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**THE EUROPEAN SPECIES OF THE GENUS VOLUCELLA (DIPTERA, SYRPHIDAE)  
AS COMMENSALS AND PARASITOIDS IN BUMBLEBEE AND WASP NESTS**

- Investigations in host-finding, larval biology and mimicry

**SUMMARY**

A comparative study was conducted on four European species of hoverflies from the genus *Volucella* (*V. bombylans*, *V. pellucens*, *V. inanis*, *V. zonaria*), whose larvae live in the nests of bumblebees and social wasps, in order to analyse their development and way of life with respect to their host relationships.

The aims of the study were to discover:

- the host specific adaptations and strategies for host-finding
- the location of the larva within the nest of the host and larval feeding behavior
- possible species specific differences with respect to host relationship
- the significance and effectiveness of the resemblance of adult flies to their hosts - bumblebee and wasp mimicry - was studied with respect to their successful infiltration of the host nests

The *Volucella* (imagines), as well as bumblebee and wasp nests, were collected in the field. The hosts were bred in wooden boxes. The fly larvae were kept in the laboratory as well as in host nests.

Host-finding and nest-visiting by female *Volucella* were investigated through field observations of behavior as well as through experimentation. Imaginal antennae, eggs, larval cuticula and cephalopharyngeal skeletons were investigated morphologically using light microscopy and SEM.

Host-finding: To lay their eggs, *Volucella* females seek out the nests of their hosts. Finding and recognizing the host is made possible by the triggering stimulus of the host odor in combination with the appearance of host imagines.

- The odor of bumblebee workers and/or nests triggers the following specific behavior in females of *V. bombylans*: intensive cleaning and protrusion of the ovipositor. This behavior is also exhibited by females under natural circumstances before they enter the tunnel leading to a bumblebee nest.

- *V. bombylans* females approach such an odor source directly. Thus to find the entrance of a bumblebee nest they need only the olfactory sense (within a radius of 1 m).

- Without the presence of bumblebee odor *V. bombylans* females showed no reaction to acoustic or optical stimuli in the form of a bumblebee flying behind Plexiglass.

- *V. pellucens* and *V. inanis* females also reacted to the odor of a host wasp nest with protrusion of the ovipositor, yet they did not approach the source of the odor. Presumably these species require the appearance of wasps as an additional optical stimulus.

- No morph-specific behavior was found in *V. bombylans*. The females infiltrate the nests independent of the respective color patterns of the bumblebee species.

- The strikingly strong branching of the aristae found only in *V. bombylans* females (sexual dimorphism) is interpreted as an adaptation for staying in bumblebee nests. Apart from serving as a mechanical protection of the funiculus, this branching of an arista could aid the fly in its tactile and acoustic orientation within the passageways of the underground nests.

Nest-infiltration: Bumblebees and wasps defend their nests against invaders. The entrances of wasp nests are generally guarded by several workers. Among bumblebees, however, this is only the case in large colonies.

Volucella females of the different species have different strategies for invading nests and correspondingly trigger different reactions among the host imagines.

- Before entering a bumblebee nest, *V. bombylans* females remain in the vicinity of the entrance for an average of 25 minutes. They avoid contact with the bumblebee workers and infiltrate the nest only the passageway is free of incoming and outgoing workers. When the *Volucella* arrive in the nest, they bury themselves in the outer material of the nest. The female flies leave the nest after approx. 25 minutes.

- *V. pellucens* females enter the wasp nest with the stream of arriving wasps only a few minutes after approaching the outer entrance of the nest and remain on the outer layer of the nest for approx. 30 minutes.

- *V. inanis* females do not enter a normally busy wasp nest and remain only in the outer entrance area.

- Wasps (*Paravespula*) react to *Volucella* species in the entrance area of their nest differently:

- . *V. inanis* females are attacked aggressively and stung to death
- . *V. pellucens* females are not attacked

- It is discussed whether the protection from attack of *V. pellucens* females is due to chemical mimicry or to pheromones which repel or inhibit aggression.

Eggs: The eggs of the *Volucella* species are different with regard to size, form, structure of the chorion, number and the location they are laid in. Compared with *V. bombylans* and *V. pellucens* (approx. 60 eggs), *V. inanis* has a conspicuously large number of eggs (approx. 300). This is interpreted as a compensation for the higher risk of larvae loss. Because the eggs are laid outside the host nests, they are less protected, and the hatched larvae have to cover a large distance to arrive at the wasp brood.

Larvae: According to their location within the host nest, as well as the structure of the cephalopharyngeal skeleton and digestive tract, the larvae of *V. bombylans*, *V. pellucens* and *V. zonaria* live as saprophages. Thus, they can be characterized as commensals

in bumblebee and wasp nests. Attacks on the host brood could be observed only once the colony was dissolving and the reproductives were already hatched.

The development of *V. inanis* larvae is closely adapted to that of the host brood, on which they live as parasitoids.

- All three larval stages are of great similarity in each of the following species: *V. bombylans*, *V. pellucens* and *V. zonaria*. They have larval features typical of saprophagous syrphid larvae such as mandibular lobes, cibarial ridges and long caeca.

- *V. bombylans* were found only in ground nests of bumblebees. They are present only outside of the actual breeding area in the outer nest material as well as under the nest.

- *V. pellucens* larvae were found only in underground nests of *Paravespula vulgaris* and *P. germanica*. They were in the ground beneath the opening of the nest, where faeces and detritus fall from the breeding area.

- A description was given of the larval development of *V. inanis*. The larvae live in the underground and "attic" nests of *Paravespula vulgaris* and *P. germanica*.

The several larval instars are clearly differentiated in their appearance. Thus L2-instar is dorsoventrally flattened. This is an adaptation to living in cells with a living wasp brood.

The short caeca and the lack of a functioning cibarial filter apparatus are characteristic of parasitic feeding.

L1 and L2 larvae feed on the haemolymph of 4th and 5th instar wasp larvae without killing them. The 2nd instar larvae of the fly remains inside the cell while the mature wasp larvae spins its cocoon. Afterwards, the *V. inanis* larvae moults into the 3rd instar, which completely sucks out the wasp prepupa. The mature fly larvae leaves the cell and burrows into the ground beneath the host nest for larval rest.

Evolution: The parasitoid life habit of *V. inanis* is considered as derived feature for the genus *Volucella*, since the larval development is complex and since the cephalopharyngeal apparatus could have developed from saprophagous species.

Since the wasps show different behavior towards the females of *V. inanis* and *V. pellucens*, these reactions are attributed to the respective live style of the larvae. *V. inanis*, as a parasitoid, can reduce the reproductive success of a wasp colony. Therefore a selection pressure exists to prevent the invasion of this fly.

The saprophagous larvae of *V. pellucens* probably contribute towards nest hygiene and, therefore, they are welcomed guests.

Mimicry: The visual resemblance of *V. bombylans* to bumblebees and that of *V. inanis* and *V. zonaria* to wasps is regarded as a form of protective mimicry. No evidence was found of aggressive mimicry in which the host was deceived.

The development and stabilization of the mimetic color polymorphism in *V. bombylans* can be explained as a adaptation to locally changing models and thus as a case of protective mimicry.



L Rupp (1989)

[ The central European species of the genus *Volucella* (Diptera, Syrphidae) as commensals and parasitoids in the nests of bees and social wasps: studies on host-finding, larval biology and mimicry ]

Inaugural Dissertation, Albert-Ludwigs University, Freiburg-im-Breisgau

## 1 Introduction

Thanks to their strikingly gay colours and notable size, their characteristic hovering flight and commonness, the syrphids are the best known and best loved flies of our native fauna. While the adults of all syrphid species feed exclusively on pollen and nectar or honeydew, and after bees constitute the most significant flower visitors of all insects, their larvae possess very different lifecycles and feeding modes. Especially noteworthy are the many species whose larvae feed on aphids and other pests of useful plants. They have been many times the object of applied research (Schneider 1969; Bastian 1986) where they have great significance from the agricultural viewpoint. Many syrphids have an amazing visual resemblance to noxious insects. Especially common and striking are species with yellow and black wasp patterns, or bee-like hairs and colours. From their appearance they are easily confounded with wasps or bees.

The native representatives of the genus *Volucella* are distinguished by several characteristics. The species *Volucella bombylans* occurs in several colour morphs, each of which resembles a different bumblebee species (mimetic polymorphism). The species *Volucella inanis* and *Volucella zonaria* have an extensive similarity to wasps. Their larvae develop in the nests of their models.

Since wasps and bees defend their nests against intruders, the female *Volucella* must enter into these nests unnoticed or unrecognised. Also their larvae must run the risk of being discovered and killed by the host adults. Special morphological structures, odours and modes of behaviour can be demonstrated from the rich inquiline fauna of termite and ant nests, which represent adaptations to the host and make possible their residence in the nests of their respective hosts. In a few cases these inquilines achieve a complete integration within the host colonies (Wasmann 1925, Wilson 1971, Kistner 1979, 1982).

In his extensive monograph on the genus *Volucella*, Künckel d'Herculais (1875) set out almost every known observation and idea in the literature to that date on volucellines and their host relationships, while the metamorphosis and morphological and anatomical studies of the larvae and adults of his study remain relevant today. The state of knowledge and views on what interactions take place between volucellines and their hosts, and what their significance for mimicry is, have from that time not substantially increased or changed. Thus the larvae of native species are taken either as parasites or commensals in the host nests, although there are only sporadic observations of their lifecycle and, for example, until now only the final instar larva of *V. inanis* was known (Kistner 1982). Moreover differences between bumblebee and wasp nests have rarely been considered, for example with respect to passing the nest guards and adjusting the various "requirements" of an intruding *Volucella* female. Thus the larvae of *Volucella bombylans* are supposed to develop with both bumblebees and wasps (Spradberry 1973).

The aim of the present work is to clarify the following questions on the host relations of particular *Volucella* species:

- how do *Volucella* females find their host nests, and what stimuli of the hosts or nests play a role?
- how can females intrude into guarded host nests, and what is the reaction of host adults?
- How do the larvae of the various species live in the host nests? What is their trophic relationship - commensal or parasitic - to their hosts?
- what significance and role does the host have as a mimetic model in stabilising the mimetic polymorphism of *V. bombylans* (a question of the mimicry system)?

Using comparative studies of behaviour and morphological structures of adults, eggs and larvae, the host-specific adaptations and strategies should be exposed and possible differences made evident.

## 2 Materials and Methods

### 2.1 Provenance and maintenance of volucellines

Flies were caught from the field. The sites were flower-rich meadows and woodland edges in the region of Freiburg, at Schönberg and the foothills of Alsace (Voges) and near Münster (Braunskopf). *Volucella bombylans* came mainly from the southern Upper Rhine area, only from nature reserves and in the Vosges, but animals were also caught in Switzerland in an area (1800m) with extensive alpine meadows above the village of Mathon (Graubünden). There this species was common.

Captured flies were individually marked with a bee-tag and held individually in clear plastic containers (9 x 9 x 11 cm) with gauze lids; the floor of the containers was lined with damp filterpaper; a diagonally placed piece of pasteboard served as for climbing. Flies were fed with sugarwater several time a day.

Larvae were obtained for the most part from eggs laid by captive females, but were also taken from dug-up bumblebee and wasp nests. The larvae of *bombylans*, *pellucens* and *zonaria* could be reared in plastic containers or petri dishes



lined with filterpaper or peat dust. Bee-collected flower pollen served as food for the first two species, and the last was fed dead wasp brood. Moreover the larvae of *bombylans* were held in nests of various bumblebee species, and those of *pellucens*, *zonaria* and *inanis* in the nests of *Paravespula vulgaris* and *P. germanica*.

Mature larvae (L3) were overwintered in plastic containers (11 x 11 x 6 cms) with damp peat dust in a thermostat at 4 C, with up to 15 larvae per container.

## 2.2 Provenance and maintenance of the hosts

Bumblebees .....

Wasps.....

Hornets.....

## 2.3 Behavioural observations and experiments

Most observations and behavioural experiments on host nest finding were done in a net flight cage (4.5 x 2 x 2 m (Fig 2) placed outside. The roof and side walls were made of nylon netting stretched over a pole frame of a marquee. The corner poles were anchored to the ground; the floor of the cage was natural turf. Studies in the net cage were carried out either in the Institute garden in Freiburg, or also during a 2-month stays (summer 1983-1985) in the Alps near the Mursenas/Mathon mountain refuge (capture site for *bombylans*). Various behavioural observations were recorded with a video camera. Exact description of the study methods of the various behavioural studies follow under the relevant headings.

## 2.4 Microscopic preparation

The morphological studies of the antennae of adults, chorion structure of the eggs, and cephalopharyngeal apparatus and cuticle of the larvae were done using TEM and SEM. For TEM the object was embedded in polyvinyl-lactophenol. Histological sections in the head region of the larvae were done using ..... (technical)

## 2.5 Radioactive marking

Radioactively marked amino acids were injected into wasp larvae to prove the ectoparasitic feeding mode of *inanis* larvae. (details of amino acids used, method of injection, treatment of animals, recording of data).

## 3 European *Volucella* species - appearance, distribution, occurrence, phenology

The genus *Volucella* is worldwide with many species (Curran 1926, Sack 1932, Telford 1973). In central Europe there are only five species present: *bombylans*, *pellucens*, *inanis*, *zonaria* and *inflata*. The flies are usually large and stout. They have a barrel-like appearance and have a hairy arista. [...]

### *Volucella bombylans*

The flies are very hairy and have three distinct colour forms which each resembles a different bumblebee species (Fig 7) (cf. section 8.3). In *bombylans* the females clearly have longer and hairier arista than the males (sexual dimorphism) (Sect 4.3; Figs 15, 16). Body size ranges from 10 to 16 mm (cf. Fig 4). Since the whole length of the fly, because of unequal body positions, can only be measured and compared inaccurately, part of the wing was used to represent body size variation: the length of the costal vein from the cross-vein h to the radial vein R1 (Fig 4). It was assumed as in bumblebees (Pekkarinen 1979, Sowig 1988) that wing length was correlated with body size. The species is distributed throughout Europe and North and West Asia (Lundbeck 1916, Sack 1932). In southern Upper Rhine the first individuals appear at the end of May, first the males, as in the other species; In the middle of July only isolated females are flying (Fig 3). In the alpine site the phenology of *bombylans* was shifted by about a month (beginning of July - end of August).

Flies are found especially at woodland edges of flower-rich meadows. Whilst a preference for yellow-coloured traps has been shown in some syrphids (*E. tenax* - Kugler 1950; *M. corollae* - Peshken 1965; *E. pertinax* - Lunau 1988) and most hoverflies can be found on yellow or white flowers, *bombylans* flies almost exclusively to blue or blue-violet flowers. Of 192 flower visits, only 4 were to yellow flowers, although yellow or white flowers were not uncommon.

Males prefer to position themselves on woodland or copse edges. From a lookout position above the vegetation, the undertake reconnaissance flights to find females ready to copulate. Individually marked males (n=12) in favourable weather remained only for the length of an afternoon (3 hrs) at the same place (area), where they flew at, followed and possibly drove away other males, flies and also bees from an area of 1-2 m<sup>2</sup>. Only once did I find ~~the~~ a male in the same area for two consecutive afternoons. In *Volucella* I never saw the patrol flights at flowers known in other syrphids (Maier & Waldbauer 1979).

After several years observations in the field and the lab, only one copulation of *bombylans* (field) and two of *inanis* (lab) were seen, the pairs remaining in cop for about an hour. Presumably *Volucella* species are monandrous. Unfortunately *bombylans* never copulated in the lab or in the flight cages.

### *Volucella pellucens*

Apart from the transparent whitish 2nd abdominal segment, the thorax and other abdominal segments are black with a bluish reflection (Fig 8). There is no visual similarity with native hymenoptera. In males but not in females there is a longitudinal black midline on the 2nd abdominal tergite. Body length 13-18 mm (cf. Fig 4). The species is distributed in north and central Europe, and reaches Japan also (Lunbeck 1916, Sack 1932). *Pellucens* is the commonest native species of *Volucella*. They are found on flowers by woodland paths, in open landscapes, and in gardens. The first individuals appear in about mid-June; one can see adults (females) until the beginning of October. *Pellucens* has the longest flight period of our native species (Fig 3).

