Botany. — *"Contribution to the knowledge of the cytological development of Oenothera Lamarckiana"*, by Mr. J. M. GEERTS. (Communicated by Prof. F. A. F. C. WENT).

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Among the numerous plants, of which the cytological development has not yet been investigated, *Oenothera Lamarckiana* was the one selected, because a cytological examination of this plant would probably be of importance for the solution of other and more general questions.

Thus it may afterwards perhaps be possible, through a knowledge of the cytology of *Oenothera Lamarckiana* and its mutants, to find an explanation of mutation.

Although the mutation-theory has gained acceptance in most countries, this has not hitherto been the case in England to any considerable extent. There, BATESON and his pupils regard *Oen. Lam.* as a hybrid and attribute the origin of new species to hybrid segregation. The cheef argument in favour of this view is that *Oen. Lam.*, like many hybrids, shows a large degree of sterility in the ovules and pollen grains.

In this investigation I therefore set out to trace when and how this sterility arises, in order to be able to judge of the value of BATESON's argument.

It is quite conceivable that some day we may obtain a full insight into the conditions, through which mutations arise, and also that we may be able to bring about these conditions at will. For this purpose experiments with *Oen. Lam.* are especially desirable, in which attempts should be made to influence the origin of the mutants by subjecting the flowers to various conditions, for instance of temperature, or to various conditions of humidity, to injections etc.

In order to obtain reliable results in such experiments, it is necessary to know exactly, not only which flowers have been subjected to the influence, but also, in which developmental stage the flowers were at the time of treatment, whether before the synapsis, before or after the reducing division etc.; for then only can we determine whether a change in the number of mutants is really the result of the treatment, or is produced by other, unknown causes.

In order to make this investigation available for such experimental investigations, I have therefore also followed out the development of the flower, so as to be able to refer the principal cytological conditions to externally visible stages.

With reference to the mutants and hybrids of *Oen. Lam.* many questions arise of which the answers may be of importance to cytology itself. Especially hybrids with *O. gigas*, which mutant probably possesses twice as many chromosomes as *Oen. Lam.* itself, might provide material for interesting cytological investigations. For this purpose a knowledge of cytological development of the mutating plant itself is, however, a first desideratum.

The principal results obtained in this investigation, will now be briefly stated.

The floral development of *Oenothera Lamarckiana* was investigated in 1895 by POHL [1]; this author arrived at conclusions, differing widely from those obtained for other *Onagraceae*, for instance by BARCIANU [2]) and by PAYER [3]), for according to POHL the development is not acropetal, as found by the other investigators. It has now been found, however, that *Oen. Lam.* like other *Onagraceae* has an acropetal development of the flower. In addition the chief points observed in this development, are:

1. The corollar stamen arises from the protuberance, which the corolla forms on its inside.

2. The ovary is the floral axis, which has become hollow. Only the stigmas develop as 4 separate protuberances, alternating with the calyx stamens.

3. The ovary becomes quadrilocular by the growing inwards of 4 parietal septa, which stand before the calyx stamens and fuse in the centre.

4. The axis does not grow further; there is no columella.

5. The placentae are differentiated from the edges of the septa.

6. A corollar stamen and its petal have no common vascular bundle.

7. The ovule arises from periblem and dermatogen, except for the vascular bundle in the raphe.

The cytological development was studied both in the ovules and in the stamens. A summary of the principal phenomena observed, is here appended.

When the mother-cells go into synapsis, the nuclear network contracts, and a thin thread arises, in which chromatin particles can be seen. This thread contracts to a tangle and continually becomes shorter and therefore thicker; this is clearly visible when the tangle unrolls itself

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1) Ueber Variationsweite der *Oenothera Lamarckiana* von J. POHL Oesterr. botan. Zeitschrift Jahrgang 1895 Nr. 5 u 6.

2) Untersuchungen ueber die Bluethenentwicklung der *Onagraceae*, Inaug. diss. von D. P. BARCIANU, Naumburg 1874.

3) J. B. PAYER, Organogénie de la Fleur.

again. The thick thread now divides transversely into 14 chromosomes, the nuclear wall disappears, and the chromosomes place themselves perpendicularly to the long axis of the spindle of the heterotypic division. *In the synapsis of Oen. Lam. no double thread can therefore be seen; from that thread the vegetative number of chromosomes is formed, and just before the division these chromosomes show pairing.*

Both in the ovules and in the stamens the synapsis takes place in this manner. In the first division of the mother-cells entire chromosomes separate, which already during this division show a longitudinal splitting for the second division. After this heterotypic division the two nuclei scarcely enter upon a stage of rest, the chromosomes, split longitudinally, always remain visible, especially in the mother-cells of the embryosac. The wall between the two nuclei is often not formed completely. At the second division the halves of the two chromosomes separate, so that 4 nuclei are formed; in general but little staining substance is visible [1]).

