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# SPECIALIZED BEES FAIL TO DEVELOP ON NON-HOST POLLEN: DO PLANTS CHEMICALLY PROTECT THEIR POLLEN?

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*Abstract*. Bees require large amounts of pollen for their own reproduction. While several morphological flower traits are known to have evolved to protect plants against excessive pollen harvesting by bees, little is known on how selection to minimize pollen loss acts on the chemical composition of pollen. In this study, we traced the larval development of four solitary bee species, each specialized on a different pollen source, when reared on non-host pollen by transferring unhatched eggs of one species onto the pollen provisions of another species. Pollen diets of Asteraceae and *Ranunculus* (Ranunculaceae) proved to be inadequate for all bee species tested except those specialized on these plants. Further, pollen of *Sinapis* (Brassicaceae) and *Echium* (Boraginaceae) failed to support larval development in one bee species specialized on *Campanula* (Campanulaceae). Our results strongly suggest that pollen of these four taxonomic groups possess protective properties that hamper digestion and thus challenge the general view of pollen as an easy-to-use protein source for flower visitors.

*Key words: Apoidea; Asteraceae; bee–flower relationships;* Echium; *Megachilidae; oligolectic bees; pollenkit; pollination;* Ranunculus; *secondary compounds;* Sinapis; *toxic pollen.*

## Introduction

The great majority of flowering plants rely on insects or other animals for pollination. This interaction has shaped the evolution of both the angiosperms and their pollinators since the rise of the flowering plants in the early Cretaceous (Soltis et al. 2005). Among insects, bees are the most important pollinators. Having probably originated in the Cretaceous (Danforth et al. 2004), they share a long and intimate evolutionary history with the angiosperms. However, the relationships between bees and flowers are not merely mutualistic (Inouye 1980, Westerkamp 1996, 1997*a*, Thorp 2000, Irwin et al. 2001), but are better viewed as a "balanced mutual exploitation" (Westerkamp 1996).

Bees are above all herbivores. They store pollen and nectar as the exclusive food source for their larvae. The quantity of pollen withdrawn from flowers for bee reproduction is huge. For example, as much as 95.5% of the pollen produced by flowers of *Campanula rapunculus* was removed by bees, while only 3.7% contributed to pollination (Schlindwein et al. 2005). In another study, 85% of 41 bee species examined were found to require the whole pollen content of more than 30 flowers to rear a single larva, and some species even needed the pollen of more than 1000 flowers (Müller et al. 2006).

Bees not only collect large amounts of pollen, they also collect it very efficiently (Westrich 1989, Müller 1996*b*), which frequently conflicts with the successful pollination of the flowers. In some proterandric flowers (i.e., those with stamens coming to maturity before the pistil) for example, female bees restrict their foraging to flowers in the male phase, thereby scarcely contributing to pollination (Müller 1996*a*). Similarly, many bee species act as pollen thieves due to morphological incongruences between the flowers and the bees or inappropriate bee behavior (Minckley and Roulston 2006, and references therein). In addition, bees carefully groom their body after having visited a flower and transfer the pollen grains into specialized hair brushes, making them generally inaccessible for pollination (Westerkamp 1996). Consequently, specialized bee flowers must balance the need to attract bees for pollination, on the one hand, and to restrict pollen loss to bees, on the other. Plants are thus expected to evolve adaptations to minimize pollen loss by narrowing the spectrum of pollen feeding visitors. Indeed, several morphological flower traits can be viewed as adaptations preventing excessive pollen harvesting by bees: heteranthery, where showy anthers provide fodder pollen while inconspicuous anthers produce pollen for fertilization (Vogel 1993); concealment of the anthers in nototribic flowers (i.e., flowers in which the stamens and style are placed below the upper lip in order to come into contact with the dorsal surface of the forager's body; Müller 1996*a*), in narrow floral tubes (e.g., Boraginaceae; Thorp 1979, Müller 1995), or in keel flowers (e.g., Fabaceae; Westerkamp 1997*b*); concealment of the pollen in poricidal anthers (i.e., anthers releasing pollen through a distal opening; Buchmann 1983, Harder and Barclay 1994); and progressive pollen release to force pollinators to repeatedly visit the flowers (Erbar and Leins 1995, Schlindwein et al. 2005).

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TABLE 1. The five selected bee species, their floral specialization, the plant species used for nest establishment, and the non-host pollen onto which the larvae were forced to develop.

| Bee species | Specific host plant | Plant species for nest establishment | Non-host pollen tested |
|---|---|---|---|
| *Heriades truncorum* (Linnaeus 1758) | Asteraceae | *Tanacetum vulgare*<br>*Buphthalmum salicifolium* | *Campanula* (Campanulaceae)<br>*Ranunculus* (Ranunculaceae)<br>*Echium* (Boraginaceae)<br>*Sinapis* (Brassicaceae) |
| *Chelostoma rapunculi* (Lepeletier 1841) | *Campanula* | *Campanula rotundifolia*<br>*Campanula portenschlagiana* | *Buphthalmum* (Asteraceae)<br>*Ranunculus* (Ranunculaceae)<br>*Echium* (Boraginaceae)<br>*Sinapis* (Brassicaceae) |
| *Chelostoma florisomne* (Linnaeus 1758) | *Ranunculus* | *Ranunculus acris* | *Tanacetum* (Asteraceae)<br>*Campanula* (Campanulaceae)<br>*Brassica* (Brassicaceae) |
| *Hoplitis adunca* (Panzer 1798) | *Echium* | *Echium vulgare* | *Buphthalmum* (Asteraceae) |
| *Osmia brevicornis* (Fabricius 1798) | Brassicaceae | *Brassica napus*<br>*Sinapis arvensis* | |

