

Investigations on the autogamy in *Ophrys apifera* Hudson.

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Summary

O. apifera is well-known for its autogamy. In literature we can find different opinions about how auto-pollination actually takes place. Some authors postulate there are active, inward-curling movements of the caudicles, bringing the pollinia towards the stigmatic surface. Others believe, that external factors alone are sufficient enough to bring about autogamy. In this study we found that the wind is the crucial factor in the pollination process. A gust of wind is enough to make the pollinia, that have left the anther, swing to and fro, eventually making contact with the stigma, after which auto-pollination takes place. The caudicles of the pollinarium play a decisive role in this process. We found that they are not solid, but instead contain hollow spaces, weakening the structure of the caudicles. This enables them to swing freely in all directions. This clearly is an adaptation to the principal factor in the pollination process, the wind. Other external factors (pollinators, passing animals) play no role in this process. We found no evidence for the supposed active movements of the caudicles.

Zusammenfassung

O. apifera ist bekannt für ihre Autogamie. In der Literatur finden sich verschiedene Meinungen über den genauen Verlauf der Autogamie. Einige Autoren postulieren, dass es eine aktive, einwärts-drehende Bewegung der Pollenstielchen gibt, die notwendig ist um den Kontakt zwischen Pollinien und Narbe zustande zu bringen. Andere dagegen nehmen an, dass externe Faktoren an sich schon genügen, um die Autogamie zu erreichen. Diese Studie zeigt, dass der Wind der ausschlaggebende Faktor ist im Bestäubungsprozess. Ein Windhauch genügt, um die Pollinien, die die Anthere verlassen haben, hin und her schwingen zu lassen. Dann kommen sie in Kontakt mit der Narbe, wonach die Selbstbestäubung stattfindet. Die Pollenstielchen spielen eine entscheidende Rolle. Wir haben herausgefunden, dass sie nicht massiv sind sondern hohle Räume enthalten, die die Struktur der Pollenstielchen schwächen. Dadurch sind sie im Stande, frei in allen Richtungen zu schwingen. Dies ist eine deutliche Anpassung an den wichtigsten Faktor im Bestäubungsprozess, den Wind. Andere externe Faktoren (Bestäuber, vorbeigehende Tiere) spielen keine Rolle in diesem Prozess. Wir fanden keine Beweise für die vermeintliche aktive Bewegung der Pollenstielchen.

Keywords

Ophrys apifera, autogamy, bending of caudicles

Introduction

“... kaum eine andere Pflanze ist hinsichtlich der Selbstbefruchtung interessanter als *Ophrys apifera*” (WIEFELSPÜTZ 1964).

Ophrys apifera is an interesting species: in its normal form it is quite typical and very constant. Yet various forms can be found, varying greatly from the typical *O. apifera* (BAUM, CLAESSENS & KLEYNEN, 2002). The one thing all forms have in common is that they are autogamous.

Autogamy has always puzzled botanists, among which the famous scientist CHARLES DARWIN (1877). His entire theory of survival of the fittest was based on the belief that in the long term only the outbreeding species could survive, as expressed in the famous statement: “...nature abhors perpetual self-fertilisation”. DARWIN studied *O. apifera* for many years and described its fertilisation in great detail. He concluded that it is strictly autogamous in fact, although *O. apifera* is well adapted to cross-fertilisation. Most publications in more recent times were based on DARWIN's findings. KULLENBERG (1961) and WIEFELSPÜTZ (1964) who studied *O. apifera* also, came to contradictory conclusions about the way autogamy takes place. The aim of our study is to compare the descriptions in literature with our own findings, in order to clarify how autogamy in *O. apifera* actually takes place.

General structure of an *Ophrys* flower

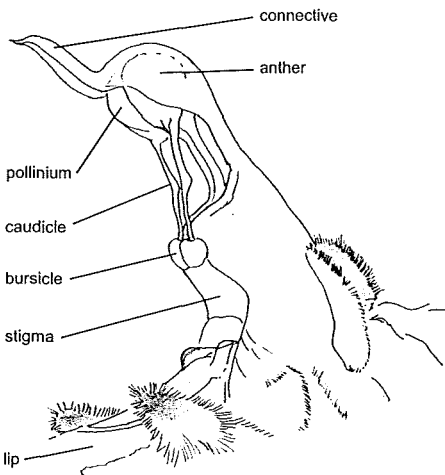


fig. 1: *O. apifera*, gynostenium side view

First of all, we will give a description of an *Ophrys* flower in general. In all *Ophrys* species, the labellum is specially formed to draw attention: greatly differing in shape, colour, scent and texture from the other parts of the flower.