### *Volucella inanis*

With its yellow appearance, the black-and-yellow thorax and a yellow-black banded abdomen, this has a great similarity to wasps (Fig 9). In comparison to other species, the abdomen is narrower. The small variation in body length is striking (15-16 mm). The length of the wing vein (costa) across numerous females of the different *Volucella* species clearly shows a smaller variance in *inanis* as compared with *pellucens* and *bombylans* (Fig 4). *Inanis* is distributed in central and southern Europe, in England only in the south (Sack 1932, Stubbs & Falk 1983). According to Lundbeck (1916) it is also in Syria. Individuals are found visiting flowers at woodland edges, woodland paths, and in city gardens, and in city areas they fly long distances to flowers of terraces and balconies. Adults of *inanis* are the latest *Volucella* species to appear, in mid-August; individuals can be found until mid-October. From groups of larvae brought to pupation in the lab, the first individuals to eclose are always males. Most of the females eclose 3-4 days later (Fig 5). This corresponds with observations from the field. This originates not from different pupal durations (both sexes have pupal durations of about 24 days), but male larvae pupate correspondingly earlier. Fig 7 shows that larvae that pupate first (up to 25.viii - [mistake on graph legend, where says month ix]) are 98% male, whilst later pupae (25.viii - 29.viii) are only 20% male and 60% [sic] 80% from graph] female.

### *Volucella zonaria*

The species is the largest and most striking of hoverflies. In contrast to *inanis*, the thorax is not black but red-brown, and the wings also have a red-brown reflection. From its size and colour pattern, the species resembles greatly the hornet (Fig 10). Body size: about 18-20 mm. *Zonaria* is distributed in southern and central Europe, and is East and central Asia (Sack 1932). In England in former times the species was a vagrant (first occurrence in 1870), and was first found more commonly in 1940 in southern England (Smith 1974, Stallwood 1979). The few captures in the field (n=7) in southern Upper Rhine were between mid-June and mid-August (Fig 3).

### *Volucella inflata*

In appearance the species is very similar to *pellucens*. Differences exist in the colour of the 2nd abdominal tergite and the dorsal edge of the thorax, which is red-brown in *inflata*. With a body size of about 12-15 mm, this is the smallest of our native species. According to Sack (1932), *inflata* occurs in Europe and Siberia. Altogether there were only three individuals of this species found in the field. There are observations that females lay their eggs in sapflows of trees, and in the same substrate larvae were found which were assumed to be *inflata* (Stubbs & Falk 1983).

## 4 Host finding

The larvae of the studied species of *Volucella* develop in the nests of bumblebees and social wasps. Females search for host nests for oviposition. They must be equipped with mechanisms for finding and recognizing suitable places for laying their eggs. This habitat selection could only occur using innate or learned behaviour. *Volucella* females could use visual, acoustic or olfactory cues from host nests and their inhabitants as signals. To clarify the host-finding mechanism, the behaviour of females was observed with host nests, and behavioural experiments carried out on the reactions to the above signals.

### Study methods

Most observations and studies were carried out in the netting cage in the field (see Section 2.3, Fig 2). Studies on *bombylans* took place mostly at the swiss site. Field-caught females were placed in the cage and kept there (holding in small containers rapidly damaged the flies wings). Large bunches of *Geranium pratense* placed in vases on the ground served as food and overnight shelter. In the cage were usually about 30 females. Daily additional catches made up for losses due to, for example, bad weather, shrews, or loss of the ability to fly. Studies with *pellucens* and *inanis* were done with the same cage in the Institute gardens. For offered hosts, bee and wasp nests were colonized in wooden boxes (see Section 2.1, Figs 1,13). These were placed in the cage for the relevant studies. The bees and wasps could leave their nest, fly inside the cage, and seek food. In the Alps, observations and studies could only be carried out during direct sunshine, since only then was the necessary air temperature reached for fly activity.

#### 4.1 Behaviour at the host nest

##### 4.1.1 *Volucella bombylans*

*Bombylans* females were found above all via their flower visiting. Occasionally they were discovered sitting on leaves near the ground. It was never observed therefore, that females fly over the ground for very long periods and long distances, a behaviour typical for, for example, host-searching cuckoo bees of the genus *Psithyrus*. Efforts to make quantitative observations [in the field] on *bombylans* females in their search for both natural bumblebee nests (a colony each of *Bombus terrestris*, *hortorum* and *mastrucatus*) and also those established in the field in wood boxes (a colony each of *B. terrestris*, *mastrucatus* and *mesomelas*) were frustrated because *Volucella* females rarely searched for the nests.

Observations were therefore carried out in the netting cage, where many *Volucella* females could be placed, so that some flies would seek the offered nests with a greater probability. The majority of *bombylans* females passed most of the day on the roof and the side-walls of the cage; for visiting flowers, in the evening, and in bad weather, they sought out the vegetation on the floor. Some individuals (about 10% of the cage population) remained on the ground during the day, without visiting flowers. Once these females came within 1 m of a bumblebee nest, they then ordinarily approached directly the hive entrance and mostly then entered the nest hole (nestbox). During the approach and the period in front of the host nest, the *bombylans* females performed characteristic behaviours:

- at greater distances ( $\leq 1$  m) from the nest-hole entrance, the fly sits with raised anterior pair of legs (Fig 11a). This posture is interrupted only by several short flights (bringing it) nearer to the hive entrance.
- in the immediate vicinity of the entrance (20 - 1 cms), females begin intensive cleaning movements of above all the wings and the now extruded ovipositor (Fig 11b,c).
- underneath the hive entrance, the *Volucella* female presses its body directly against the ground while running (Fig 11d); the ovipositor is extruded even further.

The entire approach phase lasts from 10 mins to more than an hour in different females. On average the flies enter the nest hole after about 35 mins. Timing began as soon as a *Volucella* female had landed within a distance of a metre from a bumblebee box. Table 1 lists the stay times of nine *bombylans* females in nest searching, subdivided into the different approach phases.

[ Table 1: Stay times of *bombylans* females at various distances from the entrance to the nest. (measured from the time of their appearance at a distance of about 1m from the nest entrance to entry into the nest). I: Time spent at greater distances ( $\leq 1$ m) from the nest; II: time spent  $< 20$  cms from nest entrance. S = sum of I + II. All times in minutes. ]

According to these observations (Table 1) *bombylans* females remain only up to a maximum of 15 mins at the larger distances from the nest. Mostly they fly after a few minutes to the immediate region of the nest entrance ( $< 20$  cms). More than 80% of the stay times is spent by the flies in the immediate vicinity of the entrance ( $p < 0.01$ , U-test). During this time the flies clean themselves intensively in the already described manner, or sit motionless facing the entrance to the nest. After cleaning they commonly run a short distance towards the nest entrance. The behaviour (Fig 11d) by which they move forward, with the thorax, abdomen and ovipositor in contact with the ground, was always observed when they found themselves in front of the nest-hole entrance beside the wooden floor of the antechamber of the nest. Were the nest entrance in grass (the entrance to the true nest passed over a tube which lay on the turf), this behaviour could not have been observed. In this situation, near the entrance between the short grass halms, the *Volucella* females commonly tried to reach the actual earth surface.

When they have reached the nest entrance, they disappear quickly into the hive when no bumblebee is present in the area of the entrance. After that they find then also the nest, reached when they have been through a long tube of up to 150 cms (PVC-tubing, diameter 2 cms).

No cage effect produces both the described behaviour and also the temporal course for entry into a host nest, since *bombylans* females ( $n=13$ ) behave in the same way in "free flight". Under free flight we understand the flight and nest searching of non-captive *Volucella* females to nests in bumblebee nest boxes outside the netting cage.

##### 4.1.2 *Volucella pellucens*

Observations on *pellucens* were carried out in the netting cage in the Institute garden. Wasp nests settled into wooden boxes were offered as host nests. *Pellucens* females that once came within 1 m of a wasp nest mostly then entered into it. In contrast to *bombylans*, female *pellucens* did not sit in front of nests with raised forelegs (cf. Fig 11a). However they display as before an intensive cleaning and extrusion of the ovipositor immediately in front of the nest entrance. On average female require about 7 mins to reach immediately in front of the hive entrance; already after about 3 mins they then climb into the nest box ( $p < 0.03$ , U-test) (Table 2).

[ Table 2. Stay times of *pellucens* females at various distances from the nest entrance until entry into the host nest (*Paravespula vulgaris*, *P. germanica*) ( $n=8$ ). (same as Table 1) ]

#### 4.1.3 *Volucella inanis*

Most observations were made in the Institute garden. Two natural flights to wooden nest boxes containing *P.vulgaris* colonies could be observed in *inanis* females. In *inanis* it was striking that some females showed an interest in the wasp nest already after a few minutes, after the wasps were able to fly out of their nest box. They flew in the vicinity of the wooden nest box, where they at first landed on high-up places such as the protocol-stool (the wasp nest boxes were always on the ground). In comparison to *bombylans* and *pellucens*, *inanis* females are very skilful fliers, performing sustained flights in the space of the cage. The approach phase to the nest was recorded in eight cases. The flies proceeded to oviposition in the nest boxes after an average of 15 minutes. The approach could not be split into phases, since flies in the immediate vicinity of a nest box always flew up again at the appearance of a wasp, and returned after a short time. Time in minutes: 51,18,17,15,10,5,5,3; mean = 15.5 (n=8)

In contrast to the other species, females perform no intensive cleaning behaviour. The ovipositor was equally extruded when she found the nest box. Only in a few cases did a female climb through the hive entrance to the inside of the nest box (n=3). Usually eggs were laid 5 cms in front of the hive entrance or on the edge of the top of the nest box (n=12) (cf. oviposition, Section 6.1.4). Also in free flights, female *inanis* laid their eggs outside the nest box near the hive entrance on the outside of the walls.

#### 4.2 Behavioural experiments on host recognition with different stimuli

Behavioural observations in front of the host nests do not permit any conclusions about the nature of the cues used for host finding and host recognition. For this an approach was made using suitable behavioural experiments on various signal cues. In aphidopagous syrphids colour and shape of the host habitats direct the searching behaviour of gravid females (Dixon 1959, Chandler 1968c, Sanders 1979, 1980, 1981, 1983); oviposition is released by aphid secretions perceived essentially by olfaction (Dixon 1959, Bombosch 1962, Volk 1964, Schneider 1969).

In bumblebee and wasp nests, the comings and goings of the workers provide visual, olfactory and acoustic (flight-tone) signals. In addition, various olfactory cues come from the nest entrance (marking pheromones and the nest odour). It was therefore an obvious step to test the attractivity of corresponding signals for *Volucella* females.

##### 4.2.1 Significance of olfactory stimuli in host-nest recognition in *Volucella bombylans*

Bumblebee nests represent rich sources of olfactory cues from both the pollen and nectar pots and also from the specific marking pheromones of bumblebees, cues which operate also outside the broodchamber proper, in the region of the entrance. Bees mark their nest entrance with pheromones from the mandibular gland (Cederberg 1977, 1983); they mark it also passively via the nest odour adhering to themselves, which surrounds and accompanies a bee as a scent cloud. To show the significance and effect of the bumblebee nest odour for host finding by *bombylans* females, this odour cue was offered in different studies. Their reaction to the olfactory cues should for the time being be able to be tested by this.

Preliminary studies showed that the attractiveness of, for example, honeypot materials increased considerably when the odour cues were blown out in air. Probably this is equivalent to the effect of flying bees in front of the nest entrance.

Expt 1: With an aquarium pump, a flow of air was guided through a box (20 x 10 x 5 cms) where there were nest material and brood cells of bees (*B. mastrucatus*, *B. terrestris*). On one side was a round opening 2 cms wide through which the air flowed, and through which the flies could come into in the "air box". The flies had to come by themselves into the vicinity of the air flow.

Expt 2: With the same pump, the air was sucked from a colonised bee nest, and guided further over a 150 cm long PVC tube (diam 2 cms). Over the tube opening from which the air flowed was a box without a floor (5 x 6 x 5 cms) with a hole (diam 2 cms). The *Volucella* females were released about 1 m from the odour cue onto the ground. The odour came from nests of *B.mastrucatus* and *B.terrestris*.

Both experiments were carried inside the netting cage. In a control study (null sample), only air (without bee odour) was guided through the boxes.

Under the conditions of experimental design 1, only about 10% of the females of the cage population came near the odour cue (and hence could potentially react), of which then however almost all individuals visited the box. In experiment 2, where females were released at the beginning into the vicinity of the odours, about 80% of the flies proceeded into the box. (Expt 1 - 23 visits to the box from 18 animals: Expt 2 - 40 visits to the box from 24 animals).

In all experiments female showed that once they had come into the vicinity (ca. 20 cms) of the odour cues (box), the same behavioural sequences were seen as before a bee-active nest entrance (cf. Sect 4.1.1; intensive cleaning, extrusion of the ovipositor). Most individuals moved towards the odour cues and climbed through the opening into the box.

It cannot be completely excluded that the dark entrance to the box represents at the same time a visual releaser. However, the flies also find then the odour cues when there is no box over the end of the colourless odour tube (*bombylans*, n=7). Females show no equivalent reaction when presented with only air (no bee odours) in the different

experimental setups (*bombylans*, n=28). From this it can be concluded that the scent bouquet of a bee nest alone not only triggers host-finding behaviour in *bombylans* females, but the odour cues also orient and permit discovery (of the host).

In the experiments, flies only laid eggs in the boxes in which they could come into contact with bee nest material (Expt 1). It is likely that specific, chemotactically perceived cues are necessary from the oviposition substrate for release of oviposition itself. Readiness to oviposit is different among females: under stress conditions many individuals lay their eggs without an adequate substrate, as for example in the tube after capture. One can stimulate females to lay eggs by giving them a darkened tube with bee nest material. Oviposition follows also without the above behavioural sequence (normally) observed under natural conditions before entering the host nest. This shows that this behaviour does not represent a chain reaction.

In lab studies, females reacted to hexane-extracts of heads, bodies and brood cells of bumblebees with an oviposition reaction (Fig 12). Brood-cell extract triggered a behavioural reaction (extrusion of the ovipositor) more commonly (40%) than head or body extracts ( $p < 0.01$ ,  $p < 0.025$ ; 4-group test) (Table 3).

Expt 3: *Volucella* females were placed in a petridish with a filter-paper lining, after the filterpaper had been provided with a drop of the relevant extract and the solvent evaporated. Individuals were kept for 3 mins in the petridishes. As a behavioural reaction, partial extrusion of the ovipositor (AL) and complete extrusion and probing of the ovipositor (TL) were recorded. Individual flies were tested at most twice per extract; the experiment used workers and broodcells of *Bombus mastrucatus*. Extracts: 10 heads in 100 ml n-hexane; 10 bodies (without heads) in 300 ml n-hexane; pieces of cells in 300 ml n-hexane. On the filter paper, one drop (0.1 ml) of the relevant extract was placed.

[ Table 3. Oviposition reaction of *bombylans* to extracts of bees and their brood cells (*Bombus mastrucatus*); (-) = no reaction; AL = slightly extruded ovipositor; TL = fully extruded ovipositor. n = 13 ]

[ Fig 12. *Volucella bombylans* female with extruded ovipositor (oviposition reaction) in a petridish after presentation of bee odour extract ]

From Expt 3 it cannot be determined whether females perceive the bee extracts on the filter paper using gustatory receptors on the tarsi, or olfactory receptors on the antennae. To show the significance of the antennae for the perception of the bee odour, the reaction of normal and antennae-amputated females was compared; adequate odour cues were given from an air stream (Expt 4).

Expt 4: Some *bombylans* females (n=12) had their antennae (pedicellus, funiculus) amputated. centrally from the top using a pump, air and bee odour was introduced into a round plexiglass container (diam 19 cm, ht 10 cms). Three workers of *Bombus mastrucatus* served to provide the odour cues. The flies remained about 3 mins in the vessel. As a control, only air (without bee odour) was presented. First all individuals were tested with the control, and afterwards the test with bee odours. As a reaction, a slight extrusion (AL) or a fully outstretched of the ovipositor was recorded.

All individuals with antennae reacted to the bee odour with an intensive oviposition reaction, whilst only two females with amputated antennae showed a slight reaction (Table 4). The higher number of females with antennae that showed a reaction also in the controls (39% - Table 4) is perhaps to be attributed to a stress reaction.

