FACULTATIVE PARTHENOGENESIS AND HAPLOIDY IN
EPIPACTIS LATIFOLIA

BY

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1. Introduction.

In *Orchis maculatus* it is a normal occurrence that some few ovula are not fertilised but develop haploid embryos. In addition it may happen that a few female nuclei in the same ovary are fertilised by more than one male nucleus, and in this way polyploid embryos may arise beside the normal diploid ones (HAGERUP, 1944).

It would be of interest to ascertain whether these changes in the degree of polyploidy are peculiar to this one observed species, or whether we are here concerned with universally valid laws. This can only be decided by investigations on many other species. The present notice must be regarded as part of such a series of studies which it is to be hoped can in due course be continued by investigations on other plants.

The orchids are particularly well suited for studies on fertilisation, both because they are very easy to fix and stain, and also because their ovaries contain a greater number of ovula than most other plants. To this must be added that the formation of species is probably livelier within the orchids than in any other family, for which reason we may here expect to find species well suited for researches on the significance of polyploidy for the formation of species in nature.

Next to *Orchis maculatus* among our native orchids *Epipactis latifolius* is the orchid which is most polymorphous, for there are great variations both in the form, colour, and pubescence of the floral and the vegetative organs. But notably there are striking differences in the size of the individuals, both giant and dwarf forms occurring under partly ecologically different conditions. The individuals employed for this investigation all belonged to a medium type; unfortunately I had no opportunity of observing

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extremes. But such individuals would be particularly valuable and ought to be studied.

All my material has been collected in nature, partly on Funen and partly in the environs of Copenhagen (Kongelunden). Meiosis is found in the anthers in the middle of July and the fertilisation takes place only a couple of days after the pollination in the first half of August, when the ovary is 3—4 mm. thick. The ovaries must be pruned as thoroughly as possible and treated for a short time beforehand with CARNOY's liquid, so that the ovula die quickly. Then the actual fixing is done according to LEVITZKY, by which method remarkably good results are obtained when the specimens are left for several days in the fluid, which should be renewed a couple of times.

Haematoxylin is not quite good for this object. But the sections should be treated for half an hour in normal hydrochloric acid heated to 60°, and should then be stained according to FEULGEN. After this they are stained 1$^{1}/_{2}$—2 minutes in "Lichtgrün". In this way a result approaching the ideal can be obtained, which makes both *Orchis maculatus, Epipactis,* and especially *Listera* perhaps the best suited pedagogic specimens for embryological studies (EFTIMIU-HEIM). The fertilisation itself, so difficult to observe in most other plants can also very easily be followed with the above-mentioned technique; the male nuclei are large and stain such a vivid deep red that they are at once seen already under low enlargement, because they often lie in the green cytoplasm of the pollen tube.

The embryo sacs are very large and the sections should therefore be thick (25—35 μ). So as to be able also to investigate interesting exceptional cases I examined a very large quantity of material and studied about 35.000 embryo sacs containing male nuclei, so presumably my investigations are based on a fairly reliable numerical material.

As an object of study the plant, however, suffers from an essential defect, the chromosomes being difficult to count even if they are well fixed. This is partly because they are remarkably long and provided with constrictions, partly because often the large chromosomes do not lie in the same plane but curve irregularly. In spite of this it is, however, as a rule fairly easy to see whether a less good mitosis is diploid or haploid.
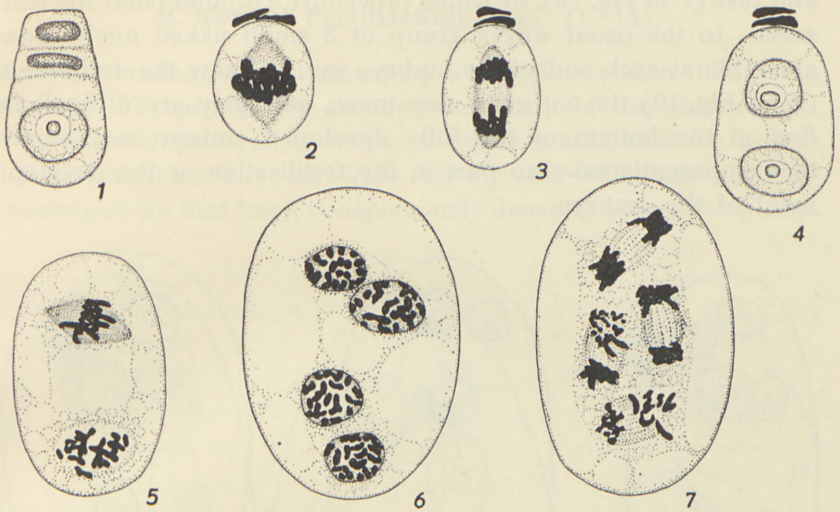
Figs. 1—7. First stages in the development of the embryo sac. Figs. 1—4 above
show the dark remnants of 2 degenerated macrospores. × 700.