The columns of the *Ophrys* species can vary but always function in the same way. Their basic structure is alike; the anther is situated above the stigma. Both anther cells contain a pollinarium, consisting of pollinium (the actual pollen mass),

a caudicle and a viscidium. Each viscidium is enclosed in a separate bursicle. The caudicles of most *Ophrys* species are rigid enough to stand upright in the anther cells, awaiting the insects which will transport them to another flower. This general pattern can also be found in the flowers of *O. apifera*. Its gynostemium is easily recognisable by its typical S-shaped connective (figure 1). *O. apifera* is, in contrast to most other *Ophrys* species, autogamous. The pollinaria, and more specifically the caudicles, play an important role in the process of auto-pollination. The caudicles are quite typical, curved forward and very flexible. We will discuss their form and function later on.

Is autogamy an active process ?

All authors agree that *O. apifera* is an autogamous species, but there is disagreement about the self-pollination process itself. Authors like DARWIN (1877), DIEUZEIDE (1922) and KULLENBERG (1961) on the one hand believe that the pollinia, after leaving the anther, hang freely in the air and need the wind or another external factor to make contact with the stigma. Others believe, on the other hand, that external factors alone are not sufficient to bring about the contact between pollinium and stigma. They believe a mechanical physiological process is involved (MARTENS 1926, SCHREMMER 1959, WIEFELSPÜTZ 1964, VAN DER CINGEL 1995).

In order to understand the pollination process, we will discuss the various stages in detail. WIEFELSPÜTZ (1964) describes exactly the pollination of *O. apifera*. In order to release the pollinia, two actions take place.

Firstly, when the pollen masses are mature the column stretches, putting the caudicles under pressure. This can clearly be observed in freshly opened flowers: the caudicles no longer follow the curvature of the column, but stretch between the bursicle and anther cells. At this stage they still contain the pollinium (fig. 1). There is more evidence that the anther stretches: sometimes the very thin, membranous walls of the anther show small cracks when ripe, proving they were pulled taut, in order to release the pollinarium. This movement of the column can be observed in more orchid genera. In *Limodorum abortivum* the anther bends forward, bringing about the contact between pollinia and stigma (CLAESSENS & KLEYNEN 1995).

The second part of the action is the opening up of the anther cells, made possible by the loosening and widening of the thin parts of their walls. During this stage the pollinium is forced in a forward and downwards direction by the sheer weight of the pollen masses, eventually hanging freely in front of the anther cells. The arguments in favour of a mechanical factor in the pollination of *O. apifera* are based on the characteristic and position of the pollinia, making it a critical factor in the observations.

MARTENS (1926), SCHREMMER (1959) and WIEFELSPÜTZ (1964) were quite convinced that the caudicles bend inwards and developed a theory to support their view. In order to make their theory fit, they started from two assumptions. First these authors stated that the pollinia themselves do not bend far enough down to reach the stigmatic surface. MARTENS (1926) stressed the importance of the position the pollinia take up after leaving the anther cells. He pointed out that the picture of DARWIN's *O. apifera* (DARWIN 1877 p.52, fig.8) was not quite correct: the ovary had been drawn in a upright position, bringing the labellum in a horizontal position (figure 2).

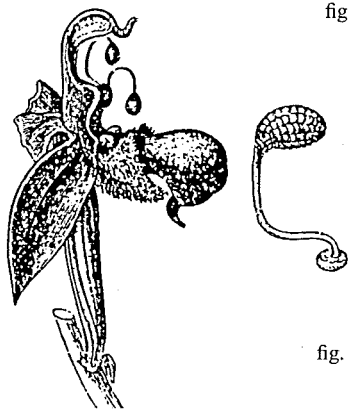


fig. 2: *O. apifera*, after
DARWIN 1877

fig. 3: *O. apifera*, gynostemium
showing the pollina hang-
ing in front of the bur-
sicles (after WIEFELSPÜTZ
1964)