Selection may as well act on pollen toxicity or on pollen nutritional quality to prevent excessive pollen collection. Although the presence of secondary compounds in nectar has recently received considerable attention (e.g., Adler 2000, Irwin et al. 2004, Adler and Irwin 2005, Johnson et al. 2006), little is known of how the chemical composition of pollen influences pollen use by bees or other insects (but see Detzel and Wink 1993, Pernal and Currie 2002). Indications exist that pollen is not an easy-to-use protein source readily digestible for all flower visitors (Levin and Haydak 1957, Roulston and Cane 2000, Cook et al. 2004). In fact, surprisingly few insect taxa rely on pollen as a sole protein source (Krenn et al. 2005). Furthermore, if pollen were a mere reward to flower visitors, it should contain extra protein. However, pollen protein content has been found to be associated with the need for pollen tube growth rather than to reward pollinators (Roulston et al. 2000).

Many bee species are pollen specialists ("oligolectic") and restrict their pollen foraging to few related plant species belonging to the same family (Westrich 1989, Wcislo and Cane 1996, Cane and Sipes 2006). In contrast, "polylectic" bees have a broader host range that encompasses at least two plant families. However, many polylectic bees still show a restricted range of pollen sources (Westrich 1989, Müller 1996*a*, Cane and Sipes 2006). If pollen were an easily digestible source of protein, the larvae of both oligolectic and polylectic bees should be able to develop on non-host pollen. Only a few observations on the performance of bee larvae on non-host pollen have been reported. Two larvae of the oligolectic bee *Nomadopsis zonalis* grew normally on non-host pollen (Rozen 1963); *Lasioglossum galpinsiae*, a bee species strictly specialized on *Oenothera* (Onagraceae), developed on pollen of *Medicago sativa* (Fabaceae) (Bohart and Youssef 1976); and the larvae of the Asteraceae specialist *Osmia californica* developed on pollen of Hydrophyllaceae and Brassicaceae (Williams 2003). Such sparse evidence led to the tentative suggestion that floral specializations in bees are not linked to the chemical composition of the pollen (Wcislo and Cane 1996, Minckley and Roulston 2006). However, the findings that the larvae of *Osmia lignaria* failed to develop on five different non-host diets (Levin and Haydak 1957) and that the larvae of *Megachile rotundata* failed to grow on pollen of Asteraceae (Guirguis and Brindley 1974) suggest that this assumption is possibly premature.

In the present study, the larvae of four oligolectic osmiine bee species (Megachilidae: Osmiini) were forced to feed on non-host pollen. Given that strict oligolectic bees may refuse to harvest pollen in the absence of their specific host plants (e.g., Strickler 1979, Williams 2003), we removed unhatched eggs from the brood cells and transferred them onto non-host pollen and nectar collected by one of the other species. From the observed patterns in larval survival, we infer possible protective properties of pollen and discuss their potential implications to our understanding of bee-flower relationships in general.

## METHODS

### *Bee species and nest establishment*

We selected five bee species (Table 1) belonging to the tribe Osmiini (Apoidea, Megachilidae). They are widespread and common throughout Europe, and their foraging behavior is well documented (Westrich 1989, and references therein). All species are strictly oligolectic, restricting pollen collection to a limited number of related plant species. *Heriades truncorum* is an Asteraceae specialist preferring flowers of the Asteroideae. *Chelostoma rapunculi*, *Chelostoma florisomne,* and *Hoplitis adunca*, which are all oligolectic at the plant genus level, collect pollen exclusively on *Campanula* (Campanulaceae), *Ranunculus* (Ranunculaceae), and *Echium* (Boraginaceae), respectively. *Osmia brevicornis* is a broad oligolege of the plant family Brassicaceae. Each bee species was allowed to build nests separately, in a cage made of gauze ($160 \times 70 \times 120$ cm), outdoors in Zurich, Switzerland. The bees originated from several different localities in Switzerland. We provided potted plants of suitable host species as pollen and nectar

sources (Table 1) and hollow bamboo stalks as nesting sites. To avoid mixed provisions, we provided only one plant species in each cage at a given time.

### *Egg transfer*

To trace larval performance on non-host pollen, we transferred unhatched eggs from the brood cells of one species to the pollen and nectar provisions of another species. We carefully moved each egg with a thin spatula onto pollen that we had taken from one nest and previously placed into an artificial cell (a predrilled clay block coated with paraffin; Torchio and Bosch 1992). Pollen attached to the egg was removed to avoid contamination. We estimated the pollen requirements of each of the differently sized bee species by comparing the dry body masses of the adult females (data from Müller et al. 2006). In the rare cases where the larva consumed the entire provision before ending its development, additional pollen was provided. We used unhatched eggs from the nests established in the gauze cages and additionally from nests collected in the wild: nests of *C. florisomne* were collected in reed stems of thatched roofs at Gletterens in western Switzerland, and nests of *H. truncorum* and *H. adunca* from bamboo stalks at different localities in northern Switzerland.

