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Is flower selection influenced by chemical imprinting to larval food provisions in the generalist bee *Osmia bicornis* (Megachilidae)?

Heidi E. M. DOBSON¹, Manfred AYASSE², Katherine A. O'NEAL^{1,3}, Jesse A. JACKA^{1,4}

¹Department of Biology, Whitman College, Walla Walla, WA 99362, USA

²Institute of Experimental Ecology, University of Ulm, 89069 Ulm, Germany

³5004 Granite Creek Court, Las Vegas, NV 89131, USA

⁴4215 Sanders View Drive, apt. 205, Colorado Springs, CO 80916, USA

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Abstract – To investigate whether flower selection in polylectic solitary bees is modulated by chemical imprinting to nest provisions, larvae of *Osmia bicornis* (L.) were reared on either *Brassica napus* L. (Brassicaceae) or *Onobrychis viciifolia* Scop. (Fabaceae). Flower preferences by adults were evaluated in multiple-choice behavioral tests based on visit number and duration, and flowers selected in the first three visits; data were compared to control bees from the wild. Females reared on *B. napus* showed only subtle increases in selection for this species, which was highly attractive to both control and experimental bees, masking any effects of imprinting; however, in the first three visits, experimental bees tended to select *B. napus* more frequently and consistently than controls. Bees reared on *O. viciifolia* were few and mostly males, which tended to visit this species more than controls. Rearing larvae on either plant affected bee attraction to other plant species. Overall, the data do not provide clear evidence of imprinting, but suggest that rearing bees on a single plant can both directly and indirectly affect flower selection by adults.

solitary bees / polylecty / flower selection / imprinting / larval food

1. INTRODUCTION

Among the close to 20,000 bee species described worldwide (Michener 2007), the vast majority live solitarily and are univoltine, such that adults emerging from nests must locate their food plants with no guidance from older adults other than possibly the larval food, consisting of nectar and pollen, gathered by their mothers. Solitary bees vary in the range of

plant species utilized as sources of nectar and pollen, with flower specialization being most pronounced—and documented—in the bees' associations with pollen. These are typically characterized by the range of flowers visited by females to collect pollen (readily quantified through pollen grain identification), which is categorized along a continuum from oligolecty, where pollen is collected from one or a few closely related plants, to polylecty, where it comes from unrelated plants in various genera and families (Cane and Sipes 2006; Müller and Kuhlmann 2008). Investigations of stimuli used by solitary bees in flower selection have focused mainly on oligolectic species, where olfactory cues (whole-flower scents, pollen odors, and even particular volatiles) have been

Corresponding author: H.E.M. Dobson,
dobsonhe@whitman.edu

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shown to be essential in attracting bees, often in varying combinations with visual cues (Dobson 1987; Dobson and Bergström 2000; Burger et al. 2010, 2012; Dötterl and Schöffler 2007; Dötterl et al. 2005, 2011; Milet-Pinheiro et al. 2012). The extent to which flower preferences by solitary bees are environmentally induced (through chemical imprinting to nest provisions) versus genetically based is not well understood.

The tendency of adult phytophagous insects to preferentially feed on their larval host plants (Hopkins Host Selection Principle, Craighead 1921) has been variously explained as having a genetic basis, as arising from olfactory learning (i.e., imprinting or conditioning) to the larval host plant, or a combination of both (see Dethier 1954; Visser 1986; Barron 2001). In oligolectic solitary bees, selection of flowers for pollen collection is typically rigid (Linsley 1958; Eickwort and Ginsberg 1980) and has been suggested to be based, at least partly, on bees becoming imprinted to chemicals in their larval food (Linsley 1978); but arguments in favor of a genetic determination (e.g., Sedivy et al. 2008) recently received empirical support in the first experimental study addressing this question in an oligolectic species (Praz et al. 2008a). It remains to be established to what extent this finding can be generalized to other oligolectic species, or to nectar foraging, or to polylectic solitary bees, which are more likely to be influenced by imprinting given their greater plasticity in host-flower selection. Insect imprinting may follow a path described by the chemical legacy hypothesis (Corbet 1985; Barron 2001), whereby olfactory learning occurs when emerging adults are exposed (coincident with a sensitive period of neuronal development) to chemicals from the larval environment (e.g., Turlings et al. 1993; Van Emden et al. 1996); this might be possible for solitary bees in which new adults remain in the nest cells for several days before emergence, where they could be exposed to odors from unconsumed larval food or larval feces (Dobson and Peng 1997). Alternatively, insects may learn scents during preimaginal, especially late prepupal, stages and carry this olfactory memory through metamorphosis

(e.g., Thorpe 1939; Gutiérrez-Ibáñez et al. 2007; Blackiston et al. 2008). While there is no evidence that solitary bees undergo imprinting to their preimaginal environment, it is plausible that either of these pathways might occur especially in polylectic bees.

Polylecty is a characteristic of species or populations, but not of individual bees; while a species may visit a wide range of unrelated plant species to collect pollen and nectar, females can show preferences that may vary from one individual to another, or from one population to another, as well as flower constancy that varies with foraging trips, times of day, and seasonal bloom (Eickwort and Ginsberg 1980; Cane and Sipes 2006). Consequently, while individual nest cells are typically provisioned with different mixtures of pollens, the proportions of each pollen species can vary from one cell to the next, with some cells being dominated by or composed almost exclusively of a single species. Nectar would be expected to vary as well, especially if it is collected on the same flowers as pollen (unfortunately, identification of nectar host plants is not as readily accomplished from nest provisions as for pollen). With this in mind, one can ask how pollen and nectar composition in the nest provisions of polylectic bees might influence flower selection by individual adults, and whether any chemical imprinting occurs to flowers species that are most abundant, or that have the most prominent profile of chemicals. Such imprinting should facilitate a bee's location of host flowers that occur reliably and in sufficient abundance in its local surroundings, thus giving it an adaptive advantage over bees that might select flowers strictly based on genetically determined preferences. Imprinting of polylectic solitary bees to nest provisions has been addressed in only one unpublished exploratory study of the alfalfa leafcutting bee, *Megachile rotundata* (Megachilidae), which is a polylectic species (Westrich 1989; Small et al. 1997) that can display a strong preference for alfalfa (Stephen and Torchio 1961). When the larvae were experimentally reared on pollen and nectar from carrot flowers and the ensuing

adults were given a choice of flowers, the adults persisted in visiting alfalfa (V.J. Tepedino, unpublished, cited in Wcislo and Cane 1996). These intriguing results suggest that flower selection has a genetic basis here too, similar to the oligolectic species studied by Praz et al. (2008a); follow-up investigations are needed to clarify how flower selection is modulated in polylectic bees.