In this position it would be fairly easy for the pollinia to reach the stigma, but MARTENS pointed out that in reality the ovary is at an angle with the rachis. Therefore the labellum is not quite horizontal but slightly facing downwards. MARTENS was right stating these facts, but then postulated that in this position the pollinia could not reach the stigma at all. A conclusion later taken on by WIEFELSPÜTZ (1964), referring to a photograph (WIEFELSPÜTZ 1964, p.58, fig. 16) which shows both pollinia hanging in front of their respective bursicles (figure 3), according to WIEFELSPÜTZ the normal position of the pollinia. WIEFELSPÜTZ however only showed the first stage of the bending. As the flower matures, the pollinia bend more and more, eventually hanging in front of the stigma (fig. 4), as will be discussed in more detail later.

The second assumption of these authors was that the wind alone would not be able to make the pollinia touch the stigma, and therefore another factor or component must be involved. MARTENS describes how he tried to blow the pollinia against the stigma, but did not succeed in making them touch. According to him the caudicles, hanging free after leaving the anther, were partially dehydrated, thereby giving them a certain rigidity and making bending more difficult. Despite this the caudicles kept turning

inwards, eventually reaching the stigma. MARTENS and WIEFELSPÜTZ (1964) mentioned this inward curving of the pollinia but did not mention the factors enabling this movement. From their papers it is not clear whether the authors actually saw the entire process of the bending of the caudicles, finally contacting the stigma. It is possible they only saw the first and the last stage of this process: first the bended, free-hanging caudicles and then the pollinia adhering to the stigma. In order to explain this, they developed their theory of the inward-curving caudicles.

Following SCHREMMER and his opinion, WIEFELSPÜTZ (1964) is convinced the movement is a mechanical physiological process, acknowledging that the exact causes are not yet known though. He speculates that the inward movement is analogous to what we can observe in many plants which have tissues under tension, like e.g. the fruit of *Impatiens*. The tropical orchids *Catasetum* and *Cynoches* have pollinia that are catapulted out of the anther onto the back of a visiting insect.

According to WIEFELSPÜTZ this active movement of the pollinia could also explain the transverse position often observed when the pollinia are stuck to the stigma (fig. 6 + 7). In nature we can often observe spiral growing movements. In conclusion, we may say that MARTENS and WIEFELSPÜTZ argue that the pollinia do not drop down far enough in front of the stigma, and that they can not be blown onto the stigma by the wind. This means that an active component must be involved. This argument seems quite convincing but is on the whole mainly descriptive, not based on experiments.

KULLENBERG's experiments

KULLENBERG (1961) studied *O. apifera* mainly in western Morocco and came to different conclusions in regards to its pollination process. DARWIN views *O. apifera* as being almost entirely autogamous. KULLENBERG observed how various insects (*Eucera* and *Tetralonia* males) were attracted to the flower and attempted copulation (see plate 85-89 in VAN DER CINGEL 1995). He does not describe an actual case of the removal of the pollinia. KULLENBERG compared the Moroccan plants with three plants sent to him from southern England, but fails to mention their state (cut or potted). Yet this information is crucial for the importance and interpretation of the experiment. Cut flowers do not always pass through all the normal stages of development.

We were able to observe some cut flowers kept in a vase and found that the pollinia did not leave the anther in the normal way, but kept sticking to it even when the flower had withered. In an old flower one expects that the membranous part of the anther would weaken, thus releasing the pollinia. But apparently this does not always take place in cut flowers. Kullenberg found that plants, kept indoors in a low and fairly constant level of humidity, did not drop their pollinia; they stayed in the anther until the flowers had dropped off.

Our results were in line with KULLENBERG's description, so it is possible that the English plants observed by him were cut and put in a vase, diminishing the importance of his observation. KULLENBERG was aware of the fact that the number of plants from England was too small to draw conclusions from it.

KULLENBERG mentioned that the plants from England were more slender, had narrower, weaker caudicles and less taut anther walls. He suggested, that those characteristics indicated that the English plants were better equipped for auto-pollination. We mentioned before, that the caudicles play a vital role in the auto-pollination process. In order to test the bending capacity of the caudicles KULLENBERG carried out some little experiments on them.