[ Table 4: Oviposition reaction of *Volucella bombylans* with and without antennae (amputated) to bee odour (*B.mastrucatus* workers). A: with antennae. O: without antennae. (-) = no reaction. AL = ovipositor slightly extruded. TL = ovipositor fully extruded. n=28 (A) and 12 (O) ]

From these results it cannot be excluded that the amputation operation caused a behavioural impairment, and hence these results can only be an indication of the significance of the antennae as the bearers of the olfactory receptors tuned into host finding.

For qualitative determination of the effectiveness of different odour components as cues, EAG were carried out using the third antennal segment (funiculus) in *bombylans* females.

The measurements followed those of Herrn Dr E Priesner of the Max Planck Institute in Seewiesen. The studies used three *bombylans* females. The experimental technique corresponds to the standard procedures for EAG measurement (Priesner 1973). It could be derived from it that either the pure test substance or the odour mixtures (freshly pulverized flowers of *Geranium pratense*, flower odour, bee-collected pollen, freshly killed bee workers, and brood cells of *Bombus terrestris*) were provided in sufficient odour concentration.

In comparison with corresponding inferences from the antennae of butterflies and bee, cue responses from fly antennae were very weak (low amplitude) (Priesner, pers.comm.). The possible relevant test substances for host finding and oviposition, such as odours of bees, brood cells or various terpenes (marking substances of bees) produced no EAG responses. In addition, flower and pollen odour resulted in no reaction. A pulverized flower and a few pure substances (such as hexenol and amyl acetate: Table 5) present as short-lived elements of most green plants produced slight reactions.

[ Table 5: EAG reaction of *Volucella bombylans* females to various odours. (-) = no reaction; (+) = low amplitude on the oscilloscope ]

Henderson & Wellington (1982) obtained likewise from EAG of aphidophagous syrphids (*Metasyrphus venablesi* and *Eupodes volucris*) responses to 6-carbon-chain unsaturated and saturated alcohols and amylacetate, but no response to aphids and their honeydew, whilst the flies reacted to these cues in the olfactometer. From this we conclude that in *Volucella* as in the aphidophagous syrphids studied, the olfactory receptors for the perception of the specific odour substances for oviposition substrates are to be found on the antennae (see Sect 4.3), but it appears from these results that EAG responses are not a suitable method for showing the effectiveness of odour components as cues. Kaib (1974, 1984, pers.comm.) also could obtain only reaction from individual cells to flesh and flower odours in the fleshfly *Calliphora vicina*. Henderson & Wellington (1982) discuss to what extent EAG responses to plant substances (e.g. hexenol, amyl acetate) support the observations of Chandler (1968b), according to whom aphidophagous syrphids occasionally lay eggs on plants without aphids. It is possible that these are also reactions which are relicts derived from an original phytophagous habit (see Sect 7).

#### 4.2.2 Reactions of *Volucella bombylans* females to the visual stimulus "flying bumblebee"

Bumblebee nests are generally found in the ground. Their nest entrances are not obvious, so that for a *Volucella* female, only the bumblebees flying in and out come into question as potential visual signals. To test the visual effect and significance host searching behaviour in *V. bombylans*, they were presented with bumblebees flying in front of a nest entrance but with odour perception prevented.

Expt 5: Bumblebee nest boxes were placed in an airtight transparent plexiglas cage (Fig 4). The flight platform was through a tube to the outside so that the bees could continue their collecting operations. To reach or leave the nest, the bees had to cross the antechamber between the nestbox and the opening of the tube to the outside (30 cms distance). They could be seen in this area. Since they crossed the distance mostly by flying, this corresponds to the normal situation of flying to and from the nest entrance. When a fly came within about 20 cms in front of the antechamber of the outer cage, this was scored as a potential flight to the bee nest.

In no case could it be observed that a female *V. bombylans* near the outer cage showed the behavioural modes which are characteristic for a host nest in the presence of bumblebees (intensive cleaning, extrusion of the ovipositor) (Table 6).

[ Table 6: Reactions (typical behavioural mode in nest searching) of *Volucella bombylans* females to bumblebees (*B. macstrucatus*) flying to and from the nest entrance with no scent cues. Frequency of bee flights = about 2 per minute. n = 23 *V. bombylans* ]

[...flowers in cage...]

According to the results of the experiment, visual cues (bees flying at the nest entrance, with no odour) have no effect in eliciting host-finding or oviposition behaviour in *Volucella bombylans*.

#### 4.2.3 Visual significance and effect of bumblebees in association with odour

*V. bombylans* females that had already approached a source of bumblebee odour (test 1 & 2) and already shown an oviposition reaction, turned and moved in the direction of a bumblebee flying at the entrance, when these were offered as alternatives. Perhaps a bumblebee has a visual effect as a cue in addition to its odour, whereby this combination is more attractive than odour alone. [...] In their first visit most of the flies proceeded to the odour source in the transparent cages with bumblebees ("p < 0.25, 4-fields test, null hypothesis of equal proportions" - [actually  $\chi^2_1 = 4.3$ , p < 0.05]). In further tests, however, the same individuals also chose the odour opening of the transparent cage without bumblebees (Table 7). Perhaps for the flies the visual perception of flying bumblebees behind plexiglass (e.g. via reflections) is reduced and falsified so that they can only work as weak visual releasers, or the plexiglass can be recognised as an obstacle.

#### 4.2.4 Significance of bumblebee flight tone in the search for host nests

In bumblebee nests a number of tones have been recorded (Schneider 1972, Fisher & Weary 1988) produced by bumblebees by the wing beats (flight tone) or thoracic vibrations (modulated tone). In connection with the host-finding behaviour of *V. bombylans*, the flight tone of bumblebees flying in and out of the nest and/or the tone in the nest area might play a role as acoustic signals.

The perception of acoustic signals has been demonstrated in different cyclorrhaphan Diptera. A well-studied example is the love-songs of *Drosophila* (Bennet-Clark & Ewing 1970). In different members of the Sarcophagidae (*Euphasiopteryx ochracea*; *Colondamyia auditrix*) whose larvae feed as parasitoids on gryllids and cicadas, the viviparous females recognise the host from their specific songs. Using a tape-recording of the host song, the female flies can be enticed right up to the loudspeaker (Cade 1975, Soper et al 1976).

To test the effect of bumblebee buzzing in front of the nest entrance on *V. bombylans*, the corresponding tape-recording was played. On the basis of such acoustic cues, no phonotactic reaction could be established in the flies. Analogous to the study of visual effects of bumblebees, the attractiveness of flight buzzes in connection with odour was tested.

Expt 7: Two similar loudspeakers were placed on the ground of the net tent. From both, identical bumblebee odours were

released. From one loudspeaker, a sequence of bumblebee flight tones was played, at a volume subjectively corresponding to the flight buzzing at the nest entrance. The recording was taken at the entrance of a ground nest of *Bombus terrestris* (gear: Uher-Report; microphone: Uher M516). Before each test, the flies were placed at the same distance (30 cms) from both odour sources and the loudspeakers. A reaction was recorded when a fly proceeded to one of the sources/loudspeakers.

[ Table 8: Reaction of female *Volucella bombylans* to bumblebee odour in combination with or without bumblebee flight tones. n=9 individuals ]

In combination with odour, the flies showed no preference for the combined olfactory-acoustic cue (n.s.; 4-fields test, null hypothesis = equal proportions) (Table 8) [...]

#### 4.2.5 Reaction of *Volucella pellucens* and *Volucella inanis* to wasp odour and appearance

Corresponding to the tests with *V. bombylans* on the significance of bumblebee odour, *V. pellucens* and *V. inanis* were tested for the effect of wasp odour on the host-finding behaviour and the oviposition reaction.

If female flies were only offered odour from a wasp nest (cf expt 2, p.26), they approached the source of the odour and showed the oviposition reaction (V.p., n=16; V.i., n=8). In contrast to *V. bombylans*, they did not climb up the box from where the odour came out. Towards wasps presented in a transparent cage without any odour cues, female *V. pellucens* and *V. inanis* showed no reaction (cf. expt 5, p 32).

The combination of the visual effect of wasps with wasp-nest odour could only be tested in very few experiments. A small nest of *P. vulgaris* (ca. 10 cm diameter) with a few workers (12) was placed in a corner of the transparent cage, whilst the nest odour was released from the corner diagonally opposite. Some female *Volucella*, perhaps stimulated by the odour, proceeded straight to the site of the cage contained the wasp nest (V.p. n=4, V.i. n=2).

In a further variation of the experiment, up to 5 *V. inanis* females were placed in a transparent, rectangular plexiglass cage (100 x 40 x 50 cms); on one side wasp odour was released, which stimulated the flies to an oviposition reaction. Outside, a wasp colony (in a nestbox) was presented on different sides, without the flight traffic at the entrance being significantly disturbed. It was recorded whether the *Volucella* females proceeded to the cage side where the wasp nest was presented. The animals were left in the cage for 20 mins.

Of 14 *V. inanis* females, eight proceeded immediately to the wasp nest, whilst the others mostly remained sitting in the middle of the cage. Only in two cases did a female change to the side of the cage with the wasps after the wasp nest had been removed.

Perhaps the animals habituated after a certain time to the odour and lost their motivation to seek out the host nest.

A further pointer to the significance of the visual effect of wasps on *V. inanis* females showed itself in their flight to the nestboxes in the net tent: they approached them first after the boxes had also had wasp fly to them. In one case a *V. inanis* female followed wasps even to a box which was only visited for taking up offered sugar water.

The results of the test and observations suggests that in female *V. pellucens* and *V. inanis* searching for the host nest, the host odour alone is not sufficient, and flying wasps play a role as visual cues.

#### 4.2.6 Is there morph-specific host-finding by *Volucella bombylans* ?

In connection with the mimetic polymorphism of *V. bombylans* (see 10.3), the question of a morph-specific host-finding arises. Do the different colour morphs of female *V. bombylans* search out those bumblebee models that they mimic (morph specificity) ?

Apart from the colour pattern of their covering of hairs, the bumblebee species differ in many ecological and biological characters. One can classify the species into different distributional types. This many species prefer open and dry habitats, and other are present mainly in woods (Pittiono & Schmidt 1942, Postner 1952, Reinig 1970). The nests are placed either above or under the ground in a species-specific manner. The different species also have different colony sizes. In the form of the nest position and the provisioning of the larvae, the more primitive species, the pocket-makers, differ from the more derived species, the pollen-storers. Apart from a special colony-specific nest odour, each species has also a species-specific odour (Cederberg 1983).

In the following, the nest visits of *Volucella* females were compared with reference to the colour pattern of host and fly. Possible preferences of the flies for particular bumblebee species were not taken into consideration.

Most of the central european bumblebee species can be arranged into three colour types, which at the same time represent the models for the three colour morphs of *V. bombylans* (Table 9).

[ Table 9. Colour morph types of native bumblebees and colour morphs of *V. bombylans* ]

To test for a morph-specific behaviour of *Volucella bombylans* females in host-searching, the observed visits of females in bumblebee nests and the offered odour sources (expts 1, 2: see 4.2.1) were analyzed according to the morph of the fly and the colour pattern type of the relevant bumblebee species.

In Table 10 are listed all the recorded nest visits of *V. bombylans* morphs and visited bumblebee species; they contain also



cases in which the flies were placed in the antechamber of the bumblebee nestbox and then went into the nest from there. Most of the nest visits occurred inside the net tent. The visits delineated in a box in Tables 10-12 represent cases in which *Volucella* colour morph and colour pattern of the bumblebee correspond.

In Table 11 are enumerated the visits of individual colour morphs of female *V. bombylans* in bumblebee nests and in the "odour boxes". Cases of nest visits were only included in which the flies approached the nests by themselves. The different bumblebee species are pooled according to colour pattern.

Because the various bumblebee species were offered for various lengths and frequencies, and the number of *Volucella* morphs was different in each situation, the number of visits in Tables 11 and 12 are not comparable with one another.

[ Table 10. Nest visits by *V. bombylans* females of different colour morphs to different colour pattern types of bumblebees. \* = flies placed in the antechamber of the nestbox. (F) = free flight to the box ]

[ Table 11. Visits of individual colour morphs (*V. bombylans*) to different colour pattern types (bumblebee nests and 'odour boxes'. A = visits to active bumblebee nestboxes. B = visits to 'odour boxes' (without bumblebees). ]

The cases in which the female flies before a nest visit had had a choice between at least two different bumblebee species and colour pattern morphs are listed in Table 12. Here also are the number of visits from the experiments with the 'odour boxes' shown, in which the bumblebee nest odour was taken from two different alternative bumblebee species.

[ Table 12. Visits of colour morphs of *Volucella bombylans* when offered simultaneously the nests of different colour pattern types of bumblebees (A) or nest odours (B) ]

Whilst in most tests the female *Volucella* had no choice between different bumblebee species, in the case of morph specificity it would be expected that each female which had the corresponding pattern to the offered bumblebee (mimetic model) would more frequently seek out the offered nests or its odour than flies with other colour patterns.

In Table 13, the number of visits of each colour morph in the odour boxes and nestboxes of various bumblebee colour types is listed next to the percentage in the course of the experiments in the net tent. In addition, the corresponding expected values are given, which come from visits irrespective of the bumblebee colour type, as a null hypothesis.

[ Table 13. Observed and expected visits of different morphs of *Volucella bombylans* in the nests and odour boxes of bumblebees of different colour pattern types, as a percentage of the individual morphs in the experiments. Each block gives: number of colour morphs used in the tests (as percentages), visits to one/two colour types, expected number of visits, calculated from the % in the test

A: Visits to bumblebee boxes with flying bumblebees (*B. mastrucatus*)

B: Visits to 'odour boxes' (*B. mastrucatus*)

C: Visits to 'odour boxes' (*B. terrestris*)

D: Visits to odour boxes with an alternative presented: odour of *B. terrestris* and *B. mastrucatus* ]

## Summary of the results

- In 32 nest visits to active bumblebee nest boxes, 25 *Volucella* females (72%) went to bumblebee species that did not correspond to their mimetic models. 7 individuals (22%) sought their correct model (Table 10)
- In 7 free flights (flies in the field visiting nests in wooden nestboxes), 3 individuals chose their non-models, and 4 went into nests with bees that were similar in colour pattern (Table 10)
- In test in which only bumblebee odour was offered as a cue, in 52 cases (67%) the *Volucella* females visited the odour source of a species of bumblebee that did not correspond to its mimetic colour morph (Table 11)
- Also in situations in which the *V. bombylans* females could choose between different colour types, they did not seek out the nests or the odour of their corresponding mimetic models (Table 12)
- If we consider the ratio of *V. bombylans* colour morphs in the experiments, then the number of visits expected under the assumption that no colour morph has a preference for any bumblebee type (the null hypothesis) corresponds approximately to the observed visits.

The results presented provide no indication at all that there is a morph-specific host-finding behaviour in *V. bombylans*. Females enter nests unconnected with the colour pattern type of the bumblebee species.

Because under natural conditions as a rule we do not find two bumblebee nests next to one another, the *Volucella* females could not choose directly between two colour types. They could only decide whether they enter a nest or not. In so far as the experiments, in which only one bumblebee species was offered, represent the natural situation (Table 10,11), each 'incorrect' decision is an indicator against any morph-specific host finding.

When given the possibility of choosing themselves between model and another colour type, the *Volucella* females also choose non-models (Table 12).

The proportion of visits of different fly colour morphs in nests or to corresponding odour sources corresponds largely to the distribution of morphs in the fly population in the net tent, and produced no preference for a colour pattern

(Table 13).

The objection, that the behavioural reactions of the fly females were a cage artefact, can be rejected because there was no difference in the behaviour of the flies in a nest visit inside the net tent and in the field, and also in the latter cases nests of non-models were visited.

In morph-specific host-finding by *V. bombylans*, the characteristics of the development of each colour pattern of the hairs should be genetically correlated with the corresponding host finding or recognition mechanism. An (environmental) entrainment on the correct host e.g. via the larvae (the adults have no contact with the host nest, since the larvae pupate outside the nest) is not guaranteed, since the colour morph is genetically determined (Gabritchevsky 1924), and corresponding to the alleles of the parents, from one egg-batch other morphs than that of the mother can develop (see 10.3).

