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PLANT-ANIMAL INTERACTIONS - ORIGINAL PAPER

# Specialist *Osmia* bees forage indiscriminately among hybridizing *Balsamorhiza* floral hosts

**James H. Cane**

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**Abstract** Pollinators, even floral generalists (=polyleges), typically specialize during individual foraging bouts, infrequently switching between floral hosts. Such transient floral constancy restricts pollen flow, and thereby gene flow, to conspecific flowers in mixed plant communities. Where incipient flowering species meet, however, weak cross-fertility and often similar floral traits can yield mixed reproductive outcomes among pollinator-dependent species. In these cases, floral constancy by polyleges sometimes serves as an ethological mating barrier. More often, their foraging infidelities instead facilitate host introgression and hybridization. Many other bee species are oligolectic (taxonomic specialists for pollen). Oligoleges could be more discriminating connoisseurs than polyleges when foraging among their limited set of related floral hosts. If true, greater foraging constancy might ensue, contributing to positive assortative mating and disruptive selection, thereby facilitating speciation among their interfertile floral hosts. To test this Connoisseur Hypothesis, nesting females of two species of oligolectic *Osmia* bees were presented with randomized mixed arrays of flowers of two sympatric species of their pollen host, *Balsamorhiza*, a genus known for hybridization. In a closely spaced grid, the females of both species preferred the larger flowered *B. macrophylla*, evidence for discrimination. However, both species' females showed no floral constancy whatsoever during their individual foraging bouts, switching randomly between species proportional to their floral preference. In a wider spaced array in which the bouquets reflected natural plant spacing, foraging oligolectic bees often transferred pollen surrogates (fluorescent powders) both between conspecific flowers (geitonogamy and xenogamy) and between the two *Balsamorhiza* species. The Connoisseur Hypothesis was therefore rejected. Foraging infidelity by these oligolectic *Osmia* bees will contribute to introgression and hybridization where interfertile species of *Balsamorhiza* meet and flower together. A literature review reveals that other plant genera whose species hybridize also attract numerous oligolectic bees, providing independent opportunities to test the generality of this conclusion.

**Keywords** Megachilidae · Asteraceae · Hybridization · Floral constancy · Ethological mating barrier

## Introduction

Among flowering plants, natural hybridization and introgression are recognized as potent and pervasive sources of genetic novelty and evolutionary diversification (Rieseberg et al. 2003; Wissemann 2007). Hybridization is widespread, though apparently sporadic, among many closely related species of flowering plants (e.g., 22% of the British flora; Stace 1975). Different interfertile species may hybridize in sympatry only when pollen is moved from the anthers to stigmas of each others' flowers by either wind or pollinators (Regal 1982), bees being the predominant pollinating animals (Neff and Simpson 1993). However, detecting the transfer of congeneric pollen among stigmas of potentially interfertile species is commonly frustrated by

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J. H. Cane (✉)
USDA Bee Biology and Systematics Lab,
Utah State University, Logan, UT 84322-5310, USA
e-mail: Jim.Cane@ars.usda.gov

the indistinguishable morphologies of their pollen. Conversely, stigmas of animal-pollinated plants rarely receive much pollen from flowers of species in other genera that are in flower owing to isolation by mechanical and ethological mating barriers (Grant 1994) that result from divergent floral structures and specialized pollinator behaviors, respectively.

