

## *Pollination in the European orchids: four examples*

### Pollinisation chez les Orchidées européennes : quatre exemples

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#### **Abstract**

*Four examples of pollination in European orchids are given. Column morphology is emphasized. In the genus *Cephalanthera* a membrane covering the upper stigmatic rim prevents autogamy. In *Ophrys apifera* the construction of the caudicle is essential for achieving autogamy. Pollination mode and fruit set in *Corallorhiza trifida* and *Epipogium aphyllum*, two rare European orchid species, are discussed.*

#### **Résumé**

Le gynostème est important en taxonomie, sa morphologie détermine la façon dont la pollinisation s'effectue. La plupart des descriptions de gynostèmes des orchidées européennes ont été faites d'après des préparations dans l'alcool, nous avons travaillé sur du matériel frais. Notre présentation concernera plusieurs genres d'orchidées européennes, *Cephalanthera*, *Corallorhiza*, *Epipogium* et *Ophrys*. La forme et la fonction de la fleur sont étroitement liées. Des exemples sont donnés pour démontrer comment des changements mineurs dans le plan de construction du gynostème peuvent déterminer de quelle manière la pollinisation se produit.

Pollination of orchids is a fascinating subject; pollinator activity as well as flower and gynostemium morphology can determine the way pollination takes place and can mutually influence each other. This article has two objectives: first we want to demonstrate how minor changes in gynostemium morphology drastically change the pollination pattern in the genus *Cephalanthera* and in *Ophrys apifera*. Secondly we want to describe the relatively unknown aspects of pollination of *Corallorhiza trifida* and *Epipogium aphyllum*.

#### **Pollination in the genus *Cephalanthera***

In Europe seven *Cephalanthera* species are distinguished, all but one are allogamous. A *Cephalanthera* flower is characterised by the following features: the flowers are rather large, not opening very wide. The lip has longitudinal ridges, imitating extra pollinia, tempting visiting insects to enter the flower. The lateral lobes of the lip are erect, making the entrance to the long, slender gynostemium more or less tubular. The anther is hinged, movable, connected to the gynostemium base with a long filament. The pollinia are banana-like, formed at the ventral side of the anther.

*Cephalanthera* is a primitive genus, lacking a viscidium. The pollinia are fixed to the pollinator's back by means of stigmatic fluid. The insect visitor searches

inside the flower for nectar and, due to the tube-like construction of the lip, is forced to leave the flower the same way as it came in. When retreating, it first touches the stigma, is smeared with stigmatic fluid and then touches the hinged anther that lifts, releasing the pollinia, which then adhere to the insect's back. This pollination mode is only moderately successful, and hence the allogamous *Cephalanthera* species show a low fruit set: in *C. longifolia* it varies from 2.6 to 17.4%, in *C. rubra* from 1.2 to 28.3% (Künkele and Baumann, 1998) and original observations (unpublished).

*Cephalanthera damasonium* has cream-coloured flowers that often hardly open and is the only autogamous *Cephalanthera* species. Fruit set is very high, varying from 82 to 96% original observations (unpublished).

When examining the gynostemium of *C. damasonium*, its morphology precisely resembles that of the allogamous species. When the anther opens, at the beginning of anthesis, the pollinia are released. Unlike the allogamous species they do not keep standing upright after falling out of the anther, but instead fall downward on the upper stigmatic rim and soon touch the stigma. Once they contact the stigma, a visiting insect cannot anymore remove them, this could at best remove some pollen fragments. Normally the pollinia of *C. damasonium* stick to the stigma as a whole, and autogamy is achieved (Claessens and Kleynen, 1995).

How is such a difference in pollination syndrome between allogamous and autogamous *Cephalanthera* species possible? When carefully examining a flower of an allogamous species, e.g. *Cephalanthera longifolia*, one can find a thin membrane, covering the upper stigmatic rim. This membrane is well visible in older flowers, because later in anthesis it turns brown. This membrane prevents the pollinia from contacting the stigma. Other allogamous species examined also possess this membrane, preventing autogamy. Even in old, wilted flowers the pollinia still are in the anther and do not contact the stigma. This minor change in gynostemium morphology is responsible for the difference in pollination syndrome. The change in pollination method is advantageous for *C. damasonium*: autogamy makes it independent of insects and allows this species to inhabit heavily shaded forests where little pollinators are available.



