VARIABILITY OF THE FEMALE REPRODUCTIVE ORGANS IN GINKGO BILOBA L.

by

W. K. H. KARSTENS

(Botanical Laboratory, University of Leyden).

Introduction.

In 1915 Worsdell signed: "The object of botanical investigation, in whatever department, should be to determine, as far as possible, the interrelationship of the various facts which are accumulated and arrange them accordingly; and not merely, as has for so long been the custom, to pile them in chaotic heap."

This is quite true; only too often the facts have been piled in a chaotic heap. This has been the case with investigations on the nature of the reproductive organs, both female and male, in Ginkgo biloba. An extenuating circumstance, however, is the great variability in the aforesaid organs; so great a variability indeed that one hesitates to tackle the subject, for fear that it will appear to be still greater than was previously supposed.

It is perfectly unnecessary to state once more that Ginkgo is a very remarkable tree, less known is the fact that the variability in the female reproductive organs is very considerable, not only in one plant at one moment, but in relation to time too. It makes a difference, whether a Ginkgo tree is young or old. So great is the variability that quite common variations have been termed "abnormalities".

Material.

From a number of trees seeds were obtained. In the first place the tree in the Botanic Garden of Leyden has to be mentioned. From this tree planted in 1850, a large number of seeds has been collected in 1940 by Jonkheer W. C. van Heurn. I could investigate these seeds by the courtesy of the curator of the Rijksherbarium at Leyden Dr S. J. van Ooststroom, to whom they were originally presented. In 1943 a still greater quantity was collected from the same tree. In the same year seeds were also received from trees growing at Slikkerveer, 's Graveland, and Maastricht. For the receipt of these seeds I should like to thank Jonkheer W. G. Groeninx van Zoelen van Ridderkerk, Mr P. G. van Tienhoven, and R. Schoenmakers respectively. The tree at Slikkerveer was the same from which in 1914 Affourtit and La Rivière (1915) received their material.

Furthermore the Leyden tree supplied plentiful material for the investigation on the variability of the female "inflorescences".

Variability of the female "inflorescences".

Since many years the nature of the female reproductive organs of Ginkgo has been a subject of much discussion. Pilger (1926) gives a review of the literature on this subject, while Zimmermann (1930) discusses the "flower" of Ginkgo as an example of a phylogenetic problem. This author prefers to call the female reproductive organs "Makrosporangienstände", macrosporangiophores, and says: "Wir wenden uns gegen den weit verbreiteten Glauben, ein Problem wie die Phylogenie der Ginkgosporangienstände sei dadurch zu lösen, dass man derartige primitive Bildungen hineinpresst in Begriffe, welche von typischen oder höheren Pflanzen gewonnen sind, das sind in diesem Falle die uns geläufigsten Pflanzen, die Koniferen und die Angiospermen."

We shall take Zimmermann's advice to heart and call the "flower" of Ginkgo a macrosporangiophore.

a. *Number of macrosporangiophores on the brachyblasts.*

The Ginkgo tree bears branches with both long and short shoots, the latter are the so-called brachyblasts. Generally, it takes some years before the brachyblasts become fertile. It occurs rather frequently that the brachyblasts form a long shoot at their top. Next year the buds of these secondary long shoots develop into fertile brachyblasts. These we will call "young brachyblasts" in contradistinction with the ordinary fertile short shoots, which we will call "old brachyblasts".

Some authors have procured information as to the number of macrosporangiophores on the brachyblasts. According to Carothers (1907) the number of macrosporangiophores varies from one to six. De Haan (1920) mentions this number also, but says that it is mostly five.

In the present investigation, however, a somewhat greater range was found, the number varying in older brachyblasts from one to eight, in younger ones from one to six (cf. *Table I*).

TABLE I (cf. *Fig. 1*).

Number of macrosporangiophores on the brachyblasts.

Number of macrosporangiophores on brachyblast.	1	2	3	4	5	6	7	8
old brachyblasts	2	4	5	9	13	13	9	2
young brachyblasts	2	5	6	6	5	2	—	—

From this table and from *fig. 1* it may be seen in the first place that the number of macrosporangiophores may be higher than six. Probably this number will appear to be still higher on studying very old and well outgrown short shoots. Secondly it appears that the variability in young short shoots is much less than in older brachyblasts — a phenomenon we will encounter again — and that the maximum lies at 3—4, against 5—6 in the older brachyblasts.

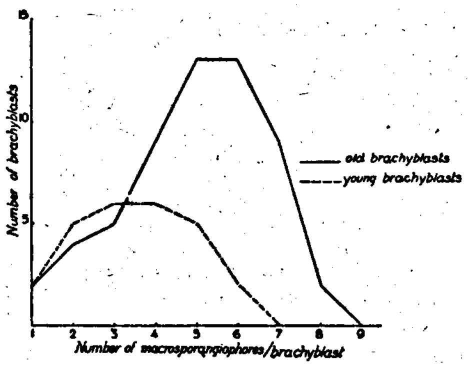

Fig. 1 — Number of macrosporangiophores in young and old brachyblasts.

b. *Variation in the shape of the macrosporangiophores.*

It is a well-known fact that the shape of the macrosporangiophores may be very different. However, the various authors do not agree as to this point. The reason of this dispute is the great variability in relation to the age of the Ginkgo trees investigated.

Strasburger (1879) described female sporangiophores in which the stalk bore four ovules supported on slender stalklets.

Von Wettstein (1899) makes a subdivision of his material into two groups, normal flowers (von Wettstein calls the female reproductive organs of Ginkgo flowers) with two sessile ovules, and abnormalities. These abnormalities can, according to von Wettstein, be arranged into four categories:

a. flowers with two more or less stalked ovules,
b. flowers with more than two sessile ovules,
c. flowers with more than two stalked ovules,
d. flowers with only one ovule.

Representatives of the first category are, according to von Wettstein, restricted to the axils of leafy budscales, and nearly always a little bud is found between the stalklets of the ovules. Representatives of the second category, however, show a positional preference for the axils of the uppermost normal fertile leaves. Mostly there are three, more rarely four sessile ovules.

Von Wettstein considers the first group primitive and atavistic in relation to the so-called normal flower, while the second category is interpreted as a splitting and an advanced character. The third and fourth group do not seem to be very important to von Wettstein, since they are not mentioned in detail. The following quotation gives an impression of von Wettstein's point of view: "Der im Vorstehenden beschriebene Bau der normalen Blüte und der von mir untersuchten Abnormitäten spricht unbedingt dafür, dass die normale Blüte von Ginkgo aus einem Gebilde besteht, das einem blos zwei transversale Fruchtblätter tragenden Sprosse gleichwertig ist. Durch Auseinanderweichen der beiden Blätter und stielartige Ausbildung ihrer Basis entstehen die gestielten Samenanlagen, durch Theilung der Fruchtblätter 3—4-samige Blüten, durch Ausfall eines der Fruchtblätter 1-samige Blüten."

Celakovsky (1900) also studied the macrosporangiophores and does not agree with von Wettstein regarding the following point. According to Celakovsky three ovules do not originate by a dédoublement of one of the two ovules of the normal flower, but in the case of three ovules these are always equally stalked, while von Wettstein only saw three sessile ovules. In our opinion the solution of this controversy is that both authors did not see sufficient material, probably since the trees were too young. In material obtained from more aged trees both cases occur.