### *Non-host pollen tested*

We compared the larval performance of *H. truncorum* and *C. rapunculi* on the same five pollen types (Table 1). Larvae of *C. florisomne* were reared on three different non-host pollen diets and larvae of *H. adunca* on pollen of *Buphthalmum* (Asteraceae). We obtained too few eggs of *O. brevicornis* to assess its development on different pollen diets, and thus used this species as a source of Brassicaceae pollen only. As controls, we transferred eggs of each bee species onto its host pollen following exactly the same procedure as for egg transfer onto non-host pollen.

The pollen for our experiments originated from the bee nests in the gauzed cages. Additionally, we used pollen of *Ranunculus acris, Echium vulgare,* and Asteraceae from nests collected in the wild of *C. florisomne*, *H. adunca,* and *H. truncorum*, respectively (see *Methods: Egg transfer*). The use of pollen of Asteraceae from nests in the wild was restricted to control diets for *H. truncorum*. Whenever pollen from natural nests was used, the brood cell provisions were analyzed microscopically to confirm pollen purity. The pollen was stored at 4°C until use.

We acknowledge that our method confounds the impact of pollen and that of nectar on larval performance. However, as the pollen provisions collected by *H. truncorum, C. rapunculi, C. florisomne,* and *O. brevicornis* contain only little amounts of nectar, we postulate that the impact of nectar on larval performance was negligible. In contrast, the pollen provisions of *H. adunca* contain considerable amounts of nectar. In this species, we cannot exclude that nectar might have influenced larval performance.

### *Larval development*

Egg hatching and larval development took place in the artificial cells in a climate chamber in the dark at 26°C and 60% relative humidity. The eggs and larvae were checked every two or three days. At each occasion, we recorded whether the egg had hatched, whether the larva was alive and feeding, and whether the larva had defecated or started to spin a cocoon. Eggs that did not hatch were excluded from all analyses. Dead larvae were also excluded if they had undoubtedly died from external factors such as mites, fungal growth in the pollen provision, or marked changes in the consistency of the pollen. As bee larvae go through several instars that are difficult to separate, we only distinguished four developmental stages: (1) feeding, non-defecating; (2) feeding, defecating; (3) non-feeding, spinning cocoon; and (4) immobile, diapausing in completed cocoon. Cocoon spinning was discriminated from the production of silk strands to fix feces during the feeding phase. After the cocoon had been completed, cells were kept for 15 days in the climate chamber and then stored at 4°C for overwintering. Although Williams (2003) recommended larval weight as the best surrogate measure of fitness to assess the performance of bee larvae on different pollen diets, we refrained from weighing the larvae as preliminary trials indicated that such handling induced higher mortality.

### *Data analysis*

For each individual, we determined the hatching date as the average of the two observation dates between which the egg hatched. We followed a similar protocol to assess the date of the onset of cocoon spinning and the date at which the larva entered diapause. *Development time* was calculated as the number of days between hatching and onset of cocoon spinning, and *pre-diapause life length* as the number of days between hatching and diapause. For those individuals that died before the end of their larval development, pre-diapause life length was calculated as the number of days between hatching and death.

We used Kaplan-Meier statistics to compare survival of a bee species on host and non-host pollen following Lee and Wang (2003). We considered the parameter pre-diapause life length as "censored data": individuals that died before the end of their larval development were the exact observations for which the event (death) occurred, while those that reached the diapausing stage were the censored observations. The latter were thus withdrawn from survival calculations once the development was completed, which reflects the fact that diapausing larvae are much less exposed to mortality risk than feeding larvae. To test for differences between survival distributions, we used the log-rank test when comparing two groups and the $k$ sample test implemented in R

TABLE 2. Larval survival of the four oligolectic bee species when reared on their host pollen (controls shown in boldface) and on non-host pollen.

| Bee species | Pollen diets | No. bees | No. survivors | Survivors (%) | Survival time (d) | Group heterogeneity | |
|---|---|---|---|---|---|---|---|
| | | | | | | *P* | Groups |
| *Heriades truncorum* | **Asteraceae** | 21 | 19 | 90.5 | 45.67 ± 2.53 | <0.001 | a |
| | *Campanula* | 30 | 23 | 76.7 | 34.97 ± 2.61 | | a |
| | *Ranunculus* | 19 | 0 | 0.0 | 6.84 ± 0.78 | | b |
| | *Echium* | 19 | 15 | 78.9 | 35.50 ± 1.64 | | a |
| | *Sinapis* | 25 | 18 | 72.0 | 38.61 ± 4.31 | | a |
| *Chelostoma rapunculi* | ***Campanula*** | 23 | 19 | 82.6 | 20.26 ± 1.49 | <0.001 | a |
| | *Buphthalmum* | 18 | 0 | 0.0 | 12.42 ± 1.80 | | b |
| | *Ranunculus* | 20 | 0 | 0.0 | 4.83 ± 0.55 | | c |
| | *Echium* | 14 | 0 | 0.0 | 8.64 ± 0.71 | | d |
| | *Sinapis* | 21 | 1 | 4.8 | 13.81 ± 1.24 | | b |
| *Chelostoma florisomne* | ***Ranunculus*** | 25 | 21 | 84.0 | 25.22 ± 1.53 | <0.001 | a |
| | *Tanacetum* | 18 | 0 | 0.0 | 9.42 ± 0.74 | | b |
| | *Campanula* | 25 | 2† | 8.0† | 15.10 ± 0.49 | | c |
| | *Brassica* | 11 | 6 | 54.5 | 28.47 ± 2.08 | | a |
| *Hoplitis adunca* | ***Echium*** | 7 | 6 | 85.7 | 18.75 ± 2.10 | <0.001 | a |
| | *Buphthalmum* | 19 | 0 | 0.0 | 5.45 ± 0.43 | | b |