Firstly, the caudicles of four pollinia already adhering to the stigma were cut off at the base of the pollinium (the actual pollen mass). They straightened immediately, although not completely. Two caudicles of pollinia still remaining in the anther cell were cut off in the same way but did not bend over at all. KULLENBERG concluded that the weight of the pollinia made them bend over.

In another experiment KULLENBERG shook flowers where the pollinia had been released but had not dropped yet: the pollinia swayed in all directions. So the weight of the pollinia determines their movement towards the stigma.

In the study on the Moroccan *O. apifera*, plants with pollinia hanging down were placed in a calm (wind free) environment, but the pollinia did not show any sign of movement towards the stigma. KULLENBERG also touched several anther cells still containing pollinia with his finger tip or a steel pen, but the pollinia did not fall out of the anther cells.

We repeated this experiment many times, but most of the pollinia remained in the anther cells. The pollinia normally fall out of the anther in the morning, as discussed in more depth later on.

The process of the pollinia being released from the anther is unique, interesting and important. We already described the process of the pollinia being released from the anther and finally hanging freely in front of the stigmatic surface. At this stage the pollinia can be removed with a pencil or similar object. Once the pollinarium is removed from the anther and sticks to the needle, the pollinarium bends forward and not backwards as might be expected (DARWIN 1877). If not removed, the pollinarium moves in the other direction. We may therefore conclude that in this stage the viscidium is fully effective and the pollinaria could be removed successfully by a visiting insect, although they are never actually removed, as will be discussed later on.

KULLENBERG studied the influence of the wind on pollination: in Morocco an *O. apifera* plant placed indoors did not drop any pollinia from the anther; five plants growing outdoors dropped their pollinia which then stuck to the stigma. He also observed the activities of snails on the plants: in some flowers the pollinia had been eaten; in others the pollinia were completely detached. KULLENBERG notices that pollinia were removed but does not give any exact number. In his study he did not observe an actual pollination by an insect.

Investigations

In order to investigate how pollination of *O. apifera* takes place we made studies in three different European regions. In the north of its distribution area lies the nature reserve “Roodborn”, near Wittem in the province of Limburg (The Netherlands). For the central regions we studied various sites in the Drôme region (France). Finally in the southern region we studied *O. apifera* at two sites in Sicily, where the species is rather rare.

	Roodborn (Wittem)	Drôme	Sicily	total
number of plants	103	87	17	207
total number of flowers	515	530	85	1130
buds	175	298	17	490
flowers open, pollinia in anther	59	16	20	95
flowers open, pollinia hanging free	58	39	4	101
flowers open, pollinia missing	2 (*)	11 (**)	1 (***)	14
flowers pollinated, pollinia alongside	93	67	15	175
flowers pollinated, pollinia crosswise	68	52	14	134
not recognisable	60	47	14	121

tab. 1: observations of the pollination of *O. apifera* in different regions

- (*): one missing pollinium (the actual pollen-mass) was eaten by a snail (viscidium still in bur-
sicle), the other pollinarium (the entity of pollinium, caudicle and viscidium) stuck on a
nearby grass-stalk
- (**): in 9 cases the caudicles (and parts of the pollinium) were eaten by snails, one pollinarium
stuck on a nearby grass-stalk, one pollinarium was actually missing.
- (***): one pollinarium missing.

We also made observations in the Eifel (Germany), but here we studied only the pol-
lination process, and no data are available. The following table shows which factors
were examined and observed. Special attention was paid to the pollination process.

We investigated mainly two things: in open flowers we looked at the percentage of pollinia that either still remained in the anther, were hanging free in front of the stigma or actually stuck onto the stigmatic surface. Secondly we noted the way the pollinia were deposited on the stigmatic surface: alongside or crosswise. This was important because it gave an indication of the influence of the wind. The data in table 1 give us an idea of the time it takes before the auto-pollination process is completed. Out of 1130 flowers, 640 flowers (57 %) had opened. Only 95 flowers, that is 15 %, still had the pollinia in the anther, in 83 % of all open flowers the pollinia had either left the anther (15 %) or had already adhered to the stigma (67 %). In the remaining 2 % of the flowers the pollinaria were missing. We may conclude that pollination indeed takes place rapidly.