2. The Development of the Embryo Sac (Figs. 1—10).

VERMOESEN (1911) as well as BROWN and SHARP (1911) have
already described the development of the embryo sac and found
almost the same as I have. It will therefore suffice here to give
a brief account supported by the subjoined figures.

Nearly always three macrospores only are formed (Fig. 1), of
which the inner one develops into an embryo sac. This mother
cell gradually increases considerably in size and soon supplants
the other two macrospores, which can long be seen above the
embryo sac as two compressed dark remnants (Figs. 2—4).

When the mother cell of the embryo sac has attained a certain
size its nucleus will divide in two in the usual way (Figs. 2—4),
though no cell wall is formed between them. The two daughter
nuclei soon continue the division (Fig. 5), young embryo sacs
being thus produced first with 4 (Fig. 6), later with 8 nuclei
(Figs. 7—8). Originally there is a group with 4 nuclei at each
end of the young embryo sac (Fig. 8), but from each of these
two groups a nucleus soon wanders towards the centre of the
embryo sac (Fig. 9). These two nuclei draw near each other

and fuse (P in Fig. 10), by which procedure a diploid polar nucleus arises. In the usual way a group of 3 small naked nuclei now assemble at each end of the embryo sac; of these the antipodals (A in Fig. 10) do not grow any more, and they are difficult to find at the bottom of the fully developed embryo sac, where they apparently take no part in the fertilisation or the development of the embryo.
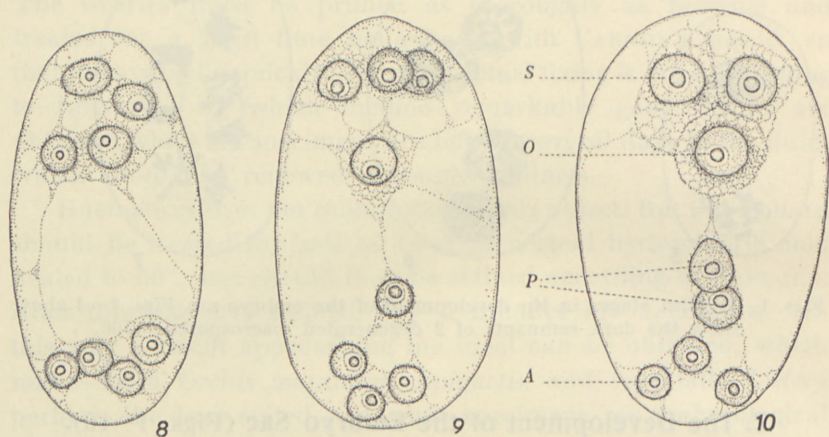

Figs. 8—10. Young embryo sacs with 8 nuclei, which are taking their final positions. S, synergid. O, egg. P, polar nucleus. A, antipodal. × 700.

When the embryo sac is fully developed so that it can be fertilised it is often fairly easy to identify the different nuclei by means of their size, colour, and position. The synergids (S in Figs. 10, 11, 13) are both the smallest nuclei at the upper end of the embryo sac, they are almost globular and slightly stained. A little below these lies the female nucleus itself (O in Figs. 10, 11, 13) which is comparatively large and also stains relatively faintly. It is surrounded by plenty of cytoplasm but has no wall. The polar nucleus (P in Figs. 10, 11), on the other hand, is comparatively dark, often somewhat flat and situated below the egg cell and not exactly in the middle of the embryo sac. In contrast with the other nuclei it contains 2—3 nucleoli. It can be decided whether the female nucleus is fertilised by ascertaining how many nucleoli it contains, since every nucleus brings with it its nucleolus; thus the fertilised female nucleus will contain 2 nucleoli.

