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ANT, BEE AND FLY POLLINATION IN EPIPACTIS PALUSTRIS (L.) CRANTZ (ORCHIDACEAE)

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SUMMARY

In a semi-wild population in Haren, The Netherlands, *Epipactis palustris* (L.) Crantz was visited by honeybees, sawflies, parasitic hymenopterans, ants, hoverflies and other flies. Bumblebees rejected the flowers. Of the hoverflies only *Lejogaster metallina* and males of *Syritta pipiens* were pollinators. *S. pipiens* was common enough (20% of the visits) to effect substantial pollination. Small flies of several families were effective pollinators but in low abundance (1%). Ants were the most frequent visitors (50%) and effected self- and cross-pollination. Honeybees (25%) pollinated and collected pollinia for larval food. Other hymenopterans were very rare on the flowers (1%). *Vespula vulgaris* neglected the flowers. *E. palustris* was not autogamous here. The epichile bent down under heavy insects, and worked as a 'springboard' for light hymenopterans. On the epichile a taste nectar guide was found, effective for flies, ants, experienced honeybees and other hymenopterans.

1. INTRODUCTION

In his detailed study of the pollination of *Epipactis palustris* (L.) Crantz (Orchidaceae) NILSSON (1978) noted the absence of facultative autogamy, which had been recorded by WIEFELSPÜTZ (1970). The visitors found by NILSSON (1978) differed substantially from those mentioned by other authors (DARWIN 1869, GODFERY 1933, SUMMERHAYES 1951, WERTH 1952, WIEFELSPÜTZ 1970, PROCTOR & YEO 1973). The findings of all these authors differ especially in relation to the role of bumblebees and honeybees. To see whether these differences depended on local conditions, we decided to study the pollination of *E. palustris* in The Netherlands. As shown below, several deviations from the observation made by NILSSON (1978) were discovered.

NILSSON (1978) observed ants as flower visitors. Pollination by ants exists (HAGERUP 1943, HICKMAN 1974), but is rare. Because we also observed ants taking nectar from *E. palustris*, we undertook a special investigation of their role as pollinators.

2. MATERIALS AND METHODS

Epipactis palustris grew in a semi-wild population in 'Hortus de Wolf' in Haren, the botanical garden of the State University of Groningen. Observations started when the flowers were in full bloom, on 30 July, 1979 and lasted until 20 august 1979; they were always made in the mornings because after 1 p.m. hardly any animals visited the flowers. Nectar was located using Glucostix®, Ames Comp., which reacts positively at concentrations exceeding 0.25% glucose. We counted all the visitors on five pre-selected inflorescences, during a total of 9½ hours. On all other occasions we collected all the insects bearing the conspicuous bright yellow pollinia.

In pollination experiments inflorescences with only buds were individually enclosed in cages (15 × 10 × 10 cm) of fine netting. One month later, seed set was determined by counting the number of developed fruits. Each developed fruit was filled with seeds, and microscopic examination showed that every seed contained an embryo.

Drawings a – e were made with a camera lucida and f and g from photographic transparancies.

The insects have been deposited in the collection of the Rijksmuseum voor Natuurlijke Historie, Leiden, Netherlands; the collection number is given in brackets (*table 1*). Identifications were kindly made by Dr. P. J. van Helsdingen (Diptera), Dr. C. van Achterberg and Dr. G. van Rossum.

3. OBSERVATIONS

3.1. Nectar

In the flower (see NILSSON 1978 and *fig. 1a*), the lip has a basal cup-shaped part (the hypochile), and joined onto it by a hinge is a distal part (the epichile) with a fringed upcurved margin and with a central group of calli. Wiefelspütz and Nilsson mention nectar production only on the yellow raised spots inside the hypochile cup. However, nectar is also produced on the epichile, at the yellow bases of the calli. This was first apparent from the behaviour of flies, ants, experienced bees and other Hymenoptera which all started licking here, and then continued by licking the hypochile. The location of this epichile nectar was confirmed by confining several inflorescences in plastic bags, where in the high humidity the water did not evaporate from the nectar, which formed visible drops, which gave a colour reaction on glucose test strips. The epichile drops did not originate from the hypochile, because the petal surface remained dry on the hinge and just below it.

Each day the number of visits decreased around noon, presumably because there was no longer any nectar in the flowers.

3.2 Visitors and pollination

Not all visitors obtained pollinia (*table 1*). Those that picked up pollinia can be regarded as effective pollinators. Their contribution to the pollination must depend on their numbers (*table 2*) and on their behaviour.