Photo. 1 – *Cephalanthera damasonium*, gynostemium. Pollinia have already connected the stigma (cl. J. Claessens and J. Kleynen).

## Pollination in *Ophrys apifera*

*Ophrys apifera* is an autogamous species, although it has many features of an allogamous species. How is pollination achieved in this species? At the beginning of anthesis the anther opens a little, releasing the caudicles. The gynostemium stretches somewhat, tightening the caudicles. *O. apifera* is functionally prepared for allogamy: it has two bursicles, both containing a functional, sticky viscidium. If the pollinia are manually removed, they bend backward (in direction of the stigma), just as all allogamous *Ophrys* species do. Yet autogamy is the rule. Soon after anthesis the pollinia fall forward and downward, eventually hanging in front of the stigma, but later on sticking onto the stigmatic surface (Claessens and Kleynen, 2002).

When the pollinia contact the stigma, they are drenched with stigmatic fluid and soon the pollen tubes start growing, ensuring a very high fruit set, varying





Photo. 2 – *Ophrys apifera*, gynostemium. Pollinia are dislodged; one pollinium already sticks onto the stigma (cl. J. Claessens and J. Kleynen).



Photo. 3 – *Corallorhiza trifida*, flower shortly after anthesis. The pollinia are attached to the stipes and have partly rotated (cl. J. Claessens and J. Kleynen).

from 72 to 89 percent (Künkele and Baumann, 1998). We were puzzled by the fact that the caudicles bend so easily, whereas in other *Ophrys* species the pollinaria keep standing upright after anthesis. We also wondered how the contact between pollinia and stigma is brought about. The morphology of the caudicle plays a critical role in the pollination process: although it is capable of performing the same movement as other *Ophrys* species, it always bends just the other way around, in addition to which the viscidium stays in the bursicles.

In order to find out what was typical about the caudicle of *O. apifera*, we compared the caudicle of *O. kotschyi* with that of *O. apifera*. Microscopic sections revealed, that the caudicle of *O. kotschyi* is firm and solid. The sections of the caudicle of *O. apifera* showed that in this species the caudicles are not solid, but instead contain hollow spaces, that might weaken the structure of the caudicle (Claessens and Kleynen, 2002). This could explain why the pollinia fall downward so easily, and also could give the clue to the mechanics of pollination: a gust of wind is sufficient to make the pollinia sway until they contact the stigma and stick onto it. As in *Cephalanthera*, a small morphological change makes autogamy possible, enabling *O. apifera* to conquer new appropriate habitats very fast.

### Pollination of *Corallorhiza trifida*

*Corallorhiza trifida* is a quite inconspicuous orchid, usually growing in damp pinewoods, often in considerable shade. The tiny flowers have a white lip with small crimson dots at the lip base.

In European literature there are various mentions of autogamy in *C. trifida* by means of pollinia falling onto the stigma. But no detailed descriptions of gynostemium morphology and process of pollination were given. We studied *Corallorhiza* flowers in early development, before anthesis (Claessens and Kleynen, 1998 b). In this stage, when the anther is still closed, the rostellum develops a stalk-like protuberance, the so-called hamular stipe or hamulus (Rasmussen, 1982). A stipe is the connection between viscidium and caudicles. The *Corallorhiza* anther opens early in development, mostly already before anthesis. At this stage the structure of the flower can well be observed: the anther contains four superposed pollinia. They are connected to the stipe by means of very short, thin and very flexible caudicles, that can be drawn out up to several times their own length, without breaking.

