MANY WAYS TO GET HAPPY: POLLINATION MODES OF EUROPEAN EPIPACTIS SPECIES

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Summary

The European members of the orchid genus *Epipactis* shows a great variety of pollination modes, ranging from allogamy (reproducing by cross-fertilization) to autogamy (pollination of a flower by its own pollen) and a mixture of both. In this article the structure of the flower, the adaptations to the pollinators, as well as the changes in the column structure that enable self-pollination are discussed.

Introduction

The species of the genus *Epipactis* are not among the flashiest orchids that draw the attention of enthusiasts, like the genus *Ophrys* or the tropical orchids. Yet they are a quite interesting group. They show remarkable adaptations to their pollinators and have also adapted their mode of pollination to environmental conditions. In this article, we will examine several species of the genus *Epipactis* and discuss their pollination mechanisms.

Pollination Mechanism of Allogamous Species

For better understanding of the changes in the construction and pollination mode of the flowers we will take *Epipactis helleborine*, the Broad-leaved Helleborine, as an example (fig. 1). This is a European species that has colonized North America successfully: in Wisconsin, it is considered a weed! It was first found in the USA in 1879, after which its population expanded rapidly. In Europe, it is widespread and frequent. It prefers moderately shaded places and can be found in a wide variety of habitats: woods, bushes but also, man-made environments like parks, lanes, sidewalks and cemeteries.

The flowers are rather inconspicuous, possessing typical drab wasp-colors of reddish brown and dull purple, as can also be found in the genus *Scrophularia* (figworts), which have typical wasp flowers too. The perianth segments, the sepals and petals, are wide open (fig. 2). The lip is divided in two parts, the outer part

Fig. 01: E. helleborine.

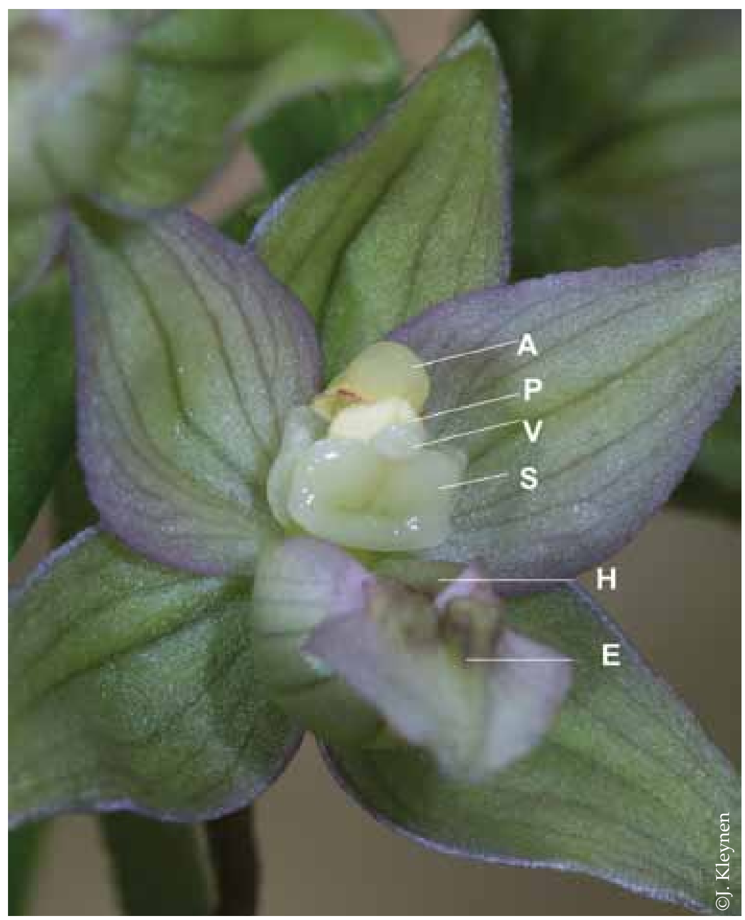


Fig. 02: *E. helleborine*, flower showing the anther (A) with the pollinia (P), the viscidium (V) and the two parts of the lip, the hypochile (H) and the epichile (E)