*Notes:* Survival time gives the Kaplan-Meier survival time (mean ± SE) of the larvae on each pollen diet. Group heterogeneity was tested either by the log-rank test (*Hoplitis adunca*) or the *k* sample test (other species). Diets sharing the same letter did not differ significantly at $P < 0.05$ (post hoc tests: pairwise log-rank tests using Bonferroni corrections).

† Mortality attributed to fungal infection; see *Results: Chelostoma florisomne*.

(command *survdiff*) when comparing more than two groups. For statistical analyses, we used the statistical package R (R Development Core Team 2006) and SPSS 11 (SPSS 2005) for Macintosh OS X.

## RESULTS

In total, we transferred 405 bee eggs, of which 315 (77.7%) hatched. The survival of the individuals transferred onto their host pollen (controls) amounted to >80% for each of the four species (Table 2), indicating that egg transfer had little negative impact on larval development. The development time on host pollen significantly differed between the bee species (ANOVA with Duncan post hoc tests, $F = 29.821$, df = 3, 57, $P < 0.001$; Fig. 1). It was longest for the Asteraceae specialist *Heriades truncorum*, by far the smallest of the bee species tested, and shortest for *Chelostoma rapunculi* and *Hoplitis adunca*, the latter being the largest species investigated.

### Heriades truncorum

Larval survival in the Asteraceae specialist *H. truncorum* significantly differed between pollen diets (Kaplan-Meier analysis, *k* sample test, $\chi^2 = 104$, $P < 0.001$; Table 2). No single larva reached the diapausing stage when reared on *Ranunculus* pollen (Fig. 2), and survival was significantly lower than on any other pollen diet (Table 2). The larvae fed *Ranunculus* pollen grew normally for an initial period of 3–4 days, then mostly turned green and eventually died after 5–7 days. In contrast, larval survival did not significantly differ between diets of host pollen and of those of the other non-host pollen species, namely of *Campanula, Echium,* and *Sinapis* (Table 2). On these non-host diets, the larvae were not visibly different from the controls in their size or general appearance. Development time was significantly longer on pollen of *Sinapis* than on the three other pollen types (ANOVA with Duncan post hoc tests, $F = 7.526$, df = 3, 73, $P < 0.001$; Table 3).

### Chelostoma rapunculi

The larvae of the *Campanula* specialist *C. rapunculi* had a substantially lower capacity to develop on non-host pollen (Table 2) than *H. truncorum*. Different pollen diets had a significant effect on larval survival, which was always lower on non-host than on host pollen (Kaplan-Meier analysis, *k* sample test, $\chi^2 = 93.8$, $P < 0.001$; Table 2). One single larva developed on a non-host pollen diet (*Sinapis*). It required 25 days to reach

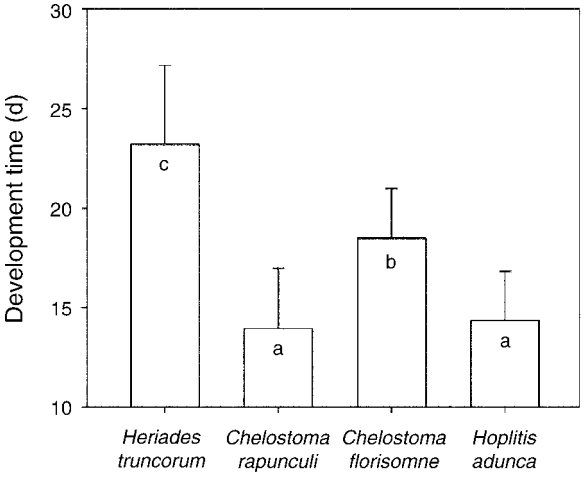


FIG. 1. Development time (number of days from egg hatching to onset of cocoon spinning) of the four oligolectic bee species *Heriades truncorum, Chelostoma rapunculi, Chelostoma florisomne,* and *Hoplitis adunca* when reared on their respective host pollen. Different letters indicate significant differences at $P < 0.05$ (ANOVA with Duncan post hoc tests).

