Closely related pollen generalist bees differ in their ability to develop on the same pollen diet: evidence for physiological adaptations to digest pollen

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Summary

1. Given the enormous quantitative pollen requirements of bees and their high efficiency in pollen removal, flowers should balance the need to attract bees for pollination on the one hand and to restrict pollen loss to bees on the other hand. Although various morphological flower traits have been identified that reduce excessive pollen losses to bees, the question of whether pollen might also be chemically protected remains largely unexplored.

2. In this study we compared the larval performance of the two very closely related and highly pollen generalist solitary bee species *Osmia bicornis* and *Osmia cornuta* on four different pollen diets.

3. Despite their very large pollen diet breadth, the two bee species showed striking differences in their ability to develop on pollen of the same plant species. *Osmia bicornis* developed well on *Ranunculus* pollen but failed to do so on *Echium* pollen, whereas the reverse held true for *O. cornuta* with the exception of two larvae grown on *Ranunculus* pollen that developed into dwarfish adults. Both bee species performed well on *Sinapis* pollen, while neither of the two species managed to develop on *Tanacetum* pollen.

4. The observed differences in larval survival of these two *Osmia* species when reared on the same pollen diet as well as their failure to develop on *Tanacetum* pollen clearly demonstrate that bees require physiological adaptations to cope with the unfavourable chemical properties of certain pollen.

5. Our results show a remarkable analogy of bee–flower relationships with herbivore–plant interactions and possibly indicate that the pollen of certain plant taxa might be chemically protected.

Key-words: bee–flower relationship, larval development, *Osmia bicornis, Osmia cornuta,* pollination, solitary bee, toxic pollen

Introduction

The great majority of flowering plants attract animals to distribute pollen. Bees, which comprise between 20 000 and 30 000 species worldwide, are the primary pollen vectors in most ecosystems (Michener 2007). They differ from all other pollinating animal taxa except for the masarid wasps in one key aspect: pollen and nectar are not only consumed by the adults, but additionally serve as the exclusive food source for their larvae. The pollen of up to several hundred flowers is required to rear one single offspring (Müller *et al.* 2006), and bees were found to deplete more than 95% of

the total pollen amount produced by their hosts (Schlindwein *et al.* 2005). In addition, bees are highly efficient in pollen collection (Westrich 1989; Müller 1996), frequently removing 70–90% of all available pollen contained in a flower per visit (Thomson 2003). As bees store pollen immediately after its collection in specialized hairbrushes (scopae) or in the crop, where it is often inaccessible for pollination (Westerkamp 1996; Thomson 2003), pollen carryover curves of bees usually fall off very quickly (Thomson 2003 and references therein). Pollen-collecting bees are therefore often low-efficiency pollinators, which remove much pollen but deposit only little (Thomson & Thomson 1992), or in some cases even none at all, thereby acting as pollen thieves (Hargreaves, Harder & Johnson 2009). In the presence of high-efficiency pollinators, bees with low or no

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pollination efficiency become functional flower parasites and often considerably decrease pollination success (Wilson & Thomson 1991; Aigner 2001; Thomson 2003). Furthermore, pollen-collecting bees commute several times a day between nest and host plants, and hence have a limited average foraging range of a few hundred metres only (Zurbuchen *et al.* 2010), which considerably increases the quantity of pollen withdrawn by bees in the vicinity of their often aggregated nests. Thus, given the high quantitative pollen requirements of bees and their high efficiency in pollen removal, flowers should trade the need to attract bees for pollination against excessive pollen losses to pollen-harvesting flower visitors (Westerkamp 1996).