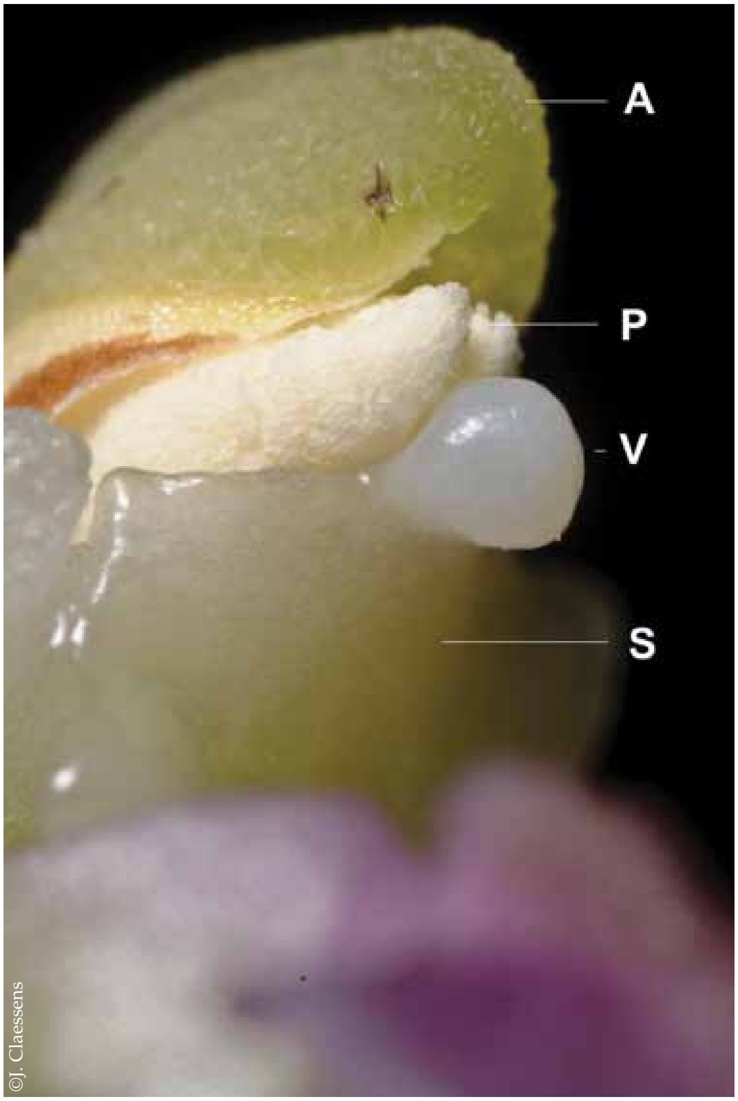


Fig. 03: *E. helleborine*, close-up showing the anther (A) containing the pollinia (P), the globular viscidium (V) and the large stigma (S) underneath

(epichile) and inner part (hypochile). The epichile is broader than long, heart-shaped with two bosses (a protuberance or roundish excrescence) at the center. The hypochile, where the nectar is secreted, is bowl-shaped and reddish-brown on the inside. In warm weather, you can see the glistening nectar it contains. Immediately above the hypochile is the horizontal column, a fusion of the stamen and stigma, topped by the yellow anther cap which contains the pollinia. At anthesis (the opening of the flowers), the anther cap opens and releases the two yellow pollinia. At this stage, they are already connected to the underlying viscidium, a globular structure consisting of milky viscid fluid covered by a thin membrane (fig. 3). The viscidium is placed at the upper rim of the large, rectangular, concave stigma, covered with sticky stigmatic fluid. The pollinia fall onto the slightly hollowed top of the column, which forms a pollen bed. The pollinia cannot fall out of this pollen bed because they are already connected to the viscidium. The powdery pollen within the pollinia coheres rather well because it is interconnected by viscid threads.

Fig. 04: *E. helleborine*, wasp bends to reach the nectar.

The shape and placement of the viscidium, stigma and pollen bed are crucial, because they can determine the pollination mode, as we will demonstrate later. In the case of *Epipactis helleborine*, the viscidium has a double function: it ensures that the pollinia are glued to a visiting insect and at the same time it prevents the pollinia from sliding forward and contacting the stigma. The large pollen bed ensures that the pollinia are in place. Thus, even if the pollinium could lose some pollen fragments, they are unable to slide onto the underlying stigma.

