THE ANNALS

AND

MAGAZINE OF NATURAL HISTORY.

[THIRD SERIES.]

No. 69. SEPTEMBER 1863.

XV.—On the Impregnation in Orchids as a Proof of the two different Effects of the Pollen. By Dr. F. HILDEBRAND, of Bonn.

Or late years most of the investigations on impregnation in plants have been directed to the pollen-tubes acting on the ovules to form the embryo. That there are a great many other interesting and important points respecting the fecundation of plants, everybody will admit who has directed attention to the two celebrated works of Darwin, 'On the Origin of Species' and 'On the Fertilization of Orchids,' bearing in mind at the same time the inquiries of Koelreuter, Sprengel, Gaertner, Herbert, &c.

Looking at some tropical Orchids cultivated in the Botanical Garden of Bonn, I found no ovules in the ovarium of the expanded flower; nevertheless I saw the enlargement of the ovarium after having applied the pollen to the stigma. This curious circumstance seemed deserving of further examination, especially as the numerous writers on the impregnation in Orchids* have made out this point imperfectly; and even Robert Brown, in his paper on the Fecundation in Asclepiadeæ and Orchideæ†, has merely alluded to it.

As my investigations are to be published at greater length in Mohl and Schlechtendal's 'Botanische Zeitung,' I shall describe only the experiments and observations made on one species, and then give the results of these and all the other experiments. The ovarium of *Dendrobium nobile* has a diameter of about

* Brongniart in Ann. des Sc. Nat. 1831, p. 117; Amici, Flora, 1847, p. 255; Mohl, Bot. Zeitung, 1847, p. 465; Hoffmeister, Entw. d. Embryo der Phanerog. p. 5; Schacht, Ann. des Sc. Nat. 1851, p. 83; Henfrey, Trans. Linn. Soc. xxi. p. 7.

† Trans. Linn. Soc. 1833.

Ann. & Mag. N. Hist. Ser. 3. Vol. xii.

 $2-2\frac{1}{2}$ millim., and a length of 10-15 millim.; its cavity is a very narrow channel, on the walls of which are to be seen three ridges, of an irregularly undulated appearance; these ridges are the placentæ that are not yet fully developed; there are no incipient ovules upon them. The stigma of the expanded flower is covered with numerous utriculi separated by a copious viscid substance. If the pollen is applied to this viscid surface of the stigma, very soon, at least after two days, the labellum of the flower folds up round the column, and the petals and sepals incline over it, and, withering in about nine days, do not fall off, but are to be found at last on the top of the ripe fruit. If the pollen is not applied to the stigma, the flower remains unchanged for a long time; in from twenty to thirty days it begins to wither, and falls off: during this time the placentæ have not grown in any way.

After the application of the pollen to the stigma, the petals and sepals soon begin to wither, as stated above; at the same time the column begins to swell hemispherically, and the pollentubes, forming a cord, pass through the channel of the column. On reaching the cavity of the ovarium, they divide into three parts, and each of these parts divides again into two, running down on each side of the placentæ. Soon after the application of the pollen to the stigma, the ovarium began to enlarge both in length and in diameter; in eleven days, the placentæ were more deeply undulated, and in twenty days they were distinctly divided into two parts, each part being fringed irregularly; no ovules were yet to be seen. Now I thought that the enlargement of the ovaria which had no ovules on their placentæ was only abnormal, and that no ovules ever would be formed. Therefore I did not examine a fruit before the 3rd of March: this fruit had originated from a flower impregnated on the 5th of January. I found the placentæ quite covered with ovules showing very different degrees of development: there were some incurved, the outer and inner coats enclosing the nucleus; some where the nucleus was still protruding; and some that appeared only as a papilla a little incurved, and surrounded at its base by the incipient coats. The cords of the pollen-tubes were in an unchanged state on both sides of the placentæ. At this time the fruit had a diameter of 20 millim., and a length of 60 millim.; it had become green and succulent, and there were some stomata on its surface; its much enlarged cavity was not vet filled up with the ovules. Next a fruit that had originated on the 10th of January was examined on the 13th of April, and all the ovules were found in a perfect state, filling up the entire cavity of the ovarium; the embryo-sac was to be seen distinctly, but no pollen-tube had reached it. On the 22nd of April, the germinal corpuscles were distinctly evident, but the cords of the pollen-tubes were unchanged as before. Finally, on the 12th of May, when the diameter of the fruit was 25 millim., and its length 80 millim., the first two or three cells of the embryo were formed, one of the germinal corpuscles had disappeared, and the pollen-tubes were seen in a decaying state between the ovules.