Therefore according to these considerations, morph-specific behaviour in female *V. bombylans* is very unlikely.

#### 4.3 Comparative antennal morphology of *Volucella*

[...]

### 5 Nest visiting

*Volucella* females must penetrate into the nest cavities of their hosts for oviposition. This is true for *V. bombylans* and *V. pellucens*, which lay their eggs on the nest envelope of underground host nests (cf 6.1). Because bumblebees and wasps defend their nests against intruders, the question arises as to how *Volucella* females are able to penetrate the nests of active host insects. In the previous chapter (4.1) we have already described the behaviour of *Volucella* females in front of the host nests from the viewpoint of host searching. In the following, their behaviour is considered under the influence of the active adult hosts.

#### 5.1 Bumblebee guards

It is generally known that the hive entrance is watched by honeybees of the so-called guard bees. They check the hive identity of the arrivals. They recognise them as nest members from their specific nest smell and by a particular behavioural reaction (Free 1958).

In bumblebee nests one finds such guards only in very dense colonies (Free 1958, Michener 1969a), and more commonly in for example *B. terrestris*. Of 26 bumblebee colonies held in nestboxes, only two *B. terrestris* and one *B. mesomelas* colonies occasionally had a guard at the entrance to their nest cavity. In five natural ground nests, no guards were found at the entrances during the observation period.

If you watch the entrance, one finds a particular bumblebee for a few hours at the entrance inside the nest cavity. Arrivals at the nest are touched/tasted with the antennae and their smell recognised as a nest member (Free 1958). Non-members are attacked with the mandibles and legs and stung (occasional observations of nest members being attacked is perhaps to be attributed to their nest odour being masked or overlaid by other odours: Free 1958, Alford 1975).

In contrast to honeybees and social wasps, bumblebees have no alarm pheromone that in a threat to the nest allows rapid mobilisation for nest defence (Maschwitz 1964).

Under massive interference in a bumblebee nest, workers fly at the attackers and use their stings, or they perform a typical defensive behaviour. In this they lie on their backs, spread their mandibles, crook the abdomen and produce a characteristic buzz with the wings and thorax ("defence buzz").

The attack readiness of bumblebees is very different in each species and each nest (Alford 1975). Thus the workers of *B. terrestris* and *B. lucorum* react with fierce attacks to irritation of the nest, whilst one can dig out the nests of *B. pascuorum* and *B. maculatus* without any special protection. Free (1958) also found different aggressiveness (willingness and duration) among the workers of a colony that perhaps is connected with the developmental condition of the ovaries.

##### 5.1.1 *Volucella bombylans* as an invader

The superficial similarity with its host leads above all to the suggestion that *V. bombylans* females are spared by their appearance from attack by their hosts (Kirby & Spence 1817, Poulton 1890, Gabritchevsky 1924). In tests in which *Volucella* females (n=8) were placed in front of the observed nest entrance of two different colonies of *B. terrestris*, the flies were attacked by the bumblebees irrespective of their colour morph. *V. bombylans* females were only twice observed to have penetrated into an observed nest (*B. mesomelas*). In both cases, the females were stung by the guard bumblebees.

Obviously *Volucella* females are not able to get into bumblebee nests, so long as there is a guard bee at the entrance. They were recognised immediately as foreigners to the nest and attacked.

Since however in most cases the nest entrances of bumblebee nests are not guarded, the female flies have the opportunity to enter them unnoticed. Thus in 29 nest searches (without guards), only three female *Volucella* were killed by the bumblebees.

Bumblebee workers returning from foraging or leaving the nest do not as a rule linger at the nest entrance. They also showed no reaction at all to various pinned insects placed next to the entrance. Also *V. bombylans* females were in most cases themselves not paid any attention by flying bumblebees near the area of the entrance (Fig 21).

In nest visits by female *V. bombylans* (n=16), on average two meetings per visit were recorded. A meeting means here the close approach to about 1 cm of a bumblebee and the fly. Number of meetings in 16 nest visits of *V. bombylans* (n=16): 0,0,2,2,3,1,1,3,1,2,2,3,3,3,3,5. Mean = 2

During about 10 observation hours in which *Volucella* females were found in front of bumblebee nest entrances, a bumblebee only pursued a fly for a short time six times. The *Volucella* avoided it each time, and flew a small circle, eventually to land again in the immediate vicinity of the entrance.

Obviously bumblebees have little respect for the intruder in this situation. Since other insects also (fleshflies, scorpion flies, crickets, longhorn beetles) are not attacked upon meeting directly with bumblebees at the nest entrance, it is improbable that in *Volucella* the visual similarity with bumblebees or an odour masking plays any role.

Not all approaches to the nest entrance by *V. bombylans* females end in a visit to the nest. Of 24 females, six abandoned the nest entrance, after which they remained in close proximity and showed the typical behaviour before a nest visit (see 4.1.1).

In almost all nest visits that could be observed, the *Volucella* females only proceeded into the entrance to the nest tube when no bumblebee was present in the area of the entrance. This was (apart from a few cases in which a guard bee was present) the time between the to- and fro-flights of the foragers.

The to- and fro-flight frequency and corresponding timespan during which there was no bee at the nest entrance was different in different colonies. It was essentially connected with the number of individuals in the colony (see Table 15). The established time interval ranged from about 18 sec to 10 mins (Table 15), but with greater variation (cf the SD of Table 15). Successful visits by *V. bombylans* females could be observed in all the specified mean flight activities of bumblebees at the nest entrances.

It was not determined in what way, e.g. by higher flight frequencies, entry into the nests was rendered so much more difficult that the flies abandoned their attempts more frequently, because there were so few observations for different strong nests.

The *Volucella* females mostly pressed forward shortly after a bumblebee flew into or out of the entrance, in order to traverse very quickly the whole length of the tunnel to the nest, so that even when the nest tunnel (plastic tube) was more than a metre long, they already had reached (the nest) after a few seconds.

In a few cases it was observed that female flies abandoned the entrance immediately when a bumblebee approached the exit from the inside of the nest.

[Table 15: Length of time at the nest entrance between the to- and fro-flights of bumblebees in various colonies ]

After the fly has reached the nest of the bumblebee, she digs herself into the material of the outer envelope (see 8.3, Fig 54), and begins to lay her eggs. She is largely protected there from attack by the bumblebees, which remain as a rule inside the wax envelope, in the brood chamber. However, if a *Volucella* female is discovered by a bumblebee female in this area, she can easily escape an attack and pursuit in the dense ("Genist").

In a *B. terrestris* nest, in which the outer nest material had been removed, the female fly was quickly noticed by its approach to the wax nest, and was stung. *V. bombylans* females that have been stung by bumblebees immediately begin to lay eggs, so that the eggs remain in the nest area even when the fly is carried outside the nest. *V. pellucens* and *V. inanis* do not show this behaviour.

Female *V. bombylans* remain for about 26 mins (n=10: 11,15,18,24,25,28,31,35,35,40, mean = 26.2 mins) in the bumblebee nest. After reaching the exit, they fly away immediately.

## 5.2 Nest guarding and nest defending by wasps

In contrast to bumblebee nests, as a rule there are several workers at the entrance to a wasp nest, as so-called guards, to taste/smell the arrivals with their antennae and check their nest odour. Guards are usually older individuals (Potter 1964); however, this task can be accepted by old workers already from their first few days (Edwards 1980). Only very weak colonies lack these guards at the entrance. Normally guards are found day and night at the entrance, although their number varies. The individual workers may remain as guards at the entrance for a few minutes or several hours (Potter 1964, Edwards 1980).

If intruders are recognised as such, the workers menace them with open mandibles, and then in larger insects they try to grasp them with their legs while individual wasps bite them hard and sting them. Smaller insects (about housefly size) are attacked by the workers immediately with their mandibles and stung; the victim then are usually dismembered and the individual pieces carried away.

Mobilisation of more workers from the nest follows in fierce attacks at or in front of the nest. This works by shaking about, which releases attack behaviour in wasps. The individuals hurry out of the entrance and hurl themselves onto the attacker. The release of an alarm pheromone may stimulate still more workers from the nest into attacking (Maschwitz 1964). Returning foragers do not notice the intruder, nor do they immediately share in nest defence (Edwards 1980).

### 5.2.1 Reaction of wasps to *Volucella pellucens* and *Volucella inanis*

A *V. pellucens* female entered right to the nest envelope unmolested and almost unnoticed by the stream of workers flying in

and out of the nest, as recorded by Nixon (1934) from his observations on a nest of *Paravespula vulgaris* and [he means “or”] *P. germanica*. My own observations also establish that *V. pellucens* females entering a wasp nest (*P. vulgaris*, *P. germanica*) are attacked neither by the foragers coming and going nor by the guard wasps, nor are they molested in any way during their visit.

Guards that have obviously noticed the flies avoid contact, or persist with raised up antennae and open mandibles directed towards the *Volucella* (Fig 24). This behaviour resembles the warning posture described by Potter (1964) and Arnold (1966) that wasps assume towards intruders.

Female *V. inanis* avoid contact with wasps; in strong nests with lots of flying and watching, they cannot get in (n=10) (cf. 4.1.3).

In order to record the reactions of wasps towards *V. pellucens* and *V. inanis* quantitatively, and estimate the chance of nest invasion, adults of both species were placed in front of the nest entrance of *P. vulgaris* and *P. germanica* nests (Fig 25, 26).

Methods: The live insects were held by the wings in a clip so that the legs, head and abdomen could still move. The clip was attached to a rod, and could be put right up at the flight platform of the wasp nest (in a nest box) (Figs 24, 26, 27).

Apart from *Volucella* species, other fly species were also offered, as well as other insects of a similar size to the wasps.

- Diptera: (Syrphidae) *Eristalis tenax*, *Arctophila bombiformis* (Tachinidae) *Echinomyia fera* (Sarcophagidae) *Phormia terraenovae*, *Sarophaga* sp.

- Coleoptera: (Scarabaeidae) *Trichius fasciatus* (Rhipiphoridae) *Metoecus paradoxus*

- Gryllidae: *Acheta domestica*

- *Bombus lucorum* (♂), *B. terrestris* (♂), *B. pascuorum* (♂)

The insects were caught from the field. Each test insect was offered for a maximum of 5 mins. After an attack by the wasps, the individual was withdrawn. An attack was recorded when a wasp bit the offered insect with its mandibles, or tried to sting it. A series of tests lasted up to 30 mins. A different test insect was used after each one. In order to demonstrate the attack readiness of the wasps, after a failure to attack an object was tested that was ordinarily fiercely attacked by the wasps. The tests were carried out on three different colonies of *P. vulgaris* and two of *P. germanica* over several weeks (27.8 - 28.9.1986, 10.8 - 29.9.1987) and at different times of the day (0800 - 2000 h). The wasp nests were located in nestboxes in the field (Institute garden), and the tests also took place there, so that the normal foraging and flight operations were scarcely affected.

[ Table 16: Reaction of worker of *P. vulgaris* and *P. germanica* to *Volucella* and other insects (test length = 5 mins) placed before the nest entrance. + = attack, - = no attack, n = number of test insects. I, II, III = different nests ]

The collated results of Table 16 show that female *V. pellucens* were not attacked at the nest entrances of *P. vulgaris* and *P. germanica*, but male and female *V. inanis*, other flies, and other insects were attacked. The few tests that could be carried out on male *V. pellucens* suggested that only females were spared from the attacks of the wasps. This is true only for live individuals.

*V. pellucens* that had been killed by CO<sub>2</sub> or in the deep freezer were gnawed at and transported by the wasps, just as other insects. Wasps showed a lower level of attack also for the syrphid *Eristalis tenax*, of which only 50% were attacked.

If we compare the attack behaviour of the wasps towards *Eristalis tenax* with that towards female *V. inanis*, the former (in the case of an attack) was mainly (70%) worked at with the mandibles (“gnawed”) (Table 17), whilst the latter was reacted to with a stinging attack in 70% of cases, which was recorded as more intensive attack behaviour. Both fly species were similar in their body size and their defensive reaction (leg movements and buzzing).

[ Table 17. Attack reactions (‘stinging’ or ‘gnawing’) of *Paravespula vulgaris* and *P. germanica* against *Eristalis tenax* and *Volucella inanis* females at the nest entrance. Exposed for 5 mins ]

Female *V. pellucens* (n=8) placed before the flight platform of a nestbox containing *Dolichovespula media* were stung by the workers. During this test (15-20.8.1987) there were only about 15 workers in the nest.

Inside the nest cavity, *V. pellucens* females go to the outer nest envelope (Fig 22), where they lay their eggs. (cf 6.1.3). Here also they are not attacked or molested by the wasps (Fig 23). The female flies remain in the wasp nest (nest box) for about 30 mins (*V. pellucens*, n=8, stay times in minutes: 20, 22, 29, 31, 33, 34, 38, 45, mean = 31.5).

Female *V. inanis* (n=3) only get into the nest cavity when no guards or foragers remain in the entrance. This was only the case in one of the test nests. But even in this case, they did not climb onto the nest envelope, but laid their eggs on the inside walls of the nest box (cf 6.1.4).

### 5.3 Discussion: host-finding, nest-visiting

Host finding can be broken down first into the distant orientation with the search for the host habitat, and secondly into the discovery and localisation of the exact oviposition site. The first is affected by various parameters of the host environment, but the latter by visual, chemical or tactile cues from the host itself (review by Vinson 1976).

In the aphidophagous hoverfly *Syrphus corollae*, Sanders (1982) was able to establish an avid searching activity towards black surfaces, which he interpreted as signal cues for plant resources; dark surfaces stimulate the *Syrphus* females even more strongly in their search when coupled with striped patterns or leaf shapes (Sanders 1983). The search area and

choice of location were here set by visual cues, whilst the exact position of oviposition was then found by olfaction of aphid odour (Dixon 1959, Bombošch 1962, Volk 1964, Peschken 1965, Chandler 1968c).

Which environmental cues could female *Volucella* use for finding the host nest? Bumblebee and wasp (*Paravespula*) prefer to place their ground nests in former ground nests of small mammals (mainly mice). In the study site (Mathon/Alps), bumblebee nests were found more commonly where there were also many mouse burrows, e.g. on shallow slopes and the sides of roads. In the early spring, one can commonly see queen bumblebees searching for mouse sites on woodland edges, fields and ditches to discover mouse dens for their own nest sites.

While nest-searching bumblebee or wasp queens may notice the entrances of mouse dens partly visually (in low vegetation), by the time female *Volucella* appear the nest entrances are normally completely covered by the vegetation and hence are hardly recognizable visually.

In contrast, the nest sites of colonially nesting solitary bees, which are mostly in open sandy places can be found by a simple optical search image. Correspondingly, some species of Bombyliidae and Miltogrammini whose larvae develop with these bees use visual releasers to find their host habitat (Bohart et al 1960, Spofford et al 1986).

The nest habitats of the host of *Volucella* cannot be described definitively apart from the soil surface, so that the question must remain largely open about the recognition characteristics for distant orientation (search image).

To recognize the host nest, olfactory cues may play a role in *Volucella*, since many hymenoptera mark their nests with odour (Butler et al 1969, Cederberg 1977, 1983, Hefetz 1987). Other odour sources may also serve here as signals, such as the nest material itself, the larval food and the comminuted frass and rejecta near the nest. The workers flying to and from may also distribute various scent components around the area of the nest entrance.

It was shown experimentally that in female *V. bombylans* host-finding behaviour could be released only by bumblebee-nest odour, and that they search for the source of the odour. Female *V. pellucens* and *V. inanis* also react to the nest odour of their hosts (wasps) with an oviposition reaction (extrusion of the ovipositor). If you provide females with only visual or acoustic cues in the form of bumblebees or wasps flying in and out or their flight tones, without any corresponding odour cues, the *Volucella* females show none of the reactions that are typical in host-finding behaviour. It must remain open in what way the particular odour components cause an attraction at a distance and perhaps other agents release the oviposition reaction. As an oviposition reaction, we understand here only the extrusion of the ovipositor. The laying of the eggs probably follows after tactile and chemical cues have been detected.

In the few studies that could be carried out on the visual cues of the host adults at the nest entrance, at least a tendency was shown for female flies to prefer nests where hosts were presented visually as against those with only odour cues. Bumblebees or wasps flying around the nest were always more attractive for *Volucella* females than just odour, or odour and host adults in enclosure cages (buzzing cues).