After the insect visitor (almost exclusively a wasp) has landed on the flower's labellum (epichile), the head is position forward towards the hypochile. In order to reach the nectar, the wasp has to bend over the edge at a steep angle before it can start licking the nectar (fig. 04). In doing so, it bumps its head against the protruding viscidium. The delicate membrane ruptures and the pollinia become instantly cemented to the wasp's head (fig. 5). This obviously bothers the wasp, because it often starts grooming and trying to get rid of the pollen load. If it does this immediately, it can remove the pollinia because the glue of the viscidium has not yet hardened completely. This is demonstrated by their pollinia, which can be seen sticking to various parts of the plants. However, within a few seconds the glue hardens and then it is no longer possible to remove the pollinia. After collecting an orchid's pollinia the wasp is not deterred from visiting more orchid flowers (fig. 6).

Attraction of Insects

The flowers of *Epipactis helleborine* are not showy and conspicuous like some orchids, but this is made up for by several other mechanisms that enhance their appeal to their pollinating insects. The flowers were thought to be scentless, but recent investigations showed that the nectar contains several compounds that are attractive to wasps (vanillin derivatives, benzene, octanal, nonanal, and decanal). The composition of the nectar is an important feature, because certain compounds can make it toxic or repellent to some visitors. Also microbes present in the nectar can change the nectar sugar composition.

Fig. 05: *Vespula germanica* with pollinia of *E. helleborine.*

Fig. 06: *E. helleborine*, visiting wasp with pollinia sticking to its forehead.

Fig. 07: *E. helleborine,* wasp with a bunch of pollinia.

Some visiting wasps can give an impression of "sluggish," intoxicated behavior: they stay on the same flower for a longer time and crawl from one flower to another, rather than flying from one to the next like they normally do. Sometimes they even fall from a flower onto the ground when crawling around (see the YouTube video: Orchid pollination 7: Pollination of *Epipactis helleborine* by wasps part 2)

Some researchers have suggested that warm weather causes the nectar to start fermenting, thus producing ethanol which intoxicates the wasps. Since they frequently visit rotting fruit containing large quantities of yeast, fungi and bacteria, they can transport them to the orchid where they contaminate the nectar while feeding. Indeed, small quantities of ethanol, produced by such micro-organisms have been found. Other researchers found narcotic substances in the nectar. The morphinian oxycodone in particular seems to play an important role in intoxicating visiting insects. It is, however, not yet clear which substances influence the visitors under which circumstances. The composition of the nectar has varied in different investigations; some researchers found yeast species in the nectar, other studies did not but found mainly microbial communities instead, mostly of the family of *Enterobacteriaceae*. Weather conditions probably play an important role in the process of intoxication because we mostly observed "drunken" wasps in warm weather. We have seen enough evidence to conclude there is a definite effect on the wasps. The cause of this is not yet clear, but lies in the composition of the nectar and the amount of contamination.

The presence of these contaminants may be advantageous to the orchid, because the insects stay longer on the flower spikes and pollinate more flowers. However, this means the percentage of geitonogamy (flowers that are pollinated with pollen from the same flower spike) is considerable. Geitonogamy is equal to autogamy, which could be adverse to the plant. *Epipactis helleborine* is self-compatible, so it produces seeds, although the seeds originating from geitonogamous pollination may be of poor quality. But that is compensated for by the very high fruit set, of 76% on average. The wasps creep from one flower to another and the glue from the viscidium is very effective, so wasps often carry a whole bunch of pollinia on their foreheads (fig. 7). Obviously this hinders them in reaching the nectar, but it is a good means of augmenting pollen deposition. Also, the load on their heads obliges the wasps to push hard to reach the nectar, and as a result the pollinia are forced into the stigmatic fluid, thus increasing the number of massulae (a coherent mass of pollen grains that are deposited on the stigma).

Epipactis helleborine has another means of luring visitors. The plant produces GLVs (Green Leaf Volatiles), chemical signaling substances that are normally produced by plants that are under attack by herbivores like caterpillars. The GLVs attract predators of the herbivores (in the case of caterpillars they are Ichneumonid wasps) which deter attackers. *E. helleborine* produces these substances although there is no predator around. This kind of attraction is called chemical mimicry and is an adaptation that is supposed to attract wasps that have not yet visited the orchid. Guided by the (false) alarm signal, the wasp inspects the flower and finds an adequate food source. The wasps learn to associate the scent with the reward, thus the loyalty of the visitors is reinforced. The shaded habitat, the scent, and specific colors make this orchid attractive to a quite specific group of pollinators, of which the main species are

Vespula vulgaris, V. germanica, Dolichovespula sylvestris, and D. saxonica.