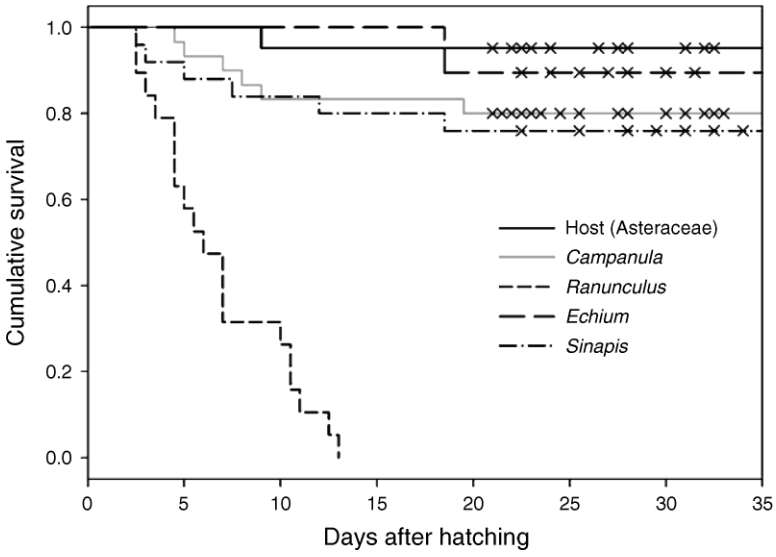


FIG. 2. Cumulative survival of larvae of the Asteraceae specialist *Heriades truncorum* when reared on host pollen (control) and on four non-host pollen diets. Crosses indicate individuals that completed development and entered diapause (censored data).

diapause, compared to 14 days only on host pollen (Table 3). As a mature larva, it was distinctly smaller (~6 mm long) than larvae grown on host pollen (~10 mm long). It spun a loose cocoon and entered diapause, but died during overwintering. On pollen diets of both *Buphthalmum* and *Sinapis*, the larvae fed for up to 30 days (Fig. 3) and most of them initiated defecation before they died. They remained visibly smaller than control individuals of the same age. Larvae reared on *Buphthalmum* pollen progressively adopted an orange hue. In contrast, larvae fed pollen of *Ranunculus* and *Echium* died within few days after hatching (Fig. 3).

### Chelostoma florisomne

Larval survival of the *Ranunculus* specialist *C. florisomne* significantly differed between pollen diets (Kaplan-Meier analysis, *k* sample test, $\chi^2 = 81.5$, $P < 0.001$; Table 2). Pollen diets of *Tanacetum* (Asteraceae) resulted in the lowest larval survival. All larvae fed *Tanacetum* pollen remained visibly small, adopted an orange hue and died, mostly after the initiation of defecation. In contrast, the larvae could develop on the non-host pollen of *Campanula* and *Brassica* (Table 2). The development time on *Campanula* pollen was significantly shorter than on either host pollen or *Brassica* pollen (Kruskal-Wallis test, $\chi^2 = 19.961$, $P < 0.001$; Table 3), but not significantly different from the development time of *C. rapunculi* on *Campanula* pollen (*t* test, $t = 0.516$, corrected df = 28.9, $P = 0.6$). However, after onset or completion of cocoon spinning, most larvae suddenly died, with black spots appearing on the otherwise ivory-colored body, a symptom typical for chalkbrood disease in solitary bees (confirmed by J. Bosch, *personal communication*). We therefore postulate

TABLE 3. Larval development time (number of days from egg hatching to onset of cocoon spinning) for all bee species when reared on host (shown in boldface) and non-host pollen.

| Bee species | Pollen diet | No. bees | Development time (d) | | Group heterogeneity | |
|---|---|---|---|---|---|---|
| | | | Mean ± SE | Median (range) | *P* | Groups |
| *Heriades truncorum* | **Asteraceae** | 19 | 23.21 ± 0.91 | | <0.001 | a |
| | *Campanula* | 24 | 22.27 ± 0.59 | | | a |
| | *Echium* | 16 | 21.00 ± 1.12 | | | a |
| | *Sinapis* | 18 | 27.47 ± 1.38 | | | b |
| *Chelostoma rapunculi* | ***Campanula*** | 19 | 13.95 ± 0.69 | | | |
| | *Sinapis* | 1 | | 25 | | |
| *Chelostoma florisomne* | ***Ranunculus*** | 21 | | 17.5 (14.5–22) | <0.001 | a |
| | *Campanula* | 12 | | 14 (9.5–16.5) | | b |
| | *Brassica* | 9 | | 16.5 (13–30) | | a |
| *Hoplitis adunca* | ***Echium*** | 6 | 14.36 ± 0.93 | | | |

*Notes:* For normally distributed data the mean value (±SE) is given; for data not normally distributed the median and range are given. Group heterogeneity was tested either by an ANOVA with Duncan post hoc tests or by a Kruskal-Wallis test with pairwise Mann-Whitney *U* test and Bonferroni correction. For each bee species, groups sharing the same letter did not differ significantly at $P < 0.05$.