Sewards and Gowan (1900) described some abnormal forms of female flowers, one in which the peduncle bears three stalked ovules and a lateral unexpanded bud, another where five ovules are extant.

Von Spiess (1902) studied the macrosporangiophores of Ginkgo in relation to Cephalotaxus and Taxaceae. According to this author there is no such thing as a small bud between two stalked ovules. His material, however, was certainly not sufficient to decide upon this point.

Sprecher (1907) published a monograph on Ginkgo. In a paragraph on the "Anomalies de la fleur femelle" he gives an exposition of the nature of abnormalities, considering two sessile ovules as normal. Concerning Ginkgo the author says: "Les anomalies dont je vais parler sont de nature atavique ou peut-être quelquefois des phénomènes de mutation; les caractères latents se sont développés sous l'influence du milieu ambiant."

Sprecher investigated a great number of female brachyblasts: 28 % had normal flowers, 72 % showed both normal and abnormal flowers. The material hailed from a relatively young tree. From a much older tree the material showed a greater percentage of abnormalities.

Ten groups of abnormalities have been described by the author:

a. three sessile ovules,
b. two stalked ovules,
c. three ovules on two stalklets, one solitary and two fused ovules,
d. one solitary ovule,
e. six stalked ovules,
f. four sessile ovules,
g. four ovules fused by twos,
h. four stalked ovules,
i. six stalked ovules, the seventh sessile,
j. three stalked ovules.

Sprecher did not see the variation described by von Wettstein, in which a small bud occurred between two stalked ovules, neither did he see the abnormality described by Seward and Gowan with three stalked ovules and a small lateral bud.

Worsdell (1916) writes in his Principles of Plant-Teratology: "The uni- or biovulate axis of the maidenhair tree (Ginkgo biloba) may proliferate in the sense that it bears a greater number of ovules which then become stalked and may be spirally arranged." Later on the same author says furthermore: "In the maidenhair tree (Ginkgo biloba) we find abnormally an increase of the number of carpels (here reduced to ovules), which also become long-stalked; doubtless a case of reversion, the normal female flower of Ginkgo being palpably a reduced structure."

De Haan (1920) is the first author, who discovered that there is a certain regularity in the occurrence of abnormal flowers, or as the author calls them "strobili". Of the 114 examined brachyblasts were 76 in which the first strobilus, standing in the axil of a budscale, had an abnormal shape, that is to say was split, or had only one terminal ovule, or was in some other way curiously formed. Their dominating feature was the division in slender stalklets with one terminal ovule, contrary to the normal strobilus with sessile ovules. According to the regularity in the occurrence of certain forms of strobili the author says: "The first strobilus always arising in the axil of a budscale, has sometimes an abnormal form, as I mentioned before. The second strobilus, also standing in the axil of a budscale is normal, in nearly all cases bearing two sessile ovules. The third one sometimes, and nearly always the fourth, standing in the axil of an ordinary leaf, bears three sessile ovules. The number of ovules on the fifth strobilus is very different and often they are less developed. The frequency of four sessile ovules is greatest on this strobilus. Sometimes there is a sixth strobilus, which again bears two normal ovules."

De Haan concludes from the fact that, as he puts it, the lowest budscales are the most primitive, that the first strobili, developing, from the axils of those primitive budscales represent the most original case. According to him, the arrangement of the ovules on the strobilus is just the same as the position of the leaves on the ordinary long shoot, the one or two lowermost pairs being decussate, the others in a spiral order. Unlike von Wettstein, De Haan says: "Therefore it is clear, that in the now existing reduced strobili, on which only the lower sporophylls are developed, these are decussate, but it may not be called the normal order, this being the spiral one."

The work of De Haan does not seem to have drawn the attention it deserves. Pilger (1926), in his revision of the Ginkgoales in Engler-Prantl, only gives the title of De Haan's thesis, but does not quote him and only mentions the incomplete work of von Wettstein. As a matter of fact I did not see De Haan's publication until after having come to similar, though in some respects less complete results. The time of getting good material was over then, as the budscales and many macrosporangiophores had fallen off by that time.

Schaffner (1927) studied the form of the young female reproductive organs in relation to their morphological nature. He arrived at the conclusion that these are megasporophylls and drew the attention to the fact, that the normal shape is dorsiventral. At one side there exist a groove between the ovules, at the other side there is a prominent flat surface, the leaf, ending in a ridge. As a matter of fact female organs of this description are often to be found.

It is, however, very difficult to explain the structure of the more complicated macrosporangiophores in the light of this hypothesis. The author says to this point: "The abnormalities which have been observed by various investigators are plainly in agreement, for the most part at least, with the view that the structures are sporophylls, although conclusions drawn from monstrosities are in general of little phylogenetic significance." It is evident that this is no great help.

Finally in 1929 Sakisaka published an article on the seed-bearing leaves of Ginkgo. This author gives some data on the frequency of the number of ovules on the macrosporangiophores. No attention, however, is drawn to the fact that macrosporangiophores with two, three or four ovules may show an entirely different structure. Furthermore the impression is gained, that in the material investigated, sporangiophores with more than five ovules were not found. The illustrations, however, show that seven and even ten ovules have been found.

The Leyden tree, which was planted in 1850, provided us with plentiful material; more than two thousand sporangiophores could be investigated. Material collected in 1913 has been conserved in the Rijksherbarium Leyden. This proves that Affourtit and La Rivière (1915) were right in saying that the tree had flowered during recent years.

When studying the fertile brachyblasts it is obvious that there exists a certain coordination between the shape of the leaves and the structure of the macrosporangiophores. As several authors have observed, the more complicated macrosporangiophores are always standing in the axils of budscales. From a budscale an uninterrupted series to a green reduced leaf may be observed (cf. *fig. 2*). This figure is, in a way, a least common

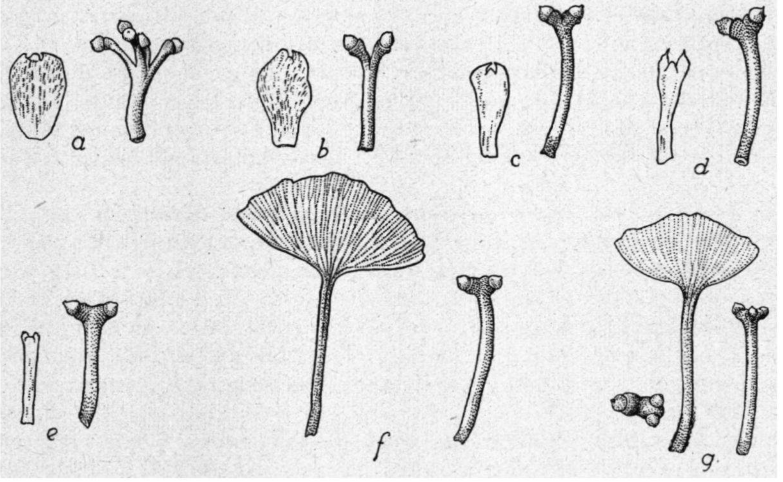

Fig. 2 — Relation between the type of macrosporangiophores and the shape of the corresponding leaves.

multiple, which in this form never occurs in nature; there are, for instance, too many budscales and too few leaves in this series.

We have subdivided the macrosporangiophores into four groups:

a. branched macrosporangiophores with 8—3 stalked ovules (*fig. 2a*),
b. macrosporangiophores with 2 stalked ovules (*fig. 2b*),
c. "normal" macrosporangiophores with 2 sessile ovules (*fig. 2c–f*),
d. "normal" macrosporangiophores with 3 or 4 sessile ovules (*fig. 2g*).