One pollen-generalist solitary bee that is a good model for examining this question is *Osmia bicornis* (Linnaeus) (= *Osmia rufa* (L.)) (Megachilidae), which is common throughout Europe, extending into North Africa and SW Asia (Ungrecht et al. 2008). It is easily reared in trap nests with preformed holes (O'Toole 2000), and has been used as a pollinator in orchards (Krunić and Stanisavljević 2006), field crops (Roth 1990; Steffan-Dewenter 2003; Teper and Biliński 2009; Fliszkiewicz et al. 2011), and greenhouses (Holm 1973; O'Toole 2000). It is univoltine, with a flight season from April to June; after mating usually only once, females typically build several nests, consisting altogether of up to 30 brood cells (Westrich 1989; O'Toole 2000). *O. bicornis* visits a large assortment of plant species (up to 140 species in 37 families) for nectar and pollen, although analysis of nest provisions indicates that individual females tend to restrict pollen collecting to only a few taxa at a time (Seidelmann 1991), resulting in nest cells often having nearly pure single-species pollen provisions. Among the most commonly encountered pollen is that from *Quercus*, *Ranunculus*, *Acer*, Rosaceae (*Rubus*, *Rosa*), and *Papaver* (Westrich 1989; Seidelmann 1991; Radmacher and Strohm 2010; Sedivy et al. 2011), but the species vary with the local flora (Westrich 1989; Teper and Biliński 2009). Given that pollen constancy (and perhaps also nectar constancy) in *O. bicornis* can vary among individual females, among sites (due to different plant compositions), and over the season (due to different bloom phenologies of host plants), do bees that emerge from nests that contained a particular pollen tend in turn to visit flowers of this same plant species, at least initially before they learn (through foraging experience) to visit

other flowers that may be more abundant and profitable?

The goal of this study was to determine whether flower-generalist bees, using *O. bicornis* as a model, can become imprinted to chemicals in their larval food provisions, as revealed by whether or not individual bees show an initial preference to visit the flower species on which they were reared. Larvae of *O. bicornis* were fed exclusively on pollen and nectar from a single species, and the flower preferences of the newly emerged bees were established in multiple-choice behavioral tests; bee responses to each flower species were analyzed in terms of number of visits, duration of visits, and sequence of the first three visits. We conducted two distinct experiments using different plant species to provision the bee nests. While both male and female solitary bees visit flowers to feed on nectar and pollen (e.g., Schäffler and Dötterl 2011), we emphasized females in our tests since they are the sex that more actively forages on flowers, and included males when female numbers were low. Flower preferences of experimental bees were compared to those of control bees reared in the wild (at the same site where flowers were collected for experiments).

2. MATERIALS AND METHODS

2.1. General

The study was conducted at the University of Vienna, in Austria: Department of Evolutionary Biology (behavioral tests), Botanical Garden (tent for experiment 1, collection of flowers, and all trap-nesting bees), and Agricultural Research Station, Institut für Bienenkunde in Lunz am See (tent for experiment 2).

Separate experiments were conducted in two different years, distinguished by the flowers used to rear the bees: (1) *Brassica napus* L. (Brassicaceae) (1997–1998) and (2) *Onobrychis viciifolia* Scop. (Fabaceae) (2001–2002). The rearing species were selected on the basis of their being documented host flowers for *O. bicornis* (Westrich 1989), the ease of growing them during the spring in a greenhouse, and

their offering sufficient pollen and nectar to meet the full needs of nesting *O. bicornis* females. Because *B. napus* was highly attractive to both experimental and control bees in experiment 1, we sought a less visited species for experiment 2; and because none of the less attractive species included in behavioral tests of experiment 1 met all of our selection requirements, a new species was chosen. The experiments also differed in the gender of bees used in the behavioral tests. We focused on female bees in experiment 1, but extended the tests to male bees in experiment 2 since very few females emerged from the experimental nests.

2.2. Experimental bees (reared on single flower species)

To obtain bees reared exclusively on *B. napus* or *O. vicifolia*, adult female bees were forced to forage only on one plant species, and use it to provision nests, by placing the bees in tents that covered an area planted exclusively with that plant species. The females were introduced in May into tents (2×2×2 m for experiment 1, 1997; 6×2×12 m for experiment 2, 2001) as preimaginal bees still in their cocoons, which were obtained from trap nests (wood blocks with drilled nesting holes 7–8 mm in diameter) in wild populations.

2.3. Control bees (reared in the wild on different plants)

Bees reared on pollen and nectar gathered by free-flying wild females were obtained from trap nests; emerging adults were used as controls in both experiments.

2.4. Bee care

All filled nests within the trap-nesting blocks (experimental and control) were collected from the outdoors at the end of January; cocoons containing adults were removed and refrigerated at 4–5°C until a few days prior to the behavioral experiments. For bee emergence, cocoons were placed in open cardboard boxes inside square screen cages (55 cm on each side) at room temperature and in a naturally lighted room. As bees emerged, they were individually marked on the thorax with different colors of liquid paper correction fluid (Gillette Co., Boston) and were

provided free access to a sugar/water solution (50:50 v/v) offered on saturated white sponges (3.5×2 cm). Males and females were kept in the same cage for 2 days after emergence to ensure that all females had the opportunity to mate, and then placed in separate cages.

2.5. Flower-preference behavioral tests

2.5.1. Location

Multiple-choice behavioral tests to determine flower preferences of *O. bicornis* were carried out for 2 weeks (May–June). In experiment 1, they were conducted in a roof greenhouse (artificial and natural sunlight, 22°C), and in experiment 2, in a ground-floor greenhouse (natural lighting, 17–25°C).

2.5.2. Test cages

Bees were tested in a small cylindrical mesh cage (height, 22 cm; diameter, 25 cm) with a wood floor. During each test, a bee was offered different flower species, which were displayed as small bouquets in water-filled vials (5 cm high) and positioned equidistantly around the inner periphery of the cage. The flower displays were of similar size and height. Flower species were arranged in a set order, with alternating flower colors (based on human vision).

2.5.3. Flower species tested

All flowers were collected daily from the Botanical Garden, except *O. vicifolia* from the Donau Insel in Vienna. The selection of flowers offered to the bees was made as uniform as possible across the two experiments, based on the availability of blooming plants. Aside from the two species used to rear bees, which were each tested in only one experiment, five plant species were identical across the two experiments, two were conspecifics, and one was used only in experiment 1.

1. Experiment 1: *B. napus*

The bees were offered a choice of flowers from nine plant species, including the rearing plant *B. napus*: *Chrysanthemum leucanthemum* L. (Asteraceae), *Echium vulgare* L. (Boraginaceae), *Cerastium biebersteinii* L. (Caryophyllaceae),

Lathyrus pratensis L., *Trifolium repens* L., and *Trifolium pratense* L. (all Fabaceae), *Salvia pratensis* L. (Lamiaceae), and *Ranunculus repens* L. (Ranunculaceae).