*V. bombylans* females showed no reaction to the flight tone of bumblebees as an acoustic cue. This cannot be completely ruled out yet as a significant (cue) in host finding, since it is possible that the recording of an effect may not be able to be adduced on methodological grounds.

The long and richly feathered arista (sexually dimorphic) of female *V. bombylans* was interpreted as an adaptation to the search for bumblebee nests. As well as a mechanical protection of the funiculus and a tactile function, it could also serve for the perception of acoustic signals from the host.

In the experiment, host odour in combination with host adults proved to be a more important releaser cue which makes host finding and host recognition possible.

Since female *Volucella* could also meet bumblebees and wasps outside the nest, e.g. in flower visiting, this situation could well differ from that at a host nest. The higher odour concentration of the host and particular odour qualities in the entrance area of the nest may play a role here.

In order to track down host nests by olfaction, female *Psithyrus* cuckoo bees fly in long straight lines over the ground. Parasitic flies such as the anthomyiid *Leucophora johnsoni* follow their host bee *Lasioglossum zephyrum* in flight and hence succeed in arriving at the nest (Batra 1965). Members of the cleptoparasitic Miltogrammini of the genus *Senotainia* also follow their host, the digger wasp *Tachysphex terminatus*, to its nest in order to deposit their larvae on the prey of the host (Spofford et al 1986). In the latter, the following of the host probably occurs visually. This is the significance of the enlarged eye facets of females in the front region of the eye.

Similar host-finding strategies could not be seen in *Volucella*. *Volucella* females seek neither in continuous search flights towards the host nests, nor by following host adults. From flights to the host nestboxes, in the net cages, and also in the field, females were seen "suddenly" near host nests, so that it had the appearance of the individual in the first instance coming more or less accidentally to the neighborhood of nests. If in this way a female *Volucella* happens to be about 1 m from a bumblebee nest that has flying (bees) in operation, then usually a directed approach to the nest follows.

How great are the chances of a female *Volucella* finding a host nest with this strategy? For a simplified consideration, we can make the following assumptions in the case of *V. bombylans*:

- one bumblebee nest on average is found in 100 m<sup>2</sup> (this corresponds to e.g. the situation in the study site in Mathon/Alps).
- a female *Volucella* could lay eggs over at least 10 days (life expectancy; weather conditions).
- within 10 days, the animals change their place of residence at least 10 times over a distance of not less than 10 m
- a female fly can discover a host nest within a radius of 1 m

Hence a female *V. bombylans* reaches 100 different places and covers an area of about 600 m<sup>2</sup>, where about six bumblebee nests are to be expected. This would suffice by far as enough hosts nests to find for oviposition.

## Nest visits

In order to reproduce successfully, a female *Volucella* must not only discover a suitable host nest, but she must get into it and lay her eggs. Since bumblebees and social wasps defend effectively their nests against intruders, female *Volucella* must have evolved particular modes of behaviour or other adaptations that make it possible for them to succeed in spite of the active host adults in their nest.

In different visitors to the nests of social insects, one finds different strategies realized to overcome the nest defences (Wilson 1971, Kistner 1979):

- waiting for a suitable moment, e.g. until the nest entrance is unguarded
- by small size and speed of movement, they can slip under the vigilance of the guards (e.g. Phoridae in wasp nests)
- with a corresponding form of protection, defiance or activity, attacks by guards can be braved (e.g. Staphylinidae: *Vatesus* in ants - Akre & Torgerson 1969)
- via deception in the form of mimicry, or with the help of chemical inhibitors, the nest guards can be passed unhindered (e.g. Scarabaeidae: *Myrmecadophodius* in ants - van der Meer & Wojcik 1982)

The very important difference between bumblebee- and wasp-nests from the point of view of entry is that there are permanent so-called guards present in the latter. This demands different behavioural strategies in the respective visitors.

If we compare the behaviour of the different *Volucella* species at their respective host nests, all three show intensive cleaning behaviour and the extrusion of the ovipositor when they arrive close to the nest entrance.

Whilst female *V. bombylans* remain on average about 25 mins (Table 1) in front of the entrance before a nest visit, *V. pellucens* females proceed inside only a few minutes (Table 2) after approaching the flight platform.

Different ideas can be adduced for the comparatively long wait before a bumblebee nest. During this time, *V. bombylans* females could locate exactly the concealed nest entrance, or achieve readiness to oviposit, and/or take up an olfactorily masking host odour. In contrast to *V. pellucens*, it could however be waiting for a suitable moment when no bumblebee remains in the entrance (determined by the flight frequency).

If a *V. bombylans* female encounters a bumblebee in the nest, she is usually recognised as an intruder and attacked. Female *Volucella* must therefore use the time between in and out flights of the worker bumblebees to avoid direct contact with a bumblebee when entering the nest.

Inside the nest cavity, *V. bombylans* digs itself into the nest covering (commonly consisting of mouse nest material) and lays her eggs there. She is largely safe here from attack. However, if a *V. bombylans* female is discovered, overwhelmed and stung by a bumblebee, she is equipped with a mechanism that still allows her to lay her eggs quickly. The ovipositor remains functional and begins or continues oviposition, probably autonomically controlled at the neuronal level. Females of other *Volucella* (species) are not able to do this.

*V. inanis* approaches a wasp nest carefully, and repeatedly avoids the wasps flying in and out. Only at a nest with few workers and without guards at the entrance do *V. inanis* females proceed into the inside of the nestbox to lay their eggs. In a nest with active foragers and guards - which depicts the normal situation - the female flies do not penetrate the nest cavity, but lay their eggs on the outside of the nestbox near the entrance.

Künckel d'Herculais (1875) also observed a female *V. inanis* at a ground nest of *Paravespula vulgaris* that flew several times to the entrance, was attacked by a guard, but then proceeded to the entrance when the guard's attention was relaxed. He did not record, however, whether the nest was continuously guarded or flown out from.

Female *V. pellucens*, in contrast to *V. inanis* females and other insects in front of and inside the nest of *P. vulgaris* and *P. germanica*, are not themselves attacked when they presented to the wasps in a conspicuous manner, fastened to a clip in or close in front of the nest entrance (Fig 25).

The different and species-specific behaviours of female *Volucella* are considered to be adaptations to the respective conditions at the nest entrance and the reactions of the hosts.

In the case of the continuous attendance of guards at the nest entrance, the strategy of waiting for a favourable moment before entering a host nest, as is possible in *V. bombylans*, does not succeed.

In spite of their size, female *V. pellucens* and *V. inanis* could succeed in being unnoticed in the nests, so that as is possible in e.g. the small (only 5 mm long) and nimble Phoridae or in *Fannia* flies, there is still distance enough for passing to happen, in spite of several entrance guards.

Obviously female *V. pellucens* are equipped with a camouflage that makes them appear neutral to the host, or inhibits their aggression and defence.

In contrast to *V. inanis*, female *V. pellucens* show no similarity to wasps in their colouration and pattern, so that visual mimicry of the host in the sense of aggressive mimicry can be ruled out. Also in their behaviour, there is nothing to signify how it could contribute to the silencing of the guard wasps.

From this, it remains possible that they deceive the recognition mechanism of the host, or stop them with an inhibition at a distance, by means of a chemical mimicry. This is all the more obvious, since wasps recognise nest membership above all by olfaction, as in other social hymenoptera.

In this connection, it has already been discussed by Edwards (1980), in what way intraspecific pheromones effective in repelling or inhibiting aggression could play a role as key factors.

The use of chemical signals and cues that play a role in the social recognition and communication of their hosts is widespread in myrmecophilous and termitophilous beetles (Wilson 1971, Kistner 1979). Holldobler (1970, 1971) was able to show that the staphylinid *Atemeles pubicollis* had three functionally different glands at different positions on the abdomen

(adoption, defensive and appeasement glands) used to integrate socially with the host. To enter an ant nest (*Myrmica*), the beetle waits until it has been noticed by an ant; in a particular behavioural sequence it presents it with different secretions which lead eventually to the ant taking it unharmed into the nest.

A staphylinid (*Trichopsenius frosti*) that lives in the nests of the termite *Reticulotermes flavipes* synthesizes the same cuticular hydrocarbon as its host (Howard et al 1980). This volatile cuticular hydrocarbon is useful also in caste- and species recognition.

A further case of chemical mimicry was found by van der Meer & Wojcik (1982) in a myrmecophilous scarabaeid (*Myrmecaphodius excavaticollis*). The beetle is able to take up passively the specific hydrocarbon of its host ant (*Solenopsis* sp). A sturdy cuticular armour protects it against the first attacks until its identity with its host odour; outside the nest it loses the odour.

The ability to produce and perceive chemical signals in the form of sexual pheromones has been demonstrated already in several Diptera (Howard & Blomquist 1982). Analogous to the described examples of ant guests, this ability could also have evolved in *V. pellucens*, by means of a specific chemical camouflage and via chemical protection, success in entering the host nests.

Since female *V. pellucens* are not attacked by the wasps when they proceed directly into the nest entrance, it is improbable that they remain unmolested because of taking up the nest smell. The reactions of the wasps resemble an inhibited aggressive behaviour, which might be released via an effective repellent or an inhibitor of aggression.

Francke et al (1978) found in pentane extracts of the abdomen of *P. vulgaris* "Spiroketale", substances that showed an aggression-inhibiting effect in corresponding behavioural experiments with wasps.

Female *V. pellucens* killed by CO<sub>2</sub> or by freezing were not avoided by wasps, and therefore the effect of a possible chemical protection occurs only in live animals.

Female flies present not near any wasp nest were preyed upon and transported by workers of *P. vulgaris* and *P. germanica* (Fig 24). The signifies either that foraging wasps behave differently, or that female *V. pellucens* only produce their chemical protective mechanism when right by a wasp nest.

Perhaps the protection against attack of female *V. pellucens* is confined to a few wasp species (hosts), and is therefore the expression of host specificity. At the entrance of a *Dolichovespula media* nest, which is not a host, the fly was fiercely attacked by the workers.

Experimental clarification for the different reactions of wasps to the females of *V. pellucens* and *V. inanis* lead to the question of the significance of their larvae to the host, and their influence on the reproductive success of the wasp colony.

## 6 Description and biology of larvae

6.1 Eggs: deposition, number, morphology

6.1.1 *Volucella bombylans*

6.1.2 *Volucella pellucens*

6.1.3 *Volucella inanis*

6.1.4 Eggs: comparison and summary

6.2 Larvae: description, location, feeding, number

6.2.1 *Volucella bombylans*

6.2.2 *Volucella pellucens*

6.2.3 *Volucella inanis*

## 7 Cephalopharyngeal skeleton and digestive tract

[literature]

7.1 The head of cyclorraphan Dipteran larvae

[literature]

7.1.1 *Volucella bombylans*

The cps consists of the fused basal sclerites (clypeal sclerite, tentorium, cibarium) and the mouthparts (labrum, mandular complex and labium - Figs 40a, 41a).

The anterior mouth cavity is bounded by two 'dredging shovel'-like, strongly sclerotised half-shells, which have robust teeth on the ventral side (Fig 44a). The teeth are organised so that from the front there are three large teeth in a row; from there a slightly squared off row of smaller teeth runs paraxially; at the front a strikingly large tooth stands out, beyond the row, to the side (Fig 44b). The interior of the half-shells has flat surfaces divided by regular longitudinal folds and ridges.

They could be compared with the mandibular lobes (Hartley 1963, Roberts 1970) or the atrial shells (Krüger 1926) which in other syrphid larvae exist as complex lobster-pot structures. Krüger (1926) and Roberts (1969) consider the mandibular lobes as structures of the exocuticle on the lateral borders of the prestomum. In the following, the aforementioned structure is described throughout as mandibular lobes.

The mandibles, partly surrounded by the mandibular lobes, are constructed as rod- (or clasp-) like sclerites each with a dorsal process. With the help of the musculature inserted onto the mandibular sclerites, the mandibular lobes are able to move. The mandibular sclerites are flexibly joined with the distal processes of the tentorium, the lower tentorial bars (the lateral rods of Krüger 1926). The tentorial bars are joined ventrally by a bar-shaped sclerite (the tentorial bridge)



immediately in front of the opening to the salivary glands. A horseshoe-shaped sclerotised rod lies anterior to the tentorial bridge, which borders an unpigmented phragma. In front of that lie a pair of sclerites which insert onto a cuticle set with spines (Fig 45). This complex is regarded as the labium. Hartley (1963) described sclerites in a similar position as the labial plate and labial sclerites.

Over the lower tentorial bars and from the tentorium run a pair of rods, which almost reach right to the dorsal processes of the mandibles, and which can be described as the upper tentorial bars (Frontalsackspangen of Krüger 1926). Dorsally the clypeal sclerites extend over the tentorium, and anteriorly they are joined with the fused labrum. The tentorial arms join with the cibarial wall. The floor and side walls of the cibarium create a boat-shaped trough, with unpigmented and poorly sclerotised walls. The roof consists of a slightly pliable phragma on which the cibarial dilator muscles insert (Fig 47a). On the floor of the cibarium stretch nine so-called T-ridges in nine longitudinal stripes. These each carry two rows of filaments diverging at an angle of 45 degrees, which meet the neighbouring filaments at their tips. In this manner the grooves or channels between two ridges are hidden like a roof; in cross-section one sees Y-shaped pillars creating eight cavities (Fig 47a, 48a,b). The outer ridges only have a single row of filaments facing inwards. These ridges, filaments and grooves represent a filtering apparatus that operates to separate solid food particles from fluid.

Apart from size, the structure of the cps is essentially the same in all three instars. In L1, a pair of tentorial bars project distally, corresponding to the upper tentorial bars from their position, but which in contrast to these reach right to the mandibular lobes and project in a common spike (Fig 46). In L1 *Eristalis* the labrum is also constructed as a long drawn out sclerite reaching to the mouth opening, and acting as an egg cutter. In the later instars it fuses with the clypeal sclerite. In the Syrphinae, the labrum is still present in the 3rd instar as a stiletto (Fig 43) (Hartley 1963).

#### 7.1.2 *Volucella pellucens*

The cps corresponds largely with that of *V. bombylans* (Fig 40b, 41b). Differences lie in the construction of the mandibular lobe complex. The mandibular lobes have tall longitudinal ridges lining the inside of the mouth cavity. They are not rigidly joined with one another. At the distal ends the sclerotised strips project out as teeth (Fig 49a). As in *V. bombylans*, the L1 larva has a pair of relatively longer tentorial bars, which create a common spike distally (see Hartley 1963).

#### 7.1.3 *Volucella inanis*

As in the other species, also here the tentorium and clypeus are well developed and strongly sclerotised. However, in *V. inanis* the upper tentorial bars are missing, and the mandibular region in comparison is only drawn out a little distally (Fig 40c; 41c). There are only two pairs of sclerotised rods which viewed from the ventral side run parallel to the distal end, and have teeth (Fig 50a). The front row of teeth together create a gable-like point (Fig 50b). The sclerotised rods are considered to be homologous structures to the mandibular lobes of the other species. Underneath the sclerites there are still a few longitudinal ridges (Fig 51a). The mandibular sclerite has a dorso-lateral process, but no ventral processes (Fig 40c).

The paired labial sclerites are conspicuous; they run ventrally as strong hooks and have muscles inserted on them. Over them there is a thickly toothed pad with proximally directed teeth; the posterior part of the pad has rounded ends to the teeth (Fig 51). The labium in comparison with the other species is much more powerfully built. It could serve to take up and transport further the inner organs and haemolymph sucked out from the host body, instead of the scarcely developed mandibular lobes. The filtering apparatus on the floor of the cibarium in the form of T-ridges is scarcely present. One finds only 4 grooves and short filaments (Fig 47b). In L1 the cps has solely a paired sclerite with a process distally and joining to a common point (Fig 52a). Posteriorly it broadens into a wing which extends dorsally. The head skeleton of the 2nd instar corresponds to that of the last instar in its construction (Fig 52b).