These groups are to be subdivided into smaller categories, group a according to the number of ovules, group b into the three following smaller groups:

1. with a small bud between the 2 ovules,
2. without such a bud,
3. with the stalk split down to the base and one of the two halves aborted.

Group c. is subdivided into two groups, one with, the other without a rudimentary fissure between the two ovules (*fig. 2c* and *d–f*). The last group, finally, is to be subdivided into two groups according to the number of ovules.

TABLE II (cf. *fig. 3*).

Number and nature of ovules in the macrosporangiophores.

Group	a						b			c		d	
Number of ovules	8	7	6	5	4	3	2 + bud	2 — bud	1	2 + fiss.	2 — fiss.	3	4
old brachyblasts	3	8	13	12	34	45	24	158	9	154	706	187	22
young brachyblasts ...					5	11	6	105	37	78	508	31	1

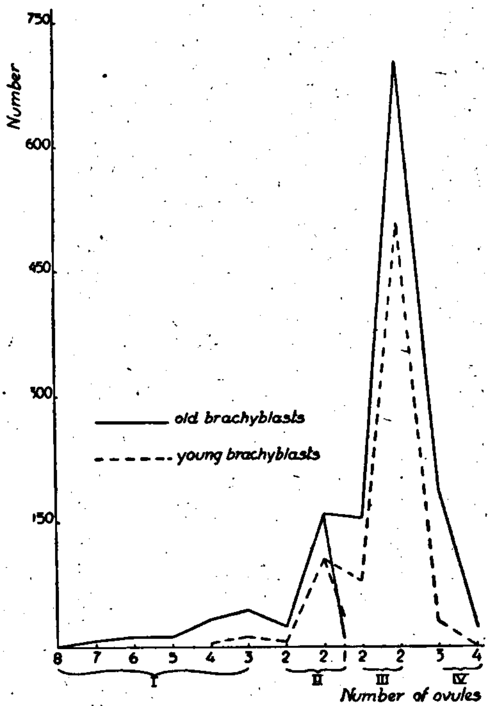

Fig. 3. — Relation between different types of macrosporangiophores in young and old brachyblasts.

From *Table II* several points are apparent. First of all the variability is not as great in young brachyblasts as it is in older ones; the age of the brachyblast seems to be an important factor. Possibly the age of the tree has a similar influence.

By plotting these data into a diagram (*fig. 3*) it is apparent that while the width of the variability is greater in the old brachyblasts, the nature of the variability is perfectly the same in both types. Another point of interest is the occurrence of single ovules. This type is to be found on a much larger scale in young brachyblasts than in older ones. Because of the great number of macrosporangiophores (1475 in old and 782 in young brachyblasts respectively) it is not very probable that this is a mere chance. Other points becoming obvious in young brachyblasts is the absence of the more complicatedly branched macrosporangiophores, while representatives of group d are rare or very rare.

By determining the nature of the leafy organ from whose axil the sporangiophores arise, we obtain more information as to the nature of the variability of the female reproductive organs of Ginkgo. It is possible to make a subdivision into a group with the sporangiophores situated in the axils of budscales (*fig. 2 a–c*) and another with the sporangiophores in the axils of leaves (*fig. 2 d–g*). The results are given in *Table III*.

TABLE III (cf. *fig. 4*).

Number and nature of ovules in the budscale- and leaf-axil macrosporangiophores.

Group	a						b			c		d	
Number of ovules	8	7	6	5	4	3	2+ bud	2— bud	1	2+ fiss.	2 — fiss.	3	4
old brachyblasts (budscales)	3	8	8	7	22	34	18	109	6	86	63	3	1
old brachyblasts (leaves)								2	2	34	446	144	17
young brachyblasts (budscales)					5	8	4	86	31	54	83	1	
young brachyblasts (leaves)										13	418	28	

This table elucidates several other points. The range of the variability for both budscales and leaves of young brachyblasts is less than is the case with the same organs of old brachyblasts. Furthermore it is obvious that representatives of group a never, those of group b are very rarely situated in the axils of leaves. On the other hand representatives of group c are preferably extant in the axils of leaves. Here again the large number of sporangiophores investigated, 368, 645, 272, and 459 respectively, are a guarantee for the correctness.

In *fig. 4* the data for the two categories of old and young brachyblasts are brought together; the group of single ovules is left out for clearness' sake.

De Haan (1920) denies the possibility, produced by von Wettstein (1899), that the decussate arrangement of the sporophylls is the normal order. According to De Haan only the first one or two pairs of sporophylls are decussate, the rest being arranged in a spiral order. Many of the macrosporangiophores investigated by us show 6 or 8 ovules in 3 or 4 decussate pairs. The morphology of the branched macrosporangiophores needs a closer investigation.

Thusfar all investigators have spoken of abnormalities. All macrosporangiophores of Ginkgo except those of group c (right hand column) have been termed abnormal. However, from the investigations by von Wettstein (1899), Sprecher (1907), and De Haan (1920) as well as from our own data it is evident that it is not allowed to speak of "abnormalities". Their occurrence is by no means a haphazard one; it is distinctly liable to definite rules. The deeper sense of these rules, however, is still unknown. With Von Wettstein we are inclined to speak of reduction in group a—c and of progression in group d as the series described is identical with that occurring in the brachyblasts.

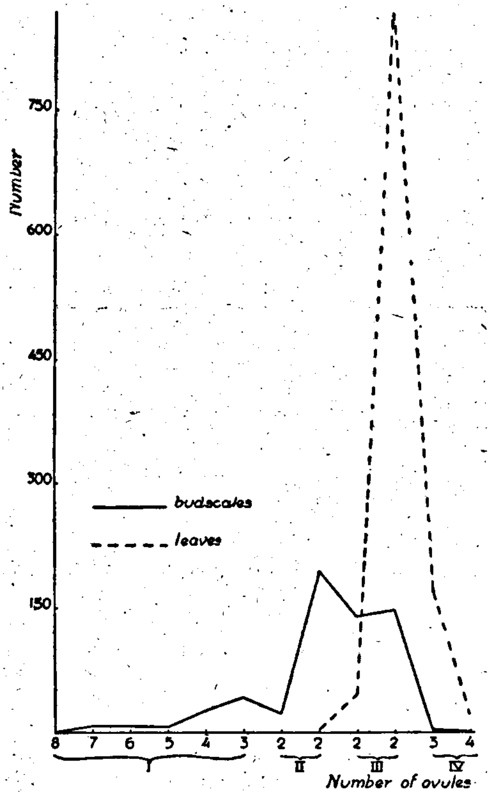

Fig. 4 — Relation between different types of macrosporangiophores and the shape of the corresponding leaves.

Variability of the seed.

a. *Differences in the shape of the seeds.*

In the autumn and the early winter of 1943 a great number, in total 3193, of ripe seeds from trees at Leyden, Slikkerveer and Maastricht have been investigated, 2474, 558 and 161 seeds respectively.