The anther soon turns brown and lifts due to dehydration, dislodging the pollinia that fall downward. This whole process takes place before or at the beginning of anthesis, ensuring autogamy. The length of the caudicles plays a role in achieving autogamy: they have exactly the right length for guiding the pollinia to the stigma: when the pollinia fall down, they inevitably fall onto the stigma that is right under the hamulus; the caudicles prevent them from falling further down. Freudenstein (1994) reports that

*C. trifida* rotates its stipe with attached pollinia very soon after anthesis, thus achieving pollination, but we cannot support this view (Fig. 1).

After contacting the stigma, the pollinia soon get soaked with stigmatic fluid, which also partly dissolves the hamulus, at that stage only visible as a small knob. The caudicles are then no longer visible. Once the pollinia stick to the stigma, they cannot be removed, nor can there be removed any fragments of pollen, because the pollinia are compact and waxy, and can only be removed as a whole.

Early in development, before anthesis, it is possible to remove the pollinia manually by means of the viscidium, placed at the base of the stipe, indicating that some degree of allogamy might be possible. We regularly found thrips as visitors of *C. trifida* that might serve as transporters of pollinia.

Examinations revealed, that in *C. trifida* only 91% (N=257) of all flowers are pollinated (Fig. 2). Although 9% of the pollinia were missing, we never found any traces of them on the lip or elsewhere, nor did we observe any pollen import: all pollinated flowers had their own pollinia sticking to the stigma. Maybe snails eat the pollinia.

Only 73% of the flowers set fruit. The top flowers mostly do not develop sufficiently, so they are not pollinated. A relatively high number of flowers are aborted: on an average of 8.5 flowers per plant 2.3 flowers were aborted, part of which of course were top flowers.

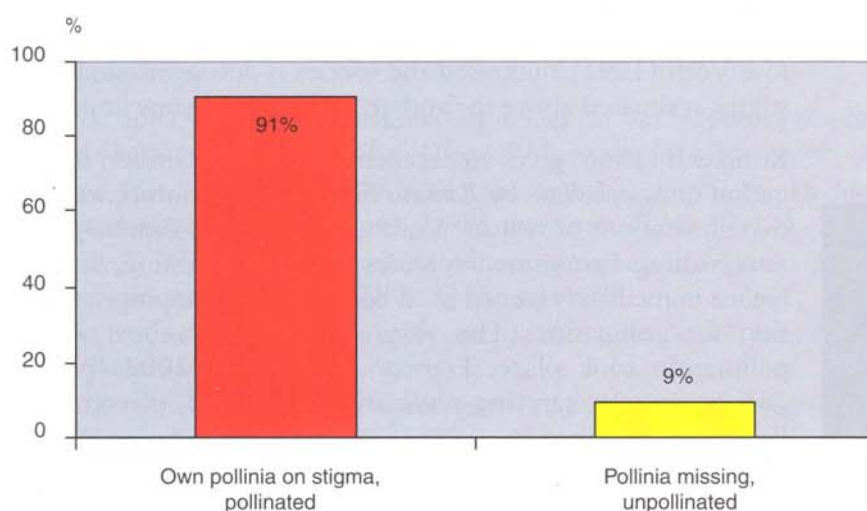


Fig. 1 – Pollination of *Corallorhiza trifida* (N=257).