2. Experiment 2: *O. viciifolia*

The bees were offered a similar choice of flowers from eight species (*L. pratensis* was not available), including the rearing plant *O. viciifolia*: *C. leucanthemum*, *E. vulgare*, *Cerastium tomentosum* L., *T. repens*, *T. pratense*, *S. pratensis*, *Ranunculus acris* L.

2.6. Bee behavioral data

Bees were tested individually. Prior to testing, each bee was acclimated to an empty test cage for 10 min. To start a test, the bee was calmly placed in the center of the flower-filled cage by allowing it to walk out of a glass vial. Each test lasted 16 min, during which the cage was rotated a quarter turn every 2 min to counter any bias caused by uneven light and air currents. Any flowers visited by the bee were replaced with fresh ones prior to testing the next bee.

During the test, all bee visits to flower bouquets were recorded as either (1) feed visits, defined as when the bee landed and extended its proboscis into a flower or (2) nonfeed visits, when a bee landed without attempting to feed (i.e., landed briefly or groomed). These two visit types were analyzed in terms of their number and duration on each flower species to determine which flowers attracted bees the most; focus was placed on feed visits, which reflected not only attraction to the flower but also stimulation to feed (no distinction was made between nectar and pollen feeding). In addition, the sequence of flowers in the first, second, and third visits was analyzed to establish to which flowers each bee was attracted initially, and in the ensuing two visits (earliest stages of foraging experience).

2.7. Statistics

Differences in mean visit number (using mean percent of feed visits per bee to account for different activity levels of bees) and mean visit duration per bee to different flowers within each bee group were tested using the nonparametric two-way ANOVA

Friedman test, followed by pairwise Wilcoxon signed-rank test for two related samples, and to single flower species between bee groups using the nonparametric Mann–Whitney *U* test for two independent samples (using percent values). Difference in activity levels (mean total number of visits per bee) between bee groups was tested using *T* test for independent groups, and equal distribution of visits or bees among the plant species using the Chi-square test. All tests were conducted with SPSS (version 17.0).

3. RESULTS

3.1. Experiment 1: *B. napus*

A total of 30 control and 21 experimental female bees were tested; all visited the flowers.

3.1.1. Number of visits

Of the total visits made by both control and experimental bees, approximately 75 % were feed visits (remaining were nonfeed). Experimental females were generally less active than controls: the mean total number of feed visits per bee in experimentals was 6.6 ± 1.1 (range, 1–16) and in controls 9.3 ± 1.0 (range, 1–21). Both bee groups distributed their feed visits unevenly among flowers ($P < 0.05$, Friedman).

In control bees, the mean percent of feed visits per bee was significantly greater on *R. repens* than all other flowers ($P < 0.05$, Wilcoxon), and on *B. napus* and *E. vulgare* except versus *S. pratensis* and *C. leucanthemum* (Figure 1). The proportion of bees that feed visited was unevenly distributed among flowers ($P < 0.05$, Chi-square), with 80 % on *R. repens*, 67 % each on *B. napus* and *E. vulgare*, and 20–53 % on others.

Experimental bees similarly preferred *B. napus* and *R. repens* ($P < 0.05$, Wilcoxon), except versus *S. pratensis* and *L. pratensis* (Figure 1). However, they differed from control bees in not including *E. vulgare* among the preferred flowers; they visited it significantly less than the top two choices ($P < 0.05$, Wilcoxon), as well as significantly less than did control bees

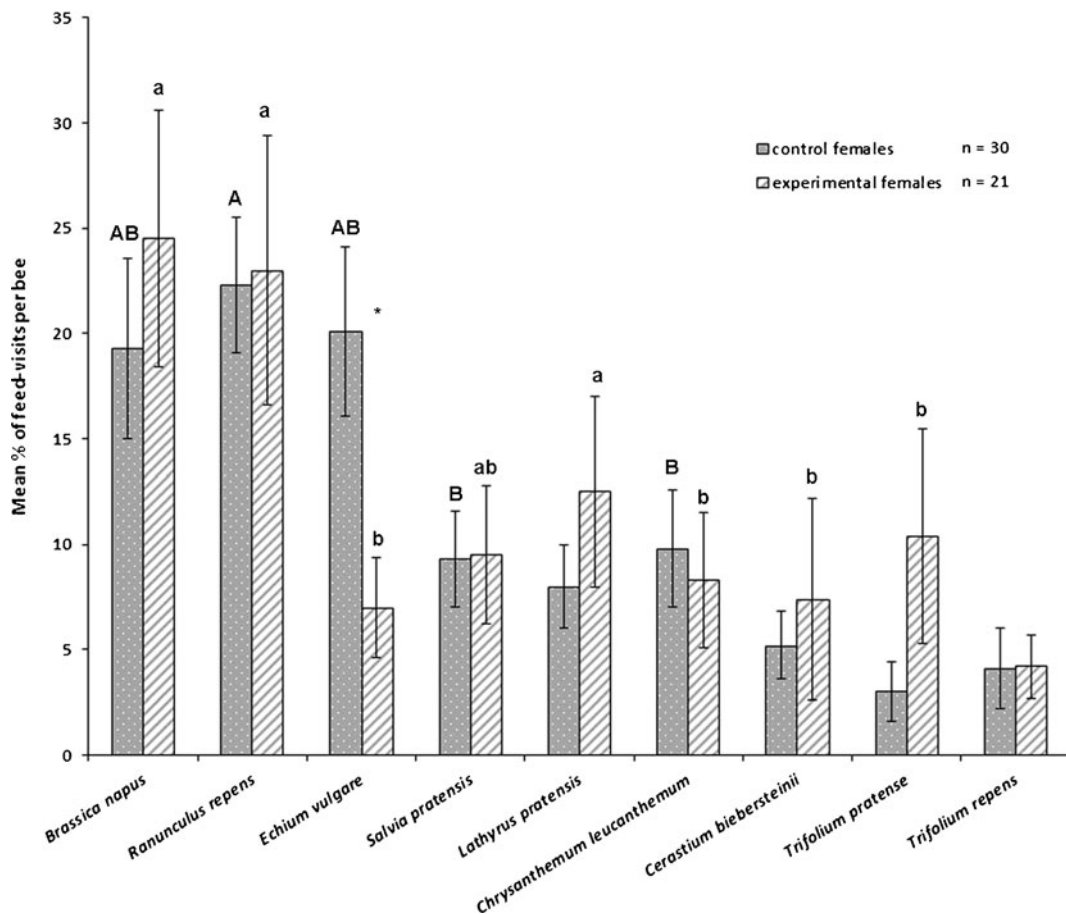


Figure 1. Mean percent of feed visits per bee (\pm SE) that were made on each plant species by *O. bicornis* females when offered a choice of flowers from nine different species. Control bees were reared on nest provisions collected by field-foraging females, experimental bees on provisions exclusively from *B. napus* (Brassicaceae). * $P < 0.05$, significant differences between control and experimental bees (Mann–Whitney *U* test); different letters above the bars indicate significant differences only among flowers with the two highest sets of values, in control (capital letters) and experimental (lowercase letters) bees ($P < 0.05$, Wilcoxon signed-rank test).