3. Normal Fertilisation (Figs. 11—14).

The fertilisation is initiated by a very fine pollen tube (*T* in Figs. 11, 13) penetrating the uppermost tip of the embryo sac. A synergid is destroyed, perhaps serving as nourishment for the pollen tube, which at any rate swells considerably with a curious bladdershaped tip that has a conspicuously dense and dark cytoplasm.
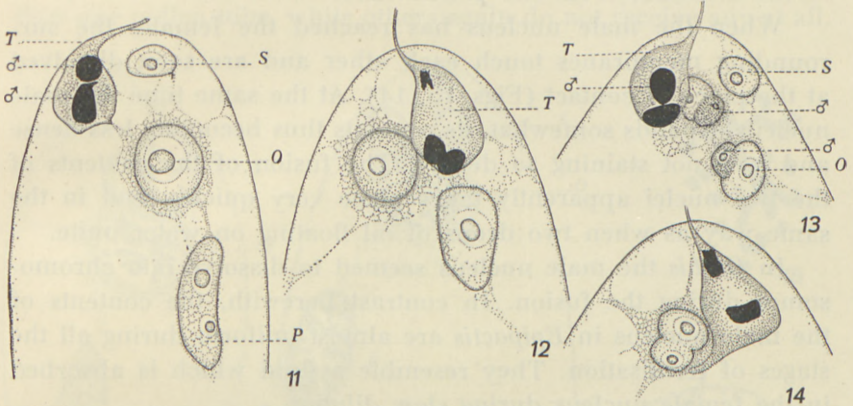
Figs. 11—14. Early stages of normal fertilisation. *S*, synergid. *O*, egg. *P*, polar nucleus. *T*, pollen tube. ♂, male nucleus. × 700. See also text.

Often the vegetative nucleus of the pollen tube does not enter the embryo sac but remains outside the ovula in the narrow part of the pollen tube. Not rarely, however, the said nucleus is found in the embryo sac with the male nuclei (♂ in Figs. 11, 13) which may give rise to confusion, since the vegetative nucleus also stains very darkly. But it is often recognisable by the difference in size (either larger or smaller); and in addition it is angular, showing that it is decaying and out of function.

The tip of the pollen tube immediately grows towards the female nucleus and soon gets into contact with the cytoplasm of the latter. The male nuclei were long and narrow when they were in the pollen tube, where there was very little room. But as soon as they enter the embryo sac, where there is plenty of room, they at once change their form, become short, discoid, and usually furnished with 1—3 angles. As soon as the pollen tube has approached the female nucleus, the male nuclei pass out through small openings in the side or tip of the pollen tube

in a place lying as near as possible to the female nucleus, in the cytoplasm of which they are soon to be found.

The conveyance of the male nuclei probably takes place by means of currents in the cytoplasm which is extended in long strands between the various parts of the embryo sac. It is remarkable that the two male nuclei nearly always reach the two nuclei to be fertilised at the same time, though these are found at different distances from the pollen tube.

When the male nucleus has reached the female, the surrounding membranes touch each other and are soon dissolved at the place of contact (Figs. 13, 14). At the same time the male nucleus expands somewhat, its contents thus becoming less dense and now not staining so deeply. The fusion of the contents of the two nuclei apparently takes place very quickly and in the same way as when two drops of oil floating on water unite.

In *Orchis* the male nucleus seemed to dissolve into chromosomes during the fusion. In contrast herewith, the contents of the male nucleus in *Epipactis* are almost uniform during all the stages of fertilisation. They resemble a fluid which is absorbed in the female nucleus during slow dilution.

In the more advanced stages of the fusion the male nucleus is placed on the outside of the female as a slight hump, the contour of which is gradually smoothed out, until it is quite flush with the membrane of the egg cell. The respective nucleoli of the nuclei do not fuse at this stage, but later.

When the female nucleus has been fertilised, there is still a live male nucleus in the embryo sac, and the later fate of this may vary a great deal. In some cases it will not function, as was also the case in *Orchis*.

But in other cases (Fig. 13) this second male nucleus will quickly reach the polar nucleus with which it fuses similarly as in the fertilisation of the female nucleus. In that case the polar nucleus will soon begin to divide, and it is common to find an endosperm consisting of either 2, 4 or 6 but very rarely of more free cells. In most other orchids the endosperm has not so many cells, and most frequently it is entirely absent, thus for instance in *Orchis* and *Listera*.