Epipactis purpurata attracts wasps in the same way. This species grows in heavily shaded woods with few other accompanying plants. The structure and function of the column is the same as in E. helleborine (fig. 8). It is frequently visited by wasps and is noted for the many cases of "drunk wasps" falling off the flowers (fig. 9 and 10, also see the YouTube video: Orchid pollination 2: "Drunk wasp" falls off *Epipactis purpurata*).

Epipactis atrorubens is a species that differs from the other allogamous species. It has wine-red flowers and a distinct vanilla-scent (fig. 11). The scent seems to be quite strong and thus an excellent means of attracting insects from afar. One researcher observed that insects were overcome if they stayed too long on the flowers; a bumblebee caged in a tube with a flower spike of *E. atrorubens* died within 15 minutes. The color deviates from the typical wasp colors, so one may expect to find a different pollinator spectrum. Indeed, the orchid is not visited by wasps but mainly by bumble bees (fig. 12) and sometimes honeybees instead. Caged flowers did not set seed, indicating this orchid relies entirely on the pollinators for its reproduction. Bumblebees are

Fig. 08: *E. purpurata*, flower.

Fig. 09: Wasp carrying many pollinia approaches *E. purpurata*.

Fig. 10: The large amount of pollinia makes it almost impossible for the wasp to reach the nectar of *E. purpurata.*

Fig. 11: *E. atrorubens*, flowers.

Fig. 12: *Bombus lucorum*, a regular pollinator of *E. atrorubens*.

excellent pollinators making very few, fast visits per plant, thus assuring a high degree of cross pollination. Because of the speed of their visits, they can pollinate many plants in a short time. Fruit set of *E. atrorubens* is high (on average 66 %).

Transitional Species

Epipactis palustris is the most exotic looking, species (fig. 13). It has purplish-white, wide open flowers with a ruffled, hinged lip. The epichile is white with yellow-orange margins, which serve as pseudo-pollen, attracting potential pollinators. The small quantities of nectar secreted in this region act as a preliminary reward for visiting insects, luring them further into the flower. Purplish lines guide the insects towards the center, where the orange hypochile produces copious nectar (fig. 14).

As in *E. helleborine*, the lip consists of two parts, but in *E. palustris* it is hinged, making the outer part, the epichile, movable. The column is horizontal and has a large stigma. The pollinia are yellow, long and stick forward, reaching beyond the upper rim of the pollen bed in which they lie. As in *E. helleborine*, there is a globular viscidium.

Darwin studied *E. palustris* and developed the "springboard" hypothesis in which he described how even the weight of a fly landing on the lip is sufficient for the articulated lip, the epichile, to bend down (fig. 15). In order to reach the nectar, the insect must then crawl forward. The hypochile, now less loaded, returns to its original position and pushes the insect against the viscidium (fig. 16). As a result, the pollinia are glued

Fig. 13: *E. palustris.*

Fig. 14: *E. palustris*, lip with orange margins at the epichile and copious nectar secretion in the hypochile.

Fig. 15: The digger wasp *Ectemnius lapidarius* has landed on the lip of *E. palustris*. The epichile bends down under its weight.

Fig. 16: *Ectemnius lapidarius* creeps towards the nectar, the hinged epichile bends upwards and pushes the hoverfly against the viscidium.

Fig. 17: While licking the nectar,
this solitary wasp pushes the pollinia into the stigmatic fluid.

to the insect (fig. 17). Our observations showed that, at least in young flowers, small insects are indeed pushed upwards where they receive a pollen load. Larger insects like honey bees have to perform a kind of balancing act, because the epichile bends down completely when they land (fig. 18). They are large enough to reach the nectar, so they do not need to bend forward. But because of the effort it takes to maintain their balance, there is a good chance they will touch the viscidium.

Epipactis palustris is well visited by insects, primarily by honey bees, solitary bees and hoverflies. In the literature, we found a total of 142 different pollinators were mentioned. And yet, if the weather conditions are bad (too hot, cold, or rainy) this orchid can resort to self-pollination. The pollinia are more friable than those of

Fig. 18: The epichile bends down under the weight of a visiting honeybee (*Apis mellifera*).

Fig. 19: Underside of the column of *E. leptochila*, showing a reduced pollen bed, a non functional viscidium and loose pollen grains that have already contacted the stigma.

E. helleborine and can crumble at the end of anthesis if the flower is not pollinated. The pollen fragments can then fall onto the stigma, assuring autogamy and a high fruit set of on average 77%.