Individual foragers of even generalist bee species, though versatile, have long been known to specialize taxonomically during a given foraging bout (Bennett 1883; Christy 1883), going from flower to flower of the same host species amid a mixed flowering community. This floral constancy is transient, lasting from a few sequential floral visits to one or more entire foraging trips. Several definitions and causal explanations have been proffered for the behavior (Waser 1986), including the simple exploitation of more rewarding species, neural limitations for motor learning memory (flower handling) (Chittka et al. 1999), or "perceptual conditioning" (Wilson and Stine 1996) that speeds floral recognition when a forager is not distracted by alternatives, an example of so-called "visual attention" (Chittka et al. 1999). Interspecific pollen flow leading to hybridization becomes possible when a bee, confronted with flowers of closely related species, nonetheless either switches its foraging focus repeatedly from one species to the other during a foraging trip (Heinrich 1979) or simply does not distinguish between flowers of the two related floral hosts. Indirect evidence for floral constancy can be seen in foragers' pollen loads (Chambers 1946; Free 1963; Free 1970; Westrich and Schmidt 1987; Macior 1994; White et al. 2001) as loads are often dominated by pollen taken from a single flowering taxon during a foraging trip. However, pollens of congeneric species are often indistinguishable and so, typically, pollinators' foraging rounds must be observed directly when studying their floral constancy amid congeneric flowers.

There is ample evidence that foraging infidelity by social bees and hummingbirds often facilitates hybridization and introgression of their floral hosts (reviewed in Grant 1994; Campbell and Aldridge 2006), raising doubts about the existence of ethological mating barriers altogether (Chittka et al. 1999). Field research with bees' foraging fidelity as it relates to plant hybridization has primarily focused on just bumblebees (*Bombus*) as the pollen vectors (e.g., Leebens-Mack and Milligan 1998). Like honey bees and stingless bees, bumblebees are broad floral generalists (=polylecty). These social bees must be able to take pollen from many hundreds of plant species (reviewed in Cane and Sipes 2007) because their colonies are active throughout the growing season. They stockpile and mix pollen and especially nectar in their colonies, freeing individual foragers to choose which resource to collect. These broadly polylectic social bees do not represent bees in general, however, as most bee species are solitary, not social, they mass-provision their progeny individually, and they have but one or a few briefly active adult generations per year. The daily foraging rounds of most solitary bees are much more constrained by their daily nest provisioning needs.

Among the non-social bees are thousands of species that are taxonomic pollen specialists (=oligolecty). All females of an oligolectic bee species forage and collect pollen from the same limited subset of related pollen hosts, often one to several genera belonging to the same plant tribe or family (reviewed in Cane and Sipes 2007). Unlike floral constancy, oligolecty is a relatively fixed attribute of a species of bee. These oligoleges are widespread and often prevalent in pollinator guilds, especially in arid or Mediterranean climates (Michener 1979).

Most of the reasons generally invoked to explain oligophagy among other herbivores—plant defensive chemistry (Feeny 1976), enemy-free space (Bernays and Graham 1988), or more efficient resource exploitation (Thompson 1994)— hold little explanatory relevance for oligolecty in bees (Wcislo and Cane 1996). Only the concept of neurological constraints (information processing bottlenecks, memory limitations) as a behavioral factor favoring oligophagy (Bernays 1996) also finds broad conceptual and empirical support for pollinator specialization, but then only in terms of individual forager constancy (e.g., Wilson and Stine 1996; Chittka et al. 1999), not species-level oligolecty. However, constancy per se is rarely measured in host specificity trials using other herbivorous insects, probably because its adaptive meaning is not obvious. In contrast, floral constancy in foraging pollinators can have profound consequences for plant reproductive fitness in terms of pollen export, male fitness, and host hybridization (Waser 1986).

Taxonomic restriction, as by oligolecty, might also be accompanied by the finer discrimination of plant hosts. For instance, some oligophagous butterflies show strong oviposition preferences, discriminating among even closely related hosts (e.g., Rausher and Papaj 1983). Moreover, in the few studies that are available, ovipositing individuals of oligophagous butterfly species have been shown to be choosier among hosts than their polyphagous relatives (Janz and Nylin 1997). If narrower host specificity of oligolectic bee species results in intensified constancy by individual foragers, then oligoleges might contribute less to host hybridization than the social, polylectic species that have been thus far studied. In this scenario, incipient floral species whose pollinator guilds are dominated by oligoleges would experience curtailed gene flow between their diverging populations. Oligolectic bees would be agents of host speciation rather than hybridization. Unfortunately, all reports implicating bees in natural plant hybridization and introgression concern polylectic bees, and in most cases, just bumble bees (Grant 1994; Campbell and Aldridge 2006), prompting Grant (1994) to lament that: "The contribution of monotropy/oligotropy [=oligolecty] to floral isolation is poorly understood."

