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Host recognition in a pollen-specialist bee: evidence for a genetic basis*

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Abstract – To investigate the effect of larval pollen diet on floral choice in a specialized bee species, we compared the floral preferences of individuals of *Heriades truncorum* (Megachilidae) reared on host pollen with those of individuals reared on two different types of non-host pollen. Females were allowed to nest in cages where both host and non-host flowers were available. All females, regardless of larval diet, restricted pollen collection to their host, although they visited the flowers of both host and non-host plants for nectar. When offered only the non-host pollen source, females ceased nesting activities. Males reared on non-host pollen exclusively restricted their patrolling flights to flowers of their normal host. This study provides the first empirical investigation of the imprinting theory in oligolectic bees, and unambiguously suggests that host recognition has a genetic basis in *H. truncorum*. We discuss the implication of this finding for the understanding of bee-flower relationships.

oligolecty / imprinting / Hopkin's host selection / *Heriades truncorum* / host recognition

1. INTRODUCTION

The majority of herbivorous insects feed on a restricted range of plants (Bernays and Chapman, 1994) and thus must have the ability to identify their hosts at some stage of their life. In most cases, host specificity is mediated by plant chemicals, which may either attract insects, stimulate feeding or elicit oviposition (Bernays and Chapman, 1994). Early observations have revealed that adult insects often show a preference for those plants on which they developed (Walsh, 1864; Hopkins, 1917). This has led to an important question in the field of plant-insect interactions, namely to what extent are the responses of adult insects genetically fixed or influenced by either learning or conditioning? Several hypotheses have been formulated to explain how these preferences can be induced. First, the Hopkins' host selection principle (Dethier, 1954; Barron, 2001)

assumes the persistence of neural changes, namely sensitivity to a chemical compound, from the larval stadium to the adult stage. This mechanism remains controversial (reviewed by Barron, 2001) and it has been demonstrated in only a few cases (Rietdorf and Steidle, 2002; Villagra et al., 2007) including a hymenopteran species (Gandolfi et al., 2003). Second, the neo-Hopkins' principle (Jaenicke, 1983) and the chemical legacy hypothesis (Corbet, 1985) suggest that the conditioning occurs during the early lifetime of the imago. This can be at or shortly after emergence, provided the pupa is in close contact with the host plant or the remains of it. There is substantially more evidence for early adult conditioning, either for host-plant preferences (e.g., Solarz and Newman, 2001; Olsson et al., 2006) or for other responses to volatiles (Jaisson, 1980; Cortesero et al., 1995; Röse et al., 1997; Bjorksten and Hoffmann, 1998; Breed et al., 1998).

Bees rely entirely on plant products for their sustenance and reproduction, and are thus herbivores. Many bee species are floral specialists

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and restrict their pollen foraging to a few related host plants. These species, the so-called oligolectic bees, show narrow relationships with their host, which is not only the sole food source for their offspring, but also often acts as a "rendez-vous" place for mating. The males of many oligolectic bees patrol the flowers of their females' pollen hosts to locate conspecific females (Eickwort, 1977; Westrich, 1989). Host recognition is therefore a key process in oligolectic bees, yet it has not been fully elucidated. In the light of earlier studies that demonstrated the importance of pollen odours in flower selection by the pollinating insects (e.g., Plateau, 1897; von Frisch, 1919), Linsley (1958) suggested that oligolectic bees could rely on pollen odours for host recognition, rather than, or in combination with, floral colour or morphology. Indeed, this was confirmed later for several oligolectic bee species (Dobson, 1987; Dobson and Bergström, 2000; Dötterl et al., 2005; Dötterl and Schäffler, 2007). The prediction of Linsley was based on the idea that "the only prior experience which the newly emerging solitary oligolege has had with its appropriate pollen source is the pollen and nectar which were stored by its parent" (Linsley, 1958). This has led the same author to the formulation of the imprinting theory in solitary bees, suggesting that bees may develop sensitivity to specific pollen volatiles either during the larval period or as emerging adults in the brood cell (Linsley, 1961). Since then, the imprinting theory is repeatedly mentioned in bee studies (e.g., Stephen et al., 1969; Dobson and Peng, 1997; Cane and Sipes, 2006), and is considered plausible by some authors (Thorp, 1969, 1979; Linsley, 1978), but challenged by others (Cruden, 1972; Westrich, 1989; Wcislo and Cane, 1996). The possibility that imprinting influences the floral choices of adult bees is of evolutionary significance: conditioned bees may remain faithful to one host and hence form a distinct host race. This is likely to be a first step towards speciation, especially if mating occurs mainly on the pollen host (Thorp, 1969; Bush, 1994; Dres and Mallet, 2002).

Although the imprinting theory has been intensively discussed in the literature, to date only two short studies have investigated it in solitary bees. Dobson and Ayasse (2000) forced the pronouncedly polylectic bee *Osmia bicornis* (= *Osmia rufa*) to provision its nest with pollen of *Brassica napus* (Brassicaceae), but observed no preference for *Brassica* in the next generation. Adult bees of the polylectic *Megachile rotundata* selected their preferred host, *Medicago sativa* (Fabaceae), even when reared on a pollen diet of *Daucus* (Apiaceae) (V.J. Tepedino, cited in Wcislo and Cane, 1996). However, no study so far has tested the imprinting theory in an oligolectic bee species. This probably relates to the methodological difficulties. First, it is challenging to force larvae of oligolectic bees to develop on non-host pollen, as strictly oligolectic species mostly refuse to collect pollen from non-host flowers (Strickler, 1979; Williams, 2003). Second, the fate of the larvae will depend on the species' ability to develop on non-host pollen, which varies among different bee species (Williams, 2003; Praz et al., 2008).