Thus we see that a space of four months (January 10 to May 12) was required in order that, after the application of pollen to the stigma of a flower (the ovarium of which contained no ovules), the ovules might attain perfection, and the formation of the embryo begin.

Having thus described the observations made on *Dendrobium* nobile, I pass over the experiments on other species, and proceed at once to give the general results of my observations.

The experiments and observations were made on thirty different species of Orchids, of which nine were tropical and twenty-one indigenous; therefore we may safely extend the results to the whole Orchidaceous family as follows :---

1. In the recently expanded flowers of Orchids the ovules are never fully developed. The degrees of development are very different: there are some species (for instance, *Listera* ovata and Neottia nidus-avis) that have almost perfect ovules, which are incurved and have both of the coats, but the outer is as yet shorter than the inner, and the embryo-sac is not yet to be seen in the nucleus; in other species (for instance, in Dendrobium nobile) the formation of the ovules has not yet commenced, even the placentæ are not yet fully developed, but appear only as three narrow undulated stripes running down the walls of the cavity of the ovarium, and the bifurcation of every placenta is only slightly indicated. Between these extremes of development there are different degrees of perfection of the ovules.

2. After the application of the pollen to the stigma, the enlargement of the ovarium begins; at the same time the ovules become more and more perfect, or if there were only the placentæ, the ovules begin to appear after a certain time as minute papillæ projecting from the surface of the placenta. The enlargement of the ovarium begins before the pollen-tubes reach the placentæ or the ovules; in the same manner the ovules begin to grow without being touched by the pollen-tubes. From this it is clearly evident that the tubes of the pollen have no direct influence on the original development of the ovules, but that these tubes first act on the enlargement of the ovules. Flowers that have no pollen applied to their stigmata do not wither so

172 Dr. F. Hildebrand on the Impregnation in Orchids

soon as those that are impregnated; their ovules show in most cases no further development; they sometimes become a little more perfect, but are always decomposed before the ovarium has withered and the flower has fallen off. After the stigmata of the flower are impregnated with pollen, in most cases the sepals and petals soon wither, but do not fall off, and are still to be found in a dry state at the top of the ripe fruit; sometimes they fall off in a few days. One curious case occurred in *Listera ovata*, where they change very little after impregnation, and were still found in a fresh and succulent state on the top of the ripe dehiscing capsule.

3. The time that elapses between the application of the pollen to the stigma and the full development of the ovules and the formation of the embryo depends on the degree of development of the ovules in the recently expanded flower. This fact might have been suspected, but it will appear to be made out sufficiently after looking at the following summary (in which ii means the integumentum internum, the inner coat; *ie*, the outer; *n*, the nucleus):—

Time from the application of Pollen to the Stigma to the incipient formation of the Embryo.	Degree of Development of the Ovules in the expanded Flower.
Neottia nidus-avis, May 24 to June 2; 8-9 days.	Ovules inverted, ii overlapping n , <i>ie</i> not yet overlapping ii .
Listera ovata, May 8 to 17; 9 days. Orchis pyramidalis, June 22 to	The same. Inverted, acorn-like, <i>ii</i> and <i>ie</i> not
July 1; 8–9 days. Orchis coriophora, June 14 to 23; 9 days.	yet overlapping n . The same.
Gymnadenia conopsea, June 8 to 23; about 2 weeks.	Inverted, <i>ii</i> beginning to appear.
Orchis Morio, May 9 to 22; about 2 weeks.	The same.
Orchis maculata, June 8 to 25; $2\frac{1}{2}$ weeks.	The same.
Orchis hircina, May 24 to June 13; 3 weeks.	Inverted, <i>ii</i> and <i>ie</i> beginning to appear.
Orchis latifolia, May 17 to June 3; $2\frac{1}{2}$ weeks.	Inverted, <i>ii</i> beginning to appear.
Ophrys myodes, May 24 to June 13; 3 weeks.	The same.
Orchis mascula. April 22 to May 22; 4 weeks.	Straight papillæ, seldom a little incurved, with the beginning of <i>ii</i> .
Orchis mascula, May 3 to 24 (?); 3 weeks	The same.
Platanthera chlorantha, May 24 to June 17; 3 ¹ / ₂ weeks.	Straight papillæ, seldom a little incurved, without the beginning of <i>ii</i> .
Orchis militaris, May 15 to June 16; more than $4\frac{1}{2}$ weeks.	The same.
Cypripedium laticolus, May 16 to June 20; 5 weeks.	Papillæ a little incurved; begin- ning of <i>ii</i> very small.