The seed of Ginkgo consists of a kernel covered with a fleshy sarcotesta. Generally the seed has the shape of a small plum. The colour is yellow to orange-yellow. The seeds of the Leyden tree are more or less apricot-coloured, the Slikkerveer and Maastricht seeds have a yellow hue. Among those from Slikkerveer a very great number — about 80 % — were much smaller than the rest, about as big as a small cherry (*fig. 5*, ns. *1* and *2*). By investigating the kernels of the latter type it appeared that these were very small indeed. There seems to be a correlation between the dimensions of the stone and of the whole seed. We did not see this variation in the Leyden material, while there was only one specimen in the material from the Maastricht tree.

Among the material from Leyden and Maastricht — and one case in the material from Slikkerveer — several seeds were found with a more oblong shape (*fig. 5*, ns. *3* and *4*), sometimes even with a more or less sharp apex (*fig. 5*, n. *5*). It appeared that the kernel was congruent to this shape, being slender and sometimes with an acutely beaked apex. Very rare is the occurrence of pear-shaped seeds (*fig. 5*, ns. *6* and *7*).

Finally we found an aberrant form (*fig. 5,* ns. *8* and *9*) 29 times in the Leyden, 7 times in the Maastricht, and only once in the Slikkerveer material. On the outside the abnormality consists of the entrance of the

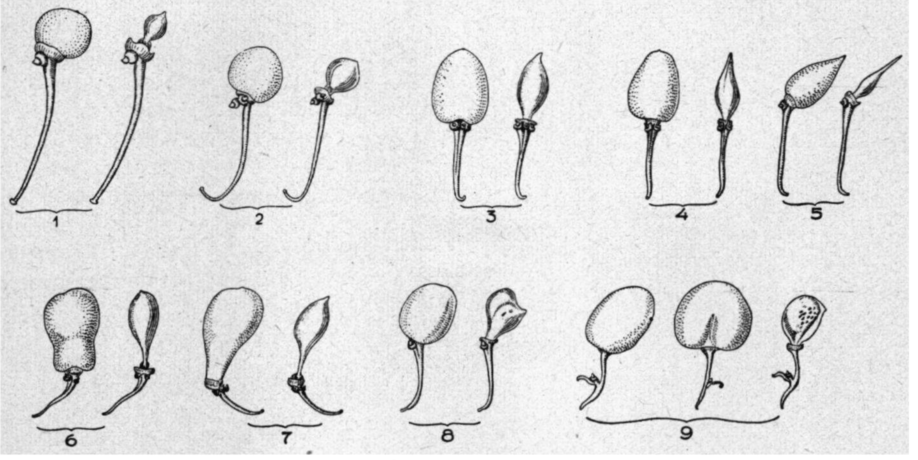

Fig. 5 — Shapes of seeds and kernels, of each pair on the left the intact seed (with sarcotesta), on the right the kernel only.
1—2 cherry-like seeds from Slikkerveer.
3—5 oblong seeds, 3—4 from Leyden, 5 from Maastricht.
6—7 pear-shaped seeds from Leyden.
8—9 seeds with lateral pollenchamber from Leyden.

pollen-chamber being laterally situated. On dissecting the seed the kernel was always found to be abnormal, in as far as its shape is irregular, while the sclerotesta — wall of the stone — is often deficient.

From the above it appears that practically always the shape of the stone may be predicted from the shape of the intact seed.

b. *Length of the seed-stalk.*

In the material investigated from the Leyden, Slikkerveer and Maastricht trees the length of the seed-stalk was most variable. It appeared that the stalks of the Slikkerveer seeds are much longer than those of the Leyden ones, the material of Maastricht taking an intermediate position (*Table IV*).

TABLE IV (cf. *fig. 6*).

Length of seed-stalk.

Origin of the material	Number of stalks	Length in cm
Leyden	34	2.7 ± .08
Maastricht	106	3.6 ± .07
Slikkerveer	250	4.1 ± .04

From *Table IV* it is obvious that the differences found are real. In order to make the graphs of the Slikkerveer and the Maastricht material comparable, the latter has been multiplied by $2^1/_3$ so as to obtain the same

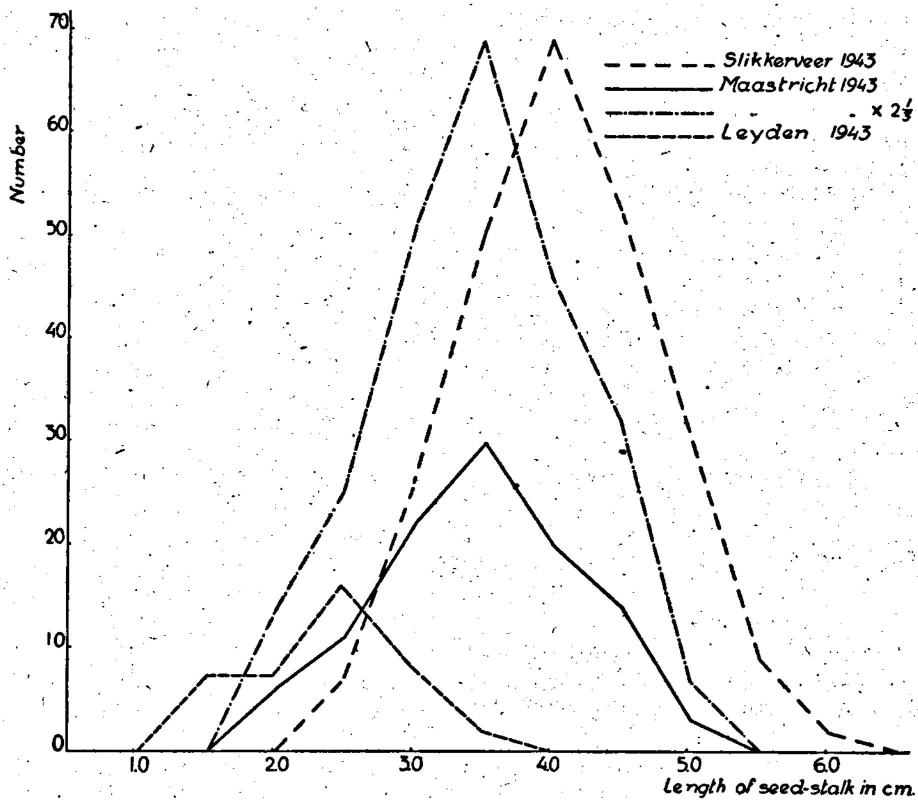

Fig. 6 — Length of seed-stalk. Material from Slikkerveer, Maastricht and Leyden.

height as the graph of Slikkerveer. The graphs appear to be normal variation curves, although the Leyden material was rather too scanty.

c. *Variation in the shape of the kernel.*

Affourtit and La Rivière (1915) studied the variations of the kernel of Ginkgo seeds on a relatively small number (117). We had an opportunity to study a much richer material (cf. *Table V*) from various sources.

It appeared that there are some variations, which thusfar seem to have escaped the attention. In addition our material enabled us to give a classification of the different possibilities in the shape of the kernel. A third point of interest is the fact that we had an opportunity to study material from the identical tree, whose seeds were studied by Affourtit and La Rivière.

Concerning the external shape of the sclerotesta the literature has been given by the above-mentioned authors. In most cases the stones are two-ribbed, but three-ribbed ones are by no means rare, one-ribbed stones are rare and four-ribbed ones extremely so.

As to the cause of the occurrence of two or three ribs, there are several authors who gave their opinion on this problem.

Carothers (1907) mentioned the following facts: "At the base of the ovule two bundles enter the watery tissue, coming up through a little gap in the tissue of the stony coat... In some cases there is a three-lipped integument and a three-angled nucellus, there being also three bundles, one ending in each angled side."