A few bumblebees (*Bombus sp.*) were present but never visited more than one to three orchid flowers before moving to other flower species in the area. Obviously they were only sampling the flowers, which thereafter were ignored. They did not contact the rostrum of the flowers, and there was no possibility of pollination.

Table 1. Visitors of *Epipactis palustris* flowers, with collection number in brackets, p= with pollinium attached.

Hymenoptera
Apidae
Bombus sp. (10036248)
Apis mellifera L. (10036221-10035227 p)
Symphyta
Tenthredo perskinsi Morice (10036228)
Braconidae, Banchinae
Lissonota sp. (10036253 p)
Ichneumonidae, Cryptinae
Trychosis legator (Thunberg) f. plebeja Tsjek (10036262)
Agrothereutes sp. (10036220)
Formicidae
Lasius niger (L) (10036232 p, 10036236 p, 10036237 p, 10036263 p)
Formica polyctena Forster (10036238 p, 10036242 p, 10036243 p, 10036244 p, 10036252 p, 10036254 p, 10036255 p, 10036256 p, 10036268 p)

Coleoptera
Oedemera femorata Scop (10036207)

Diptera
Calliphoridae
Rhinophora lepida mg. (10036231 p, 10036247 p)
Scatophagidae
Scatophaga stercoraria L, m. (10036251 p)
Anthomyidae
Nupedia aestiva Mg. (10036219, 10036229 p, 10036230 p, 10036260 p)
Tachinidae
Eriothrix rufomaculata Deg. (10036218)
Muscidae
Phaeonia fuscata Fall. (10036265)
Syrphidae
Lejogaster metallina F. (10036244 p, 10036249 p)
Syritta pipiens L. (10036214, 10036215, 10036216, 10036217 p, 10036245 p, 10036250, 10036261 p)
Eristalinus sepulchralis L. (10036213)
Eristalis horticola Deg. (10036233)
Eristalis arbustorum L. (10036201, 10036202, 10036203)
Helophilus pendulus L. (10036204, 10036205, 10036206, 10036235)
Heliophilus hybridus Lw. (10036208, 10036209, 10036210)
Heliophilus trivittatus F. (10036211, 10036212)
Metasyrphus luniger Mg. (10036239)
Paragus tibialis Fallen (10036267)

Table 2. Insect visits to inflorescences of *Epipactis palustris*.

Visitor	Number of visits to 5 inflorescences during 9.50 hours	%	Total number of indivduals with pollinia	%
Apis mellifera	25	16	9	25
Wasps	1	1	2	5
Ants	78	50	11	30
Syritta pipiens	31	20	4	11
Lejogaster metallina	0	0	2	5
Other Syrphidae	21	13	0	0
Other Diptera	1	1	8	22
Total	157	101	36	100

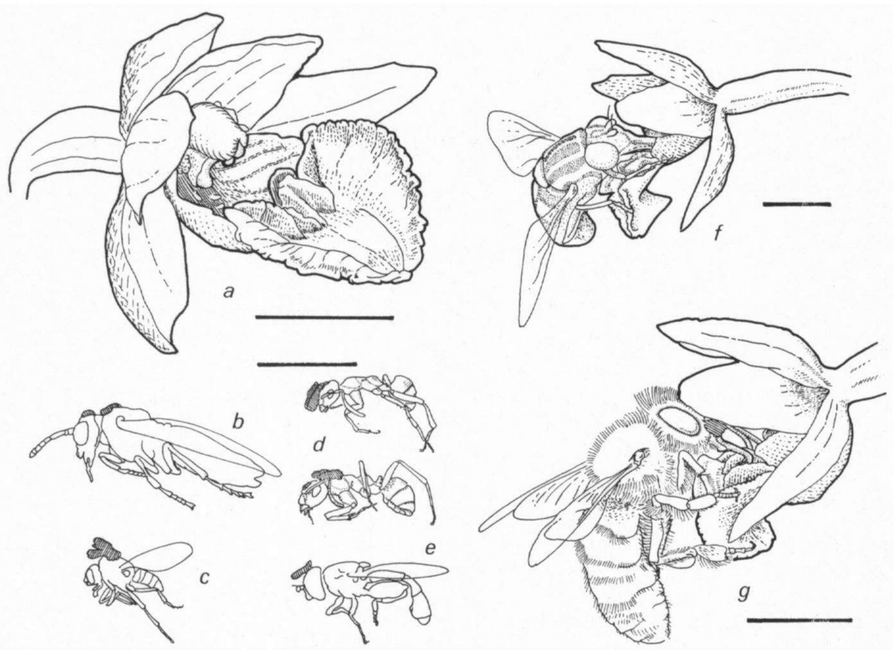

Fig. 1 *Epipactis palustris* and its visitors. Bar indicates 5 mm.

a Flower.

b *Tenthredo perkensi* (10036228) with remains of pollinia on vertex and thorax.

c *Nupedia aestiva* (10036229) with pollinium on the thorax.

d *Formica polyctena* (10036256, 10036252) with pollinium on head and on thorax.

e *Syritta pipiens* (10036217) with pollinium on the vertex.

f *Helophilus pendulus* licking the nectaries on the hypochile.

g *Apis mellifera*, just after landing on the epichile, licks the calli, probing the hypochile. There are pollinia on the clypeus.