4. Facultative Parthenogenesis and haploid Embryos
(Figs. 15—19).

At pollination an ovary as a rule receives an enormous amount of pollen. What causes the equal distribution of the pollen tubes among the ovula is not known, but at any rate this task is not always discharged without error. For many ovula receive more than one pollen tube, while others again do not receive any at all.
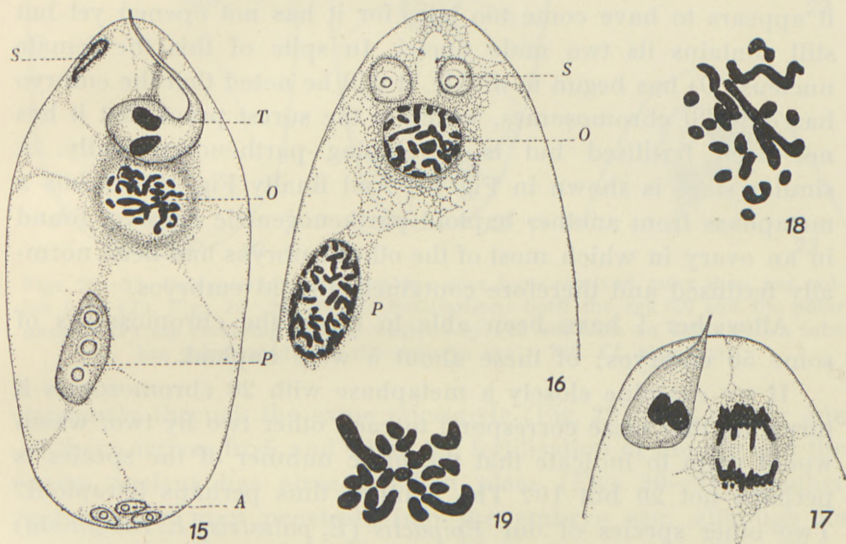
Figs. 15—19. Facultative parthenogenesis. Eggs (O) are developed without fertilisation and are haploid. Figs. 15 and 17, the embryo sac has received a pollen tube (T) after the embryo (O) has begun to develop. Fig. 16, the embryo sac has not received any pollen tube, and yet egg and polar nucleus are developing. × 700. Figs. 18—19. Metaphases with $2 n = 20$ in the first division of the egg. × 1500. Cf. text.

As is well known, the pollen tubes have a double function; not only do they accomplish the fertilisation but they also excrete a substance which makes the ovary grow. This impulse to growth affects not only the wall of the ovary and the fertilised ovula, but all parts of the ovary begin to grow and this also applies to the ovula which have not received any pollen tube and thus have not been fertilised. Such an embryo sac is shown in Fig. 16. Above it is seen that both the synergids (S) are intact, which is a sure sign that no pollen tube has penetrated into the embryo

sac. Directly below the synergids lies the female nucleus (O) which nevertheless has begun to divide. It is in prophase and contains 20 chromosomes, which is the haploid number of the species.

Below in Fig. 16 is seen the polar nucleus (P) which also develops without fertilisation, and therefore only contains 40 chromosomes and is diploid.

Fig. 15 shows another case of facultative parthenogenesis. It is true that the embryo sac has received a pollen tube (T); but it appears to have come too late, for it has not opened yet but still contains its two male nuclei. In spite of this the female nucleus (O) has begun to divide, it will be noted that the embryo has only 20 chromosomes, which is the surest proof that it has not been fertilised but is developing parthenogenetically. A similar stage is shown in Fig. 17. And finally Fig. 19 exhibits a metaphase from another haploid parthenogenetic embryo, found in an ovary in which most of the other embryos had been normally fertilised and therefore contained diploid embryos.

Altogether I have been able to count the chromosomes of some 50 embryos; of these about 5 were haploid.

If we examine closely a metaphase with 20 chromosomes it turns out that these correspond to each other two by two, which would seem to indicate that the basic number of the species is perhaps not 20 but 10? The plant is thus perhaps tetraploid? Two other species of our *Epipactis* (*E. palustris*, *E. rubiginosa*) have also $n = 20$. It would be of value to know the chromosome number of the slender *E. microphylla* as well as the more robust *E. violacea* from more southerly latitudes; it is possible that these might have other degrees of polyploidy?