In this study, we tested the imprinting theory in the solitary bee *Heriades truncorum* (Linnaeus 1758) (Fig. 1). This species, which belongs to the bee tribe Osmiini (Megachilidae), is a common bee in Central Europe and is active from summer to early fall. For pollen, it is strictly specialized on Asteraceae, with a strong preference for the subfamily Asteroideae (Maciel De Almeida Correia, 1981a; Westrich, 1989, and references therein). Because it collects pollen from several subfamilies and tribes of a large plant family, this species would be considered as mesolectic by some authors (Cane and Sipes, 2006). However, in accordance with the categorization of bee host ranges by Westrich (1989) and Müller and Kuhlmann (in press) we treat this species as oligolectic, because of its specialisation on one, albeit large plant family. Mesolecty should be restricted to polylectic species that collect pollen from a small number of plant families. Though *H. truncorum* naturally builds nests in existing burrows in dead wood (Westrich, 1989), it is a common visitor of artificial trap nests and can thus be easily reared under encaged conditions. It is capable of developing on three types of non-host pollen, namely *Echium* (Boraginaceae), *Campanula* (Campanulaceae)

Figure 1. The bee species *Heriades truncorum* is oligolectic on Asteraceae. This female is collecting pollen on *Anthemis tinctoria* (Asteroideae).

and *Sinapis* (Brassicaceae) (Praz et al., 2008). To test the imprinting theory, we compared the floral preferences of adults of *H. truncorum* that developed, as larvae, either on pollen and nectar of their normal host, the Asteraceae, or on pollen and nectar of two non-host plants, *Echium* and *Campanula*, respectively. We forced the larvae to develop on these non-host pollen provisions by transferring unhatched eggs onto pollen and nectar provisions that had been collected by two different oligolectic bee species. We thus provide the first study on imprinting in oligolectic bees and discuss the implications of the results for the understanding of the evolution of bee-flower relationships.

2. MATERIALS AND METHODS

The individuals of *Heriades truncorum* investigated here were those used in a recent study (Praz et al., 2008), which compared the larval performance of four oligolectic bee species on different pollen diets.

2.1. Larval development on host and non-host pollen diets

Unhatched eggs of *H. truncorum* were transferred in summer 2006 with a thin spatula onto the

pollen and nectar provisions collected by *Hoplitis adunca* (Panzer 1798), which is strictly oligolectic on *Echium* (Boraginaceae), and *Chelostoma rapunculi* (Lepeletier 1841), which harvests pollen exclusively on *Campanula* (Campanulaceae). Pollen provisions of *H. adunca* were collected from trap nests at one locality in northern Switzerland, where *Echium vulgare* was the only host plant available; those of *C. rapunculi* originated from trap nests of populations reared in cages, where *Campanula rotundifolia* was the only pollen source. The eggs of *H. truncorum* were obtained both from an encaged rearing population provided with *Buphthalmum salicifolium* and *Tanacetum vulgare* (both Asteraceae) as host plants, and from trap nests collected at different localities in Switzerland. Larval development took place in artificial cells (predrilled clay blocks coated with paraffin; Torchio and Bosch, 1992) in a climate chamber in the dark at 26 °C and 60% relative humidity. The larvae were kept in the artificial cells throughout their entire development until adult emergence. Diapausing larvae in these artificial cells were stored in a cold room at 4 °C for overwintering, then moved outdoors into a small insect cage made of gauze (20 × 20 × 20 cm) six weeks before the experiments, which started in July 2007.

We used separate control groups of bees for the experiments that were conducted on individuals reared on *Campanula* and those reared on *Echium* pollen. For the *Campanula* experiments (see Sect. 2.2. Choice experiments), our controls were females that had been transferred as eggs

onto host pollen, using the same methods as for bees reared on non-host pollen. For the *Echium* experiments, our controls were females and males freshly emerged from trap nests collected in the field. All bees reared on each of the different pollen types were kept in separate polyester boxes. Thus, larvae reared on non-host pollen were never in direct contact with pollen of the Asteraceae.

Seven viable females and four males emerged from a total of 15 pupae reared on pollen of *Echium vulgare*; eight females and seven males from 23 pupae reared on pollen of *Campanula rotundifolia*; and five females and three males from 11 pupae reared on pollen of Asteraceae (Praz et al., 2008). In addition, eight females obtained from trap nests were used as controls for the *Echium* experiments.

Newly emerged adults were marked individually on the thorax with enamel paint, using a code consisting of six different colours (Toepfer et al., 1999). To mark the bees, they were first immobilized in the cold room at 4 °C, and then immediately transferred into a large outdoor observation cage made of gauze (160 × 70 × 120 cm) for experiments.