Various morphological floral traits help to reduce pollen loss by narrowing the spectrum of pollen-feeding flower visitors. Flowers of many plant taxa restrict access to their pollen by concealing it within specialized anthers or flower structures (Vogel 1993; Harder & Barclay 1994; Müller 1995; Westerkamp 1997; Westerkamp & Classen-Bockhoff 2007), from where it can be efficiently harvested only by bees possessing specialized morphological or behavioural adaptations (Thorp 2000; Müller *et al.* 2006 and references therein). In addition, portioned pollen release over an extended period of time does not only increase the probability of successful pollination by enforcing repeated pollinator visits (Harder & Wilson 1994; Schlindwein *et al.* 2005), but is expected to limit pollen loss to bees as well (Castellanos *et al.* 2006). The finding that several pollen specialist and generalist bee species failed to develop on non-host pollen suggests that selection might also act on the nutritional quality or toxicity of pollen to reduce excessive pollen losses to bees (Guirguis & Brindley 1974; Williams 2003; Praz, Müller & Dorn 2008a). In fact, pollen of some plant taxa is of poor nutritional quality due to the lack of essential nutrients or the low protein content (Schmidt, Thoenes & Levin 1987; Schmidt *et al.* 1995; Roulston & Cane 2000; Génissel *et al.* 2002; Rasmont *et al.* 2005), or it contains secondary compounds, which are repellent or toxic to insects (Roulston & Cane 2000; Hargreaves, Harder & Johnson 2009 and references therein).

Patterns of host plant use by bees, such as the widespread specialization to a few closely related hosts, the occurrence of periods of expanded host range followed by respecialization, or the phylogenetically conserved host associations, display striking similarities to those of herbivorous insects (Janz & Nylin 2008; Sedivy *et al.* 2008). This indicates that the underlying mechanisms determining host plant use in both bees and herbivores might be based on similar plant characteristics, e.g. plant chemistry. While it is well known that many herbivorous insects are physiologically adapted to cope with the defensive secondary compounds of their hosts (Opitz & Müller 2009), knowledge of whether bees require specialized physiological adaptations to digest the pollen of certain plant taxa is lacking. Due to the high chemical variability of pollen with respect to its content of amino acids, lipids, starch, sterols, vitamins or secondary metabolites (Roulston & Cane 2000 and references therein), we hypothesize that bees need specialized physiological adaptations to cope with the unfavourable chemical properties of some pollen.

To investigate whether bees are physiologically adapted to digest unfavourable pollen, we compared the larval performance of the two very closely related and highly pollen generalist solitary bee species *Osmia bicornis* and *Osmia cornuta* (Megachilidae: Osmiini) (Fig. 1) on four pollen diets containing pure pollen of *Sinapis arvensis* (Brassicaceae), *Ranunculus acris* (Ranunculaceae), *Tanacetum vulgare* (Asteraceae) and *Echium vulgare* (Boraginaceae). Closely related bee species were repeatedly shown to have similar pollen preferences (Sedivy *et al.* 2008 and references therein) and highly pollen generalist bee species are able to thrive on pollen of a wide array of plant taxa (Westrich 1989). Therefore, differences in larval performance of the two tested *Osmia* species on pollen of the same plant species would provide strong evidence for the need of physiological adaptations to digest that pollen.

Materials and methods

BEE SPECIES

The two bee species *O. bicornis* (Linnaeus 1758) and *O. cornuta* (Latreille 1805) are very close relatives. Both are members of the subgenus *Osmia* and within this subgenus belong to the same monophyletic group ('*bicornis*' group), which comprises about 15 species worldwide (Peters 1978; Michener 2007). *Osmia bicornis* and *O. cornuta* are among the most pronounced pollen generalist solitary bee species in Europe, collecting pollen on at least 18 and 13 plant families, respectively (Westrich 1989). Both species are widespread in the Palaearctic and common in most parts of Central Europe. They nest in a great variety of pre-existing cavities, allowing for artificial breeding in hollow bamboo sticks. The adult females build several brood cells during their lifetime, which lasts for up to 6 weeks (Westrich 1989). Each cell is provisioned with pollen and nectar before a single egg is laid. The hatched larva feeds on the pollen–nectar mixture and develops within a few weeks to the adult insect, which overwinters inside the cell and emerges early in spring.

Fig. 1. *Osmia bicornis* female enters her nest with a full pollen load (photograph: A. Krebs).