Sprecher (1907) gives more or less the same points: "Dans les semences à trois côtes le parcours des faisseaux libéro-ligneux est pareil, avec cette différence que nous avons trois faisceaux entrant dans la même semence donnent naissance à un noyau à trois côtes prouve que celui-ci est en rapport intime avec les cordons ligneux... une ouverture bilabiale, le micropyle. Parfois cette ouverture est trilabiale; c'est probablement le cas lorsque les semences sont destinées à avoir trois côtes."

De Haan (1920) could not confirm the observation that the micropyle of Ginkgo possesses two lips, or three lips if the kernel is destined to become three-sided.

Herzfeld (1927) describes two or three "eigentümliche wellige Flügelsäume", remarkable undulate keels of the nucellus, corresponding to two or three ribs of the stone, suggesting that these keels have something to do with the germination of the seeds.

Above the one-ribbed stone-type has already been mentioned. Very often the sclerotesta has not developed completely, the result being holes or thin patches in this lignified tissue. In some cases the lignification is complete, the stones look perfectly normal, but instead of two or three ribs a single one occur. Probably the shape of the intact seed containing normally build one-ribbed kernels is the same as those containing normally build two- or three-ribbed kernels.

Table V shows the occurrence of the different types of stones in the material investigated. For comparison the values obtained in the Slikkerveer material examined by Affourtit and La Rivière have also been inserted in this table.

TABLE V.

Number of ribs in seed-kernels.

Origin of the material	Harvested in:	Number of ribs				Total number of seeds
		1	2	3	4	
Leyden	1940	92	888	265	1	1246
Leyden	1943	29	1814	630	1	2474
Slikkerveer	1914	—	47	65	5	117
Slikkerveer	1943	1	407	148	2	558
Maastricht	1943	7	116	35	3	161
's Graveland	1943	—	154	43	—	197

Before comparing the different values obtained for the four types of kernels we may draw the attention to the possibility that the material of Slikkerveer 1914 and of 's Graveland 1943 is not wholly reliable. The much deviating values obtained for the Slikkerveer harvest points in this direction. As to the seeds of 's Graveland we have to mention the fact that from this locality the seeds were not received intact, but the kernels only. This suggests the possibility of an unchecked selection.

On considering the data of table V, we notice first of all that the Leyden tree and the tree from Maastricht tend to produce a relatively large number of one-ribbed stones, while the Slikkerveer tree and possibly the tree from 's Graveland too, seldom bear this type. As to the occurrence of four-ribbed kernels, the Leyden tree is very slow in producing this type of stones. Possibly the 's Graveland tree is, in this respect, of the same type. On the other hand, the Slikkerveer and Maastricht ones carry rather often seeds with four-ribbed stones.

As to the proportion between two-ribbed and three-ribbed kernels (cf. *Table VI*) this is about 3. The ratio .72 in the 1914 material of Slikkerveer renders it very probable that the material investigated had been subject to some sort of selection.

TABLE VI.

Proportion between 2- and 3-ribbed stones.

Origin of the material	Harvested in:	2-ribbed stones	3-ribbed stones	Ratio
Leyden	1940	888	265	3.35
Leyden	1943	1814	630	2.88
Slikkerveer	1914	47	65	.72
Slikkerveer	1943	407	148	2.75
Maastricht	1943	116	35	3.31
's Graveland	1943	154	43	3.58

Affourtit and La Rivière (1915) have drawn attention to the great variability in the ribbing of the seed-stones. As a matter of fact, the angles between the ribs — the stones seen either from the top or from the base — are most variable. In order to investigate whether these are the consequence of some natural law, their angles were measured by means of a simple instrument. In the middle of the base of a graduated arc a movable needle was fixed. By holding a kernel by means of a forceps over the turning point of the needle it was possible to measure the angle between the ribs. The accuracy amounts to about two degrees. *Table VII* shows the number of seeds of which the angle between the ribs has been determined.

TABLE VII.

Number of stones in which the rib-angles were measured.

Origin of the material	Harvested in:	2-ribbed stones	3-ribbed stones
Leyden	1940	849	217
Leyden	1943	1795	606
Slikkerveer	1943	79	31
Maastricht	1943	111	34
's Graveland	1943	154	43

The great difference in the values of the Slikkerveer material in *Tables VI* and *VII* is caused by the very great number (about 80 %) of very small stones in which it was not possible to measure the angles between the ribs with sufficient accuracy.

First of all we wish to discuss the results obtained for the two-ribbed seeds. The values obtained have been brought to groups of five degrees each (*Table VIII*, page 546; the smaller angles were measured).

From *Table VIII* it appears that not all groups of angles between 1 and 180 degrees are represented in the material investigated. As the variation increases with a larger number of stones, in a still greater material probably all groups will be represented. In any case, however, there is a marked tendency towards an angle of 180°.

This tendency is still more impressively demonstrated when expressed in a graph (*fig. 7*, two harvests of the Leyden tree only). The figures of the classes 1—5 to 121—125 inclusive have been omitted because of the small number of their representatives.

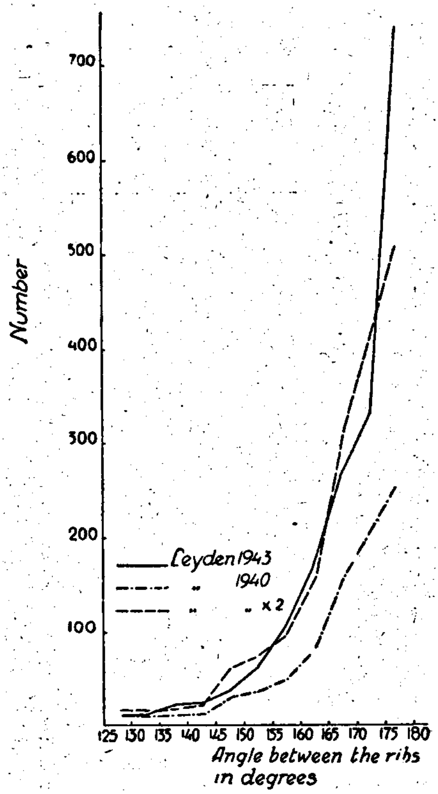

Fig. 7 — Variability in the angles of two-ribbed seed stones.

Less simple is the problem how to present the data of the three-ribbed stones in a comprehensible way. To this purpose Bakhuis Roozeboom's method was chosen, a procedure often used in physico-chemical work and introduced by Baas Becking for the description of

TABLE VIII.

Angles between ribs of two-ribbed seed-kernels (smallest angles).