($P < 0.05$, Mann–Whitney). The proportion of bees that feed visited was more equally distributed among flowers (ns, Chi-square), with about 65 % visiting both *R. repens* and *B. napus*, and 24–43 % the other species.

3.1.2. Duration of visits

Experimental bees spent significantly more time feeding (mean total duration of feed visits per bee) than control bees ($P \leq 0.001$, *T* test). Both groups fed (versus nonfed) for a similar

proportion of their visit time: experimentals 97 % (712.4 ± 48.7 s) and controls 94 % (469.8 ± 43.4 s). Both groups distributed their feed visit duration unevenly among flowers (control $P < 0.01$, experimental $P < 0.001$, Friedman).

In control bees, mean feed visit duration per bee was longer on *R. repens* and *B. napus* than all other flowers except *E. vulgare* ($P \leq 0.05$, Wilcoxon; Figure 2). In experimental bees, the duration varied more both among flowers and among individual bees, with *R. repens* and *B.*

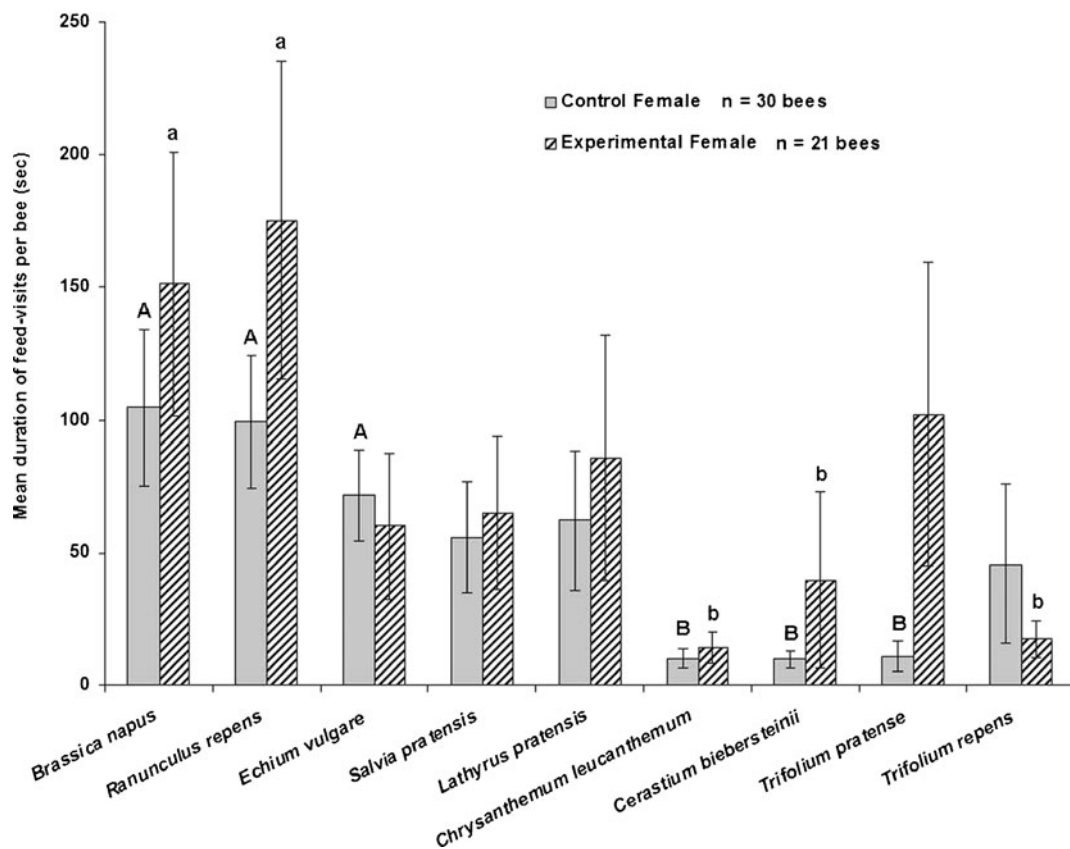


Figure 2. Mean duration (in seconds) of feed visits per bee (\pm SE) made on each plant species by *O. bicornis* females when offered a choice of flowers from nine different species. Control bees were reared on nest provisions collected by field-foraging females, experimental bees on provisions exclusively from *B. napus* (Brassicaceae). Differences were significant for the three control (*capital letters*) and two experimental (*lowercase letters*) flowers with the longest durations vis-à-vis the three flowers with the shortest durations ($P \leq 0.05$, Wilcoxon signed-rank test).

napus again receiving significantly longer times than most other flowers ($P \leq 0.05$, Wilcoxon); experimentals differed most strikingly from controls in spending markedly more time (ns, *T* test) on *T. pratense*.

3.1.3. Sequence of first three visits

The proportion of bees that made more than one flower visit (feed and nonfeed) was lower in the experimentals, with 62 % making third visits as compared to 87 % in the controls (Figure 3). In both bee groups, ≥ 75 % of bees fed during each of the first three visits.

In the number of flower species visited, experimental bees were more selective than controls after the initial visit (Figure 3). Among the nine flower species, controls visited seven to eight throughout the three visits, whereas experimentals visited nine species in the first visit, but became progressively more selective, visiting only five in the third.