## 7.2 Digestive tract

The foregut of *Volucella* larvae (L3) goes together with the already described pharynx and the oesophagus leading from it. The latter is a short narrow tube ending in the proventriculus, and projecting as a circular fold into the lumen of the midgut. The salivary glands consist of a paired tube which at first runs posteriorly, and then turns and runs in the opposite direction anteriorly. At the start of the oesophagus they join in a thin common salivary duct and lead ventral to the pharynx to the mouth cavity. In *V. bombylans* and *V. pellucens*, the salivary gland starts as thin and sinuous tubes in the first thoracic segment, bends back at the level of the proventriculus, and then runs as a thicker tube forwards (Fig 53a). *V. inanis* has a much shorter salivary gland, only about a third as long as the other species, and has the same width all the way to the common duct (Fig 53b). Shortly after the start of the midgut at the proventriculus, there are four blind tubes or diverticula, which produce digestive secretions (Wigglesworth 1984). Whilst in *V. bombylans* and *V. pellucens* the diverticula extend to about the length of the foregut, in *V. inanis* there are only 4 knob-shaped diverticula in the corresponding position (Fig 53).

At the boundary between midgut and hindgut, two Malpighian tubules on each (side) lead into the pylorus. They extend tubelike in numerous coils inside the entire body cavity (Fig 53). The hindgut emerges at the anus in the 8th abdominal segment. Anal papillae are only present in the form of small conical tubercles. In the various species of *Volucella* larvae studied, the ratio of the length of the midgut to hindgut is approximately equal: *bombylans* 4:3 mm, *pellucens* 3:4 mm; *inanis* 3:3 mm.

## 7.3 Summary and discussion

After studying the construction of the mouthparts and the digestive system in syrphid larvae, Krüger (1926) distinguished two types: saprophages and predators. In the following will be discussed in what way the larvae of the studied *Volucella*

species can be classified according to these criteria into one of these two groups.

*V. bombylans* and *V. pellucens* resemble each other generally in the construction of the mandibular lobe complex, whilst *V. inanis* demonstrates fundamental differences.

In *bombylans*, the mandibular lobes make a sclerotized pair of shovels with stronger and longer teeth (Fig 40a,44) which seem to be suitable for breaking open the bumblebee brood cells and scraping up the pollen stores, faeces and dead hosts. Mandibular lobes of this form and construction have not been described before in syrphids. Toothed mouthhooks with lobsterpot-like lobes are found also in the species *Cheilosia pallipes* (Wallace & Lavalley 1973) and *Eumerus strigatus* (Roberts 1970), which feed on decaying mushrooms and narcissus bulbs. In contrast, in these *Volucella* larvae, however, the mandibles are homologues of the toothed mouthhooks, which as a sclerite only surround and stabilize part of the body of the lobes (cf. Ch.9).

The mandibular lobes of *pellucens* are composed of sclerotized, high lobe strips which, in contrast to *bombylans*, do not create a rigid shovel (Fig 49a). Whilst these lobe strips consist only of simple raised folds, in many saprophagous larvae such as *Eristalis* and *Syrirta* they are constructed as fine ridges with additional little chitin hooks which make it possible to sieve the liquid food (cf. Krüger 1926, Hartley 1963, Roberts 1970). From the figure (Fig 42) of Wallace & Lavalley (1973) of *Volucella apicalis*, a comparable mandibular lobe apparatus could be developed. The larvae of this neotropical species develop in rotting *Opuntia*.

In the mouth cavity of *inanis* there are only very few mandibular folds (Fig 50a). Instead of which one finds two strongly sclerotized rods running distally right next to one another, and toothed at the tips (Fig 50a). With their help the larva could tear a hole in the host cuticle. Comparing the mouth structure of *inanis* with those of other predatory syrphid larvae, we find neither conformity regarding the participating elements, nor in their arrangement. The cps of aphidophage species is always constructed in the same way. The mandibular sclerite projects as a thin stiletto, the labrum and labium are drawn out further forwards, each fused into a point (Fig 43). They are used to pierce and pick up the prey which, after saliva has been injected and extraintestinal digestion taken place, are completely sucked out (Bhatia 1939; Roberts 1970). The special construction of the mouthparts can above all be seen as an adaptation to catching prey. Thus an aphid should be first pierced and lifted off the substrate before it can be sucked out without being able to escape. The prey (wasp larvae) of *inanis* larvae are, in contrast, sessile and defenceless; no particular structures are necessary, therefore, to catch and hold on to the prey.

Roberts (1970) was able to show in different syrphid larvae that the type of food and its uptake corresponded with the particular form and construction of the clypeal phragmata and tentorium. Thus species such as *Myathropa* which filter food particles from large amounts of water, have small clypeal sclerites and a long and almost horizontally lying cibarial musculature, whilst in predatory species like *Syrphus*, where small amounts of food must be taken up quickly, the tentorial arms are powerfully developed, the tentorium stands vertically, and the cibarial musculature is correspondingly shorter and arranged vertically. Larvae living in decaying substrates taking up concentrated food, such as *Eristalis*, *Syrirta* and *Rhingia*, possess likewise far drawn out clypeal phragmata and a vertically directed tentorium.

Also in *Volucella* larvae we find the latter type of construction. The upper tentorial bars are however even more strongly constructed. In *inanis* however these are completely missing, and the lower tentorial bars on which the mandibular sclerites articulate are in comparison with the other *Volucella* species only drawn out a little. Keilin (1915) thought that in cyclorraphan larvae that are saprophagous or feed on microorganisms such as bacteria, a filtering apparatus is present on the floor of the cibarium, which is built in a characteristic manner from grooves, strips and filaments. It serves to filter solid particles from the sucked up fluid. Larvae that feed on living plant material, predators or parasites possess no such filtering apparatus.

Thus in *bombylans* and also *pellucens* we find a well developed sieving apparatus with 8 ridges and corresponding rows of filaments, creating 7 grooves (Fig 47a, 48). In contrast, *inanis* only has four weakly developed grooves and short filaments (Fig 47b), which clearly are to be taken as rudiments rather than as a functioning sieve arrangement. A food sieve is useful when food particles must be filtered from a fluid. In the case of *bombylans* and *pellucens*, the food in question appears however to be of rather solid consistency, so that the sieving apparatus that is present would only be meaningful if the mixed-in saliva could be reclaimed from the food porridge and used again. In comparison with other saprophages the salivary glands of *bombylans* and *pellucens* are clearly longer, but also not so long as in the aphidophagous Syrphinae. As in the latter, perhaps also here the food is strongly mixed with saliva and perhaps already predigested.

The L3 larva of *inanis* in contrast has only a relatively short pair of salivary glands. Perhaps the inner organs of the wasp prepupa (via histolysis) are more easily sucked out of the body than these are in the case of aphid nymphs and adults. Moreover in *inanis* the labium is constructed so that with it, solid particles can be conveyed further into the mouth.

In the ratio of the lengths of the sections of the gut, Krüger (1926) found a clear difference between saprophage and zoophage syrphid larvae. The ratio of the mid- to the hindgut in the first group amounted to 3:4 or 1:1, whilst in the second group the hindgut was about a third the length of the midgut. The three *Volucella* species studied all have about the same length gut sections. Clear differences lie in the construction of the four diverticula at the beginning of the midgut. In *bombylans* and *pellucens*, these reach about the same length as the foregut, whilst at the corresponding place in *inanis* there are only conical tubercles present. Correspondingly other saprophagous syrphid larvae have likewise long diverticula, whilst carnivores have only short stumps. These gut appendages should also be recognised as caeca, which act to absorb amino acids, release digestive secretions, and store endosymbionts (Wigglesworth 1984).

In the construction and structure of the cps and the digestive tract, *bombylans* and *pellucens* show broad similarities. From the drawings of Künckel d'Herculeis (1875) this is also true of *zonaria*, which is scarcely different from *pellucens*, not only in

exterior morphology but also in anatomy. Krüger (1926) and Hartley (1963) classified the head skeleton of *pellucens* as the saprophagous type. Krüger wrote: "I did not establish experimentally that *Volucella* lives on wasp larvae, and hence I must assume from the construction of the head skeleton that it feeds on detritus of whatever sort in wasp nests". The feeding experiments in which only living host larvae were necessary describe an unnatural situation, and they allow no reliable statements to be made about the nutrition under natural circumstances. Against this, statements which follow from the construction, the presence or the absence of complex morphological structures, rest upon adaptations selected for under a well-defined nutritional mode.

In Table 23 there are placed together for comparison some specific characters and their occurrence in the studied *Volucella* species as well as in some aphidophage and saprophage syrphid species (data from the literature).

[ Table 23: Comparison of the cps and gut in larvae of the different *Volucella* species and in aphidophagous and saprophagous syrphid larvae. (compared are Mandibular lobes, Filtering apparatus, Gut ratio, and Diverticula (caeca)) ]

From this on the one hand one can conclude that clearly *bombylans* and *pellucens* are saprophagous, and on the other that there are recognisable differences between the parasitoid larva of *inanis* and aphidophagous larvae.

## 8 *Volucella* larvae as commensals and parasitoids (Summary and discussion of larval biology)

### 8.1 Host relations in social insects

Lit: Synectres, synoekes, symphiles: living in nest.

*Volucella* first found by Reaumur in bumblebee nests; then found in wasp nests. They could be commensals, or parasites/predators. No difference was seen between the various species.

In the following, the host relations of the various *Volucella* species to hand will be discussed individually on the basis of results on the lifecycle and nutrition of the larvae (section 6.2) and the different morphology of their cps and gut (ch 7), and compared to the corresponding literature and supplemented.

### 8.2 *Volucella bombylans*

The larvae are exclusively found in the nests of various bumblebee species that are in (many species) or on the ground (*Bombus pascuorum*). Until now larvae have never been found in nests of *Bombus hypnorum* or *pratorum* that are found a long way above the ground, e.g. in bird nestboxes. The assertion that *bombylans* is also sometimes in wasp nests (Verrall 1901, Kistner 1982, Stubbs & Falk 1983, Ohara 1985a) is based exclusively on the writings of Smith (1852) and Newstead (1891).

Smith (1852) who allegedly found *bombylans* larvae in a nest of *Paravespula vulgaris* did not mention whether he was able to obtain adults from them, so that a confusion with the larvae of *pellucens* cannot be ruled out. The fault of the mixup could be due to Reaumur (1738), who described *pellucens* larvae as *bombylans*. Newstead (1891) reported that the larvae of *bombylans* var *plumosa* were very common in the nests of *Paravespula germanica*. His records of the collecting dates were from August to October, which however corresponds to the phenology of *pellucens* or *zonaria* (see Fig 3); larvae of *bombylans* are in my experience only found in host nests until the end of August. In his extensive list of the fauna associated with wasp nests, *pellucens* does not occur, although usually it is common in such nests, and this suggests the conclusion that the species affiliation of the larvae cannot be correctly recognized.

From my own samples and observations on the nests of several bumblebee species, *Volucella* larvae are always found outside or underneath the actual brood nest (6.2.1; Fig 54; cf. Alford 1975). There are also never any traces of larval meals in the form of eaten bumblebee larvae or pupae (in the laboratory bumblebee colonies the disposal of injured brood from the nest was recorded).

### 8.3 *Volucella pellucens*

### 8.4 *Volucella inanis*

## 9 Model of the evolution of larval parasitism in *Volucella*

## 10 Mimicry: introduction and definition

Before describing and discussing the bumblebee- and wasp-like similarity of *Volucella*, first we describe the general assumptions and conditions of mimicry.

HW Bates (1862) outlined the deceptive similarity of unrelated butterflies with respect to their wing colours and patterns as an adaptive appearance which he called mimicry. Thus one species counterfeits the unpalatability of another by mimicking its appearance to gain the same protection from predators.

After this an abundance of cases of mimicry were discovered and described, but many similarities were misinterpreted from far too speculative and unscientific leaps, and the concept of mimicry was criticized, partly justifiably (Heikertinger 1954). To obviate these speculations, restrictive criteria were drawn up; thus in the case of so-called Batesian

mimicry, the following assumptions must be fulfilled (Rettenmeyer 1970):

- the model is unpalatable or dangerous to a predator
- the mimic is palatable, but in its appearance it matches its model so closely that it is avoided by the same predators through being confused [with the model].
- the mimic is rarer than its model, and occurs in the same habitats
- model and mimic are striking to the eye [aposematic]; a predator associates the colour pattern with the unpalatability of the model.

Today we differentiate between Batesian and other forms of mimicry, e.g. aggressive, Mertensian and automimicry, which all represent special adaptations in similarity for mimicking models, but which are the consequences of different selection pressures. Wickler (1968) gives [...]

### 10.1 *Volucella*: an example of mimicry

On sight, an unbiased observer would take most hoverflies for wasps, bees or bumblebees rather than flies. The exterior appearance of many species of this family of flies with various noxious hymenoptera suggests mimicry (Kirby & Spence 1817, Wickler 1968, Rettenmeyer 1970), which should function as protection from predators.

Recently however attention has been drawn to critics of this, that the striking colouration of syrphids might not serve as mimetic signals in every case, and that we should also seek other possible interpretations (Gilbert 1986). Thus Holloway (1976) considered the similarity of *Eristalis* to honeybees not as the result of Batesian mimicry, but as convergent evolution caused by analogous behavioural techniques in collecting food, since both are flower visitors. Burt & Gatz (1982) draw attention to the possibility of colour and pattern convergence in species exposed to similar physical and environmental conditions of selection in the same habitats.

To what extent the appearance of some *Volucella* species can be attributed to mimicry is discussed in the following from the literature and my own observations, and will be shown to correspond to the above criteria for mimicry.

### 10.2 Mimetic similarity

Reaumur (1738) had already characterised representatives of the genus *Volucella* as being similar to wasps and hornets.

*Volucella mexicana* from North America is easily confused with the co-occurring carpenter bee *Xylocopa tabaniformis*, corresponding to it in size, form and the colouration of the body and wings; in addition, in its visits to *Solanum* flowers it shows the typical bee-like 'buzzing' (Jones & Little 1983).

Of the five European species of *Volucella*, three of them have mimetic similarity to wasps or bumblebees. *V. inanis* and *V. zonaria* resemble wasps (e.g. *Paravespula vulgaris* and *P. germanica*) or hornets in their colour patterns and size. Both species have a yellow abdomen with black transverse bands. The 5-mm larger *V. zonaria* (19.5 mm) is reddish-brown on the thorax, scutellum and wings, whilst *V. inanis* is black and yellow and has lighter-coloured wings (Fig 9). *V. zonaria* has the greater similarity to hornets in its size and colouration (Fig 10).

*V. bombylans* resembles various bumblebees with its different colour morphs (Fig 7; Table 28). In contrast to the above species, *V. bombylans* bears strikingly long hairs on its thorax and abdomen. The various colour morphs are realized via distinct colours (black, yellow, red) in the hairs of the thoracic and abdominal segments. In addition to its morphological and colour similarities with bumblebees, *V. bombylans* has also behaviours typical for bumblebees and other bees. Analogous to these [bees], when threatened, male and female *V. bombylans* stretch out their anterior legs forwards and wave them about, buzzing loudly (Fig 60). In contrast to bumblebees, which raise their middle legs, the flies therefore use their anterior legs.

Bumblebees show this behaviour for example when having spent the night outside the nest they are disturbed, still numb, hanging from a grass stem. In *V. bombylans* this behaviour can always be observed when they are disturbed - perhaps by movement of the cage - and the temperature is low (10-15 C), or when individuals are found in the vegetation during equally bad weather. At higher temperatures they try to fly away immediately. This behaviour has never been seen when female *Volucella* are in the bumblebee nests and meet worker bumblebees.

In connection with this behaviour in *V. bombylans*, the buzz produced was compared<sup>able</sup> with the so-called defensive buzz of bumblebees. Bumblebees produce this typical buzz without any obvious wing movements (thorax buzz) above all when they are disturbed in the area of the nest (Schneider 1975).

### 10.3 Mimetic polymorphism in *Volucella bombylans*

The first taxonomic classification of *V. bombylans* was frustrated by the incorrect interpretation of the colour morphs. Thus in the first descriptions of Linnaeus (1758: *Musca bombylans*, *Musca mystacea*), as in numerous other treatments (described in Künckel d'Herculais 1875), the various colour morphs of *V. bombylans* were initially taken as good species.