Origin of the material	Harvested in:	1°-5°	6°-10°	11°-15°	16°-20°	21°-25°	26°-30°	31°-35°	36°-40°	41°-45°
Leyden	1940						1			
Leyden	1943	1								2
Slikkerveer	1943									
Maastricht	1943						1			
's Graveland	1943									
Origin of the material	Harvested in:	46°-50°	51°-55°	56°-60°	61°-65°	66°-70°	71°-75°	76°-80°	81°-85°	86°-90°
Leyden	1940					1				1
Leyden	1943								1	3
Slikkerveer	1943									
Maastricht	1943								2	2
's Graveland	1943									1
Origin of the material	Harvested in:	91°-95°	96°-100°	101°-105°	106°-110°	111°-115°	116°-120°	121°-125°	126°-130°	131°-135°
Leyden	1940	2	1			2	1	1	8	7
Leyden	1943	1		2	3	1	3	2	8	10
Slikkerveer	1943				1					
Maastricht	1943			1		3		1	1	2
's Graveland	1943					1	2	3	1	1
Origin of the material	Harvested in:	136°-140°	141°-145°	146°-150°	151°-155°	156°-160°	161°-165°	166°-170°	171°-175°	176°-180°
Leyden	1940	9	11	29	35	48	80	153	204	255
Leyden	1943	21	23	37	62	105	169	265	332	743
Slikkerveer	1943			2	1	6	8	23	23	15
Maastricht	1943	1	5	7	7	7	3	16	23	30
's Graveland	1943	3	2	7	10	6	15	35	31	35

the environment of organisms in brines and of the composition of natural waters (Boone and Baas Becking (1931), Massink and Baas Becking (1934)). As far as we know this method has never been used for the expression of morphological characters.

This method is based upon the fact that in an equilateral triangle the sum of any three lines perpendicular to the sides of the triangle and meeting within it, is constant (cf. *fig. 8*). The converse of this proposition is, that any point inside an equilateral triangle represents a certain proportion between the three perpendicular lines, connecting it with the sides of the triangle.

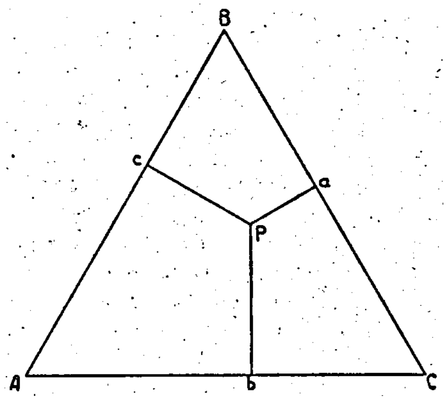

Fig. 8 — Diagram illustrating the principles of Bakhuis Roozebooms' graphic method.

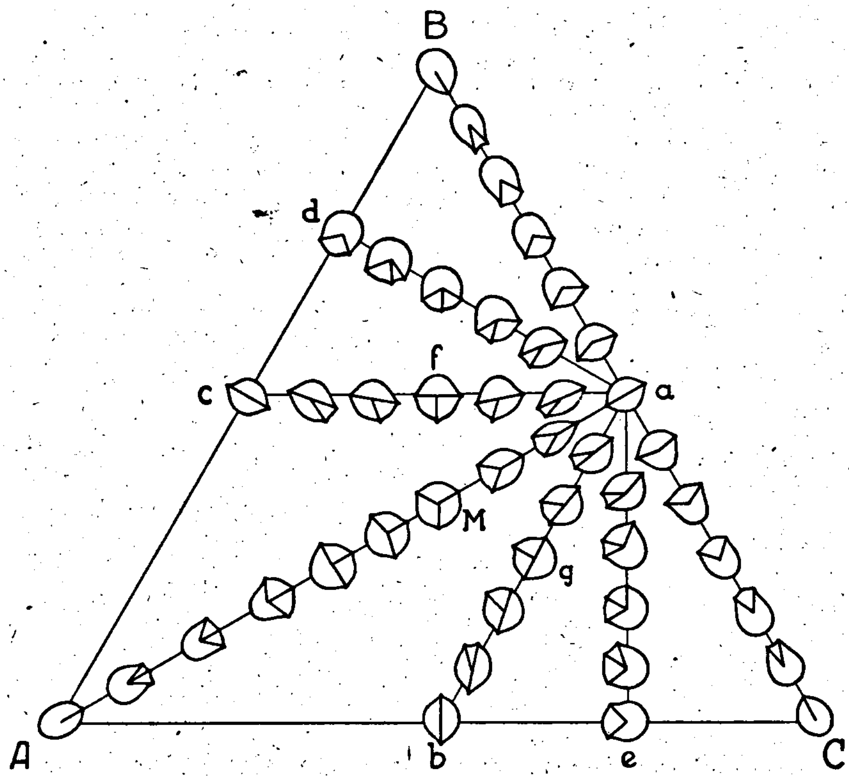

Fig. 9 — Types of seed stones plotted in the triangular diagram.

Let A, B, and C represent certain characters, which may vary from zero to a maximum, in our case angles varying from 0° to 360°. In the case that A = 360°, B and C are 0°. A point representing this situation lies in the angular point A. If A is 0° and B and C have a certain value the point representing this situation lies on the line somewhere between the angular points B and C. Generally speaking A : B : C = Pa : Pb : Pc.

Fig. 9 elucidates this method relative to our problem. Each schematical drawing of a kernel, as seen from the top, indicates a certain case, a certain proportion between the three angles A, B and C enclosed by the three ribs of the seed. On the sides of the triangle series of points are marked down, where one character is in the minimum, while the two others vary from the minimum to the maximum. If we take side BC of the triangle, all points situated on this line have this in common that the character A is in the minimum, i.e. 0°. Going from B to C the character B decreases, while C increases. This means in our case that on BC stones are placed with one angle (A) of 0°, while the two remaining angles (B and C) may vary from 0° to 360°. Thus, on the sides of the triangle — the angular points excepted — only two-ribbed seed-stones are marked down. In point a B and C are equal, which means that the kernel has two ribs with two angles of 180°.

It is clear that AB, BC, and AC give the same, corresponding points on the three sides, e.g. a, b and c indicate the same type of stone. We have completed our scheme out of one point only viz. point a. Of course the same can be done regarding b or c.

In the middle of the triangle a seed stone M is marked down with three equal angles of 120°. This type lies on the line aA, starting at a with a two-ribbed stone with angle A = 0 and the angles B and C of 180° each. A small angle A begins to develop, it grows and grows at the cost of B and C, until it has reached its maximum of 360°.

The lines a c and a b show one angle constant at 180°, in the first case this is angle B, in the second case it is angle C. Here there is a competition between A and C in the first case, A and B in the second case.

Finally there are the lines a d and a e; these show a rather surprising feature. Let us take the line a e. We start in a, angle A = 0, B and C are 180° each. Angle A is growing from 0 to 90°, angle B is very quickly decreasing from 180°, while angle C increases from 180 to 270°. At the same time we notice here the splitting of a rib, and the clockwise turning of the two products, and at last the disappearance of one of the components.

It is obvious that a f = b g, and c f = a g. Furthermore a d is the reflected image of a e, which are both again reflected images of the perpendicular lines from b on a C, and from c on a B. Finally a B = a C.

After this theoretical introduction we will try to arrange the data, obtained by measuring the three angles A, B and C, in a surveyable way.

It is, perhaps, superfluous to direct the attention to the fact that the naming of the angles is a mere agreement. For it is possible to place a certain type of stone in various points of the triangle. In *fig. 10* four diagrams are given. In these the hatched areas are modifications of one and the same geometrical pattern. In view of *fig. 9* it is comprehensible that these modifications are not meaningless; *fig. 10* gives in diagram the distribution of the types met with in the Leyden material 1943, arranged in different ways. It seems to us that *fig. 10* n. 4, by its more diffuse composition represents the arrangement in the most surveyable way, since the cases are mostly dispersed along the lines a A and a b.