In the study reported here, I experimentally evaluated floral constancy by two species of oligolectic *Osmia* bees foraging among flowers of two of their shared floral hosts, *Balsamorhiza sagittata* and *B. macrophylla.* To evaluate constancy by these bees, I tracked sequential floral visits of freely foraging, nesting females as they visited cut flowers of *B. sagittata* and *B. macrophylla* presented in randomized grids. I tested the Connoisseur Hypothesis, positing that individual females of oligolectic bee species would be discriminating foragers in a mixed array of their floral hosts. Choosy foragers might be more constant, switching hosts infrequently, and thereby limiting pollen flow between them. Such an ethological isolating mechanism could provide the assortative mating sometimes necessary to maintain the genetic integrity of nascent species in sympatry. I also tested the oligoleges' roles as pollinators, monitoring the *Osmia* bees' transfer of fluorescent powders (as a pollen surrogate) between cut *Balsamorhiza* flowers in a larger, randomized mixed species array of bouquets deployed at more natural plant spacing.

## Materials and methods

### Natural histories and sources of bees and flowers

The monophyletic genus *Balsamorhiza* (Heliantheae: Asteraceae) consists of 14 species of mostly large, herbaceous, long-lived perennials endemic to western North America (Ownbey and Weber 1943; Moore and Bohs 2009). The large (diameter 4–12 cm), upright yellow flowers of the species [Electronic Supplementary Material (ESM)] are borne in early-mid spring. Plants are slow-growing (first flowering 3–7 years after seeding) but long-lived (Treshow and Harper 1974). Multiple species pairs (often including *B. sagittata*) can occasionally hybridize, as evidenced from the widespread occurrence of individuals with clearly intermediate leaf morphologies in mixed populations (Ownbey and Weber 1943). Only the breeding biology of *B. sagittata* is known; it sets no seeds without bee visitation and sets more seed with outcrossing (Cane 2005). An ongoing survey (5 states, 30 populations) finds multiple genera of mostly non-social bees (e.g., *Andrena*, *Dialictus*, *Halictus*, *Osmia*) visiting flowers of *Balsamorhiza* (Cane, unpublished).

Both *Osmia* (*Cephalosmia*) *californica* and *O. montana* (Megachilidae) (ESM) are closely related, like-sized (10–14 mm long) oligoleges for a limited set of spring-blooming Asteraceae, particularly the closely related species of *Balsamorhiza* and *Wyethia* (Rust 1974; Torchio 1989). These non-social bees naturally nest in tunnels left in deadwood by wood-boring beetles (Rust 1974). Local nesting populations were obtained by setting out drilled-out wooden blocks of suitable dimensions ("trap-nesting"). Their progeny were overwintered and, in the spring, placed out to nest and forage at the nearby experimental floral arrays.

### Experimental foraging design

Bees were observed visiting experimental grids of *Balsamorhiza* flowers on 2 days in May 2007 at each of two locations that are 2 km apart (Logan City and Green Canyon, Cache County, UT, USA). At both sites, overwintered adults of both *Osmia* species were released at field shelters containing drilled-out wooden blocks; within 1 week (but prior to the experiment), 20–80 females of each species had commenced nesting at each site and were returning to their nests with pollen. Hence, experienced foragers visited the experimental grids. These females were seen using yellow-flowered, vernal asteraceous floral hosts (*B. sagittata*, *Taraxacum officianale* and later *Wyethia amplexicaulis*); *B. macrophylla* was not present within their flight range at either site, although these *Osmia* visit this host elsewhere in the valley.