In publications on pollination, the critical point is how pollinia are attached to the stigmatic surface. Therefore, we made many observations in the field of the movements of the pollinia. All authors cited before agree on the first stage of pollination: the withering of the outer anther walls, the pollinia being released and the bending forward after a few hours. There is disagreement about the exact position of the pollinia when the first stage of descend is completed. Our observations, completed by photographic proof, indicate that the pollinia hang free right in front of the stigmatic surface at the end of the first stage (figure 4).



fig. 4: pollina hanging in front of stigma

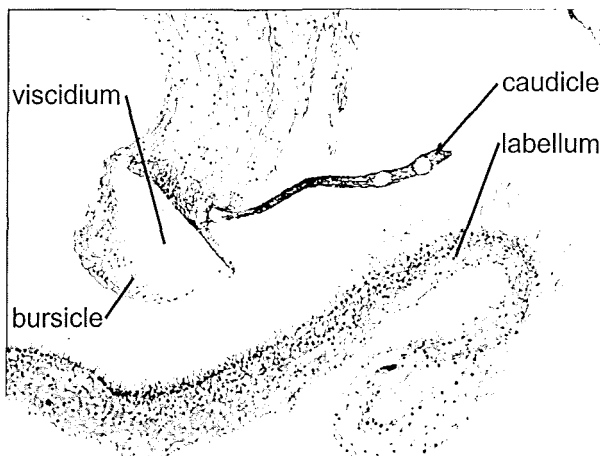


fig. 5: microscopic section of the caudicle of *O. apifera*

KULLENBERG (1961) stressed the uniqueness of the pollinia of *O. apifera*, a species well-adapted to auto-pollination. The caudicles play a vital role in the pollination process. Each caudicle has a slender, basal part and a longer sturdier part, connected to the pollinium. The upper part is slightly semi-circular. At about one third of the

caudicle a triangular thickening is formed (fig. 1), which according to KULLENBERG is a weakening of the tissue, creating a kind of a joint, which enables the caudicles to bend forward.

Furthermore, these caudicles are flexible. Their special structure enables movements of the pollinarium in all directions. During our observations we saw time and again the pollinaria, swinging to and fro in the wind, and then all of a sudden sticking to the stigma. This can be imitated by slightly tapping the flower or rachis, an experiment repeated on numerous occasions. The results were similar to field observations: the pollinia started to swing and then abruptly stuck to the stigmatic surface. Most of the time only one pollinium stuck to the stigma, on repeating the tapping action the other one soon followed.

The unique structure of the caudicles that, in contrast to the other *Ophrys*, bend forward when still being held in the anther, puzzled us. In order to find out more about the inner structure of the caudicles, we made several microscopic sections. We found, that the caudicles of *O. apifera* are not solid, but instead contain hollow spaces, as can be seen in figure 5. We examined several caudicles, in order to make sure we were not dealing with preparation artefacts. But all caudicles we examined showed those hollow spaces. Microscopic sections of an other *Ophrys* species, *O. kotschii*, revealed that in this species those hollow spaces are lacking; the caudicle is solid and shows no hollow spaces at all.

It seems to us that those hollow spaces are an adaptation, meant to facilitate auto-gamy. They seriously weaken the structure of the caudicle, enabling it to bend forward. We found no other mention of this special structure anywhere in literature. In our opinion the sturdy part of the caudicle is quite functional: it does not serve as a weakening, but instead serves as a device that sees to it that the caudicle does not bend too much. Therefore the transition between the upper and lower part of the caudicle should be strong, quite in contrast to the great flexibility of the lower, ribbon-like part. The pollinia bend down, but can not hang straight down, as they would land on the lip and not on the stigma. Moreover, when the pollinia are hanging straight downwards the wind cannot reach them without hindrance. The arched position of the pollinia is an adaptation to the wind. We believe that the sturdy, triangular part provides a distance between pollinia and gynostemium, which means that the pollinia are more exposed to the wind which, as we shall see, has quite a decisive influence on the pollination of *O. apifera*.