For the present study, cocoons of both *O. bicornis* and *O. cornuta*, originating from one population each (Konstanz, Germany), were transferred to two artificial nesting stands in Zurich containing hollow bamboo sticks. The first stand offered access to a diverse array of flowering plant species (Botanical Garden). The second stand, located inside a large (8 × 10 × 3·5 m) walk-in cage covered with gauze, limited access to a single plant species, *S. arvensis*, planted in 500 pots with two plants each (ETH Campus Hönggerberg).

ORIGIN OF POLLEN

The larvae of *O. bicornis* and *O. cornuta* were reared on four pollen diets containing pollen of a single plant species each, i.e. *S. arvensis*, *R. acris*, *T. vulgare* and *E. vulgare,* as well as on a control pollen diet. To obtain pollen diets for the experiments, we collected bamboo sticks with freshly completed nests of different bee species (see below), split them longitudinally with a knife and collected the provisions from within the brood cells. Prior to use in the experiments, the provisions were stored at −20 °C.

To obtain pollen diets of *S. arvensis*, we collected brood cell provisions from nests of *O. bicornis* and *O. cornuta* built in the walk-in cage. These brood cell provisions were frozen at −20 °C and subsequently used few days later for the experiments. To obtain pollen diets of the other three plant taxa, we collected brood cell provisions from nests of three solitary bee species, which are strict pollen specialists (Westrich 1989) and which, like *O. bicornis* and *O. cornuta*, belong to the tribe Osmiini: *Chelostoma florisomne* (specialized on *Ranunculus*), *Heriades truncorum* (specialized on Asteraceae) and *Hoplitis adunca* (specialized on *Echium*). These brood cell provisions were collected during the previous season and stored frozen at −20 °C for 6–9 months. The average quantity of nectar sugar in the brood cell provisions of *C. florisomne*, *H. truncorum* and *H. adunca,* which amounts to 54%, 62% and 64% of total dry provision weight, respectively, does not differ substantially from the average quantity of nectar sugar in the provisions of *O. bicornis* and *O. cornuta* (56% and 53%, respectively) (A. Bühler and A. Müller, unpublished data). Hence, we consider the use of brood cell provisions of these three specialist bee species as suitable for our comparative experimental approach.

Nests of *C. florisomne* were collected at Gletterens (western Switzerland), where the main pollen source was *R. acris*. Nests of *H. truncorum* were collected from a fallow at Benken (northern Switzerland), where *T. vulgare* grew at a very high density. Although *H. truncorum* is specialized at the level of plant family, microscopical analyses of pollen samples from collected brood cell provisions revealed that they all contained < 5% non-*Tanacetum* pollen. Nests of *H. adunca* were collected at several localities in northern Switzerland, where the only available host plant was *E. vulgare*.

To obtain control pollen diets, bees nesting at the Botanical Garden were allowed to collect pollen on the naturally available flower supply. The microscopical analysis of the pollen content of 12 randomly selected brood cell provisions of each species revealed that the pollen collected by *O. bicornis* consisted mainly of pollen of Rosaceae, *Fagus* (Fagaceae) and *Acer* (Aceraceae), while the pollen collected by *O. cornuta* was mainly composed of pollen of several species of Rosaceae.

EGG TRANSFER AND LARVAL PERFORMANCE

Rearing of the larvae of *O. bicornis* and *O. cornuta* on the five different pollen diets was conducted in artificial brood cells. Artificial cells were made of small blocks (4 × 2 × 2 cm) of beech wood provided with a drilled burrow (2 cm length, 0·8 cm width) open both at the top and at the front side. The openings were covered with coverslips attached to the block with transparent adhesive tape to permit free viewing into the burrow.