Climatic conditions also determine the pollination mode of another *Epipactis* species, *E. helleborine* ssp. *neerlandica*. This is a dune-form which normally depends on wasps for its pollination. But we observed a change of pollination mode in a hot and dry summer When the pollinia disintegrated and the fragments fell onto the stigma, just as in *E. palustris*. In extreme conditions in Denmark this species even becomes totally autogamous. These plants grow in dunes called “the Danish Sahara,” where a constant hard wind blows, shutting the door to all visitors, which would literally be “sandblasted.” Because of these environmental conditions and lack of pollinators this primarily allogamous species has changed its pollination strategy.

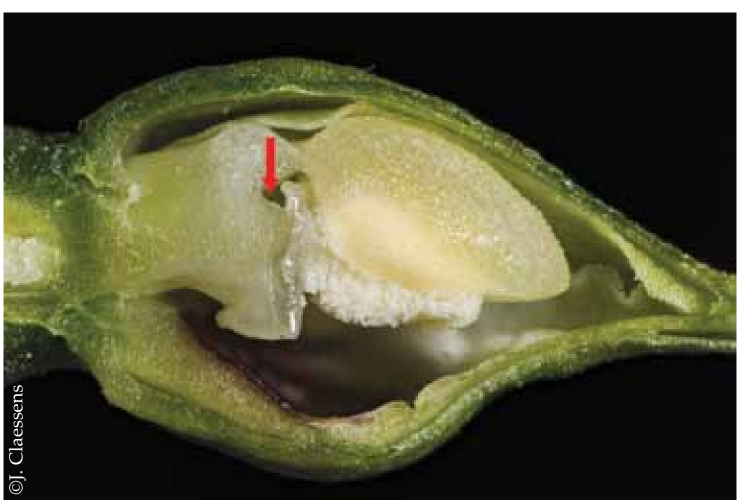
Autogamous Species

Orchids that change from allogamy to autogamy do not have to go through huge changes. Minor changes of the column are sufficient to alter the pollination mode. In order to enable autogamy, the pollen should be able to reach the stigma. This is achieved by various adapta-

Fig. 20: *E. leptochila*, an autogamous species.

Fig. 21: *E. muelleri*, another autogamous species.

tions of the column morphology. All autogamous species show a reduced pollen bed and a reduced upper stigmatic rim. Thus the large pollinia can easily contact the stigmatic fluid below. The pollinia also undergo a transformation: they lose coherence, becoming more friable, falling apart in pieces. Most autogamous species show a viscidium that is reduced or even completely disappeared (fig. 19). These changes enable easy contact between pollinia and stigma combined with a reduced chance of pollen transport by insects.



Caption: Fig. 22: Longitudinal section of a flower bud of *E. muelleri*, showing the reduced pollen bed (arrow) and the pollinia already sticking onto the stigma.

Epipactis leptochila (fig. 20) is an example of an orchid that still shows some allogamous features like nectar and scent production but is in general totally autogamous. It can even be cleistogamous, making insect visits impossible.

In *Epipactis muelleri* (fig. 21) the pollen bed has almost completely disappeared. When the anther opens, the pollinia fall down onto the underlying stigma and stick to it like two little horns (fig. 22).

But then why should an orchid turn towards autogamy, which is genetically seen as a dead end, because there is no more gene exchange? The answer is that autogamy is a good way of conquering new niches in circumstances where there are few or no pollinators. The more extreme the conditions are in which an orchid grows, the higher the chance that it will turn towards autogamy. Many *Epipactis* species have penetrated into the deep shade of forests, because there was no need to attract pollinators. Autogamy is rarely absolute; there is always a chance that an insect loaded with pollen from an allogamous species might visit the autogamous species, assuring some gene transfer. Anthropogenic factors (the destruction of the original habitats, leaving only small suitable patches for the orchids) probably also contributed to the switch of pollination mode.

The genus *Epipactis* is a great showcase to illustrate how the column structure has adapted to meet the requirements of a changing habitat. Interested? You can find more examples of the flower-pollinator relationship in our book "The Flower of the European Orchid – Form and Function."✾

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Jean Claessens and Jacques Kleynen are both teachers. They have written many articles about European orchids. Their special interest is macrophotography and the relationship between the form of an orchid flower and its pollinator. This resulted in the publication of a book: "The Flower of the European Orchid – Form and Function," which was published in 2011. The authors have spoken at many meetings of orchid societies and at conferences. Their website is: www.europeanorchids.com

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