Normally an *O. apifera* plant has only four or five flowers that are open and have not withered yet. The pollination process only takes a short time. The flowers undergo a rapid change after auto-pollination (VAN DOORN 1997): the colours become faint,

the labellum shrinks a little and the gynostemium bends a little over towards the labellum. At the same time the labellum makes a distinctive move upwards to the gynostemium. These changes appear soon after auto-pollination.

We made our observations either in the morning or in late afternoon, in order to establish if time of day is a factor. In the morning we saw many anthers with one freed pollinium, while the other one was still in the anther. In the afternoon either the flower had been pollinated, or one pollinium was hanging free, with the other one stuck to the stigma. In Wittem (Limburg) we examined 36 *O. apifera* plants in the morning. We found that in 25 flowers (out of 174) the pollinia were hanging freely, and in 46 flowers the pollinia had already stuck to the stigma. Of course we could not establish if the pollination had taken place that morning or the previous day.

Two days later, under similar meteorological conditions, we looked at 36 different plants in the evening and found 12 flowers (out of 152) with pollinia hanging free and 79 pollinated flowers. These findings indicate that the pollinia are being released from the anther cell in the morning. The process from being released to sticking to the stigma takes normally one day. In most plants the flower which had opened last, still had both pollinia in the anther, while in the previously opened flower either both pollinia stuck to the stigma or one was hanging freely and the other one stuck to the stigma.

One series of observations in the Drôme region was made the day after a violent cloudburst. Of a total of 74 open flowers there were no less than 9 flowers (12 %) that had both pollinia hanging beside the gynostemium, most of them hanging both on the same side. Normally one finds hardly any pollinia sticking to the side of the gynostemium. In all other observed cases of open flowers we found 5 cases (0,9 %) out of 566, where the pollinia stuck to the side of the gynostemium. The surprisingly large number of pollinia sticking to the side of the column stresses the importance of the wind as an important external factor. This indicates that the pollinia land on the stigma by means of an external influence, normally the wind. Other factors can play a role like a grass stalk tapping against the plant, an animal passing or activities of an insect trying to copulate with the "female".

In our observations we also examined the way the pollinia were attached to the stigmatic surface: alongside or crosswise (figure 6 and 7). In older flowers it was not possible to determine whether the pollinia stuck to the stigma alongside or crosswise, because after contacting the stigmatic surface they became soaked in stigmatic fluid. Then the caudicles adhere firmly to the stigmatic surface, partially dissolving in its fluid, a phenomena we described before in the genus *Corallorrhiza* (CLAESSENS & KLEYNEN 1998).

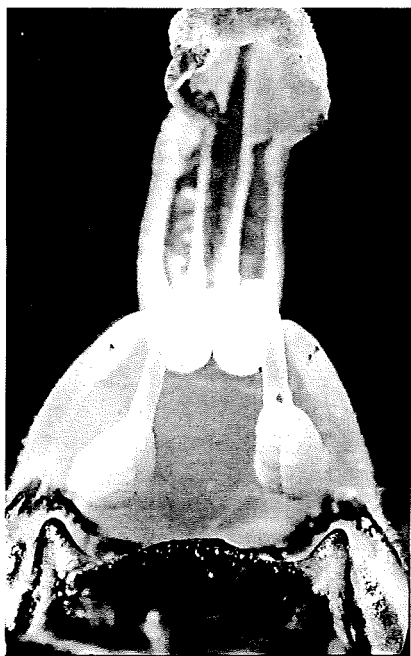


fig. 6: pollinia alongside on the stigma



fig. 7: pollinia crosswise on the stigma

WIEFELSPÜTZ also observed the two different types of adherence to the stigma, but explained it by referring to the spiral movements, often found in nature. We wanted to find out if the wind was the cause of this different deposition of pollinia. If so, then the percentage of pollinia adhering alongside compared to the pollinia adhering crosswise should be about equal. The wind makes the flowers shake, and the pollinaria, by their special structure, make unpredictable movements, so deposition onto the stigma happens at random.

When observing the *O. apifera* flowers in the field, one can see that the caudicles are very flexible indeed, allowing the pollinia to move freely. A gust of wind is enough to make the pollinia shake and swing in all directions, making them land alongside or crosswise to the stigma. This does not take into account the force and the direction of the wind, so percentages can vary. In 309 flowers we found the caudicles 175 times alongside and 134 times crosswise. Taking into account the fact that the pollinia lie alongside in the anther and first hang alongside, it is quite logical that the percentage of caudicles lying alongside is a bit higher, but our results indicate that there is no pre-programmed movement of the caudicles, as suggested by WIEFELSPÜTZ.