In *figs 11 A–F* the two- and three-ribbed kernels, mentioned in Table VII, are arranged according to the method discussed above. Of

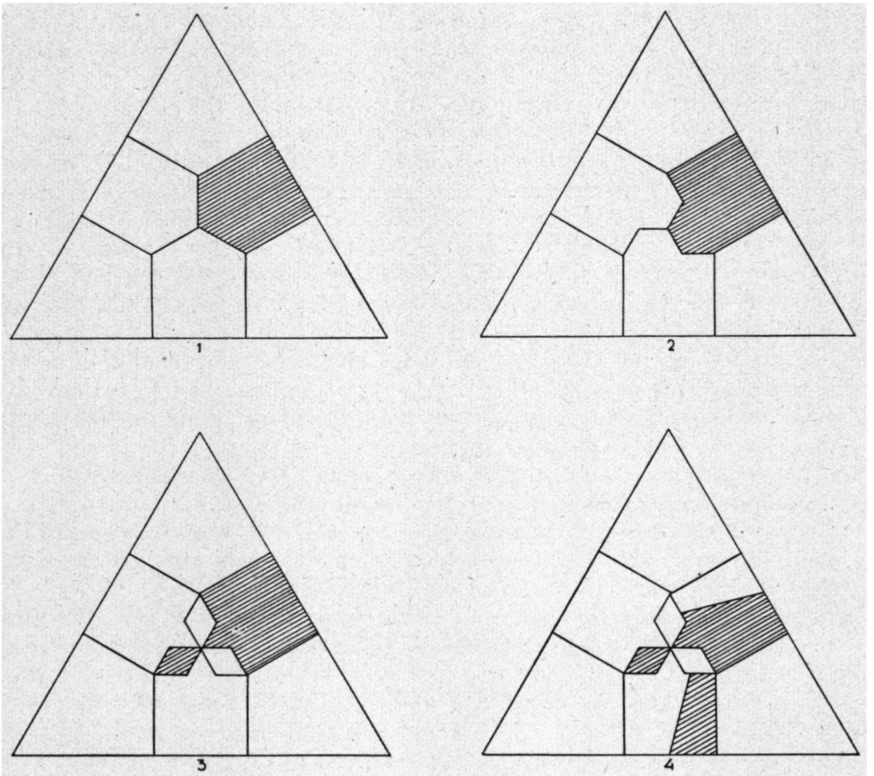

Fig. 10 — Four different diagrams so as to illustrate the possible arrangement of three-ribbed seed stones. Explanation in the text.

these the *figs 11 A, B* and *C* are the most important ones, because of the great number of measured kernels. Concerning them there are several points worth mentioning. With the exception of two points in the Leyden material, those nearest point A (*fig. 11 A*), all figures show the same feature. It is remarkable that no stones have been found along the line a A with angle A greater than 180°. In the neighbourhood of the angular points no seed stones occur, except the one-ribbed stones in the angular points and the two-ribbed ones along the sides of the triangle. Along the line a b the distribution is rather equal. Between a g and a M (cf. *fig. 9*) a great many dots are situated. The number of transitions between the types situated on these lines is very great; their distribution is rather equal. It is astonishing, however, that there are some very densely dotted areas, whereas some others show hardly any dots at all. Possibly this is due to an insufficient number of kernels.

In *fig. 11 B* the line of dots starting from b obliquely upwards to the right (reflected image of the line a e of *fig. 9*) is rather interesting. As a matter of fact this line of dots had been obtained before arrangement of types on the line a e of *fig. 9* was recognized.

Furthermore there is a transition between stones with three distinct ribs and two-ribbed kernels. The closer to a the dots are situated, the smaller becomes angle A. The dots closest to a represent seeds stones with an A angle of some degrees only, while the angles B and C are nearly 180°. The various types of two-ribbed stones have been marked down upon the line a B, only in order to show the variation; the number could not be expressed here.

Although the diagrams seem exaggerated in this respect, it is clear that the state of radial symmetry is difficult to attain.

We have taken two concentric areas around the centre M, one area representing kernels with angles varying from 108° to 132°, the other for stones with angles varying between 114° and 126°. From *Table IX* it may be taken that the number of more or less radially symmetrical stones is very small indeed.

TABEL IX.

Numbers of three-ribbed kernels with angles of 108°—132° and of 114°—126° respectively.

Origin of the material	Harvested in:	Total number of 3-ribbed stones	3-ribbed stones angles 108°-132°	3-ribbed stones angles 114°-126°
Leyden	1940	217	3	2
Leyden	1943	606	24	3
Slikkerveer	1943	31	2	1
Maastricht	1943	34	2	—
's Graveland	1943	43	—	—

In the Chinese literature the view is expressed that the two- and three-ribbed stones of Ginkgo should give rise to trees of different sex. This would be very convenient, for instance for growing Ginkgo trees from seed with the purpose to use them for street-plantation (male trees are preferred, cf. Pulle 1943). It seems to us, however, that in view of the number of transitions between typical two- and three-ribbed stones, such a difference is hardly possible.