Control bees displayed weak preferences among the flowers. The strongest was in the first visit, where the bees preferred both *B. napus* and *S. pratensis* (20 and 23 % of visits, respectively; Figure 3). Experimental bees also showed weak preferences, but these became stronger over the

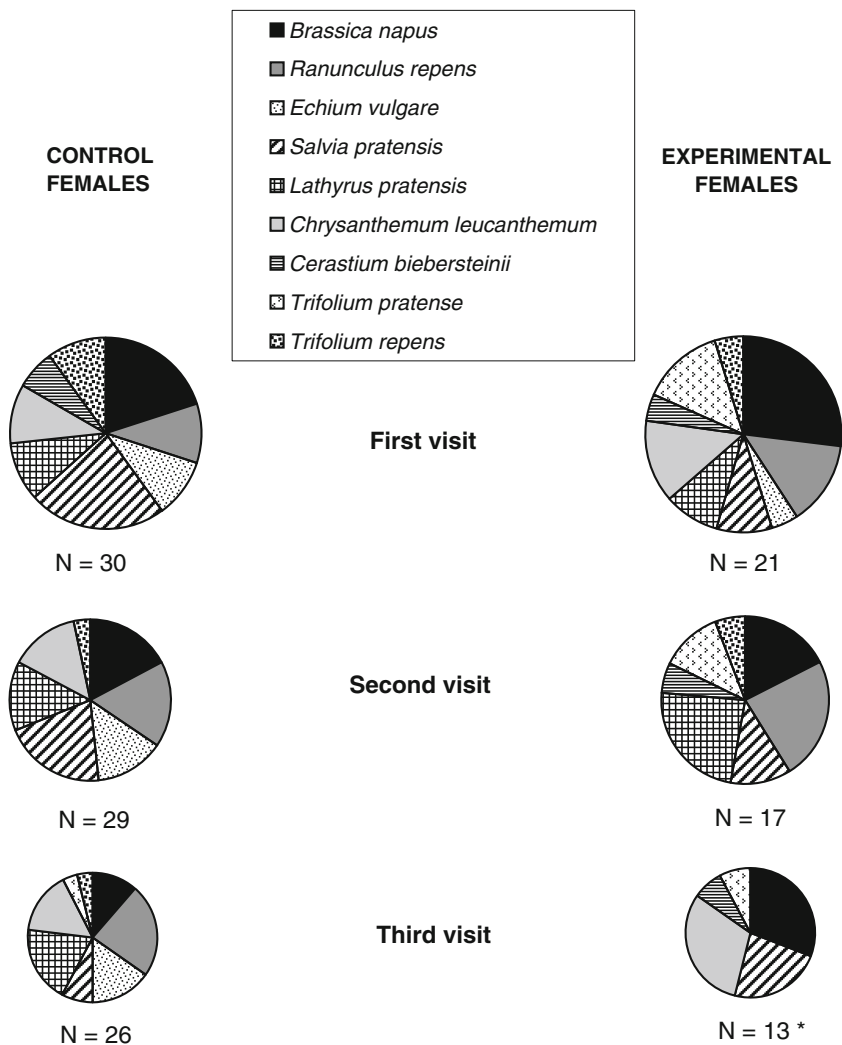


Figure 3. Distribution (percent) of the first three flower visits (feed and nonfeed) by control and experimental *O. bicornis* females among the nine different plant species offered during the behavioral tests. *N* is the number of bees that made visits during the tests: all bees made at least one visit to the flowers, but fewer made two or three visits. * $P < 0.05$, significantly uneven distribution of visits among plant species (Chi-square test).

sequential visits (third visit unevenly distributed; $P < 0.05$, Chi-square). Bees preferred *B. napus* in all three visits, together with *R. repens* and *L. pratensis* in the second, and *C. leucanthemum* and *S. pratensis* in the third. Considering only visits to *B. napus*, control bees progressively decreased their proportional visitation by half, from the first to the third visits, whereas

experimental bees included *B. napus* among their preferred flowers throughout.

3.2. Experiment 2: *O. viciifolia*

Very few nests were built by bees foraging exclusively on *O. viciifolia*: only 4 female and 12 male experimental bees emerged and survived

for testing. Consequently, this experiment focused more on males than females. For controls, 32 males and 34 females were tested; all visited the flowers.

3.2.1. Number of visits

For all four bee groups, 86–100 % of the total visits were feed visits (versus nonfeed). Bee activity levels, measured in mean total number of feed visits per bee, were similar for male experimentals (5.1 ± 1.4 ; range, 1–13) and controls (5.6 ± 0.8 ; range, 1–25), while female experimentals (2.5 ± 1.2 ; range, 1–6) were less active than controls (8.7 ± 1.2 ; range, 1–21). Between genders, control females were significantly more active than control males ($P < 0.05$, *T* test), whereas experimental females tended to be less active than males. All groups (excluding experimental females) distributed their feed visits unevenly among flowers ($P < 0.01$, Friedman) and with similar trends.

In control bees, the mean percent of feed visits per bee by females was significantly greater on *R. acris*, and by males on both *R. acris* and *C. leucanthemum*, than other species ($P < 0.05$, Wilcoxon; Figure 4). In addition, females visited *O. viciifolia* and *T. repens* significantly more than males ($P < 0.05$, Mann–Whitney). The proportion of bees that feed visited was significantly uneven among flowers for both males and females ($P < 0.01$, Chi-square), with *R. acris* having the most bees (78 and 85 %, respectively), followed by *C. leucanthemum* (71 and 56 %) and *S. pratensis* (41 and 53 %); *O. viciifolia* was visited by only 6 % of males and 25 % of females.

In experimental bees, males again significantly preferred *R. acris* over other species ($P < 0.05$, Wilcoxon), but they also directed a smaller percent of feed visits to *C. leucanthemum*, and a slightly greater percent to *O. viciifolia* than controls. The proportion of males that visited each species was significantly uneven ($P < 0.01$, Chi-square); 75 % of males visited *R. acris*, but ≤ 50 % other flowers,

including 17 % to *O. viciifolia*. The four experimental females restricted feed visits to *R. acris*, *C. leucanthemum*, and *T. repens*.

3.2.2. Duration of visits

Experimental males and females spent a similar length of time feeding (mean total duration of feed visits per bee) as control males, but a significantly shorter time than control females ($P < 0.001$, *T* test). In addition, experimental bees fed (versus nonfed) for a greater proportion of their visit duration: male experimentals fed 92 % of the time (114.5 ± 29.8 s) and controls only 62 % (111.9 ± 15.1 s), while female experimentals fed 98 % (115.5 ± 76.5 s) and controls 75 % (238.5 ± 29.5 s). All four groups distributed their mean visit duration significantly unevenly among flowers ($P < 0.05$ experimental females, $P < 0.01$ other groups, Friedman).

Variation among flowers in mean feed visit durations (data not shown) revealed trends similar to the mean percent of feed visits, but with control males spending a comparatively shorter time on *C. leucanthemum*. *R. acris* was clearly preferred over all other flowers by control bees ($P \leq 0.001$, Wilcoxon) and over all except *S. pratensis* and *C. tomentosum* by experimental males ($P \leq 0.05$, Wilcoxon), but not significantly by experimental females.

3.2.3. Sequence of first three visits

The proportion of bees that made more than one flower visit (feed and nonfeed) was lower in the experimental than control groups (Figure 5). Among control bees, 82 % of females and 59 % of males made third visits, whereas in the experimentals only 42 % of males (and 25 % of the four females) did so. In all four groups, ≥ 75 % of bees fed during each visit.

