young seedlings, one could see that many of them had two cotyledons. In July 1925, when the seedlings were being repotted, a careful examination was made, and forty-seven of the fifty-six seedlings which had survived up to this stage showed two cotyledons; three had one cotyledon, and in the other six the cotyledon situation could not be determined without sacrificing the seedlings.

The two cotyledons are shown in fig. 10, and also in A, B, and C of fig. 11. In C the embryo has been removed from the endosperm. The development of the two cotyledons proceeds as in other cycads. In the usual single cotyledon of Ceratozamia, the outline of the
cotyledon is often somewhat C-shaped in transverse section, so that by looking at the open side of the C, one might imagine that there were two cotelydons; but a section of the hybrid embryo shows the

Fig. 10.—Ceratozamia mexicana × Z. monticola, F₁ generation
two cotyledons clearly, with the vascular supply just as described by Sister Helen Angela for the dicotyl embryos developed on the clinostat.

One of the plants with a single cotyledon is shown in fig. 11 D. The stem apex lies between the leaf bud (b) and the cotyledon (c), but the suppression of the second cotyledon gives the leaf the position of the missing organ. Sections would show a few tracheids belonging to the abortive cotyledon, so that the leaf bud is really between the two cotyledons. The apogeotropic roots, shown in D and also in B, are extremely common in cycads and are not at all the result of hybridization.

The leaflets of most of the seedlings are like those of Ceratozamia in contour, and all agree with Ceratozamia in having no serration; but many leaves of Zamia monticola have no serration. However, most of the leaflets are broad and have the contour of Z. monticola.

In this hybrid, Zamia monticola, the male parent is dominant in the character of the cotyledons and in most of the leaflets, while Ceratozamia is dominant in the general topography of the seedling and the contour of some of the leaflets. Ceratozamia mexicana has
a long slender leaflet tapering to a sharp point, and, like all species of *Ceratozamia*, if there is more than one species, entirely lacking in ser-

![Diagram showing leaflets of Ceratozamia mexicana and Z. monticola](image)

**Fig. 12.**—*Ceratozamia mexicana* × *Z. monticola*: *A, B*, leaflets of two parents; *C, D*, leaflets resembling those of *Z. monticola*; *E, F*, leaflets with contour of *Ceratozamia mexicana*; one-half natural size.

...ration. In *Z. monticola* the leaflet is comparatively short and blunt at the apex. The contour of typical leaflets of the two parents and also leaflets of the hybrids are shown in fig. 12. In *C* and *D* of this
figure the leaflets have the contour of those of *Z. monticola*, and the number of veins is larger than in the adult leaflet of *Ceratozamia*; while in *E* and *F*, and also in fig. 11, the leaflets have the contour of those of *Ceratozamia*. The number of veins in leaflets of the *Ceratozamia* type, while smaller than in leaflets of the *Z. monticola* type, is nevertheless as large or larger than in adult plants of *Ceratozamia*.

Fig. 13.—*Z. pumila* × *Encephalartos villosus*, female cone shedding seeds

The histological structure of the leaflet of the hybrid resembles that of *Z. monticola* in the small number of suberized tracheids surrounding the bundle, and in the scarcity or entire lack of such tracheids between bundles. The difference here is very striking, because *Ceratozamia* has many such tracheids around the bundle and also between bundles. The mesophyll cells of the hybrid, in transverse section of the leaflet, are much elongated between bundles, while in *Ceratozamia* they are shorter and the tissue is more compact.
To summarize, the hybrid resembles *Z. monticola* in having two cotyledons and in the histological structure of the leaflet, as well as in the contour of most of the leaflets.

In this case both parents are large plants, with leaves more than a meter in length. *Ceratozamia* is arborescent, and this specimen of *Z. monticola*, a new species and the only specimen known, looks as if it might develop a trunk as large as that of *Ceratozamia*.

In the other crosses just described, all of them between different species of *Zamia*, both parents have small, tuberous, subterranean stems, and the parent with the greater display of foliage has been dominant in leaf characters.

In the cross between *Ceratozamia mexicana* and *Zamia monticola*, the latter is dominant in the characters of the leaf and cotyledons. The female cone of *Z. monticola* is not known, but the male cone, while very large for a *Zamia*, does not reach half the size of the average male cone of *Ceratozamia*. With about fifty hybrids now growing vigorously, it is quite possible that within nine or ten years there may be cones of both sexes, so that the cone character may be determined and an F2 generation obtained.

**Zamia pumila × Encephalartos villosus**

On December 12, 1924, a female cone of *Zamia pumila* was pollinated with *Encephalartos villosus*. The *Zamia* was transplanted from Hawks Park, Florida, in 1914; the *Encephalartos* was secured from the Botanic Garden at Durban, South Africa, in 1912.

Only the upper scales of the female cone opened, but the pollination seemed efficient, for the cone grew and attained the usual size of cones of *Z. pumila*, instead of degenerating when not pollinated, as do the female cones of all the species of *Zamia* described in this paper. By the middle of October, 1925, the seeds were breaking loose from the sporophylls, and two weeks later the cone fell apart and the seeds were planted (fig. 13). The fleshy layer of the seeds had less of the orange color than the usual seeds of *Z. pumila* and more of the deep red, but not so much as one usually finds in seeds of *Encephalartos villosus*. The seeds have not yet germinated, so that it is doubtful whether any F1 generation will be obtained. It is well known that pollen of cycads is easy to germinate up to a certain age
in culture solutions. It may be that the pollen germinates in the pollen chamber and stimulates the growth of the cone and ovules, but that no fertilization takes place. One could not remove material for determining such features without danger of killing the entire cone.

All cycads, like most gymnosperms, have twelve and twenty-four chromosomes as the $x$ and $2x$ numbers, which may account in some measure for the ease with which hybrids are obtained in this family.

So far as the names of the plants used in this study are concerned, I cannot speak with authority. For the two species of Zamia from Florida we have used the names $Z. \text{ pumila}$ and $Z. \text{ floridana}$, the names used in WEBBER’s\(^9\) studies of Zamia and in various papers and books from this laboratory. The $Z. \text{ latifoliolata}$ came from Porto Rico; the Ceratozamia mexicana was grown from seeds secured near Jalapa, Mexico; and the Encephalartos villosus came from the Botanic Gardens at Durban, South Africa, and is the form which occurs near Durban.

Records are being kept of the parents and hybrids described in this paper, so that data will be available when the time arrives for an F\(_2\) generation.

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