Each experimental foraging grid was assembled from a 49 × 34-cm rectangular Styrofoam block with regularly spaced holes. It was painted a mottled pattern of dull green and brown. Each foam block floated in a pan of water. A day prior to experimentation, flowering stems of both *Balsamorhiza* species (ESM) were cut at nearby sites, brought indoors, and placed upright in water. The following morning, new disk flowers had opened and were shedding pollen. These flowerheads were individually inserted into the holes in the floating foam block so that their cut stems stood in the water. Each grid held 24 fresh flowers, 12 of each *Balsamorhiza* species, distributed randomly in a 5 × 5 evenly spaced grid (one hole left empty). Experimental grids were placed on the ground 5–10 m from the bees' nesting blocks.

### Behavioral trials

Foraging trials started concurrently by 10:30 a.m. Mountain Daylight Savings Time (MDST) daily at both locations once foraging females were seen returning to their nests with loads of pollen. Recording began when a female *Osmia* first alighted on a flower in the grid. The sequence of flowers then visited by the foraging female was recorded as grid coordinates. Recording terminated when either the bee flew away to its nest (16 of 18 visitation bouts on 16 May at the town site) or we lost track of its identity (we could not track >5 foragers at one time). After 30–45 min of visitations, the grid was withdrawn, dissembled, and new flowers were arranged in a second random pattern. The trials were replicated the following day at each site, for a total of eight trials.

### Behavioral analyses

Floral preferences and degrees of foraging constancy were calculated from the floral visitation sequences of the bees. The floral preference of the two *Osmia* species was measured as the overall proportion of visits to flowers of each *Balsamorhiza* species. Since the two *Balsamorhiza* species were represented in each grid by equal numbers of flowers, a departure in visitation frequencies from the 1:1 ratio of available *Balsamorhiza* flowers in the grids would indicate foraging preference by the bees. The consistency of these bees' *Balsamorhiza* preferences among the four combinations of sites (2) and days (2) was evaluated by maximum likelihood analysis of variance (ANOVA) (Allison 1999). Before pooling data to then analyze constancy, the assumption of homogeneity for floral preference among foragers was tested (Jones 1997). For all visitation bouts of five or more flowers, the proportion of visits to *B. macrophylla* by each *Osmia* forager was compared to the overall preference for *B. macrophylla* among all *Osmia* foragers (65% of visited flowers). These measures were used to calculate the binomial test for homogeneity, comparing the test statistic with critical values for the $\chi^2$ distribution (Jones 1997). The test statistic has a $\chi^2$ distribution, assuming that bees' foraging trips are independent of one another and their movements among flowers are random.

Floral constancy (or infidelity) was calculated from transitional probabilities during foraging bouts, being the frequencies with which bees flew between sequential flowers of the same or different species. The null model posited that they would move randomly among the two species' flowers in accordance with their overall floral preference. Thus, more intraspecific moves would be expected for a more preferred floral species. For example, one bout of floral visits by a female *O. montana* consisted of the sequence: MMMSSMMSMMMMMS, where "M" and "S" refer to flowers of the two *Balsamorhiza* species, respectively. Data were evaluated using 2×2 contingency tests, one for each trial. Data for the two *Osmia* species were compared independently and then pooled when warranted.

### Experimental pollen flow design

Pollen flow was studied among flowers in more naturally spaced arrays of floral bouquets at the Logan site over a 3-day period in May 2009. Again, locally nesting *Osmia* of both species were used. Fresh flowers were place upright in water and grouped in bouquets of three conspecific flowers each to represent the multiple blooms of individual plants. Eight bouquets of each of the two *Balsamorhiza* species were randomly deployed in a 4 × 4 grid with 1-m spacing. This spacing was chosen after measuring nearest neighbor distances between individuals growing in four local wild *Balsamorhiza* populations. These patches comprised two purely conspecific and two commingled populations. To visualize pollen flow, we used six fluorescent powders, one powdered pigment, and black poppy pollen as surrogates for *Balsamorhiza* pollen. Assignments of fluorescent powder colors to species were chosen to anticipate possible difficulty in distinguishing individual particles of some colors on stigmas (e.g., orange, red, and pink).