2.2. Choice experiments

The marked bees were allowed to fly, mate and build nests outdoors in the observation cages. Males and females were introduced together into the cage to ensure mating. The cages were provided with potted plants as pollen and nectar sources, hollow bamboo stalks (10–15 cm) as nesting sites and coniferous resin (*Pinus* spp. and *Picea* spp.) in Petri dishes as material for nest construction. We used two different cages, one for the experiments with the *Echium*-reared bees (hereafter the "*Echium* cage"), and one cage with the *Campanula*-reared bees (hereafter the "*Campanula* cage").

In the first phase of the experiments ("choice phase"), we offered both host (Asteraceae) and non-host plants (either *Echium* or *Campanula*) in equal abundance. In the *Echium* cage, we provided *Buphthalmum salicifolium* and *Tanacetum vulgare* as host plants and *Echium vulgare* as the non-host plant; the plants were arranged in a random distribution. In the *Campanula* cage, *Helenium autumnale* and *Tanacetum vulgare* were offered as host and *Campanula portenschlagiana* and *C. rotundifolia* as non-host plants. Larvae of *H. truncorum* were found to be equally able to develop on both *Campanula* species (Praz et al., 2008). In contrast to the *Echium* cage, we presented the plants here in

patches: two patches with *H. autumnale* and *T. vulgare* on two sides of the cage and one larger patch composed of *C. portenschlagiana* and *C. rotundifolia* in the centre. We chose this design to ensure that the *Campanula* plants, which are distinctly smaller in height than the Asteraceae, could be perceived by the bees.

In a second phase ("non-choice phase"), which started about 4–6 weeks after initiation of the "choice phase", we removed all host plants from the cages and replaced them with supplementary non-host plants. The host plants were removed in the late evening, when the bees were already resting in their nests, and in a period of sunny weather conditions to ensure that the bees could continue nesting activity the next morning.

2.3. Data collection

2.3.1. Adult behaviour

Males of *H. truncorum* patrol the inflorescences of Asteraceae to search for unmated females (Müller et al., 1997). These patrolling flights are easily distinguished from nectar visits, because males do not land on the flowers but rather typically slow down in their rapid flights at a short distance (2–3 cm) from each flower head to spot for females. In the *Echium* cage, where both host and non-host plants were randomly distributed, we counted the number of approaches to host and non-host flowers until a total number of 100 approaches were observed. This was repeated twice on different days for each male. In the *Campanula* cage, where host and non-host plants where arranged in different patches, we assessed the time the males spent patrolling the patches of host and non-host plants, respectively, for a total of 20 minutes. This was again repeated twice on different days for each male. All observations of males were conducted at the beginning of the choice phase and during sunny weather conditions.

Females of *H. truncorum* harvest pollen directly with their abdominal scopa from the pollen-bearing flower structures by typically moving the abdomen rapidly up and down. Floral visits restricted to nectar uptake could therefore be unambiguously distinguished from visits to collect pollen. For each host and non-host plant species and in each cage, we qualitatively differentiated between pure nectar visits and combined pollen and nectar visits during daily observations throughout the experiments.

2.3.2. *Pollen composition of brood cell provisions*

We microscopically analysed the pollen provision of each brood cell built by female bees in the two observation cages. To prevent the larvae from consuming the pollen provisions during the ongoing experiments, we repeatedly removed the bamboo stalks containing completed brood cells from the cages. This was done in the late evening, when the females were already sleeping within their nests. After cooling the bamboo stalks down to 4 °C, we split them longitudinally with a knife and removed the pollen provisions from the completed cells, leaving the cell walls separating the cells intact. We then carefully closed the nest with adhesive tape, placed the female bee back into the nest, and the nest back into the cage. This procedure did not affect the nesting females, as judged by the fact that the females resumed their normal nesting activities the following morning.

The entire content of each brood cell was embedded into Kaiser's glycerine gelatine (Merck, Darmstadt, Germany) on six different slides. Before covering the provisions with a cover slip, we thoroughly mixed the pollen, using a needle, to achieve a random distribution of the pollen grains. To determine the composition of the pollen provisions, we randomly selected five spots on each slide (one in each quadrant and one in the centre) and counted all grains at a magnification of 400× (approximately 50–150 grains per spot). We calculated the ratio of host pollen to non-host pollen in each brood cell by averaging the number of pollen grains over all 30 counts. If the provisions were composed of pollen of both host and non-host plants, these ratios were corrected by their volume using the volume values given in Müller et al. (2006) to compensate for differences in pollen grain size among the different plant species.

3. RESULTS

3.1. *Echium* cage

3.1.1. *Males*

Three out of the four males of *H. truncorum* reared on the non-host pollen of *Echium* patrolled flowers in the cage to locate females. The fourth male did not perform patrolling flights but rested on the cage wall during the whole observation period and was thus excluded from the experiments. Each of the three other males strictly restricted all patrolling flights to the species-specific host plants, the Asteraceae. No single approach to the flowers of *Echium* was observed. Both host and non-host flowers were regularly visited for nectar.