Eggs used for the experiments were carefully detached with a thin spatula from the brood cell provisions in the original nest and transferred to the experimental pollen diet previously placed into the burrow of an artificial cell. Larvae were reared individually in artificial cells. The eggs of both species originated from nests of the same population (Konstanz, Germany). For each bee species, we transferred 30–33 eggs onto each of the five pollen diets. As sex could not be determined with certainty *a priori*, each larva, received the same quantity of pollen diet. To account for the different body weight of the two species, we provided a quantity of 400 and 600 mg of pollen diet for *O. bicornis* and *O. cornuta*, respectively.

Egg hatching and larval development took place in darkness in the same climate chamber (E7/2; Conviron, Winnipeg, Canada) under the following conditions: 25 °C for 16 h followed by a gradual reduction of temperature to 10 °C within 4 h followed by a gradual increase back to 25 °C within another 4 h. Relative humidity was held constant at 70%. The following parameters of larval development were recorded every second day: egg hatching, start of feeding, start of defecation, start of cocoon spinning, completion of cocoon, death. In addition, the larvae were weighed every second day to the nearest 0·1 mg (AB204-S; Mettler Toledo, Greifensee, Switzerland). To prevent mechanical damage to the fragile freshly hatched larvae, weighing started 6 days after hatching. We discontinued weighing as soon as the larvae started to spin a cocoon.

To prevent the spread of diseases, we removed artificial cells with dead eggs or larvae and, upon completion of the cocoons, cleaned the cells from faeces and leftover pollen. Unhatched eggs and larvae that died from external factors, such as foulbrood or mechanical damage, were excluded from all analyses.

Once all larvae had either died or completed their cocoons, conditions in the climate chamber were changed to 26 °C and 60% relative humidity. After 5 months, cocoons were carefully opened with nail scissors to assess survival, imaginal weight and sex.

DATA ANALYSIS

For survival analyses, we treated all larvae that had completed their cocoon as survivors irrespective of whether they later successfully completed metamorphosis or not. Cocoons were considered completed upon becoming entirely intransparent. Dates of egg hatching, start of feeding, start of defecation, start of cocoon spinning, completion of cocoon and death were determined as the average of the two observation dates between which the respective event occurred.

We used Kaplan–Meier survival statistics to compare larval survival on the different pollen diets following Lee & Wang (2003). The number of days between hatching and completion of the cocoon was considered as 'censored data': individuals that died before the completion of the cocoon represented the exact observations for which the event (death) occurred, while those that completed the cocoon were the censored observations. The latter were considered survivors and thus withdrawn from survival calculations. To test for differences between survival distributions, we applied the log-rank test with Bonferroni correction using the option 'pairwise for each stratum' implemented in SPSS 16.0 (SPSS Inc., Chicago, Illinois, USA) when comparing two groups. We tested differences between larval survival of each bee species on the five pollen diets and compared larval survival of the two species when reared on the same pollen diet. For statistical analyses, SPSS 16.0 for Macintosh OS X was used.

Results

Of a total of 308 bee eggs transferred, 258 (83·8%) hatched. Eighteen larvae that died from an infection with foulbrood (seven larvae of *O. bicornis* and five larvae of *O. cornuta* on the *Echium* and six larvae of *O. cornuta* on the control pollen diet) and one larva that died from mechanical damage were excluded from all analyses. Larval survival on the control pollen diet amounted to > 90% for both species (Table 1), indicating that both the experimental design and the handling of eggs and larvae had at most a marginal impact on mortality.

OSMIA BICORNIS

The larvae of *O. bicornis* did not perform significantly different on the *Sinapis* and the *Ranunculus* pollen diet (log-rank test, $\chi^2 = 0.47$, $P = 0.492$; Fig. 2, Table 1) and reached a similar median weight before the onset of cocoon formation of 156 and 154 mg, respectively (Mann–Whitney test, $U = 302.5$, $P = 0.397$). In contrast, all larvae reared on the *Tanacetum* pollen diet died within 10–66 days (median 23 days). Although they constantly fed and defecated, they stayed very small and reached a median weight of only 6·3 mg before they died. None of these larvae started to spin a cocoon. Twelve of the 17 larvae reared on the *Echium* pollen diet died within 16–24 days (median 19 days), reaching a median weight of 20·5 mg before death. Five larvae completed the cocoon and reached a median weight of 114 mg before the onset of cocoon formation. However, none of these five larvae completed metamorphosis to viable adults and all but one died before pupation. Survival did not differ significantly between larvae reared on the *Tanacetum* pollen diet and larvae reared on the *Echium* pollen diet (log-rank test, $\chi^2 = 0.23$, $P = 0.631$; Table 1). However, median larval weight before death was significantly less in larvae reared on the *Tanacetum* pollen diet (6·3 mg) than in larvae reared on the *Echium* pollen diet (20·5 mg) (Mann–Whitney test, $U = 12.5$, $P < 0.001$).