Whereas in most plants the lowest-cell of the tetrad grows out to form the definitive embryosac, this is not so in *Oen. Lam. Here the uppermost cell becomes the embryosac,* while the other three degenerate; the chromatin of these three cells quits the nucleus, of which the wall disappears, and the chromatin now colours the whole protoplasm dark, so that these three cells of the tetrad remain visible for a long time as a long dark band under the embryosac. In that cell of the tetrad, which grows out, three successive divisions take place in almost all plants, so that 8 nuclei are formed. In *Oenothera* I always found but 4 nuclei, which come to lie in the upper part of the embryosac. A limited number of nuclei is also known in *Helosis guyanensis,* in consequence of the researches of CHODAT and BERNARD [2]) and in *Mourera,* through an investigation of WENT [3]). In these plants the lower of the two nuclei, which are formed in the first division, probably degenerates, from the upper nucleus the

[1]) In the May meeting of the Dutch Botanical Society, in which I dealt with the greater portion of this subject, I already stated that the synapsis in *Oen. Lam.* takes place in this manner, thereby differing from what has been stated to occur in other plants. As I found in the beginning of September there appeared in the July number of the Botanical Gazette a paper by GATES on the synapsis and reducing divisions in the pollen mother-cells of *Oenothera rubrinervis.* This observer gives much the same succession of stages for the synapsis of this plant.

[2]) R. CHODAT et C. BERNARD. Sur le sac embryonnaire de *l'Helosis guyanensis.* Journal de Botanique T. XIV, 1900 p. 72.

[3]) F. A. F. C. WENT. "The development of the ovule embryo-sac and egg in *Podostemaceae.*" Recueil des Travaux Botaniques Néerlandais, Volume V, Livraison I, 1908.

three nuclei of the egg-apparatus and the polar nucleus are formed so that division nevertheless takes place three times.

In *Oen. Lam.* I never found a nucleus or remains of a nucleus in the lower part of the embryosac, whilst the four nuclei always lie in the upper portion. A few times it was possible to observe the divisions themselves. Thus I found an embryosac, in which the nucleus was dividing into two, with its spindle in the long axis of the embryosac. Further I found embryosacs with two nuclei, which were sometimes superposed, sometimes side by side. Therefore the lower nucleus probably changes its place, coming to lie higher up in the embryosac. The divisions of these two cells were also found, namely two spindles, in the upper part of the embryosac, and at right angles to each other. In the same manner the spindles are found in most plants above and below in the embryosac, at the third division in couples perpendicular to each other. The division of these two nuclei in *Oen. Lam.* is therefore pretty certainly the last division of the embryosac, in which the two synergids are formed from one nucleus, and the egg-nucleus and the upper polar nucleus from the other. In this particular embryosac, with the two spindles at right angles to each other, there was moreover no trace of nuclei at the chalazal end. We may therefore assume, that in *Oen. Lam.* the first division in the embryosac is suppressed, so that antipodal cells and lower polar nucleus are not formed at all, and that there is not even an antipodal initial cell, which occasionally degenerates again after being formed, as in *Helosis* and *Mourera*.

In the embryo-sac of *Cypripedium* the number of the divisions is still further reduced; here, according to Miss L. PACE[1]) there occurs in the embryosac only one homoiotypic division in the lower cell; the upper cell degenerates. The homoiotypic division is not followed by a cell-division. The two nuclei in the lower cell now arrange themselves at the poles; this cell grows out longitudinally and the nuclei divide again, so that they are four in number. Further divisions do not take place in the embryosac; the four nuclei become egg cell, synergids and upper polar nucleus.

When in *Oen. Lam.* the four nuclei have been formed the synergids, which lie nearest to the micropyle, surround themselves, like the egg-nucleus, with their own plasma; the upper polar nucleus remains free in the plasma of the embryosac.

The four cells, which have arisen in the pollen mother-cells through the reduction division, grow out regularly to pollen-grains; the method of origin and the structure of the walls probably agrees in

[1]) LULA PACE. Fertilization in Cypripedium. Botanical Gazette. XLIV. 1907 p. 353.