Experimental bees were more selective than controls in the number of flower species visited across all three visits (Figure 5). Among the eight flowers, male controls visited six to seven and experimentals only four to six; female

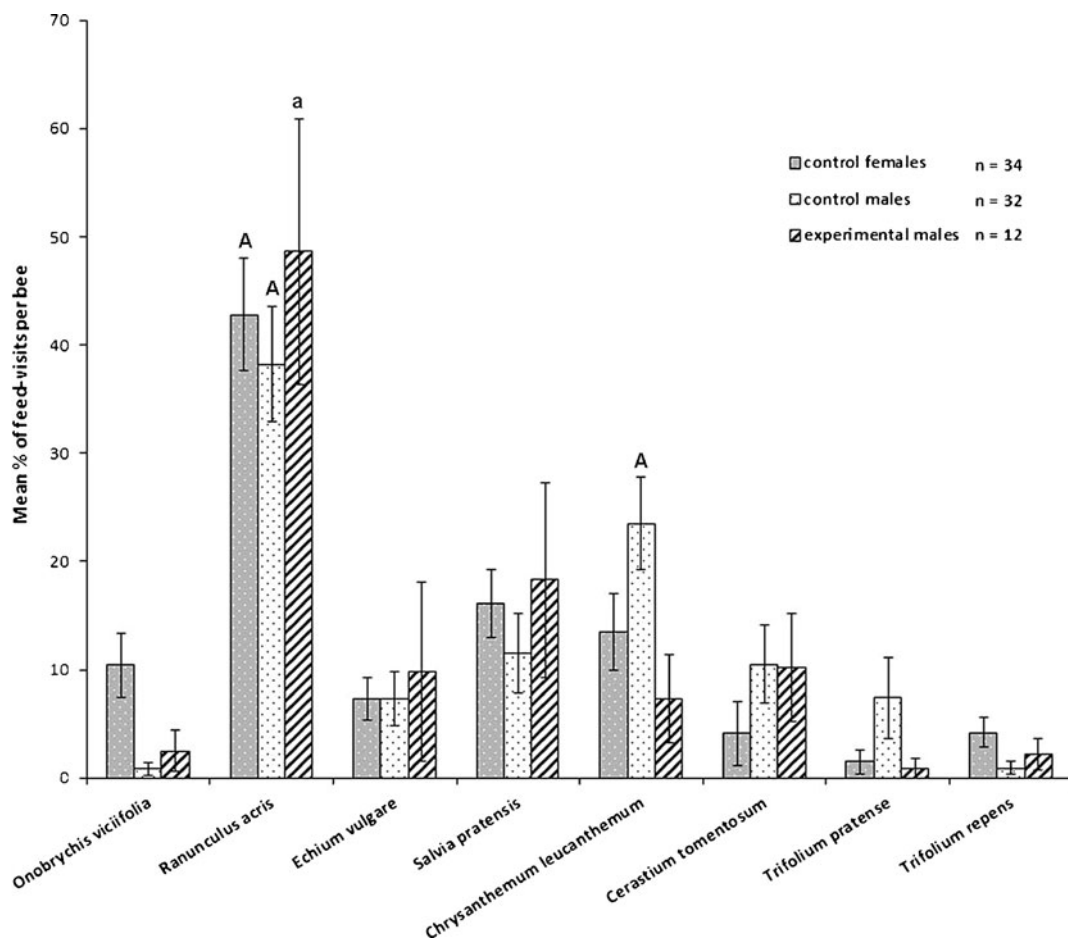


Figure 4. Mean percent of feed visits per bee (\pm SE) made on each plant species by *O. bicornis* control bees (males and females) and experimental bees (males only) when offered a choice of eight different flower species. Control bees were reared on nests provisions collected by field-foraging females, experimental bees were reared on provisions exclusively from *O. viciifolia* (Fabaceae). Letters above the bars indicate the flowers that had significant differences vis-à-vis all other flowers, in control (*capital letters*) and experimental (*lowercase letters*) males ($P < 0.05$, Wilcoxon signed-rank test).

controls were initially least selective, visiting seven to eight species in the first two visits, but decreased to four in the third, whereas experimentals visited only three species.

Control bees showed clear preferences among flowers (all three visits, except the third by males, were unevenly distributed, $P < 0.001$, Chi-square). In the first visit, both males and females preferred *R. acris* (40 % visits), followed by *C. leucanthemum* and *S. pratensis* (Figure 5). In the second and third visits, males

preferred *R. acris* and *C. leucanthemum*, whereas females continued to prefer *R. acris*.

Experimental bees displayed significant preferences in the first visit (unevenly distributed in males, $P < 0.05$, Chi-square) for *R. acris* (50 % visits), but not so clearly thereafter, partly because they visited fewer species (Figure 5). Females visited *R. acris* and *C. leucanthemum*, then *R. acris* and *T. repens*.

Considering only visits to *O. viciifolia*, control males made none, whereas experimental

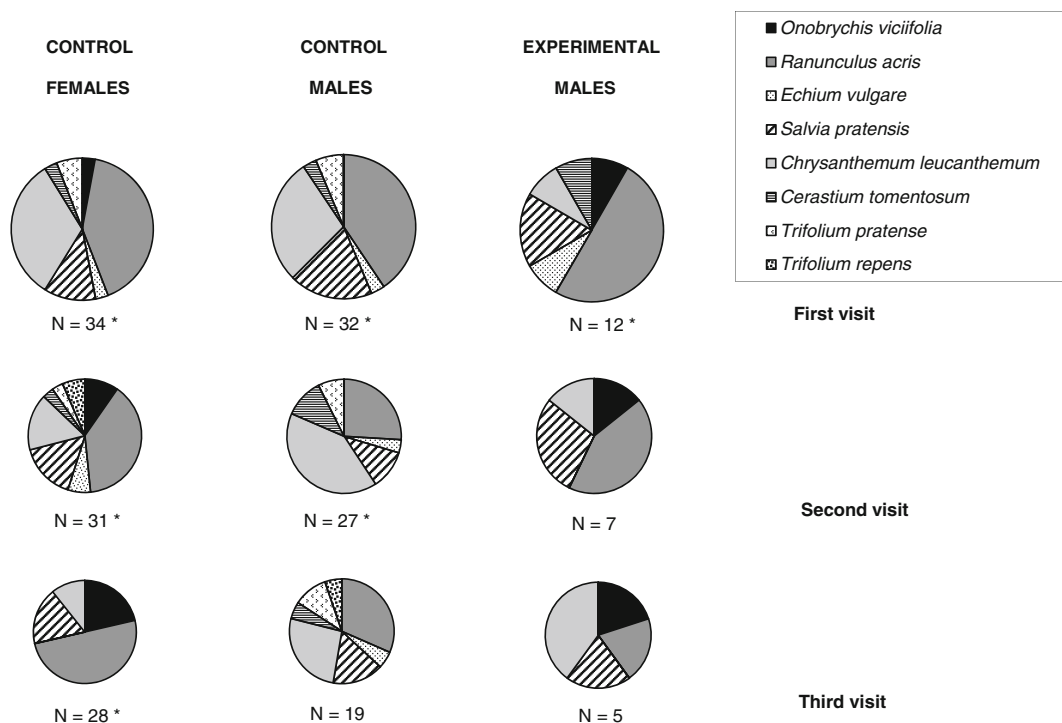


Figure 5. Distribution (percent) of the first three flower visits (feed and nonfeed) by *O. bicornis* control bees (males and females) and experimental bees (males only) among the eight different plant species offered during the behavioral tests. *N* is the number of bees that made visits during the tests: all bees made at least one visit, but fewer made two or three visits. * $P < 0.05$, significantly uneven distribution of visits among plant species (Chi-square test).