We were able to compare the caudicles of *O. apifera* with those of *O. holoserica* and of their hybrid. It proves that the caudicles of *O. holoserica* are not flexible and are clearly designed for transportation by an insect: after being attached to the insect's head they make the usual forward move in order to be in the right position for reaching the stigma. Then they stay in this position without making any further movement. It was interesting to see that the hybrid is very much an intermediary in its features: normally the pollinia stay in the anther until they are transported, but we observed that after a heavy rainfall the pollinia of a newly opened flower behaved exactly the way as described about *O. apifera*: they fell out of the anther and swung onto the stigmatic surface. In the older flowers the pollinia stayed in the anther and did not fall out at all; they could still be seen after the flowers had withered. On a slide, kindly put at our disposal by Mr. FELIX BAETEN, we observed the same phenomena. In a hybrid between *O. apifera* and *O. scolopax* the caudicles of some flowers also bent forward. Those observations show that the flexibility of the *O. apifera* caudicles is even passed down to hybrids with *Ophrys* with non-flexible caudicles.

WIEFELSPÜTZ stated that he never saw any differences in the auto-pollination process of flowers from different regions, whereas KULLENBERG stresses the differences in auto-pollination between flowers from Britain and Morocco. We were able to compare various populations and came to the conclusion that differences do exist, although mainly on the morphological level. The Sicilian *O. apifera* plants were smaller but sturdier than those of the Drôme or Limburg. Their sepals were less colourful, more greenish-rose; the labellum drawing was very greenish too and the gynostemium was more stocky.

Twice we saw a plant with the four upper flowers opened, and found that both upper, newly opened flowers had both pollinia still in the anther. The third flower had been pollinated and the fourth flower had the pollinia still in its anther. We also observed that the pollinia in the Sicilian plants stay longer in the anther: we saw several plants with 2 or 3 open flowers which still had both pollinia in the anther. In the Drôme, the Eifel and in Limburg the pollinia normally stick to the stigma within 24 hours. It is true that the Sicilian plants keep their pollinia longer in the anther, but the actual pollination of the Sicilian plants takes place in the same way as in the other regions we studied.

In order to study the pollination process in depth we placed one *O. apifera* plant under a glass jar, an experiment already carried out by DARWIN (1877). The jar was put on some stones, so as to allow circulation of air. The plant was put under the jar after its first flower had opened normally. The flowers were hanging perfectly motionless in the glass jar. The second and third flower opened normally, the pollinia fell out of the anther and swung down until they hung in front of the stigma. Then

no further movements were observed and they stayed in the same position until the end of the experiment, when all flowers had withered.

One aspect puzzled us: the plant under observation under the jar had 7 flowers, but flower 4 and 5 only opened slightly, keeping the pollinia in the anther until the flowers had dried out completely. Flower 6 and 7 did not even resupinate and behaved in the same way as flower 4 and 5. Perhaps the humidity levels influenced the results: the flowers showed a similar behaviour as those put in a vase. KULLENBERG also found that plants, placed in a low air humidity did not release their pollinia. As pointed out before, flowers in a vase do not always develop in the regular or normal way. Maybe, in this experiment, the exclusion of UV-radiation played a role as well.

One year later we repeated the experiment. The second time we tried to take into account certain experiences from the previous experiment. We put two plants under UV-permeable plastic domes, assuring sufficient air humidity by making a large slit at the base. The flowers opened in the normal way; the pollinia dropped and stayed hanging in the position as described in the previous paragraph. When half of the flowers had dropped their pollinia we removed the plastic cover of one of the plants and found the next day that all flowers had been pollinated. When the plant was covered up again, the remaining flowers opened up and dropped their pollinia as before, but were not pollinated at all. The plant which remained covered over all the time had none of its flowers pollinated after dropping its pollinia !