### Pollen flow trials

The arrangement of flowers serving as unique sources of pollen surrogate markers was chosen to quantify geitonogamy, conspecific outcrossing, and interspecific pollen flow. For half of the bouquets of each species (4 each), one of the three flowers was dosed with its own unique marker powder. Bouquets and colors were randomly assigned. Pigment was applied to the ring of freshly opening florets that morning, daubing the pigment on the pollen-presenting surfaces beneath the stigmas at the hour that the style was pushing through the staminal tube. Thus, the pollen surrogate was applied only to the pollen-bearing surfaces of individual fresh florets, leaving stigmas uncontaminated.

Bouquets with both marker-dosed and unmarked flowers were placed in their array positions, where females of both *Osmia* species that were nesting 10 m distant could forage freely amid the flower "patches". Experiments started on both days by 9:30 a.m. MDST. After 5 h of *Osmia* foraging activity, flowers were brought inside. For each of the 40 unmarked flowers, stigmatic surfaces were viewed microscopically (40×), first illuminated with a portable 365-nm LED ultraviolet light source (Nichia Corp, Tokushima, Japan) for particles of fluorescent powder, and then by white light (for other markers). The counts of flowerheads whose stigmas received particles of each color of pollen surrogate were summed and grouped as indicative of geitonogamy, conspecific outcrossing, and interspecific pollen flow.

In summary, a pollen-flow array consisted of 48 *Balsamorhiza* flowers grouped in 16 bouquets of three flowers each. Four of each species' 24 flowers received one of eight uniquely colored pollen surrogate pigments. At the end of each day's experiment, stigmas of each of the 40 unmarked flowers were examined for particles of each of the eight pollen surrogates. The experiment was repeated once using the same marked flowerheads but with freshly dehiscing disk flowers to receive and later be viewed for pollen surrogates.

## Results

### Behavioral trials

Overall, 133 foraging bouts consisting of 571 visits by *Osmia* bees were recorded at the experimental foam block grids. During the use of any one grid, the average *B. sagittata* flower received four visits from *Osmia* (range 0–9), and the average *B. macrophylla* flower was visited seven times (range 0–14). All *Osmia* visitors were females. As they walked about a flowerhead, these females invariably probed for nectar and actively collected pollen, the latter indicative of their nesting status. A female accumulates fresh loose pollen in the scopal hairs beneath her abdomen. Pollination seems inevitable because females always removed pollen by rapidly drumming or patting their abdomens up and down against the pollen-bearing floral styles, as reported by Rust (1974), thus tapping the pollen-carrying surface against the stigma surfaces (ESM).

Foraging *Osmia* females were collectively homogeneous in their floral preferences for *Balsamorhiza*. For the 46 visitation bouts that included five or more flowers, there was no significant heterogeneity among *Osmia* females in the proportions of their visits to the two *Balsamorhiza* species ($\chi^2_{[46]} = 47.6$, $p = 0.5$). Therefore, I accepted the null hypothesis that their visits all belonged to the same binomial distribution.

Foraging *Osmia* females visited flowers of *B. macrophylla* more frequently than those of *B. sagittata* ($\chi^2_{[1]} = 72.6$, $p < 0.0001$) (Fig. 1). Their preference for *B. macrophylla* (62% of visits) was consistent during experiments at both sites (Fig. 1) and during both days (data not shown) ($\chi^2_{[4]} = 1.8$, $p > 0.7$), with no interaction ($p > 0.6$). Floral preference alone, however, implies neither constancy nor an ethological mating barrier.

Foragers of the two *Osmia* species displayed no floral constancy in their visits among *Balsamorhiza* flowers. As females foraged from flower to flower in the randomized grids, they were as likely to move between *Balsamorhiza* species as to visit another flower of the same *Balsamorhiza* species during a foraging bout (210 vs. 220 moves overall) (Fig. 2). This was true at both the canyon ($\chi^2_{[1]} = 0.74$, $p > 0.4$, 53 bouts) and town ($\chi^2_{[1]} = 0.07$, $p > 0.8$, 80 bouts) sites of experimentation (Fig. 1). The bees' floral infidelity was not merely a function of limited nearest neighbor choices in the randomized grids, as most visits (68%) were not to neighboring flowers.