OSMIA CORNUTA

The larvae of *O. cornuta* did not perform significantly different on the *Sinapis* and the *Echium* pollen diet (log-rank test, $\chi^2 = 0.12$, $P = 0.731$; Fig. 3, Table 1) and reached a similar median weight before the onset of cocoon formation of 195 and 190 mg, respectively (Mann–Whitney test, $U = 151.0$, $P = 0.102$). In contrast, all larvae reared on the *Tanacetum* pollen diet died within 6–46 days (median 16 days), reaching a median weight of only 7·1 mg before death. All but two larvae reared on the *Ranunculus* pollen diet died within 10–18 days (median 14 days), reaching a median weight of 60 mg before death. The two surviving larvae successfully developed into adult females. However, with a weight of only 68 and 97 mg, these two females were distinctly
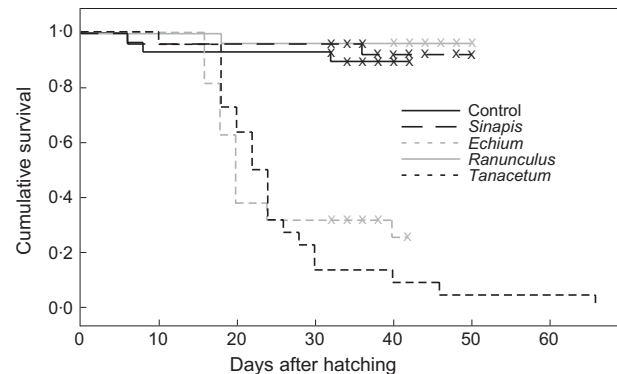

Fig. 2. Cumulative survival of larvae of *Osmia bicornis* when reared on pollen collected on the naturally available flower supply (control) and on four experimental pollen diets. Crosses indicate individuals that reached the cocoon stage (censored data).

Table 1. Egg and larval survival and number of viable adults of the two solitary bee species *Osmia cornuta* and *Osmia bicornis* when reared on freely collected pollen (control) and on four experimental pollen diets

Bee species	Pollen diet	No. eggs hatched (unhatched)	Surviving larvae			Group heterogeneity		No. viable adults
			No.	%	Survival time (days)	P	Groups	
O. bicornis	Control	31 (0)	28	90·3	39·42 ± 1·56	<0·001	a	28
	Sinapis	27 (5)	25	92·6	47·78 ± 1·68		a	24
	Ranunculus	29 (0)	28	96·6	48·90 ± 1·08		a	28
	Tanacetum	22 (7)	0	0	25·82 ± 2·52		b	0
	Echium	17 (7)	5	29·4	26·59 ± 2·63		b	0
O. cornuta	Control	20 (6)	20	100	NA*	<0·001	a	17
	Sinapis	28 (3)	27	96·4	40·71 ± 1·26		a	23
	Ranunculus	25 (7)	2	8	16·32 ± 2·27		b	2
	Tanacetum	23 (7)	0	0	18·78 ± 1·61		b	0
	Echium	17 (8)	16	94·1	42·82 ± 1·14		a	12