There were many workers of *Apis mellifera* on the flowers. The bees landed on the epichile, which then bent down. The bees extended the proboscis, which touched the calli (*fig 1g*), and then probed the hypochile cavity. The bees often touched the rostellum with the clypeus, and they all had pollinia here. Often, after a visit, the bees groomed the head intensively and rubbed off most of the pollinia. The fragments of the pollinia were then transferred to the corbiculae in flight. This is a rare case where the here rather easily fragmented pollinia of orchids are indeed collected by bees for food. Parts of the pollinia always remained on the head, probably because the centre of the clypeus is out of reach of the front legs, and so forms a 'safe spot' for pollen transference. Only the ends of the pollinia were removed and with the remaining pollen the bees pollinated the stigmas. Because of their high mobility and abundance, honeybees are probably the main pollinators here.

The sawfly *Tenthredo perkinsi* landed and walked over the flowers, started licking at the callus on the epichile and then pushed its head into the cavity. The epichile was not pressed down. The sawfly contacted the rostellum with the dorsum of the thorax, which got glue on it (*figs. 1b, 2A*). This sawfly visited several inflorescences and therefore may have been an effective pollinator, although present in low numbers.

The ichneumonid, *Agrothereutes* sp. (*fig. 2C*), was seen to walk from flower to flower on one inflorescence and to fly between inflorescences. It entered every flower, but was too small to contact the rostellum. As the ichneumonid backed out of the flower, the epichile made a rapid upward movement and the ichneumonid with it. There was no sign of panic in the insect, which continued to back out. The same upward movement was seen at the end of the visits of another ichneumonid, *Trychosis legator* f. *plebeja*. These two small species did not obtain pollinia, perhaps because the flowers they visited were already empty. Another small wasp (the braconid *Lissonota* sp.) was found creeping around, unable to fly because a pollinium on the dorsum of the thorax hindered its wing movements. This inability to fly while bearing pollinia, and their low abundance, made these small hymenopterans insignificant pollinators.

Table 3. Pollination by ants.

	Treatment	number of enclosed inflorescences	total number of fruits developed
A	Underside of cage closed around the stem of the inflorescence	4	0
B	A smal opening left around the stem of the inflorescence giving access to ants only	5	4
C	Closed cage with, on several days, an ant imprisoned inside	2	5
D	Control: open pollination	5	13