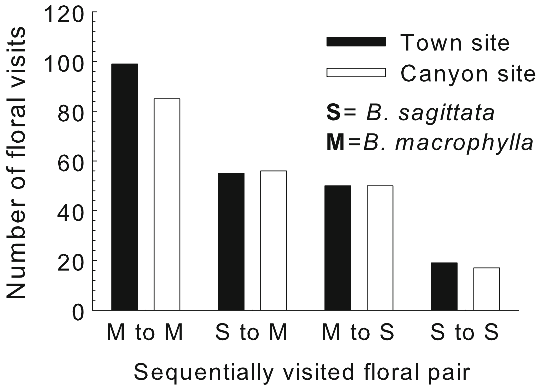

**Fig. 1** Sums of pairwise sequential floral visits during foraging bouts of *Osmia* females to floral grids tested at two locations

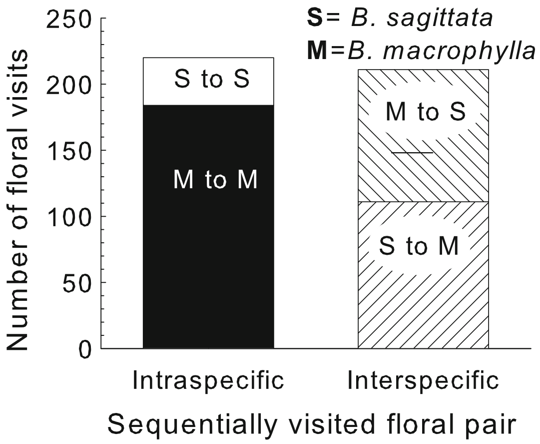

**Fig. 2** Comparisons of the sums of intra- versus interspecific floral moves by foraging female *Osmia* to floral grids during 2 days of experiments at two locations

Floral grids attracted a few individuals of several other bee species, all of them polyleges. Grids at Green Canyon attracted four foraging honey bees (18 floral visits), one bumblebee (8 visits), and nine *Halictus* bees (14 visits). These taxa each showed the same rank order of sequential visitation preference (MM > MS = SM > SS) as the two *Osmia* species (Fig. 2), with a two- to three-fold greater preference for *B. macrophylla*. However, there were too few visits for a meaningful analysis of constancy.

### Pollen flow trials

Foraging oligolectic female *Osmia* moved fluorescent markers to stigmas of other flowers within a bouquet (geitonogamy), and between bouquets of both conspecific flowers (xenogamy) and different species (potential hybridization) (Table 1). The nearby nesting *Osmia* were the only insects seen visiting the flowers on both days; in good weather, two to five pollen foragers were seen at the array at any one time. Transfer frequencies of specific

**Table 1** Intra- and interspecific movement of pollen surrogate markers between cut flowers of two *Balsamorhiza* species arrayed in a naturally spaced 4×4 grid of 16 bouquets being visited solely by oligolectic *Osmia* bees

| *Balsamorhiza* species | Number of pollen surrogate transfer events indicative of | | |
|---|---|---|---|
| | Geitonogamy | Outcrossing | Hybridization |
| *macrophylla* | 15 | 12 | 23 |
| *sagittata* | 14 | 26 | 19 |

markers could not be calculated entirely as planned, for as expected, the fluorescing hue of individual particles of several fluorescent powders on stigmas could not be reliably distinguished, nor was the black poppy pollen readily seen. Fortunately, look-alikes had all been assigned to one or the other of the *Balsamorhiza* species, so it was nonetheless evident that these pollen surrogates were transferred from pollen presenter surfaces to stigmas of flowers in the same and different bouquets by the foraging *Osmia*. Pollen surrogates were moved between different species' bouquets as frequently as among conspecific flowers of different bouquets (Table 1).