Survival time gives the Kaplan–Meier survival time in days (mean ± SE) of the larvae on each pollen diet. Group heterogeneity was tested with the pairwise log-rank test between all treatments. Diets sharing the same letter did not differ significantly at $P < 0.05$ (*post hoc* test: pairwise log-rank test using Bonferroni corrections).
*Survival time could not be computed as all larvae survived until the cocoon stage (censored data only).
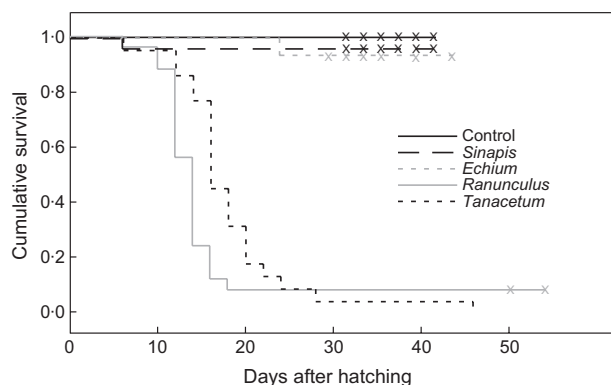
Fig. 3. Cumulative survival of larvae of *Osmia cornuta* when reared on pollen collected on the naturally available flower supply (control) and on four experimental pollen diets. Crosses indicate individuals that reached the cocoon stage (censored data).

lighter than average-sized adult females of *O. cornuta,* which typically weigh about 150–200 mg (C. Sedivy, unpublished data). In addition, these two individuals needed 50 and 54 days to complete their cocoon, whereas larvae of *O. cornuta* reared on the *Sinapis* and the *Echium* pollen diet completed their cocoon already after a median of 38 and 33 days, respectively. Survival did not differ significantly between larvae reared on the *Tanacetum* pollen diet and larvae reared on the *Ranunculus* pollen diet (log-rank test, $\chi^2 = 4\cdot27$, $P = 0\cdot39$ after Bonferroni correction; Table 1). However, median larval weight before death was significantly higher in larvae reared on the *Tanacetum* pollen diet ($7\cdot1$ mg) than in larvae reared on the *Ranunculus* pollen diet ($6\cdot0$ mg) (Mann–Whitney test, $U = 158\cdot0$, $P = 0\cdot019$).

POLLEN DIETS IN COMPARISON

Survival of the larvae of *O. bicornis* and *O. cornuta* did not significantly differ on the *Sinapis* pollen diet (log-rank test, $\chi^2 = 0\cdot38$, $P = 0\cdot539$) and the larvae of both species invariably died when reared on the *Tanacetum* pollen diet. In contrast, larval survival differed significantly between the two bee species when reared on the *Ranunculus* (log-rank test, $\chi^2 = 49\cdot70$, $P < 0\cdot001$) and the *Echium* pollen diet (log-rank test, $\chi^2 = 14\cdot57$, $P < 0\cdot001$).

Discussion

Our comparative experimental approach provided first evidence that larvae of two closely related generalist bee species differ in their physiological ability to digest pollen from the same host plant. *Osmia bicornis* failed to survive on pollen of *E. vulgare* but developed on pollen of *R. acris*, whereas the reverse held generally true for *O. cornuta*. This difference is striking for highly generalized congeneric bee species and clearly shows that the larvae of *O. bicornis* are physiologically adapted to digest *Ranunculus* pollen, whereas the larvae of *O. cornuta* are adapted to digest *Echium* pollen. Conversely, both *Osmia* species coincided in their inability to develop on

pollen of *T. vulgare*, which is an unsuitable pollen source for many unspecialized bee species (Müller & Kuhlmann 2008; Praz, Müller & Dorn 2008a), but not for specialist species (Westrich 1989), again indicating the necessity for physiological adaptations.