3.1.2. *Females*

Choice phase

Only two out of the seven females reared on *Echium* pollen provisioned brood cells. The first female completed one cell and was subsequently lost. The provision of this cell consisted of 99.3% Asteraceae and 0.7% *Echium* pollen (Tab. I). The second female completed eight cells, all consisting of more than 99% pollen of Asteraceae with only trace amounts of *Echium* pollen. Out of the eight control females reared on Asteraceae pollen, only two nested. The first female built two nests with four and six cells, respectively (Tab. I). All four cells of the first nest contained almost pure host-pollen provisions (all over 99%). In the second nest, two cells contained considerable amounts of *Echium* pollen (8% and 11%, respectively). This was most likely due to pollen contaminations during the very frequent nectar visits to *Echium* that occurred during an accidental two-day shortage of flowering Asteraceae in the cage. This female was never observed to actively collect pollen on the flowers of *Echium*. Once new Asteraceae were introduced into the cage, the female again exclusively harvested host pollen: the provisions of subsequent cells were composed of more than 99% Asteraceae pollen. The second control female constructed five cells, each containing over 99% Asteraceae pollen (Tab. I).

Non-choice phase

Immediately after all host plants were removed from the cage and replaced by additional flowering plants of *Echium*, all three females still nesting at this time (one *Echium*-reared and two controls) discontinued provisioning their brood cells. Within three days, two females (one *Echium*-reared and one control) sealed the entrance of the nest with a

Table I. The pollen composition of brood cell provisions in the choice experiment, where both host plants (Asteraceae) and non-host plants (either *Echium* or *Campanula)* were available to the adult females, which in turn had been reared as larvae on either host or non-host pollen. The average proportion of Asteraceae pollen (in % volume) per nest is given.

Non-host plant tested	Female id.	Larval diet	Nest	Number of cells in nest	Mean % Asteraceae in pollen provision
Echium	1	Asteraceae	1	4	99.5
			2	6	96.9
	2	Asteraceae	1	4	99.3
			2	1	99.5
	3	*Echium*	1	1	99.3
	4	*Echium*	1	8	99.8
Campanula	5	Asteraceae	1	7	100.0
	6	*Campanula*	1	4	100.0
	7	*Campanula*	1	1	100.0

plug of resin, although the bamboo stalk they nested in still had enough space for additional brood cells. During this non-choice phase no attempt to collect pollen on *Echium* was observed, although all three females regularly visited *Echium* to feed on nectar.

3.2. *Campanula* cage

3.2.1. *Males*

Four out of the seven males reared on the non-host pollen of *Campanula* exhibited patrolling flights. These four males exclusively patrolled Asteraceae during 100% of the observation time. No single approach to the flowers of *Campanula* was registered. Nectar uptake took place predominantly on the Asteraceae; nectar visits to *Campanula* flowers were very rarely recorded.

3.2.2. *Females*

Choice phase

Two out of the eight females reared on *Campanula* started nest construction. They built four cells and one cell, respectively. The provisions all consisted of pure Asteraceae pollen (Tab. I). Of the five control females, only one built a nest. This was composed of

seven cells, each containing pure pollen provisions of Asteraceae (Tab. I). We did not detect any traces of *Campanula* pollen in the cells of these three females (*Campanula*-reared and control), which is consistent with the observation that the females made very few nectar visits to the flowers of *Campanula*.

Non-choice phase

After the host plants were removed from the cage, all three females (two *Campanula*-reared and one control) ceased nesting activity and, within three days, sealed their nests with a plug of resin. Again, none of their bamboo stalks was completely filled with cells at this time. All three females were regularly observed feeding on nectar in *Campanula* flowers, but no single attempt to collect pollen was observed.

4. DISCUSSION

Our study provides the first empirical investigation of the imprinting theory in an oligolectic bee species. Both females and males of *Heriades truncorum* recognised their host plant even when they had never been in contact with its pollen in the natal cell, which is in contrast with the prediction of Linsley (1958, 1961, 1978). Thus, neither preimaginal learning nor early adult conditioning is likely to be the basis of host recognition in this species.

It may appear surprising that so few females started to construct nests in our experiments. This low nesting rate was observed both in the individuals reared on non-host pollen (four out of 15 females built a nest) and in the controls (three out of 13). In nature, solitary bees tend to nest close to their old nests, and often prefer to renest in the type of material from which they emerged (Stephen et al., 1969). Similarly, megachilid bees are attracted to the odours of old nests (Pitts-Singer, 2007), and females of *Megachile rotundata* prefer to nest in already occupied nesting sites rather than in new ones (Fairey and Lieverse, 1986). In our experiments, the low rate of nest establishment by *H. truncorum* may be due to the relative small size of the cages, the absence of previously occupied nests in the cage, as well as to the fact that the tested bees had been brought to the cage as adults and not in the preimaginal phase. In fact, Maciel De Almeida Correia (1981b) observed that artificially reared individuals of this species nested in a cage only when they had developed and hatched within the same cage. However, such an approach was not compatible with the purpose of the current study, which required that the eggs be transferred and each individual adult be marked. In addition, cocoons of *H. truncorum* are very loose and can not be transferred to suitable nesting sites without damaging the bees.