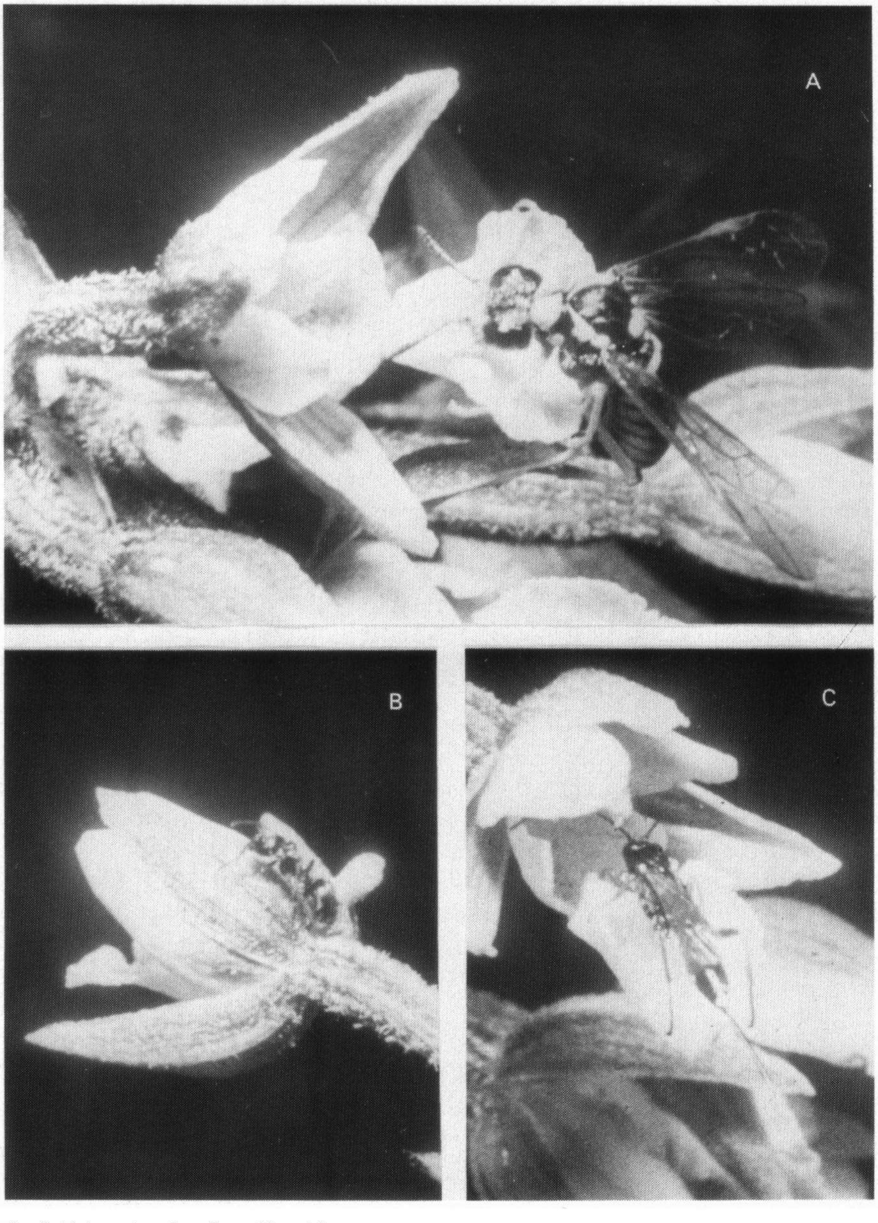

Fig. 2 *Epipactis palustris* and its visitors.

A *Tenthredo perkensi* (10036228), licking the calli. Glue is visible on head and thorax.

B *Lasius niger* (10036232) walks over the calyx, prior to entering the flower. A pollinium is situated on the gaster.

C *Agrothereutes* sp. (10036220) entering the flower.

Ants were the most abundant visitors (*table 2*), and also the insect group with most pollinia. These ants walked over the inflorescences and often entered the flowers from the side. While sitting below the column and drinking, they often touched the rostellum and obtained pollinia.

On *Lasius niger*, a small ant, the pollinia always became glued onto the gaster (*fig. 2b*). On the larger species, *Formica polyctema*, the pollinia stuck to the vertex or the thorax (*fig. 1d*). Ants with pollinia on the thorax, gaster or head seldom groomed these regions. The ants could transfer pollen onto the stigmas and were efficient pollinators, see *table 3*. The ants effect not only geitonogamy, but also xenogamy: Several times an ant with a pollinium was seen to descend one inflorescence and then climb into another. The nuber of visits to flowers by ants decreased in the second half of August, at the time when black aphids appeared on the inflorescences and were tended by the ants. Obviously the ants preferred the aphids to the floral nectar.

The most abundant flies were Syrphidae. All the large species (marked L in *table 1*) landed on the epichile, which bent down under the visitor. The flies then took several steps into a position with the head in front of the hypochile and the longitudinal body-axis vertical (*fig. 1f*). From this position the proboscis could easily reach the nectar in the hypochile, but remained one or more millimeters below the rostellum. Such hoverflies never obtained pollinia. Because of the absence of pollination these visits can be regarded as illegitimate.

Two small species, *Lejogaster metallina* and *Syritta pipiens*, also landed on the epichile, without pressing it down.

The *S. pipiens*, all males, licked the base of the calli on the epichile and then left the flowers. But sometimes these flies approached the hypochile, and inserted the head into the cavity, where the head touched the rostellum. For this reason many of these flies had pollinia on the vertex or on the eye (*fig. 1e*). Only pollinia on the eye were groomed; those on the vertex were not. These *S. pipiens* males approached all sitting insects but flew from flowers as soon as they were approached by others. Possibly this behaviour was related to mate-finding. Because of its ability to transport pollen and its abundance, *table 2*, this fly is a substantial pollinator. *Lejogaster metallina* was present in low numbers and effected pollination. It entered deeper into the cavity than *S. pipiens*, and the pollinia stuck to its vertex and its thorax.

On 14 August, one *Paragus tibialis* Fallen laid eggs on an inflorescence with aphids. While this fly was present the ants appeared very agitated, and attacked the fly whenever it landed (more than 20 times).