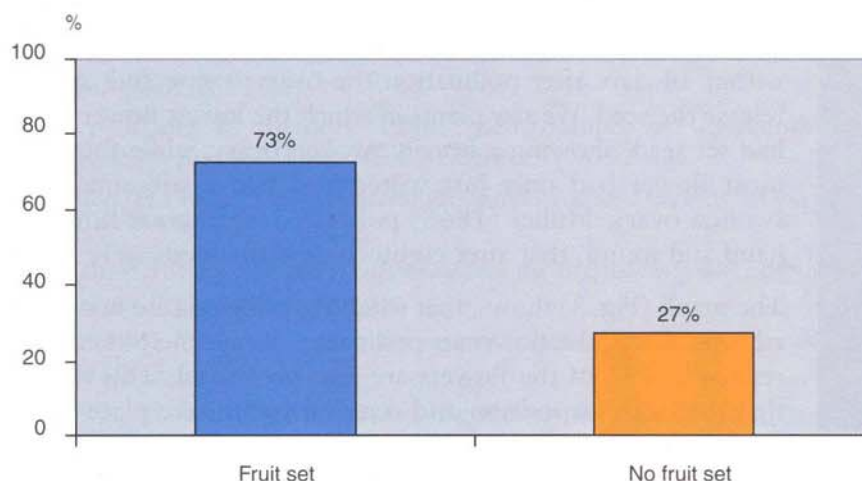


Fig. 2 – Fruit set of *Corallorhiza trifida* (N=230).





Photo. 4 – *Epipogium aphyllum*, flower, not resupinate (cl. J. Claessens and J. Kleyne).



Photo. 5 – *Epipogium aphyllum*, gynostemium. Caudicles are not yet attached to the viscidium (cl. J. Claessens and J. Kleyne).

## Pollination of *Epipogium aphyllum*

*Epipogium aphyllum* is a rare, intriguing orchid species with a very short life cycle and irregular appearance pattern. The plants are brownish-yellowish, rather inconspicuous. The flowers are not resupinate, so the lip is turned upward. The lip is the most conspicuous part; white with four deep rose crispate ridges. The large, sack-shaped, rounded spur turns upward; in the spur some nectar is secreted.

The gynostemium is quite typical: the anther is large, solid for the greater part. Pollinia and caudicles are formed on the underside of the anther, and lie in a deep clinandrium. The caudicles are attached to the back of the pollinia, and run under the pollinia (Claessens and Kleyne, 1998a). The pollinia are typical and unique for this genus, not consisting of one layer of pollen, as is the rule in the *Orchideae*, but instead are made up of more layers of pollen (Vermeulen, 1965).

The heart-shaped viscidium is situated right under the anther. The stigma does, unlike in most other European orchid species, not form an entity with the viscidium, but is separated, placed near the gynostemium base. The styler canal is very short.

There are hardly any records of pollination of *E. aphyllum*; therefore Vöth (1994) suggested the species is autogamous. But the stigma is situated above the anther, making autogamy impossible.

Rohrbach (1866) gives an excellent, precise description of pollination of *E. aphyllum* by *Bombus lucorum*. In literature we found two illustrations of visitors: Vöth (1999) depicts *Bombus pascuorum*, visiting *Epipogium*. He states that, after alighting, the bumblebee immediately turned head downward, the appropriate position for pollination. The visit was only very short and no pollination took place. Francon, in Berger (2003) portrays *Bombus proteus*, carrying pollinaria on its head, placed just as Rohrbach described it.

Fruit set is indeed very rare: Reineke and Rietdorf (1998) found out of 2,000 examined flowers only 4 that were pollinated.

We had the rare chance to observe a population (Hochobir, Austria) that was very well pollinated. The life cycle is very short: within 14 days after pollination the ovary is ripe and opens to release the seed. We saw plants in which the lowest flower already had set seed, showing a heavily swollen ovary, while the uppermost flower had only just wilted and had a still small, little swollen ovary. Müller (1868) pollinated *Epipogium* flowers by hand and found, that after eighteen days the seeds were ripe.

The graph (Fig. 3) shows, that when the pollinaria are not removed, only 5% of the flowers is pollinated. When the pollinaria are removed, 43% of the flowers are also pollinated. This suggests that pollinaria deposition and removal partly take place successively, during one visit. In 19% of the flowers the pollinaria were removed, but the flower was not pollinated. In total a surprisingly high number of 48% of all flowers were pollinated.