Though the number of brood cells provisioned by the *Echium*- and *Campanula*-reared females was admittedly small, we nevertheless think that our results allow rejection of the imprinting hypothesis in *H. truncorum* for three reasons. First, each cell provisioned by a bee represents a large number of floral visits: females of *H. truncorum* require 30–50 foraging trips to provision one cell and each trip consists of 15 floral visits on average (Maciel De Almeida Correia, 1981b). Second, the females reared on non-host pollen consistently ceased to provision their cells when offered only this pollen, although they had experienced it in the brood cells. If imprinting had influenced the bees' floral choices, these females would have been expected to collect pollen, at least partially, from the new hosts. In contrast to the study by Williams (2003), who observed the oligolectic bee species *Osmia californica* col-

lecting pollen on non-host flowers as long as the species-specific host plants were present, we did not observe a single attempt to harvest non-host pollen, neither during the choice phase, when bees were provided both host and non-host plants, nor during the non-choice phase, when bees were offered only non-host species. Third, both the *Echium*- and *Campanula*-reared males of *H. truncorum* exclusively searched for females at the inflorescences of the Asteraceae. Interestingly, all individuals of *H. truncorum* visited the non-host flowers for nectar, indicating that they are able to perceive these flowers as a potential source of food. In conclusion, our results indicate that the preference by *H. truncorum* for Asteraceae is innate and, therefore, must have a genetic foundation.

4.1. Host recognition in solitary bees

In bees, host recognition probably relies on olfactory or visual cues, or on a combination of both (Linsley, 1958; Wcislo and Cane, 1996). The role of pollen or floral odours in host recognition has been documented for several oligolectic bee species (Dobson, 1987; Dobson and Bergström, 2000; Dötterl et al., 2005; Dötterl and Schäffler, 2007), but could not be definitely shown for other species (Dobson and Bergström, 2000). In contrast, little is known of the importance of visual cues in host recognition by solitary bees. Visual cues might include colour, patterns of UV reflection or floral shape. Indeed, colours were shown to influence host choice in two polylectic species of *Megachile* (Michener, 1953; Golpen and Brandt, 1975), and an innate preference for yellow flowers was found in three oligolectic bee species (Dobson and Bergström, 2000).

In *H. truncorum*, host recognition is unlikely to rely on visual cues alone. This species forages on a variety of different species of the Asteraceae. Pollen hosts in central Europe include, among many others, *Buphthalmum salicifolium*, *Tanacetum vulgare* and *Achillea millefolium* (subfamily Asteroideae), *Centaurea* spp. and *Cirsium* spp. (Carduoideae) as well as *Cichorium* spp. (Cichorioideae) (Westrich, 1989). These hosts distinctly differ in shape and colour. Thus, we postulate that

host recognition in *H. truncorum* must rely predominantly on olfactory cues. But so far, no clear preferences for pollen or flower odours could be shown in foraging-naïve *H. truncorum* (Dobson and Bergström, 2000).

4.2. Implications for bee-flower relationships

Though pollen preferences are generally highly conserved within specialist bee clades, in some lineages closely related species were found to be oligolectic on unrelated floral hosts (Wcislo and Cane, 1996). These "sequential specialists" may have descended from an oligolectic ancestor, having retained the narrow diet breadth but switched host (Minckley and Roulston, 2006). The host-switching phenomenon has initially been cited as evidence that interspecific competition drives specialization through resource partitioning, yet this hypothesis remains untested and controversial (Bernays and Wcislo, 1994; Minckley and Roulston, 2006). Thorp (1969) formulated a hypothesis of speciation mediated through host switches in groups of closely related bee species of the subgenus *Diandrena*. In his model, an oligolectic bee species may include a new host into its pollen diet due to a shortage of its normal host. This plasticity is indeed observed in some oligolectic bees (Minckley and Roulston, 2006, and references therein). Subsequently, conditioning in the natal cell acts to fix or maintain this host shift in succeeding generations and thus contributes to the first steps toward reproductive isolation, as the specific pollen hosts often act as rendez-vous places for males and females. If imprinting does occur in solitary bees, this scenario could happen in a particularly short time and, in theory, even in sympatry (Bush, 1994). However, the results of the present study do not support imprinting in bees. Therefore, the above scenario of a sympatric speciation mediated through host shift appears to be unlikely. Other isolating mechanisms must be involved, such as geographic isolation and adaptations to the new hosts under disruptive selection, for example for foraging efficiency, digestion efficiency or phenological synchronisation (Thorp, 1969; Wcislo and Cane, 1996;

Minckley and Roulston, 2006). The scenario would thus approach the general mosaic theory proposed by Thompson (1994).

An additional important aspect that may influence diet breadth and host shift are sensory limitations of the adult bee, which were suggested to influence host breadth in phytophagous insects (reviewed in Bernays, 2001). In our experiments, *H. truncorum* refused to collect non-host pollen in spite of its suitability to support larval development. This suggests that neural constraints are more important than nutritional constraints in shaping the host range of this species. This neural fixation may lead to fitness advantages that compensate for the dependency on a limited range of host plants (Bernays, 2001).

4.3. Future research

Though the pollen specialization in *H. truncorum* has most probably a genetic basis, the possibility of imprinting in other bee species can not be excluded. As suggested by Dobson and Peng (1997), a hypothetical conditioning to pollen volatiles during early life stages may strongly vary among different bee-flower pairs. Many lipid-soluble volatiles, which might be involved in imprinting, are included in the pollenkitt (Dobson, 1987, 1988). The amount of pollenkitt strongly varies depending on the plant taxon, as does the degree to which solitary bees digest it (Dobson and Peng, 1997; Dobson and Bergström, 2000). Thus, the presence or absence of pollen volatiles in the natal cell may strongly influence the possibility that conditioning could occur at the time of adult emergence. Therefore, the imprinting theory has to be tested in other bee species before it can be generally rejected.