However, this behaviour did not prevent the fly from ovipositing a few centimeters from the aphids. The fly was not seen to drink from the flowers, and the inflorescences with the aphids were already in fruit. This situation therefore differs substantially from that of *Epipactis veratrifolia* (= *E. consimilis*, DAFNI & IVRI 1980) where *Sphaerophoria rueppellii* oviposits in, and pollinates flowers, (IVRI & DAFNI 1977).

Flies of several families were found on the plants with pollinia on the thorax. Because of their small size and short proboscis they had to enter the hypochile chamber, below the rostellum. All these flies began by licking the calli on the epichile, did not move under their low weight. Although they could transfer pollen, these flies were too few to be regarded as important pollinators.

DISCUSSION

Bagged inflorescences formed no fruits, and therefore autonomous autogamy is absent (see NILSSON (1978) versus WIEFELSPÜTZ (1970) and others).

The composition of the visitor population in our area differed substantially from that found in Sweden (NILSSON 1978). The geographical ranges of the insect species can only partly explain this. Bumblebees were abundant in our area, but they neglected *E. palustris* flowers, which is in accordance with GODFREY (1933) and SUMMERHAYES (1951), but in contrast to the situation in Sweden. Honeybees were much more abundant on the flowers, and made a larger contribution to pollination than in Sweden. Because of their grooming behaviour, each bee carries only one pollinium. Therefore Nilsson underrates their contribution when he uses the average number of attached pollinia as an index of an insect's contribution to pollination. *Apis mellifera* is an introduced species in The Netherlands. This may explain why *E. palustris* is ill-adapted to this pollinator, which even collects the pollen for larval food. That there is pollination, is a case of short-circuiting (see discussion in FAEGRI & VAN DER PIJL 1979). Eumenid wasps were not found, perhaps because they were absent in the location. Several species of Vespidae were present, and there was a nest of *Vespula vulgaris* L. 30 metres away, but in contrast to the situation in Sweden none visited *E. palustris* flowers. Most visiting Syrphidae do not pollinate, only *S. pipiens* and *L. metallica* do. Both here and in Sweden *S. pipiens* was abundant.

As on other plants, in our location e.g. *Commelina communis* (BRANTJES 1980), species of Syrphidae differ in their behaviour and in their flower preferences (see discussion by STELLEMAN 1979). A remarkable feature was the absence from *E. palustris* of *Episyrphus balteatus*, which was present here on many other flower species. In general, small flies are effective pollinators. Their contribution depends on local abundance.

Open pollinated plants produced more fruits than plants to which only ants had access. Therefore, ants effected only part of the pollination, mostly geitonomany, but perhaps also cross-pollination between neighbouring plants. As in all orchids, the small seeds are dispersed by wind over large distances and this may compensate for the short distance of gene-flow during pollination by ants.

Other researchers (NILSSON 1978, WIEFELSPÜTZ 1970) have not observed the nectar on the epichile, around the calli. This was present in smaller quantity than that in the hypochile and it seemed to be of nutritive value only for the *S. pipiens* males that often restricted their licking to this nectar. The pollinating ants, sawflies, parasitic hymenopterans and flies were seen to start licking at this epichile nectar before entering the hypochile chamber. The nectar functioned as a 'taste nectar guide' and helped to induce the proper orientation for pollination.

Visual nectar guides (MANNING 1956, KUGLER 1971) and olfactory nectar guides (LEX 1954, VON AUFSESS 1960) are well documented, and appeal to 'higher' pollinators (FAEGRI & VAN DER PIJL 1979, p. 85). Taste nectar guides have not been described before. VOGEL (1950) suggests that flies are able to smell the presence of nectar. It is significant that taste guides were used by 'lower' pollinators (sawflies, parasitic hymenopterans, ants and flies) as well as by experiences honeybees.

The hinged epichile of *E. palustris* can be moved up and down and its possible function for pollination has often been discussed (see NILSSON 1978). Here it appeared that only heavy insects (*Apis mellifera* and large hoverflies) could press the epichile down. Since these large hoverflies did not pollinate, and *Apis mellifera* is an introduction, the epichile is unlikely to be an adaptation to these insects. More probably the downward movement is an adaptation to pollination by large solitary wasps, as NILSSON (1978) suggested.

When small hymenopterans visited our orchids the epichile moved upwards, taking the insects with it. In neither of the two cases observed did the insect carry a pollinium, but insects thrown against the column in this way might act as pollinators, as DARWIN (1869) and others have proposed in the 'springboard' theory. A comparable mechanism is found in many *Bulbophyllum* species (CARR 1928, VAN DER PIJL and DODSON 1969, SAZIMA 1978).

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