Oen. Lam. with what R. BEER [1]) has described for *Oenothera longiflora.*

When the pollen-grains are almost mature, the generative and the vegetative nucleus are formed by division; the generative nucleus, which is the smaller, and is surrounded by a quantity of plasma, then applies itself to the wall; the vegetative nucleus remains in the middle of the grain. The division of the generative nucleus into two probably takes place in the pollen-tube.

The pollen-tube penetrates through the micropyle and through the nucellar tissue. It seems as if in this place the nucellar tissue already becomes in advance disorganized. Since, when the pollen-tubes penetrate, the synergids are already completely disorganized and stain very deeply, and since in the nucellus, at the spot where the pollen-tube has penetrated, dark coloured remnants are everywhere visible, I have not succeeded in observing the division of the generative nucleus. Fertilisation itself was however clearly observed. A double fertilisation takes place; one nucleus penetrates into the egg-cell and applies itself against the nucleus; the generative nucleus is at that time round, but smaller than the egg-nucleus, although it probably becomes somewhat larger before fusion. The other generative nucleus, which presents the same shape, applies itself to the polar nucleus. The fusion between the polar nucleus and its generative nucleus takes place more rapidly than that of the other generative nucleus with the egg-nucleus. The fertilized polar nucleus now soon divides, so that frequently there are already a number of endosperm nuclei before the egg-nucleus has completely coalesced with the generative nucleus, and before the egg-cell has a wall of its own. The fertilized egg-cell grows out to a short suspensor, and an embryo with distinct octants. Endosperm nuclei then already lie along the whole of the wall of the embryosac. Afterwards this endosperm however again disappears.

In Oenothera Lamarckiana the endosperm is therefore formed from one fertilized polar nucleus.

In mature stamens very many sterile grains are found in the pollen; in young fruits there occur between the developing seeds a fairly large number of ovules, which are not undergoing development. In both cases the sterility arises after the reduction division. Whilst in all embryo-sac mother-cells the reduction division takes place normally, there are still many ovules in which the upper tetrad-cell also degenerates, this degeneration being accompanied by much the same phenomena as occur regularly in the three lower cells of the

[1]) On the development of the pollen grain and anther of some Onagraceae, RUDOLF BEER. Beih. zum Bot. Centr. blatt 1905, 19 I.

tetrad. In the pollen mother-cells also the divisions take place regularly, but after 4 tetrad cells have been formed, only two cells, in general, develop properly, the other two only partially, so that from them grains are formed, from which the contents gradually disappear, although the wall is fairly normal in structure.

Since the divisions are normal, the appearance of this sterility need not at all be the result of a hybrid nature. It follows from the literature on sterility, that, besides in hybrids, sterility occurs in many other plants, and that in most hybrids, which have been examined cytologically, sterility was already present in one or both parents. And since an examination of one hundred species showed sterility to be pretty common among *Onagraceae*, we are not, in my opinion, justified in deducing from the sterility of a plant that it is a hybrid. If the above-mentioned objection of Bateson against the mutation-theory is to have value, it will be incumbent upon him to name the presumptive parents of *Oen Lam.*

In mature ovaries those ovules, which are not destined to develop, may be recognized by their more transparent nucellus, not containing an embryosac. In such ovulus the penetration of a pollen-tube was never observed, whereas this was repeatedly found in the normal ovules of the same section. *It would appear, therefore, that the normal embryosac exerts an attraction on the pollen-tubes.*

By combining the results of the investigation of the ontogenetic, with those of the cytological development, it appears, that *Oen. Lam.* is very suitable for the experiments, referred to in the introduction, because the cytological development of the pollen and of the ovules are sharply separated in point of time. In flowers of 30 mm. the development of the pollen is almost complete, while in the same flowers the development of the mother-cells in the ovule is only beginning. It would therefore be possible to influence the pollen and the ovules separately, and the flowers which should be selected for this purpose, can be easily recognized after a little practice. Should one wish to influence the pollen before the synapsis, then flowers 10—11 m.m. long should be selected, in which the stamens have a length of 3 mm., since the synapsis of the pollen takes place in flowers of 12—13 mm , in which the anther and also the filament have a length of 4 mm. The synapsis of the embryosac mother-cell takes place in flowers of about $3^1/_2$ cm. In order to influence this, flowers of 3—$3^1/_2$ cm. should therefore be selected, in which the ovary and the calyx tube have about the same length i.e. $4^1/_2$ mm.

I hope soon to be able to publish a more detailed description, with plates, of the results of this investigation in the Recueil des Travaux Botaniques Néerlandais.