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Reconnaissance de l'hôte par une abeille spécialiste par rapport au pollen : preuve d'une base génétique.

Heriades truncorum / **Megachilidae** / **oligolectie** / **reconnaissance de l'hôte** / **empreinte** / **séection de l'hôte selon Hopkin** / **pollen** / **Asteraceae**

Zusammenfassung – **Wirtspflanzenerkennung bei einer pollenspezialisierten Biene: Hinweis auf eine genetische Grundlage.** Auf bestimmte Pollenarten spezialisierte Bienen zeigen bereits als naive Imagines Präferenzen für den Pollen ihrer Wirtsarten oder für spezifische Pollenduftstoffe. Eine wichtige Frage ist hierbei, ob bei diesen solitären Bienen die Präferenzen für Pollenduftstoffe angeboren sind und damit eine genetische Grundlage haben, oder ob sie das Ergebnis einer präimaginalen oder frühimaginalen Konditionierung sind (Prägungstheorie). In ihren Brutzellen, in denen die Entwicklung vom Ei bis zur Imago erfolgt, sind diese Bienen in der Tat sowohl als Larven als auch als frischgeschlüpfte Adulte ständig in Kontakt mit Pollenduftstoffen. Obwohl die Prägungstheorie in der Literatur verschiedentlich angesprochen wurde, wurde sie noch nie an einer pollenspezialisierten Biene überprüft. Wir untersuchten die Rolle der larvalen Pollennahrung auf die Wirtpflanzenwahl bei *Heriades truncorum*, einer auf Asteraceen spezialisierten Biene. Dabei verglichen wir die Blütenpräferenzen von Bienen, die auf Wirtspollen aufgezogen wurden, mit denen, die auf Nichtwirtspollen aufgezogen wurden, insbesondere auf Pollen von *Echium* (Boraginaceae) und auf Pollen von *Campanula* (Campanulaceae). Zur Aufzucht von Larven auf diesen Nichtwirtspollen übertrugen wir noch ungeschlüpfte Eier von *H. truncorum* auf Pollenvorräte, die von zwei anderen oligolektischen Bienenarten angelegt worden waren. In den künstlichen Zellen wuchsen die aus den transferierten Eiern geschlüpften Larven heran, überwinterten und entwickelten sich zu Puppen. Nachdem die Imagines geschlüpft waren, konnten sie sich verpaaren und dann in Käfigen nisten, in denen ihnen sowohl Wirts- als auch Nichtwirtspflanzen angeboten wurden. Unabhängig vom Pollen, auf dem sie als Larven aufgezogen worden waren, sammelten alle Weibchen auschliesslich auf ihrer Wirtsart Pollen, während sie Nektar sowohl auf der Wirts- als auch den Nichtwirtsarten sammelten. Wenn ihnen nur Nichtwirtspflanzen als Pollenquelle angeboten wurden, stellten die Weibchen sofort die Nistaktivitäten ein. In ähnlicher Weise führten auch die Männchen ihre Patrouillenflüge ausschliesslich auf Wirtspflanzen durch, selbst wenn sie auf Nichtwirtspollen aufgezogen worden waren. Unsere Er-

gebnisse sind demzufolge ein eindeutiger Hinweis auf eine genetische Grundlage der Wirtserkennung bei *H. truncorum* und haben damit wichtige Implikationen für unser Verständnis der Evolution von Bienen/Pflanzen-Beziehungen. Unter der Annahme, dass die Wirtserkennung auf Prägung beruht, sollten Adulte, die sich z.B. infolge aus Mangel ihrer Wirtsart auf Nichtwirtspollen entwickelt haben, die Wirtsart schnell wechseln und dieser neuen Art dann treu bleiben. Dieser Prozess könnte zur Bildung isolierter Wirtsrassen führen, da die Männchen solch spezialisierter Bienen dann auf der neuen Wirtsart nach Weibchen suchen würden. Im entgegengesetzten Fall einer genetischen Basis der Wirtserkennung, wie wir sie für *H. truncorum* in der dieser Studie nachweisen konnten, würden einem Wirtswechsel andere Mechanismen zugrundeliegen, wie z.B. geographische Isolation oder neurale Fixierung. In der Tat weigerten sich die Weibchen von *H. truncorum*, auf Nichtwirtspflanzen Pollen zu sammeln, obwohl sich dieser als für die Brutaufzucht geeignet erwiesen hatte. Dies ist ein Hinweis darauf, dass für diese Art neurale Beschränkungen für die Ausprägung des Wirtsspektrums wichtiger sind als Einschränkungen in der Ernährung.

Oligolektie / **Prägung** / **Hopkinsche Wirtsselektion** / *Heriades truncorum* / **Wirtserkennung**

REFERENCES

Barron A.B. (2001) The life and death of Hopkins' host-selection principle, J. Insect Behav. 14, 725–737.

Bernays E.A. (2001) Neural limitations in phytophagous insects: Implications for diet breadth and evolution of host affiliation, Annu. Rev. Entomol. 46, 703–727.