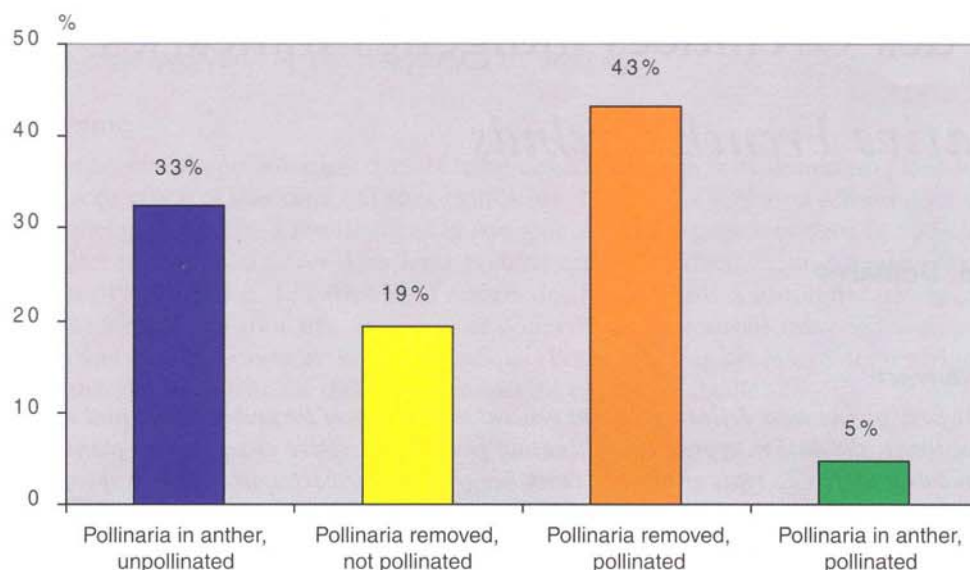


Fig. 3 – Pollination of *Epipogium aphyllum* (N=83).

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## References

- Berger, L. 2003. « Stratégies et mécanismes utilisés par les Orchidées européennes pour attirer leurs pollinisateurs ». *Bull. Group. Rhône-Loire-Isère-Ain. Soc. fr. Orch.* 8.
- Claessens, J. and J. Kleynen. 1995. "Die Systematik der europäischen Orchideen, illustriert an Hand von Makro-Fotos." *Journ. Eur. Orch.* 27 (1): 93-124.
- . 1998 a. "Column structure and pollination of *Corallorhiza trifida* Châtelain (Orchidaceae)." *Journ. Eur. Orch.* 30 (3): 624-628.
- . 1998 b. "Die Säulchenstruktur der europäischen Orchideen." *Jahresber. Naturwiss. Ver. Wuppertal* 51: 23-42.
- . 2002. "Investigations on the autogamy in *Ophrys apifera* Hudson." *Jahresber. Naturwiss. Ver. Wuppertal* 55: 62-77.
- Freudenstein, J.V. 1992. *Systematics of Corallorhiza and the Corallorhizinae* (Orchidaceae). Ph. D. dissertation, Cornell University, Ithaca.
- Künkele, S. and H. Baumann. 1998. "Orchidaceae." In: Sebald *et al.*: *Die Farn- und Blütenpflanzen Baden-Württembergs*. Verlag Eugen Ulmer.
- Müller, H. 1868. "Beobachtungen an westfälischen Orchideen." *Verh. Nath. Ver. Preuss. Rheinl. Westf.* 25: 1-62.
- Reineke, D. and K. Rietdorf. 1998. "Bemerkungen zu *Epipogium aphyllum* in Südbaden." *Ber. Arbeitskrs. Heim. Orchid.* 15 (2): 52-62.
- Rasmussen, F. 1982. "The gynostemium of the neottiid orchids." *Opera Botanica* 65: 1-96.
- Rohrbach, P. 1866. *Über den Blütenbau und die Befruchtung von Epipogium gmelinii*. Gekrönte Preisschrift, Göttingen.
- Vöth, W. 1994. "Sind Blüten von *Epipogium aphyllum* Sw. entomogam oder autogam?" *Orchidee* 45(6): 248-251.
- . 1999. "Lebensgeschichte und Bestäuber der Orchideen am Beispiel von Niederösterreich." *Stapfia* 65: 1-257.
- Vermeulen, P. 1965. "The place of *Epipogium* in the system of *Orchidales*." *Acta Botanica Neerlandica* 14: 230-241.