males increased their proportional visitation over the three successive visits (but each consisted of only a single visit by the same bee). Control females progressively increased visitation from 3 % in the first visit to 21 % in the third, but the few experimentals made no visits.

4. DISCUSSION

The findings from this study neither clearly support nor negate the possibility that flower preferences by adults of the polylectic solitary bee *O. bicornis* can be modulated by the floral sources of the bees' larval food, through chemical imprinting to the nest provisions (pollen and nectar). Nonsignificant trends in some of the behavioral data suggest that bees reared exclusively on provisions from *B. napus*, and possibly

O. viciifolia, exhibited increased attraction to these flowers, at least in their initial visits.

O. bicornis bees that were reared exclusively on *B. napus* pollen and nectar showed only suggestive evidence that females (no males were tested) became imprinted to *B. napus* flowers, given that *B. napus* was among the flowers most preferred by both experimental (i.e., reared on *B. napus*) and control bees. Indeed, when females were offered a choice of nine flower species, both bee groups preferred *B. napus* and the generally attractive *R. repens* (buttercup) significantly more than other flowers, making any effect of imprinting difficult to uncover in terms of the number and durations of feed visits by individual bees. However, data on the sequence of the first three flowers visited suggest that experimental bees tended to have a heightened preference for *B. napus*, which was

either the most or among the few most heavily visited flowers by experimental bees in the three visits, whereas control bees included *B. napus* among their preferred flowers only in the first visit. Experimental bees thus were consistently more strongly attracted to their larval host flowers in their initial three flower visits compared to control bees. Records of the first flower visits may be key to uncovering any effects of imprinting to either pollen or nectar in larval food since both male and female solitary bees feed on pollen as well as nectar during their first visits to flowers following emergence (Schäffler and Dötterl 2011; Cane, Dobson and Boyer, unpublished). While firmer conclusions could have been reached had the number of experimental females been larger, these data support the possibility that the bees' initial flower choices might be influenced by imprinting to nest provisions.

Our use in experiment 2 of a less attractive rearing plant (*O. viciifolia*) in order to more readily measure possible changes in the bees' flower preferences raised new issues that make studies of bee imprinting challenging: only few and mostly male bees were produced in the nests provisioned exclusively with *O. viciifolia* and tested. Solitary male bees, similarly to females, must visit flowers to feed, but might not demonstrate as strong foraging associations with certain flower species as do females, which also actively visit flowers to collect nest provisions. However, in *O. bicornis*, some males also use flowers as secondary mating sites, in addition to patrolled nests (Seidelmann 1999), suggesting that flower selection would be similar in the two sexes. When males were reared on *O. viciifolia*, they showed a slight but not significantly greater visitation to its flowers compared to control bees. The first three visits also suggest that experimental males were more attracted to the flowers, but the data are insufficient to reach conclusions. Thus, the most pronounced effect of rearing bees on *O. viciifolia* is a suggested trend of enhanced visitation to the flowers by males.

In addition to these subtle effects of larval host flowers on flower selection by adults, rearing bees exclusively on either *B. napus* or

O. viciifolia pollen and nectar influenced the bees' selection of other flower species offered in the choice tests, implicating cross-induction (Jaenike 1983). Thus, females reared on *B. napus* visited *E. vulgare* significantly less compared to controls, and males reared on *O. viciifolia* visited *C. leucanthemum* markedly less than controls. Curiously, suggestions of cross-induction at the sensory detection level were observed in earlier exploratory imprinting experiments, where solitary bees that developed from prepupae into adults within environments artificially enriched with single volatiles showed higher electroantennographic responses than control bees to volatiles that were not present in the enriched environments (H. Dobson and L. Ågren, unpublished). The underlying causes of our cross-induction effects are not clear, but exposure to chemicals within the nest cell may have influenced bee responses to floral scent compounds through either interference or reinforcement at the level of chemical perception, olfactory processing in the brain, and/or integration of these with behavioral responses.

Data from both experiments in this study reveal that *O. bicornis* has innate preferences for certain flowers, perhaps founded on genetically determined responses to specific visual and olfactory floral stimuli. All experimental and control bee groups demonstrated a strong attraction to flowers of *Ranunculus* spp., the pollen of which is frequently prominent in *O. bicornis* nest provisions (Seidelmann 1991; Radmacher and Strohm 2010). Similarly, their high visitation to *B. napus* in experiment 1 also implies a strong preference, although *Brassica* pollen is not particularly abundant in nests in the wild (Westrich 1989; Maciel de A. Correia 1994), except when *O. bicornis* is pollinating *Brassica* crops (Teper and Biliński 2009). On the other end of the attraction spectrum, *T. repens* and *Cerastium* spp. received consistently few visits. In the middle preference range are flowers whose visitation rates varied, both between control and experimental bees (discussed above), as well as between control bees of the two experiments. Significant differences in visitation to *E. vulgare*, which was among the

most preferred in experiment 1, but only moderately visited in experiment 2, suggest that some preferences may be context dependent, varying with the composition of flowers present. Since the eight to nine species offered to the bees were nearly identical across the two experiments except for the rearing flowers, changes in the occurrence of only single or few species seem sufficient to cause shifts in the bees' rankings of moderately attractive flowers. Thus, flower selection by generalist bees may depend partly on the assemblage of flower species available to them.