Bernays E.A., Chapman R.F. (1994) Host-plant selection by phytophagous insects, Chapman & Hall, London.

Bernays E.A., Wcislo W.T. (1994) Sensory capabilities, information processing, and resource specialization, Q. Rev. Biol. 69, 187–204.

Bjorksten T.A., Hoffmann A.A. (1998) Plant cues influence searching behaviour and parasitism in the egg parasitoid *Trichogramma nr. brassicae*, Ecol. Entomol. 23, 355–362.

Breed M.D., Leger E.A., Pearce A.N., Wang Y.J. (1998) Comb wax effects on the ontogeny of honey bee nestmate recognition, Anim. Behav. 55, 13–20.

Bush G.L. (1994) Sympatric speciation in animals – new wine in old bottles, Trends Ecol. Evol. 9, 285–288.

Cane J.H., Sipes S. (2006) Characterizing floral specialization by bees: analytical methods

and a revised lexicon for oligolecty, in: Waser N.M., Ollerton J. (Eds.), Plant-pollinator interactions: from specialization to generalization, The University of Chicago Press, Chicago and London, pp. 99–122.

Corbet S.A. (1985) Insect chemosensory responses – a chemical legacy hypothesis, Ecol. Entomol. 10, 143–153.

Cortesero A.M., Monge J.P., Huignard J. (1995) Influence of two successive learning processes on the response of *Eupelmus vuilleti* Crw (Hymenoptera, Eupelmidae) to volatile stimuli from hosts and host plants, J. Insect Behav. 8, 751–762.

Cruden R.W. (1972) Pollination biology of *Nemophila menziesii* (Hydrophyllaceae) with comments on the evolution of oligolectic bees, Evolution 26, 373–389.

Dethier V.G. (1954) Evolution of feeding preferences in phytophagous Insects, Evolution 8, 33–54.

Dobson H.E.M. (1987) Role of flower and pollen aromas in host-plant recognition by solitary bees, Oecologia 72, 618–623.

Dobson H.E.M. (1988) Survey of pollen and pollenkitt lipids – chemical cues to flower visitors, Am. J. Bot. 75, 170–182.

Dobson H.E.M., Peng Y.S. (1997) Digestion of pollen components by larvae of the flower- specialist bee *Chelostoma florisomne* (Hymenoptera: Megachilidae), J. Insect Physiol. 43, 89–100.

Dobson H.E.M., Ayasse M. (2000) Does larval pollen diet influence host-flower selection in solitary bees? Meeting of the Botanical Society of America, Am. J. Bot. 87, 6, 42.

Dobson H.E.M., Bergström G. (2000) The ecology and evolution of pollen odors, Plant Syst. Evol. 222, 63–87.

Dötterl S., Schäffler I. (2007) Flower scent of floral oil-producing *Lysimachia punctata* as attractant for the oil-bee *Macropis fulvipes*, J. Chem. Ecol. 33, 441–445.

Dötterl S., Füssel U., Jürgens A., Aas G. (2005) 1,4-Dimethoxybenzene, a floral scent compound in willows that attracts an oligolectic bee, J. Chem. Ecol. 31, 2993–2998.

Dres M., Mallet J. (2002) Host races in plant-feeding insects and their importance in sympatric speciation, Philos. Trans. R. Soc. Lond. B 357, 471–492.

Eickwort G.C. (1977) Male territorial behaviour in the mason bee *Hoplitis anthocopoides* (Hymenoptera: Megachilidae), Anim. Behav. 25, 542–554.

Fairey D.T., Lieverse J.A.C. (1986) Cell production by the alfalfa leafcutting bee (*Megachile rotundata* F.) in new and used wood and polystyrene nesting materials, J. Appl. Entomol. 102, 148–153.

Frisch K. von (1919) Über den Geruchsinn der Biene und seine blütenbiologische Bedeutung, Zool. Jahrb. Abt. Allg. Zool. Physiol. Tiere 37, 1–238.

Gandolfi M., Mattiacci L., Dorn S. (2003) Preimaginal learning determines adult response to chemical stimuli in a parasitic wasp, Proc. R. Soc. London B 270, 2623–2629.

Golpen B.P., Brandt S.A. (1975) Alfalfa flower color associated with differential seed set by leaf-cutter bees, Agron. J. 67, 804–806.

Hopkins A.D. (1917) A discussion of H. G. Hewitt's paper on "Insect Behaviour", J. Econ. Entomol. 10, 92–93.

Jaenike J. (1983) Induction of host preference in *Drosophila melanogaster*, Oecologia 58, 320–325.

Jaisson P. (1980) Environmental preference induced experimentally in ants (Hymenoptera, Formicidae), Nature 286, 388–389.

Linsley E.G. (1958) The ecology of solitary bees, Hilgardia 27, 543–599.

Linsley E.G. (1961) The role of flower specificity in the evolution of solitary bees, XI. Int. Kongr. Entomol. Wien, Vol. 1, pp. 593–596.

Linsley E.G. (1978) Temporal patterns of flower visitation by solitary bees, with particular reference to the southwestern United States, J. Kans. Entomol. Soc. 54, 531–546.