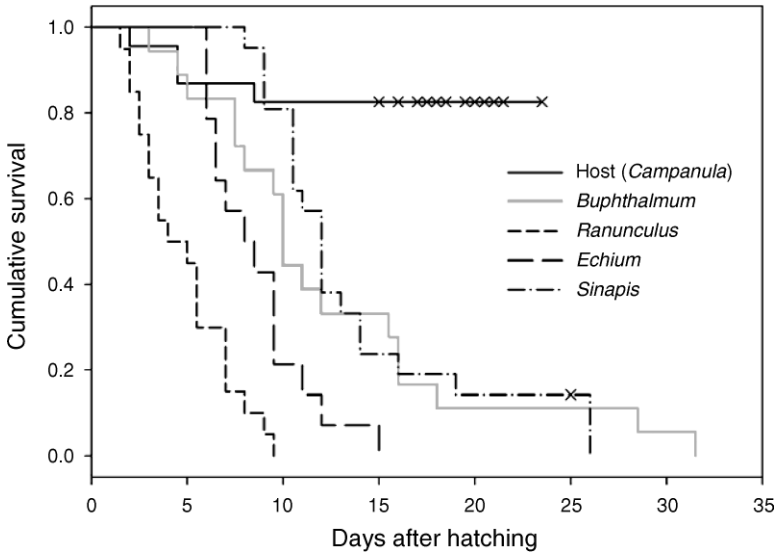


FIG. 3. Cumulative survival of larvae of the *Campanula* specialist *Chelostoma rapunculi* when reared on host pollen (control) and on four non-host pollen diets. Crosses indicate individuals that completed development and entered diapause (censored data).

that *Campanula* pollen is an appropriate diet for *C. florisomne*, the observed mortality being an artifact caused by fungal infection.

### Hoplitis adunca

The *Echium* specialist *H. adunca* failed to develop on pollen of *Buphthalmum* (Table 2). The larvae hatched and started to feed, but all died within a few days, before reaching the defecation stage.

## DISCUSSION

This study clearly shows that pollen does not represent a universally exploitable resource for bees. Pollen of Asteraceae did not allow for the development of *Chelostoma rapunculi*, *Chelostoma florisomne,* and *Hoplitis adunca*, and pollen of *Ranunculus* was an inadequate diet for both *Heriades truncorum* and *C. rapunculi*. In addition, *C. rapunculi* failed to develop on pollen of *Sinapis* and of *Echium*. Thus, pollen originating from five different plant families strongly differed in its suitability as larval food for bees, and the four bee species differed in their ability to use non-host pollen for their development.

Several pollen traits may underlie these differences. Protein content is known to vary widely among pollen types (Roulston et al. 2000), and among the ones included in our study, two are particularly rich in protein: pollen of Campanulaceae (47.9–55% protein; Roulston et al. 2000) and pollen of *Echium vulgare* (Somerville and Nicol 2006). Asteraceae pollen is relatively protein poor (11.7–34.4%, mean 24.4%; Roulston et al. 2000), while the pollen of both *Sinapis* and *Ranunculus* has intermediate protein contents (Wille et al. 1985, Roulston et al. 2000, Somerville and Nicol 2006). These differences might explain why the *Campanula* specialist *C. rapunculi* performed badly on all non-host pollen types tested. It may similarly account for the high flexibility of the Asteraceae specialist *H. truncorum* in using a broad array of pollen sources. The long development time of *H. truncorum* despite its small size may also relate to the low protein content of its host pollen. Compared to *H. truncorum*, *C. rapunculi* and *H. adunca* (specialists on *Campanula* and *Echium*, respectively) had a distinctly shorter development time.

However, protein content of pollen alone can not account for the failure of *H. truncorum* larvae to develop on pollen of *Ranunculus*, the protein content of which is higher than that of most Asteraceae (Wille et al. 1985). Similarly, the larvae of *C. rapunculi* died sooner on the protein-rich pollen of *Echium* than on pollen of *Sinapis* and *Buphthalmum*. Moreover, bee larvae are known to develop on diets strongly differing in their protein concentration. The generalist bee *Lasioglossum zephyrum* successfully developed on pollen diets with a protein content ranging from 20% to 39%, resulting in adults of strikingly different size (Roulston and Cane 2002). Thus, if protein content were the only factor influencing pollen nutritional quality, we would expect the bee species tested in our study to be capable of developing on non-host pollen but to reach only a small size.

Three further factors may influence the suitability of pollen as a food source (Roulston and Cane 2000). First, secondary compounds contained in the pollen grains may be either directly toxic for the bee larvae or interfere with nutrient assimilation. Second, the absence of essential nutrients in the pollen may prevent or prolong larval growth. Third, the lack of specific enzymes, which are necessary for digestion of the intine, might inhibit the extraction of nutrients.

### *Toxic pollen*

Freshly hatched larvae of both *H. truncorum* and *C. rapunculi* initiated feeding on *Ranunculus* pollen but died within few days, indicating a toxic effect of the pollen on the larvae. Indeed, pollen of *Ranunculus* has been shown to be toxic to the honey bee: honey bee workers foraging on *Ranunculus* for pollen suffered dramatic mortality, a phenomenon known as the "Bettlacher May sickness" (Morgenthaler and Maurizio 1941). Most species of the Ranunculaceae contain the toxin protoanemonin, which is poisonous to vertebrates and shows insecticidal and antimicrobial activity (reviewed in Jürgens and Dötterl 2004). In *Ranunculus acris*, protoanemonin was the most abundant volatile in pollen, but proportionally less represented in other floral parts (Bergström et al. 1995). These authors postulated that protoanemonin acts as a chemical defense against herbivores in plant tissue, but that its high concentration in pollen plays a role in attracting specialized bees. In contrast, Jürgens and Dötterl (2004) hypothesized that the high protoanemonin concentration deters the collection of pollen from flowers that also offer nectar as a food resource. They compared the chemical composition of anther volatiles in 12 Ranunculaceae species belonging to six genera, and found protoanemonin to be especially abundant in pollen of the bee-pollinated genera *Ranunculus* and *Pulsatilla*, but less abundant in genera pollinated mainly by other insects. *Anemone sylvestris* was an exception: the pollen of this bee-pollinated but nectarless species was found to have a low concentration of protoanemonin, which might reflect the fact that pollen is the only reward offered to pollinators. Our results demonstrate the toxicity of the pollen of *Ranunculus* to some bees nonspecialized on this pollen, and thus support the hypothesis of Jürgens and Dötterl (2004). However, a few bee species, such as the broad polyleges *Osmia cornuta* and *Osmia bicornis*, can utilize the pollen of *Ranunculus* (Westrich 1989, Nepi and Pacini 1997). This suggests that these two bee species, as well as the *Ranunculus* specialist *C. florisomne,* have the physiological ability to metabolize *Ranunculus* pollen.