One consequence of rearing bees on *B. napus* was an apparent decrease in general flower visitation by experimental females compared with controls, suggesting lower vigor (this did not occur in males reared on *O. viciifolia*). Furthermore, it should also be noted that females from *B. napus* nests seemed to display more difficulty in learning flower handling skills, particularly on *T. pratense*, than control bees, based on our qualitative observations and visit duration data. These behavioral impacts are intriguing, and raise the question of whether females suffered from their pure *B. napus* larval provisions being of limited nutritional value, either quantitatively or qualitatively. Quantitative limitation typically translates into smaller body size (Radmacher and Strohm 2010), which was not measured here. Qualitative limitations are more complex to identify. *B. napus* is generally attractive to bees (Delaplane and Mayer 2000; Westcott and Nelson 2001), including *O. bicornis*, which uses it when abundant to provision nests (Teper and Biliński 2009), and the pollen and nectar provide sufficient nutrients for successful brood rearing in polylectic bees (Abel and Wilson 1998; Soroka et al. 2001), including *O. bicornis* (Holm 1973). Nevertheless, potential impacts of pure *B. napus* provisions on bee activity and cognition have not been examined. If different pollen diets do in fact lead to different behavioral and physiological attributes in bees, such as activity levels and learning abilities, and given that *O. bicornis* nest provisions are often dominated by single pollen types (Seidelmann

1991; Radmacher and Strohm 2010), individual bees with a propensity (genetic or environmental) to forage on flowers with the highest nutritional value would have a selective advantage. This, together with spatial variations in plant distributions, might partly underlie some of the intraspecific and interpopulational variation in ranges of host plants used by polylectic bees.

A major challenge in this study was selecting rearing host flower species that elicited nest provisioning by female bees in outdoor enclosures, while meeting our cultivation constraints. Keeping *O. bicornis* within a greenhouse can lower the ratio of females to males (Holm 1973), which occurred in both of our experiments. However, while use of *B. napus* resulted in bees building nests that provided moderate numbers of female bees for testing, use of *O. viciifolia* yielded few and mainly males. In a second attempt to rear bees on *O. viciifolia*, we obtained even fewer bees (H.E.M. Dobson and M. Ayasse, unpublished), which implies that some constant factor was limiting reproduction of *O. bicornis* on *O. viciifolia*. High bee density relative to floral resources can negatively affect nest building (Pitts-Singer and Bosch 2010), but we kept bee density purposefully low. Since *O. viciifolia* blooms a couple weeks later than *B. napus*, high ambient temperature may have limited the activity of *O. bicornis*, which flies mainly during earlier cooler weather (Maddocks and Paulus 1987); it is also possible that temperature in the large tent may have on occasion reached 30°C, which can increase *O. bicornis* larval mortality (Radmacher and Strohm 2010). Nevertheless, the combination of the overall low number of offspring and high male to female ratio (4:1) implies that availability of food resources was most likely the core problem (Ulbrich and Seidelmann 2001; Ivanov 2006).

Several factors may have contributed to food scarcity for *O. bicornis*, including the possibility that *O. viciifolia* has a low attractiveness to the bees. *O. viciifolia* attracts a variety of social and solitary bees (Richards and Edwards 1988; Hanley et al. 2008; Rozen et al. 2010; but see

Clement et al. 2006), and although *O. bicornis* is reported to visit it (Westrich 1989), it is not clear to what extent it does so. In *O. lignaria*, which is also polylectic, females cease provisioning nests altogether when rejected, novel plants are offered in a no-choice situation (Williams 2003), and it is possible that *O. bicornis* responded similarly vis-à-vis *O. vicifolia*. The few nests built in the tent enclosure imply that only few females persisted in nest provisioning, which suggests that *O. bicornis* females rarely forage exclusively on *O. vicifolia*. The food rewards (pollen and nectar) of *O. vicifolia* may be insufficient in quantity or in quality to meet the needs of *O. bicornis*. Pollen varies in its nutritional value to bees (Roulston and Cane 2000; Praz et al. 2008b), and feeding larvae of polylectic *Osmia* spp. pollen that is not normally collected by the females can lead to decreased larval mass, increased development time, and decreased survivorship (Williams 2003; Sedivy et al. 2011). Interestingly, *O. bicornis* has been shown to develop well on pure pollen diets of *Ranunculus* (Sedivy et al. 2011), to which it was highly attracted in our study, but poorly on *E. vulgare*, which had variable attractiveness (high in experiment 1, low in experiment 2). Nectar chemistry also varies among plants, but its effects on bees are not well studied (Nicolson and Thornburg 2007). Taken together, these findings demonstrate that restricting the nest provisioning by *O. bicornis* to only one plant source can variously impact successful nest building and brood rearing, depending on the plant species. It would be interesting to examine the extent to which bee reproduction is affected in the wild when fluctuations in the availability of preferred host plants require that these polylectic bees forage on typically less frequented species.

Raising bees on nectar and pollen exclusively from a specific plant species is essential to study bee imprinting, but methodological challenges can pose major obstacles. Our method of forcing females to forage on single species was chosen over manually introducing the desired pollen and nectar into nests in order to minimize invasive handling of nests, risk of

introducing pathogens, and negative impacts on larval survival through omission of secretions that females may add to provisions (e.g., Sommeijer et al. 2009). One alternative approach is that followed by Praz et al. (2008a, b) and Sedivy et al. (2011), who reared bees on single pollen species by transferring eggs to provisions collected by oligolectic bees; this ensures purity of pollen but not necessarily of nectar. Its applicability to imprinting studies of *O. bicornis* deserves future exploration.

In response to the question of whether flower selection in solitary polylectic bees can be altered by changing the floral sources of the larval nest provisions, the findings here suggest that this might be possible to some extent, depending on the flower species. Determining how flower selection is modulated in these bees has important implications in commercial pollination; even though several species are currently utilized (Richards 1993; Delaplane and Mayer 2000; and see chapters in James and Pitts-Singer 2008), a major obstacle to developing a greater reliance on their service is overcoming their limited flower associations (Torchio 1990; Bosch and Kemp 2002). While solitary polylectic bees, such as *O. bicornis*, display flower preferences that appear to have some genetic basis, our results suggest that there is also some intraspecific variation in host plant acceptance among individual bees, which would allow different plants to be used as forage by different subgroups of bees in the absence of more preferred hosts. This has evolutionary implications: if increased visitations to less preferred plants become manifested in the next generation through modification of floral preferences, as suggested by some trends in our study, this might represent an evolutionary path to populational divergence and eventual speciation (Dethier 1954; Gutiérrez-Ibáñez et al. 2007). We show that the composition of larval provisions can have some direct and indirect effects, even though subtle, on flower selection in adults, but their actual causes, as well as their ecological and evolutionary consequences on the foraging and reproduction of bees, remain to be established.

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Le choix des fleurs est-il influencé par l’empreinte chimique présente dans les provisions de nourriture destinées aux larves, chez l’abeille généraliste *Osmia bicornis* (Megachilidae)?

Abeille solitaire / polylectisme / choix de la fleur / empreinte / nourriture larvaire

Wird die Blütenauswahl der generalistischen Biene, *Osmia bicornis* (Megachilidae), durch chemische Prägung auf das larvale Futter beeinflusst?

Solitäre Biene / Polylectie / Blütenauswahl / Prägung / Larvenfutten

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