Time.	fron	the 1	Applic	ation	of Pollen
to	the	Stign	na to	the	incipient
for	mati	on of	the E	mbry).

- Cephalanthera grandiflora, end of May to beginning of July; 5–6 weeks.
- Eria stellata, Feb. 13 to April 15; 2 months.
- Bletia Tankervilliæ, Jan. 26 to end of March; more than 2 months.
- Dendrobium nobile, Jan. 10 to May 12; 4 months.
- Cymbidium sinense, Dec. 9 to beginning of June (?); 6 months (?).

Degree of Development of the Ovules in the expanded Flower.

Papillæ a little incurved; beginning of *ii* very small.

Placentæ irregularly fringed, with very small wartlike projections.

The same.

Placentæ undulated, not fringed.

The same.

It appears that those ovules which were most developed in the expanded flower (for instance, in *Listera ovata* and *Neottia nidus-avis*) wanted only nine days to attain perfection and to be impregnated by the pollen-tubes; while, on the other hand, in those cases where even the placentæ were not as yet developed (*i. e.* in *Dendrobium nobile*) an interval of four (in one case perhaps of six) months elapsed before the embryo began to be formed. Respecting the differences of temperature in the different years, it will scarcely be necessary to add that the abovementioned intervals of time will not be quite the same every year.

Soon after the commencement of the formation of the embryo, the six cords of the pollen-tubes disappear. R. Brown says (l. c.p. 707) that they are to be met with even in the ripe capsule; but I could not find them anywhere. I saw them very often in a decaying state, some time after the impregnation of the ovules.

4. From these observations it follows that, in the formation of the fruit in Orchids, the pollen acts in two different ways: on the one hand, it effects the enlargement of the ovarium and the development of the imperfect ovules without the pollen-tubes directly touching the ovules; on the other hand, it impregnates the ovules, directly touching the embryo-sac, and determining the development of one germinal corpuscle into an embryo. It is not necessary to allude further to the observations on this last point, as they only confirm known facts.

Having given the results of a long series of observations and experiments made on Orchidaceous plants, I may be allowed to add a few words on the impregnating action of pollen in general. It is a question, often spoken of among botanists, whether the pollen acts only in impregnating the ovules, or whether, independent of this power, it has yet another, and what this other power might be, and in which way it might act. Professor Treviranus, in a treatise recently published *, inclines to answer the last question in the affirmative, but he says that he cannot give any positive proof. I am therefore the more pleased to have made the foregoing observations, which seem to give such a proof in an incontestable manner. The pollen applied to the stigma of an ovarium containing no ovules, making this ovarium swell, proves that the pollen may act on the ovarium independently of the ovules; and if this is the case in Orchids, why should it not be the same in all other phanerogamous plants? If we admit that the ovules are enclosed more or less in a dark cavity, that of the ovarium, and therefore have not the power of preparing the nutritive substances themselves, but must receive them from the exterior green parts of the ovarium, we can easily imagine how the pollen, besides the direct action of its tubes on the formation of the embryo within the ovules, effects in the same direct manner the enlargement of the ovarium. We even see that, in Orchids, this last-mentioned action on the ovarium is primary-that not until this action has taken place do the ovules attain perfection and become suited for the other, embryo-forming power of the pollen-tubes. If the first power has not acted, the second cannot act. Whether the same may be the case in all other phanerogamous plants, we must leave to further but rather difficult observations.

Finally, it may be repeated that, at least in Orchids, if not in all plants, the pollen acts in two different ways: it effects the enlargement of the ovarium, and impregnates the ovules.

I close these short notices with the very just remarks of Robert Brown which are to be found at the end of his treatise on the fecundation of Orchideæ and Asclepiadeæ :—"I even venture to add that, in investigating the obscure subject of generation, additional light is perhaps more likely to be derived from a further minute and patient examination of the structure and action of the sexual organs in Asclepiadeæ and Orchideæ than from that of any other department either of the vegetable or animal kingdom."

XVI.—On the European Species of the Genus Labrax. By Dr. A. GÜNTHER.

M. BARBOZA DU BOCAGE, Director of the Museum at Lisbon, has directed my attention to a remarkable difference in the dentition of the vomer, by which he was enabled to distinguish two forms of *Labrax* inhabiting the sea at Lisbon, viz. the true *Labrax Lupus* and a second, spotted species. Fortunately

* Verhandl. d. naturhist. Ver. für Rheinland u. Westph. 1862, p. 299.