In the pollen of *Ranunculus*, protoanemonin is the main volatile included in the pollenkit (Bergström et al. 1995), an oily substance coating the pollen grains of many plant taxa. The degree to which the pollenkit is digested by bees varies among species (Dobson and Peng 1997, and references therein). Dobson and Peng (1997) found that the pollenkit of the pollen of *Ranunculus* was completely digested by larvae of *C. florisomne*. In contrast, Williams (2003) suggested that the pollenkit in pollen of Heliantheae (Asteraceae), or chemicals within it, could interfere with the nutrient assimilation process, rendering its digestion by larvae of *Osmia lignaria* difficult. In our study, the failure of *C. rapunculi* to develop on pollen of *Buphthalmum* and *Sinapis* might similarly relate to the high amounts of pollenkit typical of Asteraceae and Brassicaceae (Williams 2003). The pollen grains of the Campanulaceae, the specific host plants of *C. rapunculi*, contain only little pollenkit (A. Müller, *unpublished data*). While it is recognized that the oily nature of the pollenkit contributes to the adhesiveness of the pollen, resulting in the formation of pollen clumps or in a better adherence to the body of the pollinators (reviewed by Pacini and Hesse 2005), the function of the volatile compounds present in the pollenkit is not fully understood. As these volatiles are responsible for the specific pollen odor, they are assumed to function either as a chemical cue to attract pollinators (Dobson 1988) or as a deterrent against non-pollinating pollen feeders (Detzel and Wink 1993, Dobson and Bergström 2000). The fact that these volatiles occur in seemingly high concentrations in the pollen of some bee-pollinated flowers (von Aufsess 1960) might suggest a defense mechanism against excessive pollen collection by bees, rather than an adaptation to attract them. Similarly, the pigments occurring either in the pollenkit or in the pollen wall might contribute not only to the protection of the pollen grains against fungi, bacteria, or UV radiation (Stanley and Linskens 1974), but also to pollen defense against pollen feeders. Indeed, the larvae of both *C. florisomne* and *C. rapunculi* adopted an orange hue when raised on Asteraceae pollen, suggesting that the pollen pigments had accumulated in their body. Further studies are needed, however, to clarify to what extent the failure of these two species on Asteraceae pollen was due to these pigments.

Similar pollen defense mechanisms may also underlie the rapid mortality of the larvae of *C. rapunculi* fed pollen of *Echium vulgare*. This pollen is particularly rich in protein (Somerville and Nicol 2006), but contains toxic pyrrolizidine alkaloids in very high concentrations (Boppré et al. 2005). The fact that *H. truncorum* could successfully develop on these provisions again demonstrates the varying physiological abilities of different bee species to use non-host pollen.

### *Lack of essential nutrients*

Essential nutrients might be lacking or underrepresented in some pollen types, thereby limiting full development of the bee larvae. Lack of essential sterols in the pollen was postulated to account for the observation that honey bees did not forage on *Arbutus unedo* (Ericaceae) for pollen, although they collected nectar from it (Rasmont et al. 2005). Similarly, pure pollen diets of *Taraxacum officinale* (Asteraceae) proved to be inadequate for both honey bee adults and larvae (reviewed in Roulston and Cane 2000). The low nutritional value of *Taraxacum* pollen is probably due to deficiencies in several essential amino acids, as pure *Taraxacum* diets that were experimentally supplemented with the essential amino acid arginine proved to be appropriate for larval development of the honey bee (Herbert et al. 1970).

To determine whether similar deficiencies in the content of essential amino acids occur in the pollen of

other species of the Asteraceae, we analyzed the large data set of Wille et al. (1985), which provides the concentrations of nine essential amino acids in the pollen of 99 plant taxa belonging to 46 different plant families. Of the 13 Asteraceae taxa included, nine were severely deficient in arginine, including all members of the Asteroideae. Among the 20 taxa having the lowest arginine content, 15 were Asteraceae. Similarly, the content of phenylalanine in the pollen of Asteraceae was in the lower range of the observed values. Thus, deficiencies in the content of essential nutrients, possibly of arginine, may substantially contribute to the failure of the larvae of *C. rapunculi, C. florisomne,* and *H. adunca* to develop on Asteraceae pollen. Indeed, the long survival of these larvae in combination with their very slow growth points to the possible absence of essential substances in the Asteraceae pollen they were fed.