Then Macquart (1834) first described *V. plumata* (*mystacea*) on the basis of morphological correspondence as a 'constant variety' of *V. bombylans*. From observations of copulation between morphs (Zeller 1842), discovery of rare intermediate morphs (Erichson 1842) and emergence of the entire spectrum of morphs from a single bumblebee nest (Drewsen 1847), the various morphs were later recognised as varieties of a single species.

The simultaneous presence of several genetic different discontinuous mimetic phenotypes within one population was labelled as mimetic polymorphism. The rarest morph must appear frequently enough that it is not regarded as the

result of repeatedly occurring mutation (Dietrich & Stocker 1968).

EB Ford (1965, 1975) and Clarke & Sheppard (1960a,b) developed a model of the genetics of mimetic polymorphisms from the results of crossing experiments with the polymorphic Swallowtail *Papilio dardanus*. In this the expression of the colour pattern occurs via a polygenic system whose function is turned off or on by a regulatory gene. Mimetic colour patterns do not develop from a single mutation, but evolve gradually via the accumulation of genetic modifiers which alter the expression of the gene responsible for the pattern.

The initial development of the genetic basis of the colour polymorphism in *V.bombylans* was done by Gabritchevsky as early as 1924, and is still valid today. He raised the F1 offspring from known parents and reconstructed a combination of possible genotypes and a genetic mechanism on the basis of the resulting distribution of colour patterns. Keeler (1926) could show that the morph frequencies found by Gabritchevsky corresponded to those predicted from Mendelian genetics.

The colour morphs appear essentially in three patterns (Fig 7) which are clearly different from one another and are similar to different bumblebee species (Table 28):

Vbb (*V.b.bombylans*) = black-red = *lapidarius* type

Vbp (*V.b. plumata*) = yellow-black-white = *terrestris* type

Vbh (*V.b.haemorrhoidalis*) = yellow-black-red = *pyrenaicus* type

Gabritchevsky described other variants, but in compared to the above types these only show small differences in characters and can be clearly classified with these three.

The mimetic colours arise from four alleles on two non-sex-linked gene loci. The hairs of the thorax and first abdominal segment are either black (S) or yellow (G), and the colour of the last abdominal segments is either red (R) or white (W): these are the individual factors. Table 26 shows the corresponding genotypes of the three phenotypes Vbb, Vbp and Vbh. In the expression of these characters, black is dominant to yellow. The dominance of the tail colour is connected with allele combination of the thoracic hairs (epistasis). In homozygotes of the thorax allele (yellow), the abdomen becomes light, and also when the heterozygote is present; however, if the black S allele is present (heterozygote), the abdomen tip is always red. Examples where the tail is coloured light red or yellowish (instead of white) witness the intermediate expression of the heterozygote. Evidently not all theoretical genotypic possibilities are realised as phenotypes, since no *V.bombylans* have been found with black thoracic hairs and white tails, which should have the allele combination of SS/WW.

With the 6 genotypes shown in Table 26, there are 21 different pairing combinations possible. From these all three phenotypes could appear together in the F1 generation from two of these pairings (figure shown).  
[...not so easy in *Merodon* - see Conn ...]

The phenotypic spectrum of morphs of *V.bombylans* is determined from the genotype frequencies in the parental population. Under the assumption of the same proportions and panmixis of the different genotypes, we could expect the following phenotypic frequencies of the colour morphs according to the mapping of genotypes to phenotypes in Table 26: *bombylans* 53%, *plumata* 33%, *haemorrhoidalis* 14%

The relative frequencies of the different morphs in different regions (Table 27) deviate from this, sometimes considerably. This in the Swiss Alps (Mathon) the yellow-white morph (*plumata*) was captured about 20% more commonly than the black-red morph (*bombylans*), whilst this ratio was reversed in the southern Rhineland and the yellow-red morph (*haemorrhoidalis*) was represented only by a single individual. In this region only a few flies were caught each year, and thus the catch of several years was added together.

Gabritchevsky mentioned that he was not able to find any individuals of the *bombylans* morph in various regions of Russia (around Moscow, and Voronezh in southern Russia) even though he had caught more than 100 individuals. One of the reasons for these differences in phenotype frequencies could be the consequences of different selection pressures on individual colour morphs in different areas. Since the similarity of the flies to bumblebees is understood as imitation or mimicry, the frequency of the models should exercise a real influence on the presence of the corresponding mimic. A colour morph whose model is very rare or not present in the same habitat will in the case of protective mimicry suffer a greater predation pressure as against a morph with a greater model density, so that its frequency will reduce in the population.

[... Papilio case from Ford 1975...]

If we compare the situation in the Mathon study site with the Rhineland, it is striking that the yellow-red bumblebee models of the *haemorrhoidalis* morph constitute a large proportion in the Alps (up to 50%), whilst on the plain [of the Rhine] they are represented at most by one bumblebee species (*B.pratorum*) at a small proportion of the model numbers (up to 6%) (Table 28). Moreover, *Bombus pratorum* is a bad model of this colour morph, since the yellow thoracic ring is scarcely formed in many workers of this species, so that as models they are classified rather as the black-red colour morph. In England and Denmark also there is no suitable model for the *haemorrhoidalis* morph apart from *B.pratorum* (Alford 1971, Plowright & Owen 1980).

Corresponding to this model situation, the *haemorrhoidalis* morph of *V.bombylans* was present only in the Alps at a higher frequency (up to 20%), whilst in the Upper Rhineland (Table 27) and according to the data of other authors (Verrall 1901, Lundbeck 1916, Stubbs & Falk 1983) also in England and Denmark there are almost exclusively the two morphs *bombylans* and *plumata*.

Since the various bumblebee species have different ecological preferences, we find different species spectra and

relative abundances of colour types in the various habitats such as woods, wood edges or fields (Postner 1952, Reinig 1970). Thus within a region there can be strong heterogeneity in the model community. In Table 28 in the area around Erlangen the frequency of the various bumblebee species has been added together from various habitats. These together should represent the general species and colour-type composition of central Europe.

The frequency of different fly morphs is not directly proportional to the frequency of the corresponding models. Thus in the Swiss Alps the *haemorrhoidalis* morph was a bit less common than the other morphs, whilst the proportion of bumblebees of the yellow-red colour type (*B.pyrenaicus* type = *haemorrhoidalis*) was to some extent greater than the other colour types.

If all models were equally common and equally unpalatable or noxious to predators, then all mimics should get the same protection. Corresponding to the described inheritance method in *V.bombylans* with different allelic dominance, this leads to unequal phenotypic frequencies. The unexpectedly low number of the red-black *bombylans* morph in Mathon could be explained for example by a lower protection provided by its corresponding model. This the bumblebee *B.mastrucatus* commonly present in the Alps, which can be assumed to be the model there, is far less aggressive than its colour-type equivalent in the plain *B.lapidarius*, or than the yellow-white morph pattern of *B.terrestris* and *B.hortorum*.

The influence of the model on morph development is particularly clear in the Caucasus and North America, when compared to the model-mimic situation of *Volucella* in Europe. In the Caucasus the black-red *bombylans* morph common in Europe is missing, and instead we find a colour type with white hair on the thorax and first abdominal segment and a red tail, called '*caucasica*' by Gabritchevsky (1924). This morph resembles the common bumblebees there, namely *B.erophorum* and *B.niveatus*.

The bee-like *Volucella* in North America, which other than the colour morph show no differences in either lifecycle or morphology from *V.bombylans*, were classified by Johnson (1916) into three so-called subspecies with different varieties. The *Volucella facialis* group occurs in the West and in Canada, *V.evecta* in the East, and *V.arctica* in the arctic zone. North American bumblebees are yellow-black or yellow-black-red in colour, and the black-red or yellow-black-white patterns typical of Europe do not occur here. Gabritchevsky (1926) found correspondence in the various regions between *Volucella* and the common bumblebees. Thus *V.evecta americana* occurs on the East coast and with a typical yellow-black pattern resembles the bumblebees present there, such as *B.vagans*, *B.affinis* or *B.impatiens*. In the mountains of Colorado, Utah and New Mexico *V.facialis rufomaculata* has the same yellow-black-red-yellow bands as the bumblebees there, such as *B.sylvicola* and *B.melanopygus*.

Gabritchevsky (1926) showed the similarity of different bumblebee species to one another (mimicry rings) and with other flies of different families occurring in the same areas. In what respect there is a comparable colour polymorphism in the american *Volucella* to that of *V.bombylans* in Europe or in the Caucasus is not assessable from the descriptions of Gabritchevsky.

The origin and development of a mimetic colour polymorphism appears above all to stand in contradiction to the purifying selection (lit. 'canalisation effect'), which predators exert on models and mimics, and which leads to a signal standardisation (Fisher 1958, Wickler 1968, Nur 1970, Huheey 1976). While it is plausible that a mimetic species that imitates different models can with part of its population escape for example the search image of a predator and with it in the sense of spreading of risk possesses a better chance of survival (Rettenmeyer 1970), we must present the selective forces for an evolutionary understanding, under which these different mimetic colour forms come into being convergent [??] and are able to be stabilised.

A well-studied example of a sympatric polymorphic mimetic colour pattern is the South American butterfly species, *Heliconius numata* (Brown & Benson 1974). [...]

A similar case was described by Plowright & Owen (1980) in the dimorphic bumblebee *Bombus rufocinctus*. [...]

Also in *V.bombylans* the origin of the mimetic polymorphism can be attributed to different and changing model ratios, from which distinct mimics are selected and improved by disruptive selection. Since in *V.bombylans* no morph-specific host-finding behaviour could be found (see 4.2.6), it is probable that the mimetic polymorphism is stabilized by predators in the setting of a Batesian mimicry.

#### 10.4 Predators and models

Of the group of potential predators such as insectivorous birds, small mammals, lizards, frogs, spiders and predators insects (such as robberflies and wasps), birds should play the main role as predators of large insects (Rettenmeyer 1970, Evans & Waldbauer 1982). In the case of Batesian mimicry, effective models must be noxious and/or unpalatable to these predators. This is sufficient already since this model gets relative protection by this. From specialized wasp- and bumblebee predators such as Honeybuzzard, Bee-Eaters, Spotted Flycatchers and Red-Backed Shrikes, wasps and bumblebees get no absolute protection (Mostler 1935).

Experimentally it could be shown that insectivorous birds avoid hymenoptera after they have experienced them. From feeding studies, birds spat out again their food when this contained material from inside the abdomen of wasps (*Paravespula*) and honeybees (*Apis mellifera*) (Mostler 1935, Liepelt 1963); Evans & Waldbauer (1982) found the same reaction in feeding *Bombus pennsylvanicus* to Red-Winged Blackbirds (*Agelaius phoeniceus*). Even when the poisonous sting was not used immediately, they were not eaten by the birds. In Mostler's (1935) experiments, different birds (list) showed no rejection or avoidance reactions after eating the abdominal contents of worker bumblebees (*B.rajellus*, *B.variabilis*), but naive birds were exhausted after eating an entire bumblebee, and later showed no further interest in other bumblebees.

Davies (1977) found that a Spotted Flycatcher (*Muscicapa striata*) which could eat noxious hymenoptera, took only

1.4% bumblebees in their diet, although they represented 10.1% of the available insect prey. Davies attributed this to the large time cost that the birds would have to put up with in removing the sting from bees and wasps ("optimal foraging").

The birds learn to avoid these insects as unpalatable prey objects, and associate their negative experiences with recognizable signals such as the colour, patterns, sounds and behaviour of the supposed prey (Wickler 1968, Edmunds 1974, Vane-Wright 1980). Particularly striking colour patterns such as the so-called warning colours could facilitate this association (Rettenmeyer 1970). As well as a learned avoidance reaction, there could also be innate avoidance tendencies present. Thus in experiments young starlings rejected warningly coloured black-yellow pattern insect models from the beginning more commonly than unicoloured green, brown or yellow ones (Schuler 1982). In choice tests, at first inexperienced chicks picked up yellow-black or green painted mealworms, but only ate the green ones. The observed inhibiting reaction against yellow-black prey could be attributed to the visual effect of the colours, but not to differences in taste. In training tests, chicks learned very easily to reject black-yellow mealworms rendered bitter-tasting, and to eat palatable green ones. In the reciprocal experiment, they did not avoid the bitter green mealworms, but took them in equal amounts to the palatable yellow-black patterns (Schuler & Hesse 1985). Typical warning colours like black-yellow or red-black are above all directed to the attention of vertebrates.

### 10.5 Effects of deception

Mostler (1935) was able to establish for the first time convincingly the effectiveness of visual imitation of a wasp-, honeybee- or bumblebee pattern by syrphids in the sense of the mimicry hypothesis. In his studies different songbird species avoided wasp-, honeybee- and bumblebee-like syrphids after having had experience of their corresponding models (cf. description of methods). Still inexperienced young birds ate almost 100% of the various hymenopteran mimics. Since the mimetic syrphids were obviously palatable to the birds, there can be no question of Müllerian mimicry.

As bumblebee mimics, Mostler used the *plumata* (*terrestris* type) and *bombylans* (*lapidarius* type) morphs of *Volucella bombylans* in his experiments. Although he only had 25 flies for use in these experiments, and consequently only could carry out a small series of tests, the results were clear in their direction. For illustration they are shown summarized in Table 29. [...]

Mostler commented on his results as follows: "The convergence in form, colour, type of flight and flight tone evokes the same mistakes as in impartial human observers".

Already Pocock (1911) was convinced of the effectiveness of the deception by *V. bombylans*. In his experiments also, different birds avoided the flies after they had had experience of the model *Bombus hortorum*. A critical objection to his studies was, however, that he had used tropical birds from the zoo, the test insects were offered by hand or by forceps to the tame birds, and he conducted few experiments. In new tests, Evans & Waldbauer (1982) demonstrated the effectiveness of mimetic similarity. The bumblebee mimic *Mallota bautias* (Syrphidae) was avoided to the same degree as the model *Bombus pennsylvanicus* by wild-caught Red-Winged Blackbirds and young birds that had experienced the models.

Not only in birds, but also in toads, the effectiveness of the mimicry has been demonstrated. Toads (*Bufo terrestris*) that had had experience of the model *Bombus americanorum* avoided the mimetic fly *Mallophora bomboides* (Asilidae) significantly more frequently than inexperienced animals (Brower et al 1960).

In addition to the effect of visual signals, the influence of acoustic cues can also be assumed in all these cases. In tests of Brower & Brower (1965) hoverflies (*Eristalis*) with amputated wings (and hence no buzz) were eaten more frequently than buzzing flies. The flight tone of *Eristalis tenax* is only slightly lower in frequency than that of honeybees (Busnel 1963). Gaul (1952) saw a case of audio mimicry in the correspondence in flight tone frequency between the wasp-like hoverfly *Spilomyia hamifera* (147 Hz) and the wasp *Dolichovespula maculata* (150 Hz).

The consequence of deception is connected with the ability of the predators to differentiate between model and mimic. This is influenced by the availability of non-mimetic alternative prey. When prey density is high, predators concentrate on the palatable alternative. But when these prey first become scarce, a predator differentiates more carefully between model and mimic, by which the protection of the mimic probably decreases more strongly than that of the model. From this derives an increased selection pressure on the mimic, which then leads to an improved similarity to the model (Schuler 1974).

Mimic and model could also be separated in time. Waldbauer et al (1971, 1977, 1985) found that in different places in North America most of the wasp and bumblebee mimicking flies appeared in spring, whilst at this time the numbers of the corresponding models were low. The authors interpreted this asynchronous phenologies as the result of selection by fledgling birds. Mimics have already completed their activity maximum when young birds are leaving the nest. These are then mainly confronted with the unpalatable models. This acquired experience is already present when the birds return from their winter quarters and this then protects the mimics that are present.

### 10.6 The question of aggressive mimicry

According to the results of the experiments carried out with potential predators and wasp and bumblebee mimics (see 10.4, 10.5), the development and effectiveness of Batesian protective mimicry can be assumed for the mimetic species of *Volucella*. However with regard to the particular situation of oviposition and larval development in *Volucella*, it seems first of all obvious to interpret the mimicry of *Volucella* species also as a special mimicry of the host.

Thus the striking similarity of flies with wasps or bumblebees had already been discussed by Kirby & Spence



(1817), Wallace (1871) and Kunckel d'Herculeis (1875) in connection with entry of the flies into the host nest. They supposed that perhaps *Volucella bombylans* could get into the host nest unmolested because of their similarity to bumblebees. For this reason Poulton (1890, 1892a,b) classified the resemblance of *Volucella* as a case of aggressive mimicry (Upon the criticism of Bateson 1892 and after some of his own later observations, he described *Volucella* as an example of Batesian mimicry in his 1904 work).