Maciel De Almeida Correia M.d.L. (1981a) Contribution à l'étude de la biologie d'*Heriades truncorum* L. (Hymenoptera ; Apoidea ; Megachilidae). II. Aspect écologique, Apidologie 12, 3–30.

Maciel De Almeida Correia M.d.L. (1981b) Contribution à l'étude de la biologie d'*Heriades truncorum* L. (Hymenoptera ; Apoidea ; Megachilidae). III. Aspect éthologique, Apidologie 12, 221–256.

Michener C.D. (1953) The biology of a leafcutter bee (*Megachile brevis*) and its associates, Univ. Kans. Sci. Bull. 35, 1659–1748.

Minckley R.L., Roulston T.H. (2006) Incidental mutualisms and pollen specialization among bees, in: Waser N.M., Ollerton J. (Eds.), Plant-pollinator interactions: from specialization to generalization, The University of Chicago Press, Chicago and London, pp. 69–98.

Müller A., Kuhlmann M. (in press) Pollen hosts of western palaearctic bees of the genus *Colletes* (Hymenoptera: Colletidae): the Asteraceae paradox, Biol. J. Linn. Soc.

Müller A., Krebs A., Amiet F. (1997) Bienen – Mitteleuropäische Gattungen, Lebensweise, Beobachtung, Naturbuch Verlag, Augsburg.

Müller A., Diener S., Schnyder S., Stutz K., Sedivy C., Dorn S. (2006) Quantitative pollen requirements of solitary bees: Implications for bee conservation and the evolution of bee-flower relationships, Biol. Conserv. 130, 604–615.

Olsson P.O.C., Anderbrant C., Lofstedt C. (2006) Experience influences oviposition behaviour in two pyralid moths, *Ephestia cautella* and *Plodia interpunctella*, Anim. Behav. 72, 545–551.

Pitts-Singer T.L. (2007) Olfactory response of megachilid bees, *Osmia lignaria*, *Megachile rotundata* and *M. pugnata*, to individual cues from old nest cavities, Environ. Entomol. 36, 402–408.

Plateau F. (1897) Comment les fleurs attirent les insectes. Recherches expérimentales 5, Bull. Acad. R. Sci. Lettres Beaux-Arts Belg. 34, 847–880.

Praz C.J., Müller A., Dorn S. (2008) Specialized bees fail to develop on non-host pollen: Do plants chemically protect their pollen? Ecology 89, 795–804.

Rietdorf K., Steidle J.L.M. (2002) Was Hopkins right? Influence of larval and early adult experience on the olfactory response in the granary weevil *Sitophilus granarius* (Coleoptera, Curculionidae), Physiol. Entomol. 27, 223–227.

Röse U.S.R., Alborn H.T., Makranczy G., Lewis W.J., Tumlinson J.H. (1997) Host recognition by the specialist endoparasitoid *Microplitis croceipes* (Hymenoptera: Braconidae): Role of host- and plant-related volatiles, J. Insect Behav. 10, 313–330.

Solarz S.L., Newman R.M. (2001) Variation in host plant preference and performance by the milfoil weevil, *Euhrychiopsis lecontei* Dietz, exposed to native and exotic watermilfoils, Oecologia 126, 66–75.

Stephen W.P., Bohart G.E., Torchio P.F. (1969) The biology and external morphology of bees, Agricultural Experiment Station, Oregon State University, Corvallis.

Strickler K. (1979) Specialization and foraging efficiency of solitary bees, Ecology 60, 998–1009.

Thompson J.N. (1994) The coevolutionary process, The University of Chicago Press, Chicago and London.

Thorp R.W. (1969) Systematics and ecology of bees of the subgenus *Diandrena* (Hymenoptera: Andrenidae), Univ. Calif. Publ. Entomol. 52, 1–146.

Thorp R.W. (1979) Structural, behavioral, and physiological adaptations of bees (Apoidea) for collecting pollen, Ann. Missouri Bot. Gard. 66, 788–812.

Toepfer S., Gu H., Dorn S. (1999) Spring colonisation of orchards by *Anthonomus pomorum* from adjacent forest borders, Entomol. Exp. Appl. 93, 131–139.

Torchio P.F., Bosch J. (1992) Biology of *Tricrania stansburyi*, a Meloid beetle cleptoparasite of the Bee *Osmia lignaria propinqua* (Hymenoptera, Megachilidae), Ann. Entomol. Soc. Am. 85, 713–721.

Villagra C.A., Pennacchio F., Niemeyer H.M. (2007) The effect of larval and early adult experience on behavioural plasticity of the aphid parasitoid *Aphidius ervi* (Hymenoptera, Braconidae, Aphidiinae), Naturwissenschaften 94, 903–910.

Walsh B.D. (1864) On phytophagic varieties and phytophagic species, Proc. Entomol. Soc. Philadelphia 3, 403–430.

Wcislo W.T., Cane J.H. (1996) Floral resource utilization by solitary bees (Hymenoptera: Apoidea) and exploitation of their stored foods by natural enemies, Annu. Rev. Entomol. 41, 257–286.

Westrich P. (1989) Die Wildbienen Baden-Württembergs, Eugen Ulmer GmbH & Co, Stuttgart.

Williams N.M. (2003) Use of novel pollen species by specialist and generalist solitary bees (Hymenoptera: Megachilidae), Oecologia 134, 228–237.