### *Extraction of pollen nutrients*

Pollen nutrients are generally found within the protoplasm, which is well protected by two different layers, the intine and the exine, the latter remaining intact after pollen digestion (Suárez-Cervera et al. 1994). Bees must therefore extrude the protoplasm through the pollen pores by degrading the intine (Suárez-Cervera et al. 1994), and there is evidence that they very efficiently extract nutrients from their host pollen (Wightman and Rogers 1978, Suárez-Cervera et al. 1994, Dobson and Peng 1997). To what extent these processes involve pollen-specific enzymes is not known. Interestingly, an important proportion of the pollen grains of *Taraxacum* were found to be still intact in the feces of adult honey bee workers (Peng et al. 1985), leading to the conclusion that *Taraxacum* pollen grains could not be completely emptied by the honey bee. The failure of *C. rapunculi, C. florisomne,* and *H. adunca* to develop on Asteraceae pollen might be related to similar difficulties in extracting nutrients from the pollen protoplasm.

### *Asteraceae as a pollen source for bees*

Our results are consistent with other studies suggesting that Asteraceae pollen is difficult to utilize as a protein source by bees not specialized on this family. Pollen of Asteraceae was shown to be of poor quality for the honey bee (Herbert et al. 1970, Rayner and Langridge 1985, Somerville and Nicol 2006), for bumblebees (Rasmont et al. 2005) as well as for two polylectic solitary bee species (Levin and Haydak 1957, Guirguis and Brindley 1974). Of 153 polylectic bee species observed visiting native sunflowers (*Helianthus*), the great majority (86%) were casual visitors exploiting sunflowers in small numbers (Hurd et al. 1980). Similarly, a quantitative survey of the pollen preferences of 60 western Palaearctic bee species of the genus *Colletes* by means of microscopical analysis of 1330 pollen loads (A. Müller and M. Kuhlmann, *unpublished data*) revealed that the majority of the polylectic species do not collect Asteraceae pollen at all. Therefore, the utilization of Asteraceae pollen seems to require special physiological adaptations, e.g., to detoxify toxic compounds, to compensate for the lack of essential nutrients or to degrade the intine of the pollen grain. As the Asteraceae are ubiquitous in most temperate habitats and yield high pollen and nectar rewards, selection should favor the evolution of such physiological adaptations. Indeed, the Asteraceae host a large number of specialized bee species (Hurd et al. 1980, Westrich 1989, Müller 1996*b*).

### *Pollen quality and oligolecty*

Phylogenetic analyses have shown that the basal clades of most bee families include a high proportion of oligolectic species (Westrich 1989, Wcislo and Cane 1996). The two most basal bee families, Dasypodaidae and Mellitidae, are predominantly composed of pollen specialists, indicating that oligolecty might be the ancestral state in bees (Danforth et al. 2006). In the western Palaearctic anthidiine bees, several transitions from oligolecty to polylecty were found, but none from polylecty to oligolecty (Müller 1996*b*). Similarly, a recent study on the evolution of host plant choice in bees of the genus *Chelostoma* (Megachilidae), which consists mainly of oligoleges, revealed that the few polylectic species evolved from oligolectic ancestors (C. Sedivy, C. Praz, A. Müller, and S. Dorn, *unpublished data*), and in the pollen collecting masarine wasp *Ceramius caucasicus* polylecty is assumed to be a derived trait (Mauss et al. 2006). However, there do exist some clear examples of transitions from polylecty to oligolecty, e.g., in the genus *Lasioglossum* where oligolectic species have evolved twice within clades of polylectic species (Danforth et al. 2003). Nevertheless, growing evidence suggests that many generalist bee species have evolved from oligolectic ancestors. Given the high quantity of pollen required to rear a single bee larva (Müller et al. 2006), strong selection pressure is expected to act on oligolectic species to reduce their heavy dependence upon a limited number of pollen sources. However, pollen specialists are widespread among the bees, with up to 60% oligoleges in Californian deserts (Minckley and Roulston 2006, and references therein). Therefore, oligolecty in bees may be considered as an evolutionary constraint that has been repeatedly overcome by polylectic species, rather than a property favored under certain environmental conditions. The results of the present study suggest that nutritional suitability or protective properties of the pollen may represent such a constraint preventing bees from becoming polylectic. Hence, we urge careful reconsideration of the assumption that bees are not specialized due to the chemical composition of pollen (Wcislo and Cane 1996, Minckley and Roulston 2006). As already suggested by Dobson and Peng (1997), both the nutritional value of the pollen or the ability to metabolize toxic pollen chemicals may underlie floral associations in bees.

## Conclusions

The general view of pollen as an easy-to-use protein source that is readily digestible for all flower visitors should be considered with caution. The results of our rearing experiments suggest that pollen might be protected chemically by secondary compounds (e.g., *Ranunculus*), by the lack of essential nutrients (e.g., Asteraceae), and/or structurally by pollen walls resistant to digestion. We postulate that the enormous pollen requirements of bees may have selected for such protective properties in pollen. These conclusions open a new field of research in the study of insect–flower relationships. In future, more attention should be paid to both the chemical composition of the pollen and the physiological capabilities of the pollinators to digest and utilize the pollen.

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