5. Polyspermaty (Figs. 20—26).

The distribution of the pollen tubes among the ovules is also irregular seeing that more than one pollen tube can penetrate into an ovulum. This is so frequent that even about half of the ovula of an ovary can be "superfertilised" in this way. Quite frequently 3 pollen tubes penetrate into an ovulum, and once I have even found four. 2 pollen tubes may very well pass simul-
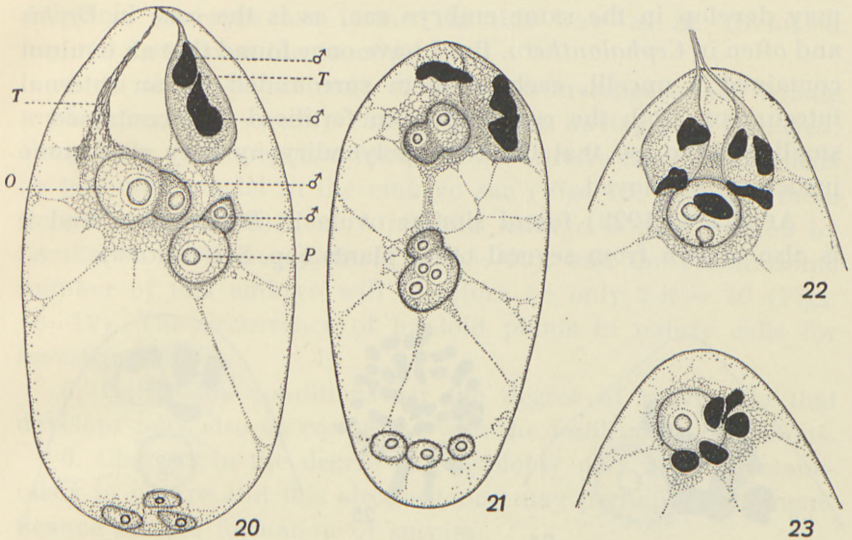
Figs. 20—23. Polyspermaty. The embryo sacs have received more than one pollen tube (*T*). Figs. 20—21. Double fertilisation: both the egg (*O*) and the polar nucleus (*P*) are receiving a male nucleus (♂); in addition an extra pollen tube has penetrated into the embryo sac. × 700. Cf. also text.

taneously through the same micropyle (Fig. 22); but mostly one of them arrives first, and when the fertilisation of the egg and the polar nucleus has already taken place (Figs. 20—21) another pollen tube may penetrate into the embryo sac, although its content of male nuclei will not perhaps come to function. Thus Figs. 22, 23, 24, 26, show examples of how the female nucleus may be surrounded during the fertilisation by 5—8 active male nuclei. How many of these fuse with the female nucleus I have not been able to observe. The only sure proof that a superfertilisation may facultatively take place is in the chromosome number of the embryos, but this is very difficult to establish in *Epipactis*, especially as regards high numbers. The species therefore is not well suited for elucidating the problem as to whether polyploid embryos can arise from superfertilisation. That this may in fact happen was, however, shown by *Orchis* in which I was able to observe that 2 male nuclei may penetrate simultaneously into the same egg; and polyploid embryos also occurred.

I have never in *Epipactis latifolia* seen that two embryos may develop in the same embryo sac, as is the case in *Orchis* and often in *Cephalanthera*. But I have once found that an ovulum contained 2 nucelli, each of them surrounded by an internal integument. Both the eggs had been fertilised and contained a small embryo, so that the term polyembryony may with some justice be employed.

AFZELIUS (1922) found similar ovula in *Platanthera*, and it is also known from several other plants (cp. SCHÜRHOFF).

Figs. 24—26. The egg is surrounded by several active male nuclei derived from several pollen tubes. × 700. Fig. 25. Metaphase from the first division with 2 n = 40 × 1500. See also text.

These investigations on the rise and disappearance of polyploidy in nature will be continued with other plants (*Listera, Papaver*).

I owe cordial thanks to Professor C. A. JØRGENSEN for examining several of my preparations and for his critical remarks.

My respectful acknowledgements are due to the Carlsberg Foundation which has supported my studies for several years.

Summary.

1. Every fruit contains several thousand ovula which are fertilised in very different ways.

2. Most of the eggs are normally fertilised (Figs. 11—14) by a single male nucleus and the embryo will then have the normal diploid number of chromosomes, 2 n = 40 (Fig. 25).

3. Often more than one pollen tube enters an ovulum (frequently 2—3). Therefore there will be several male nuclei at

disposal to carry out the fertilisation (Figs. 20—26). Whether polyploid embryos are formed in this way—as in *Orchis*—it has not been possible to observe.

4. In about 10 per cent. of the cases investigated the female nucleus begins to develop embryos without having been fertilised. Frequently another pollen tube will then arrive later which sheds its two male nuclei in the embryo sac (Figs. 15, 17); but these do not come to function. A haploid embryo is then formed by facultative parthenogenesis (Figs. 15—19), and the chromosome number of this embryo will therefore be only $2 n = 20$ (Figs. 18—19). The occurrence of haploid plants in nature calls for investigation.

5. Hence the conditions for the degree of polyploidy that develops may also be connected with the fertilisation conditions.

6. Changes in the degree of polyploidy may arise spontaneously in nature and this circumstance may perhaps be of significance for the formation of species.

While this paper was in press I collected living material of *E. microphylla* in Møn. This species too has $n = 20$.

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