In aggressive mimicry with two contributing species, the model is deceived by the mimic. Model and signal received are identical (Wickler 1968). [Examples ...]

In the following, both mimicry systems, protective and aggressive mimicry, are described schematically for the case of *Volucella*.

A mimicry system can only be keyed in when the individual elements and their roles are known. For this, the deception or the signal receiver take a central position. A clarification requires both comprehensive knowledge of the life cycle and the interrelations of the partners, and also information about the particular perceptual abilities of the receiver. This was however not wholly possible in the course of this work. However, the present results of the study (host finding, nest searching and larval development) allow a discussion of the significance and effectiveness of any aggressive mimicry in *Volucella*.

For aggressive mimicry, the following criteria and conditions should occur:

- 1) Host wasps and bumblebees recognise their nest membership, above all from the appearance or behaviour, and hence differentiate at the nest entrance between nest members and intruders.
- 2) Mimetic *Volucella* species will not be attacked, or will be attacked less commonly, by the corresponding hosts than non-mimics.
- 3) In the case of *Volucella bombylans*, the corresponding colour morph seeks out the host that is an adequate model (morph specificity)
- 4) The larvae have a predatory or parasitic lifestyle and harm the host

wrt 1) Whilst in social bees a complex shape and colour vision has been established (review by Menzel 1987), which should be the same in wasps, recognition of nestmates at the entrance occurs in the first instance by olfaction of the specific nest odour (Free 1958, Free & Butler 1959, Edwards 1980). It is a reasonable assumption that intruders also can be recognised in the same manner. Therefore it would be expected that female *Volucella* would use an imitation nest odour as a mimetic signal for invading the nest.

The use and significance of visual mimicry of *Volucella* females in the course of nest searching to the time spent by the flies in the area of the nest entrance are limited, since perception and effect of the colour pattern is not possible in view of the darkness of the nest tunnel and nest hole.

wrt 2) *V. bombylans* females linger about 24 mins immediately in front of the nest entrance before entering into the nest space (see 4.1.1). The reaction of the bumblebees during this situation in the form of flying towards the flies (without landing) could be observed only extremely rarely, but this was also the case for insects not resembling bumblebees, such as scorpionflies, fleshflies and crickets. Also pinned hoverflies with wasp markings, wasps and other insects placed near the nest entrance (about 3 cms from the flight aperture) were not noticeably paid any attention by the bumblebees (see 5.1.1). Since worker bumblebees in the area of the outer entrance of their nest also showed so little interest in non-mimetic insects, a possible outcome of deception of *Volucella* females as "flies in bumblebee clothing" could not be proved. In this situation, deception is probably not required at all.

wrt 3) A possible significance of visual mimicry in considering aggressive mimicry could in the case of *V. bombylans* also be demonstrated in the behaviour of the female flies. Females of the different colour morphs should seek out those species of bumblebees that correspond to their own colour pattern. In various choice tests, however, no indication of morph specificity could be established (see 4.2.6). *Volucella* females sought out just as frequently and successfully bumblebee species of inadequate or dissimilar colour-pattern types.

wrt 2) According to the present observations and studies (see 5.1.1), female *V. bombylans* only enters successfully into bumblebee nests when they find no bees in the area of the entrance or the tunnel. The flies avoid direct contact with the workers. In individual observations where female *V. bombylans* encountered bumblebees in the nest area, the flies were attacked. When they did not succeed in arriving safely into the nest material, they were stung by the bumblebees.

It seems very improbable that the hairs play a role as a tactile signal, or the buzz as an acoustic cue, in the sense of aggressive mimicry in encounters within the nest, but with these observations alone nothing can yet be concluded.

Thus Gabritchevsky (1924) assumed that as a result of the buzzing of an attacked female *Volucella*, bumblebees reacted less aggressively. Fisher & Weary (1988) could record a "buzzing" of *Psithyrus* females in the host nest, and supposed that this noise was used to subordinate the worker bumblebees.

wrt 2) The wasp-like *Volucella inanis* searches for the nests of *Paravespula* for oviposition. In the presence of guards, which is normally the case, the flies do not enter into the inner nest, but lay their eggs in the area of the outer entrance where there are no guards found (see 5.2.1). The nimble flies flee away at the close approach of a wasp. This behaviour becomes understandable when one contemplates the different reactions of the wasps towards females of *V. inanis* and *V. pellucens*.

While the non-mimetic female *V. pellucens* could remain in the nest entrance or inside the nest space in immediate contact with the guards without being attacked by the wasps, female *V. inanis* were killed with fierce intensity (see 5.2.1).

From this we can deduce a requirement for *V. inanis* to reduce this pressure of attack via visual similarity to wasps, mainly when they are still outside the nest. Since wasps commonly inspect the further surroundings (up to 10 cm) of the outer nest entrance, beyond the "circle of guards", as is the case in bumblebees, large insects which remain in this area are noticed and attacked. However discovery mostly takes longer for both mimetic and non-mimetic "intruders" than for example a female *V. inanis* needing to lay her eggs. In a few cases, pinned insects were paid no attention at all (2 hours). It seems improbable here also, therefore, that *V. inanis* females have a better chance of oviposition because they resemble wasps.

wrt 4) The vigilance of a signal receiver and with it the selection pressure on the mimic is also determined by the losses it (the receiver) suffers from the deception. Hence this brings into question in the mimicry system between *Volucella* and bumblebees or wasps the problem of the loss that the host suffers, and with it the life cycle of the fly larvae and their connection with the host. The development and host relations of the larvae is different in the various *Volucella* species. Whilst the larvae of *V. inanis* are parasitoids feeding on wasp larvae, the larvae of *V. pellucens* and *V. bombylans* mainly feed on detritus produced in bumblebee or wasp nests (see section 8). The harm to the host is obvious in *V. inanis*, but rather unlikely in *V. pellucens* and *V. bombylans* (we lack quantitative evidence).

Probably the latter in fact promote the cleansing of the nest space and a reduction in pathogenic infection with host flagellates, so that their presence could provide a benefit to the host (already JH Fabre expressed this view, but he applied it to the larvae of *V. inanis*: cited in Sharp 1901). From this, only in *V. inanis* are the necessity and conditions for the evolution and maintenance of a host deception present, whilst in *V. bombylans* and *V. pellucens* there is no selection pressure on the host to defend themselves against them.

The striking protection against attack of female *V. pellucens* (see 5.2.1) could then also not be understood as aggressive mimicry with e.g. olfactory signals. In known cases of aggressive mimicry, in which the mimics imitate the host smell, these live as predators of their hosts, such as the staphylinid *Trichopsenius frosti* with termites (Howard et al 1980), the scarabaeid *Myrmecaphodius excavaticollis* (van der Meer & Wojcik 1982) or the larvae of some species of the syrphid genus *Microdon* (Garnett et al 1985) with ants.

There is also no evidence of the possibility of host mimicry by the *Volucella* larvae, as in for example the larvae of the wasp *Echthrodape africana* in the nests of a south african anthophorid (*Braunsapis*) (Michener 1969), or in the *Microdon* larvae already mentioned before.

In contrast to the situation of ant- [orig. "wasp-"] and *Microdon* larvae, whose larvae and pupae imitate those of their host so perfectly that the adults treat them as their own brood, the adult hosts avoid *Volucella* larvae. The larvae of *V. bombylans* and *V. pellucens* remain outside the true brood chamber. *V. inanis* larvae are indeed found in the occupied brood cells, and here they can hide themselves underneath the wasp larvae so that they do not come into direct contact with the worker wasps (see section 6).

For the mimetic *Volucella* species, the effectiveness of an aggressive mimicry is neither demonstrable nor probable (cf. Speiser 1908, 1939). Also in other examples where both protective and aggressive mimicry appears possible, proof of aggressive mimicry has not yet been forced upon us. This is similar to a case comparable to the situation in *Volucella*. It is known that a representative of the african Asilidae, *Hyperichia*, resembles *Xylocopa* carpenter bees, and which at the same time number among their prey, and the *Hyperichia* larvae feed on the bee brood in their nests (Poulton 1927a,b). However, to what extent here the model also functions as the signal receiver is completely unclear. Thus it may also be shown that mimetic asilids of the genus *Mallophora* do indeed prey successfully on their model bumblebees (Brower et al 1960), but it remains open whether the bumblebees are deceived victims.

Since the hosts of mimetic *Volucella* species are at the same time their models, it appears at first glance plausible to see this similarity in connection with the host relationship, and to interpret it as aggressive mimicry. On the other hand, this host specificity also postulates a corresponding model situation for Batesian mimicry, since it is only possible for *Volucella* to develop and multiply where their hosts (models) are also present in abundance. It is therefore sensible to imitate their host, since they are present together with them with greater probability.

This operates in a similar manner for the various *Psithyrus* species, which are also very similar to their host bumblebees in colour pattern, each behaving host-specifically, and whose convergence in colour pattern is considered to be Müllerian mimicry (Reinig 1935, 1969; Plowright & Owen 1980).

The wasp and bumblebee similarity in *Volucella*, just as in the numerous other wasp- and bumblebee-imitating syrphids which have no direct connection with their models, has been clarified as protective mimicry. This is also true for the mimetic polymorphism of the colour pattern in *V. bombylans*. A number of other syrphid species (*Criorhina ranunculi*, *Eristalis intricarius*, *Merodon equestris*) have a similar polymorphism of colour pattern, although their larvae develop not in bumblebee nest but in plant substrates.

### The European species of the genus *Volucella* (Diptera, Syrphidae) as commensals and parasitoids in the nests of bumblebees and social wasps: studies on host-finding, larval biology and mimicry

A comparative study was conducted on four European species of hoverflies from the genus *Volucella* (*bombylans*, *pellucens*, *inanis*, *zonaria*), whose larvae live in the nests of bumblebees and social wasps, in order to analyse their development and way of life with respect to their host relationships.

The aims of the study were to discover (a) the host-specific adaptations and strategies for host finding (b) the location of the larva within the nest of the host and larval feeding behaviour (c) possible species specific differences with respect to host relationships (d) the significance and effectiveness of the resemblance of adult flies to their hosts - bumblebee and wasp mimicry - studied with respect to their successful infiltration of the host nests.

*Volucella* adults as well as bumblebee and wasp nests were collected in the field. The hosts were bred in wooden boxes. The fly larvae were kept in the laboratory as well as in host nests. Host-finding and nest-visiting by female *Volucella* were investigated through field observations of behaviour as well as through experimentation. Adult antennae, eggs, larval cuticular structure and cephalopharyngeal skeletons were investigated morphologically using light microscopy and SEM.

**Host-finding.** To lay their eggs, *Volucella* females seek out the nests of their hosts. Finding and recognizing the host is made possible by the triggering stimulus of the host odour in combination with the appearance of host adults. The odour of bumblebee workers and/or nests triggers the following specific behaviour in females of *V. bombylans*: intensive cleaning and protrusion of the ovipositor. This behaviour is also exhibited by females under natural circumstances before they enter the tunnel leading to a bumblebee nest. *V. bombylans* females approach such an odour source directly. Thus to find the entrance of a bumblebee nest they need only the olfactory sense (within a radius of 1 m). Without the presence of bumblebee odour, *V. bombylans* females showed no reaction to acoustic or visual stimuli in the form of a bumblebee flying behind Plexiglass. *V. pellucens* and *V. inanis* females also reacted to the odour of a host wasp nest with protrusion of the ovipositor, yet they did not approach the source of the odour. Presumably these species require the appearance of wasps as an additional visual stimulus. No morph-specific behaviour was found in *V. bombylans*. Females infiltrated nests independent of the respective colour patterns of the bumblebee species. The strikingly branched arista found only in *V. bombylans* females (sexual dimorphism) is interpreted as an adaptation for staying in bumblebee nests. Apart from serving as a mechanical protection for the funiculus, this branching of the arista could aid the fly in its tactile and acoustic orientation within the passageways of the underground nests.

**Nest infiltration.** Bumblebees and wasps defend their nests against invaders. The entrances of wasp nests are generally guarded by several workers. Among bumblebees, however, this is only the case in large colonies. *Volucella* females of the different species have different strategies for invading nests and correspondingly trigger different reactions among the host adults. Before entering a bumblebee nest, *V. bombylans* females remain in the vicinity of the entrance for an average of 25 mins. They avoid contact with the bumblebee workers and infiltrate the nest only when the passageway is free of incoming and outgoing workers. When the *Volucella* arrive in the nest, they bury themselves in the outer material of the nest. The female flies leave the nest after approximately 25 mins. *V. pellucens* females enter the wasp nest with a stream of arriving wasps only a few mins after approaching the entrance of the nest, and remain on the outer layer of the nest for approx 30 mins. *V. inanis* females do not enter a normally busy wasp nest, and remain only in the outer entrance area. Wasps (*Paravespula*) react to *Volucella* species in the entrance of their nest differently: *V. inanis* females are attacked aggressively and stung to death; *V. pellucens* females are not attacked. It is discussed whether the protection from attack of *V. pellucens* females is due to chemical mimicry or to pheromones which repel or inhibit aggression.

**Eggs:** The eggs of *Volucella* are different in size, form, structure of the chorion, number and the location in which they are laid. Compared with *V. bombylans* and *V. pellucens* (approx 60 eggs), *V. inanis* has a conspicuously large number of eggs (approx 300). This is interpreted as compensation for the higher risk of larval loss. Because the eggs are laid outside the host nests, they are less protected and the hatched larvae must cover a large distance to arrive at the wasp brood.

**Larvae:** According to their location within the host nest, as well as the structure of the cps and digestive tract, the larvae of *V. bombylans*, *V. pellucens* and *V. zonaria* live as saprophages. Thus they can be characterised as commensals in bumblebee and wasp nests. Attacks on the host brood could be observed only once the colony was dissolving and the reproductives had already hatched. The development of *V. inanis* larvae is closely adapted to that of the host brood, on which they live as parasitoids. All three larval stages are very similar in *V. bombylans*, *V. pellucens* and *V. zonaria*. They have larval features typical of saprophagous syrphid larvae such as mandibular lobes, cibarial ridges and long caeca. *V. bombylans* were only found in ground nests of bumblebees. They are present only outside the actual breeding area in the outer nest material, as well as under the nest. *V. pellucens* larvae were only found in underground nests of *Paravespula vulgaris* and *P. germanica*. They were in the ground beneath the opening of the nest, where faeces and detritus fall from the breeding area. A description was given of the larval development of *V. inanis*. The larvae live in the underground and 'attic' nests of *P. vulgaris* and *P. germanica*. The different instars are clearly differentiated in appearance. Thus L2 is dorsoventrally flattened, an adaptation to living in cells with a living wasp brood. The short caeca and the lack of a functioning cibarial filter are characteristic of parasitic feeding. L1 and L2 larvae feed on the haemolymph of 4th and 5th instar wasp larvae without killing them. The 2nd instar of the fly remains inside the cell while the wasp larva spins its cocoon. Afterwards the

*V.inanis* larva moults into the 3rd instar, which completely sucks out the wasp prepupa. The mature fly larva leaves the cell and burrows into the ground beneath the host nest for diapause.

**Evolution:** the parasitoid life habit of *V.inanis* is considered a derived feature for the genus *Volucella*, since the larval development is complex and since the cps could have developed from saprophagous species. Since the wasps show different behaviours towards the females of *V.inanis* and *V.pellucens*, these reactions are attributed to the respective life styles of the larvae. *V.inanis*, as a parasitoid, can reduce the reproductive success of a wasp colony. Therefore a selection pressure exists to prevent invasion of this fly. The saprophagous larvae of *V.pellucens* probably contribute towards nest hygiene, and therefore they are welcomed guests.

**Mimicry:** The visual resemblance of *V.bombylans* to bumblebees and that of *V.inanis* and *V.zonaria* to wasps is regarded as a form of protective mimicry. No evidence was found for aggressive mimicry in which the host was deceived. The development and stabilization of the mimetic colour polymorphism in *V.bombylans* can be explained as an adaptation to locally changing models, and thus a case of protective mimicry.