KULLENBERG mentions the actions of insects, but he never saw an actual case of insect-pollination, nor did DARWIN. Despite thorough investigations, we found only three reports of an insect visiting *O. apifera*. GUMPRECHT (1978) observed a *Zygaena*-male trying to copulate with the labellum, but the insect did not adopt the normal position in which the pollinia could be removed, but instead embraced the labellum mainly from its side. GUMPRECHT concludes that the "position-commands" are insufficient for this insect. KULLENBERG also describes the behaviour of visiting insects and notices, that the males (*Eucera* and *Tetralonia*) either remained sitting on the back of the labellum or executed sideways movements, but in both situations the insects were unable to reach the bursicles (images in VAN DER CINGEL 1995, plate 86 and 89). PAULUS (2000) saw a *Eucera punctulata*-male attempt to copulate, but its tactile orientation was not great, as it moved in a turning-dancing fashion on the labellum, although it succeeded in removing one pollinium in the end. Paulus concludes that allogamous pollination of *O. apifera* could be possible, although the chances of the same insect visiting and pollinating another flower are very small. In our opinion there is probably stimulation through scent, as described by KULLENBERG, but the tactile stimuli do not seem to be directed at visiting insects.

Although DARWIN never observed an actual visit of an insect, he believed that from time to time pollinia were transported by insects from plant to plant, creating a certain degree of genetic diversity. It is remarkable indeed that *O. apifera* has preserved its features so well; apart from some slight differences it is extremely stable throughout its whole distribution area. There are deviating forms, described at very different taxonomic levels, that enlarge the description of *O. apifera*, but appeared to be mere forms of *O. apifera* (BAUM, CLAESSENS & KLEYNEN 2002). These forms can be regarded as the expression of the perpetual self-pollination, as they often show ancestral features like sepal-like petals.

Conclusions

From our observations we may draw several conclusions: first of all, it shows how important the conditions of the observations are. Flowers in a vase may respond differently compared to those in the open air.

Several authors have suggested that the pollinia of *O. apifera* execute an inward turning movement after falling out of the anther. They based their opinion on two assumptions: first of all the pollinia would hang in front of the bursicles and not in front of the stigmatic surface after leaving the anther; and secondly, the pollinia would not be able to reach the stigma by external factors, like wind or a passing animal. Our observations showed that both assumptions are false: the pollinia do bend further down until they reach a position in front of the stigma; and the influence of the wind and other factors can indeed cause the pollinia to stick to the stigma.

Our experiments provide no arguments for the assumed “active autogamy” as described by several other authors. Our studies confirm the findings of DARWIN and KULLENBERG. They also found that the pollinia, if placed in a wind-free environment, stay immobile after descending from their anther, indicating that external influences form an essential part of the pollination strategy of *O. apifera*. The wind plays a decisive role in the self-pollination process of *O. apifera*.

Out of a total of 232 flowers we found only two flowers actually lacking a pollinium, but be careful before drawing conclusions. We found that snails are responsible for a major part of the missing pollinia, as caudicles seem to be one of their favourite dishes. In Beaufort-sur-Gervanne (France) we closely inspected 87 *O. apifera* plants, totalling 232 open flowers, of which 18 were missing one or both pollinia. After examination under a microscope we noticed that, in all but two cases, snails had been eating at the pollinia. They mainly ate caudicles, in some cases caudicles as well as pollinia were missing. In the remainder of these cases the two viscidia

were still in their bursicle. Only two pollinia were actually missing; they could have been removed by a pollinator, although this seems unlikely. In the Wittem observation area a grass-stalk had touched the bursicle because of strong winds, thereby removing the pollinia. Therefore, before coming to conclusions about the seeming removal of pollinia, other factors should be taken into account.

As there are hardly observations of visiting insects in *O. apifera*, their role in the pollination process is practically zero. A passing animal could cause the movement of a plant, but from our observations we can conclude that the wind is the principal factor in the pollination of *O. apifera*. The caudicles can be found both alongside as well as crosswise, reflecting the unpredictable directions of the wind and therefore unpredictable movements of the flowers. The special structure of the caudicles, with their hollow spaces that weaken the internal structure, seems to be unique to *O. apifera*, facilitating autogamy. These hollow spaces are an adaptation to the decisive external factor: the wind. Because of this internal weakening, the caudicles can swing freely in all directions, and almost invariably make contact with the stigmatic surface. This makes the auto-pollination of *O. apifera* highly effective.

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