Among the *O. cornuta* larvae feeding on *Ranunculus* pollen, two individuals reached the adult stage, whereas all other larvae died. The fact that these two individuals reached only a very low adult weight in spite of their exceedingly long development time adds further evidence for the unfavourable chemical properties of *Ranunculus* pollen. Obviously, these two larvae possessed the physiological machinery to cope with *Ranunculus* pollen, while all other tested larvae of the same population did not. This intrapopulational variation in pollen digestion ability is intriguing as such a variation is the prerequisite for selection acting towards a broader diet by including new pollen hosts. The inclusion of a new pollen host, however, does not only require the overcoming of physiological constraints to successfully utilize the new pollen but also the overcoming of neurological constraints related to the recognition or handling of flowers (Williams 2003; Praz, Müller & Dorn 2008b; Sedivy *et al.* 2008).

In both bee species tested, larval mortality patterns differed considerably between the pollen diets, indicating that the unfavourable properties of these pollen affected the larvae in different ways. When feeding on *Tanacetum* pollen, all *O. bicornis* and *O. cornuta* larvae remained very small, even those that constantly fed and defecated for more than 2 months and 1 month, respectively. This finding is in line with similar inhibitory effects of Asteraceae pollen on bee larval growth observed previously (Levin & Haydak 1957; Guirguis & Brindley 1974; Williams 2003; Praz, Müller & Dorn 2008a). The mortality pattern on the *Tanacetum* pollen diet is suggestive of either the interference of toxic pollen compounds with nutrient digestion, an insufficient quantity or quality of nutrients in the pollen of *Tanacetum*, such as amino acids or sterols (Pilorget, Buckner & Lundgren 2010), or of difficulties in extracting essential compounds from the pollen grains. Deficiencies in the content of essential nutrients were also hypothesized by Praz, Müller & Dorn (2008a) to be a possible cause for the failure of three pollen specialist bee species to develop on Asteraceae pollen. In contrast, when feeding on *Ranunculus* pollen, all but the two surviving *O. cornuta* larvae died very soon and attained a significantly lower weight than larvae reared on *Tanacetum* pollen. This finding is compatible with the assumption that *Ranunculus* pollen contains toxic compounds. In fact, anther volatiles of *Ranunculus* were found to be dominated by protoanemonin (Bergström, Dobson & Groth 1995; Jürgens & Dötterl 2004), a potential flower defence compound against destructive feeding by phytophagous insects (Jürgens & Dötterl 2004). Extracts of aerial parts of *Ranunculus sceleratus* containing protoanemonin were indeed found to exhibit insecticidal activity in biotests (Bhattacharya, Nath & Bordoloi 1993); however, whether and the extent to which protoanemonin affects bee larval development remains to be elucidated. When feeding on *Echium* pollen,

the mortality pattern of the *O. bicornis* larvae differed substantially from that on *Tanacetum* pollen and from that of *O. cornuta* on *Ranunculus* pollen. The *Echium*-fed larvae that did not reach the cocoon stage grew normally, attaining a significantly larger weight than larvae reared on *Tanacetum* pollen before they suddenly ceased to grow and died. This mortality pattern might possibly be explained by the accumulation of toxic pollen compounds, which, after reaching a lethal threshold, caused the sudden death of the larvae. Possible candidates for such toxic pollen compounds in *Echium* pollen are pyrrolizidine alkaloids. These alkaloids, which are contained in high concentrations in the pollen of *Echium* and some Asteraceae species (Boppré *et al.* 2008), are known to be toxic or deterrent for generalist herbivores (Van Dam *et al.* 1995; Narberhaus, Zintgraf & Dobler 2005). Although Reinhard *et al.* (2009) did not find adverse effects of pyrrolizidine alkaloids on adult honeybees when provided in naturally occurring concentrations, the authors hypothesized that these alkaloids, due to their mutagenic effects, might be a threat to the more vulnerable honeybee larvae.