The fact that a certain aversion may be stated against a radial

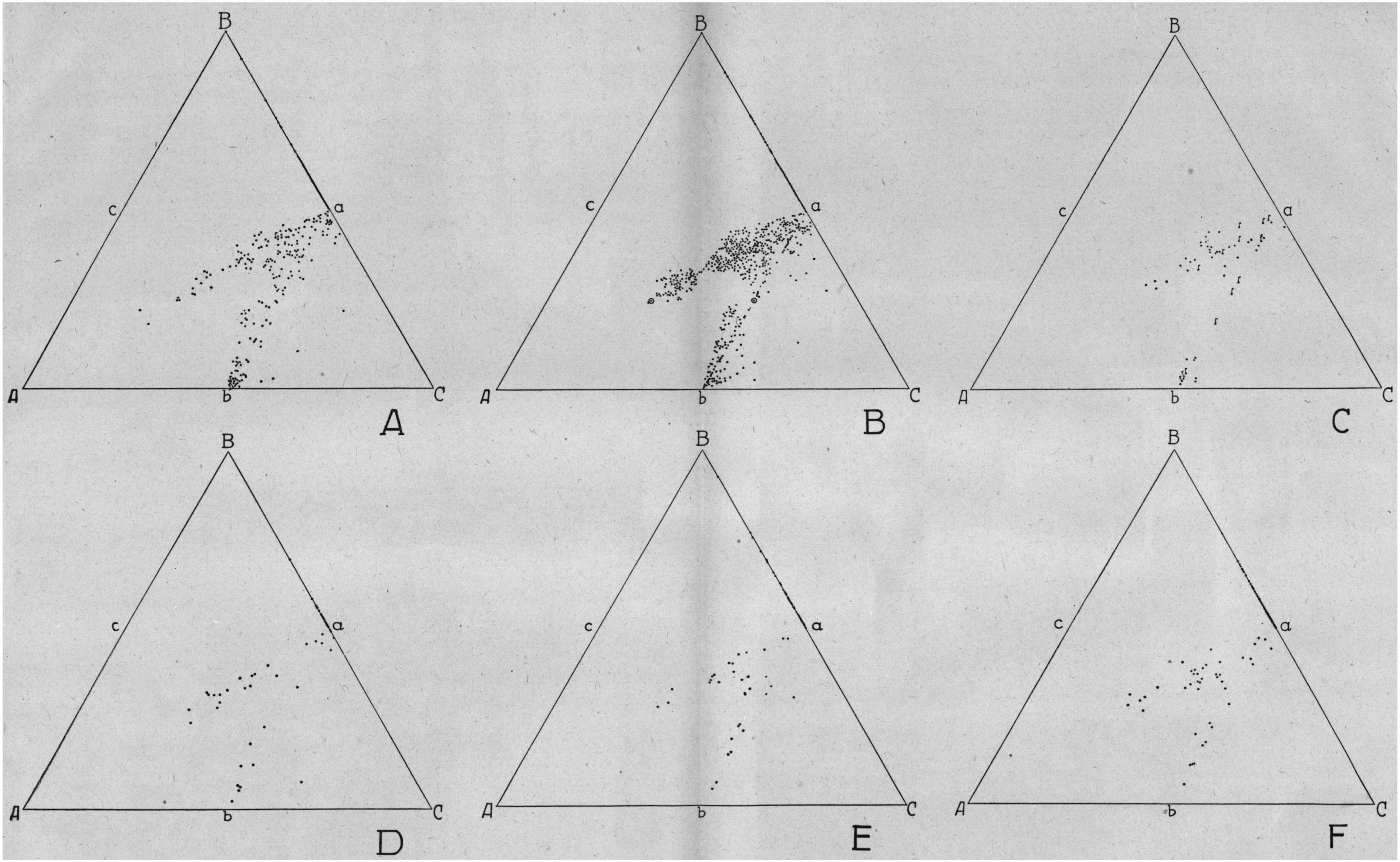

Fig. 11 — Diagrams showing the graphic position of three-ribbed seed stones. The dots along the line aB represent two-ribbed stones.

A. Leyden 1940. ⁚ dots for two identical kernels. B. Leyden 1943. ⊙ represent the same seed stone.

C. All dots represent identical kernels of diagram B. • dots for two identical kernels, ⁝ dots for three identical kernels, ⁞ dots for four identical kernels.

D. Slikkerveer 1943. E. Maastricht 1943. F. 's Graveland 1943.

symmetry of three-ribbed stones may be important regarding the opinion on the possibility of three-ribbed stones being primitive.

Salisbury (1914) writes the following sentences in relation to radiospermy and platyspermy: "Our knowledge of the structure of Conostoma and Gnetopsis has shown how narrow is the dividing line between radiospermy and platyspermy. Also the recent discoveries of Aneimitis fertilis and Pecopteris Plukeneti, together with the obvious relationships between Pteridosperms and the Cordaiteae, render it necessary to consider the possibility of deriving bilateral form from a trigonous group...... To come much nearer, the fructifications of Ginkgo biloba have been found with three ribs in place of two, a variation that may even be a reversion."

In 1916 the same author gives a short addition to the above-mentioned publication in connection with the paper of Affourtit and La Rivière: "In the taxonomically more important features of general organization the ovules of Ginkgoales, Cycadales and Trigonocarpales exhibit a uniformity of construction difficult to explain except on the basis of affinity...... On such a view, the large proportion of Ginkgo ovules with three ribs recorded by Affourtit and La Rivière has an added significance."

In 1920 De Haan again adds some points to support this view: "Salisbury has already published about the relation between Ginkgo and Trigonocarpus. Here I may add a point of apparent resemblance, which Salisbury did not notice, viz. the clearly visible sutures at the places of the ribs in the apical region in a young stage of development of the ovule of Ginkgo. These sutures support the view of possible connection between the seeds of Ginkgo and Trigonocarpus, which had been supported by the great frequention of three- angular seeds, investigated by Affourtit and La Rivière."

The regrettable selection of Ginkgo seeds, which were the object of the investigation of Affourtit and La Rivière, has given an unauthorized support to the views of Salisbury and De Haan.

The results, mentioned in *Table IX*, lend, in the trend of thought of Salisbury, sufficient support to the opinion that the regularly three-ribbed stone of Ginkgo is certainly not the more primitive form.

There remains, however, another possibility. Van Heurn and Lam (1937) investigated the occurrence of pleiomery and meiomery in the fruits of two Canarium species. These phenomena are, according to them, "to be considered as what Eichler called an 'originäre Variabilität', a variability inherent to the species. Yet this variability may have a certain phylogenetic significance; both the amplitude and the frequency are reflecting the degree of fixation of the equilibrium ('normal') stage and prevalence of one side of the variation over the other may indicate whether or not the 'average' has, phylogenetically speaking, already passed the fixed stage."

In the case of the investigated Canarium species a number of phases of regression were established, considering meiomery as a future phenomenon, pleiomery as an atavistic one. It appeared that the species in question show different phases of regression.

This might be true for Ginkgo too. As it is very difficult to decide from the data obtained from one species only how the more primitive form looked like and till now nothing is known about the relatives of Ginkgo, it is, at present, not allowed to propose even a suggestion.

Summary.

From a number of Ginkgo trees kernels were examined. The investigation of the variability of the material was greatly favoured by the large number of stones, in total about 4700. In addition, one tree, grown in the Botanic Garden at Leyden, supplied the material for an investigation of the variability of the female "flowers", in total about 1700.

1. The number of female "flowers" or rather macrosporangiophores on the brachyblasts (short shoots) proved to be most variable, showing a correlation with the age of the shoot (*Table I, fig. 1*).

2. A subdivision of the macrosporangiophores into a series of types proved to be possible (*Table II, fig. 2–3*).

3. A certain relation between the shape of the macrosporangiophore and the shape of the leafy organs from whose axil it arises, was stated. Here again the age of the brachyblasts plays a part. It should be emphasized that the term "abnormality" is misleading. A great number of so-called abnormalities in the macrosporangiophores of Ginkgo prove to form part of a normal series of gradating variations (*Table III, fig. 4*).

4. There proved to be a relation between the shape of the seed and the shape of the kernels (*fig. 5*). Oblong seeds give long, pointed stones, while pear-shaped seeds contain club-shaped kernels. Furthermore very small seeds with normally shaped, but very small stones were found. Finally seeds are found in which the pollen-chamber is situated laterally instead of apically. In these seeds the stone is abnormal in shape, its sclerotesta mostly being incompletely lignified.

5. A further point of investigation was the length of the seed stalk (*Table IV, fig. 6*). This shows a considerable variation, the Leyden material possessing very short seeds stalks, while the Maastricht material had intermediate, that from Slikkerveer long stalks.

6. Finally the variation of the shape of the kernel was investigated. First of all a subdivision into stones with 1, 2, 3 or 4 ribs was made (*Table V*). The Leyden tree produced relatively many stones of the first group, but four-ribbed kernels are very rare, two- and three-ribbed ones being in the majority. The ratio two-ribbed stones: three-ribbed stones proved to be ± 3 (*Table VI*). It is probable that the material of Affourtit and La Rivière has been subject to some sort of selection, on account of which their results are not fully trustworthy.

7. There proved to be a strong variation in the angles between the ribs in two- and three-ribbed seed stones (*Tables VII* and *VIII*). In the two-ribbed kernels a tendency towards angles of 180° was stated (*fig. 7*), the most frequent shape being that of the kernel of a prune.

8. The graphic expression of the variability of three-ribbed stones presented some difficulties. To their solution Bakhuis Roozeboom's triangle-method was chosen (*figs 8–11*). The most important result is the

extreme rarity of regular seedstones with three angles of about 120° (*Table IX*).

9. It is certainly very remarkable that so ancient a plant as Ginkgo biloba shows such a variability in so many respects.

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