Pollen of different plant taxa is highly variable with respect to its chemical composition (Roulston & Cane 2000 and references therein). Thus, pollen does not appear to be an easy-to-use source of protein for bees. In fact, larval growth and adult life span of bumblebees and honeybees were found to substantially differ among different pollen diets (Schmidt, Thoenes & Levin 1987; Schmidt *et al.* 1995; Génissel *et al.* 2002). This finding, however, was generally attributed to quantitative or qualitative differences in the protein content of the tested pollen. To our knowledge, no study has ever shown that bees possess physiological adaptations to digest pollen. The results of our experiments indicate that such adaptations might possibly be widespread in bees, mirroring the situation in herbivore–plant interactions (Opitz & Müller 2009). Interestingly, herbivorous insects may lose the ability to efficiently utilize alternative hosts after having adapted physiologically to the secondary chemistry of their hosts, a phenomenon known as the physiological efficiency hypothesis (Singer 2008 and references therein). Analogously, we hypothesize that the widespread specialization of solitary bees to a restricted number of closely related pollen hosts (Westrich 1989; Michener 2007) may in part be explained by the inability to digest alternative pollen after the bees' physiology became optimized to cope with the chemistry of a specific pollen host. This hypothesis is in line with the finding that several pollen specialist bee species failed to develop on non-host pollen (Praz, Müller & Dorn 2008a). However, there are also examples of pollen specialist bees, which are able to sucessfully develop on non-host pollen (Williams 2003; Praz, Müller & Dorn 2008a,b). Possible evidence for physiological adaptations to digest pollen also comes from a recent study on the evolution of host plant choice in bees of the genus *Chelostoma*, which mainly consists of pollen specialist species (Sedivy *et al.* 2008). The only two pollen generalists that evolved from specialized ancestors broadened their host plant spectrum by incorporating pollen hosts that are the exclusive host plants of closely related

pollen specialist species. This suggests that the physiological or neurological capabilities to cope with some of the newly added hosts were inherited from a common ancestor.

Given the evidence that bees need physiological adaptations to digest some pollen, the essential question arises as to whether unfavourable pollen properties have evolved as protection against pollen-collecting bees, whether they are by-products of the plants' physiology serving other primary goals or whether they are a pleiotropic consequence of chemical defence against herbivores in other tissues (Hargreaves, Harder & Johnson 2009). We hypothesize that the high quantitative pollen requirements of bees might have selected for protective properties of the pollen, which serve to filter pollen-consuming floral visitors. This view is supported by the following lines of reasoning: (i) As selection has shaped the morphology of flowers to reduce pollen loss to bees (for references, see Introduction), selection may be expected to act on the nutritional quality or toxicity of pollen as well. (ii) An increasing number of studies report on the occurrence of secondary compounds in pollen with insecticidal properties (Detzel & Wink 1993; Jayanth *et al.* 1993; Datta & Saxena 2001; Pimentel De Carvalho & Message 2004; Hargreaves, Harder & Johnson 2009 and references therein). The extent to which these insecticidal pollen compounds affect bee larval development on the one hand and improve plant fitness on the other hand remains to be elucidated. The findings that the concentration of pyrrolizidine alkaloids in *Senecio jacobaea* (Asteraceae) is distinctly higher in pollen compared to stems and leaves (Budde *et al.* 2004) and that the pollen of *Lupinus polyphyllus* (Fabaceae) and *Brugmansia aurea* (Solanaceae) contains higher amounts of some alkaloids than leaves or flowers (Detzel & Wink 1993) clearly suggest that some of these insecticidal pollen compounds may indeed exert a protective function. (iii) All pollen types experimentally found so far to possess unfavourable properties for bee larval development (several species of Asteroideae and Cichorioideae, *Echium*, *Ranunculus*, *Sinapis*, *Stryphnodendron*; Loper & Berdel 1980; Williams 2003; Pimentel De Carvalho & Message 2004; Praz, Müller & Dorn 2008a; this study) originate from flowers with freely accessible pollen that can easily be harvested by any flower visiting bee. Conversely, we hypothesize that pollen of flowers, which is protected from unspecialized bees within specialized flower structures such as keels, does not possess chemical properties impeding its digestion by unspecialized bees.

In conclusion, the present study provides first evidence that bees need physiological adaptations to cope with the unfavourable chemical composition of some pollen and suggests that the underlying mechanisms causing the observed larval mortality vary among different pollen.

Acknowledgements

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