The spacing of bouquets in the pollen flow arrays proved realistic for these *Balsamorhiza* species. Nearest neighbors of wild *B. macrophylla* were separated by 37 ± 2 cm (mean ± standard error, $n = 51$ pairs) and those of *B. sagittata* by 45 ± 3 cm ($n = 60$). In two commingled populations, nearest heterospecific neighbors were 38 ± 3 cm apart ($n = 40$). In a mixed population of *B. sagittata* and *B. hookeri* that included individuals with clearly hybrid leaf morphologies (near Holbrook, ID), nearest heterospecific neighbors were 72 ± 16 cm distant ($n = 10$). Foraging *Osmia* therefore encountered bouquets in the arrays that were spaced at least as widely apart as *Balsamorhiza* plants growing in the field.

## Discussion

The Connoisseur Hypothesis was only partly supported by the experimental evidence. Both species of *Osmia* did forage preferentially at larger flowered *B. macrophylla* (ESM), suggesting that they responded to differences between flowers of the two *Balsamorhiza* hosts. However, individual foragers showed absolutely no constancy during their foraging bouts. They frequently switched between flowers of these two *Balsamorhiza* hosts in the mixed species grids. Neither labile preference nor fixed oligolecty led to floral constancy. Foraging *Osmia* often moved pollen surrogates from pollen presenters to stigmas of both conspecific and heterospecific flowers, indicative of these bees' roles as pollinators of *Balsamorhiza*. Their indiscriminate foraging should result in the transport of pollen between intermingled plants of the two *Balsamorhiza* species. Bee oligolecty per se does not provide an ethological mating barrier to hybridization among species of *Balsamorhiza*.

Plant spatial distributions, their densities, and the extent of intermixture of interfertile hosts in populations undoubtedly influence how often foraging bees switch floral hosts during a foraging trip and thereby facilitate hybridization. Flowers of these two *Balsamorhiza* species would never be as closely intermixed as they were in the randomized foraging grid, but their 1-m spacing in the pollen flow array did represent natural spacing and commingling of the two species. Single foraging bouts by these two *Osmia* offer many opportunities for outcrossing and hybridization, as they must visit >50 flowers and therefore >15 *Balsamorhiza* plants per foraging trip (Cane 2005). The potential spatial scale for *Osmia*-mediated gene flow among neighboring populations of *Balsamorhiza* is large, roughly bounded by the 0.5-km foraging distance observed for these two *Osmia* species (Cane 2005). If plants of two *Balsamorhiza* species are at least moderately intermixed, as was shown here, then the foraging infidelities of these oligolectic *Osmia* bees will manifest. Interspecific pollen flow is then inevitable.

Host preferences by both *Osmia* in these experiments might reflect flower size or bee experience. Petals of *B. macrophylla* are often longer (3.5–6 cm) than those of *B. sagittata* (2.5–4 cm). The greater attraction of *Osmia* to the typically larger flowers of *B. macrophylla* is consistent with reports from polylectic bees, which prefer to forage at larger conspecific flowers (Inoue et al. 1995; Conner and Rush 1996). From this perspective, these *Osmia* may simply prefer larger *Balsamorhiza* flowers, perceiving the two species as a single entity. In contrast, the results of my study did not support the contention that bees are preferentially constant to the floral species of a hybridizing pair with which they have more local experience (Hersch and Roy 2007). The host species that both *Osmia* preferred, *B. macrophylla*, was beyond their flight range from their nesting blocks and so absent from these individual bees' adult experiences. These hypotheses were not explicit parts of the experimental design, but did not confound the observed lack of constancy by *Osmia*.

Foraging infidelity (or constancy) among oligolectic bees could be consequential for hybridization and introgression of other genera of floral hosts at which such oligoleges prevail (Table 2) and floral traits have diverged moderately. The reported constancy of oligolectic *Dufourea* bees foraging among *Phacelia* species holds such promise. The genus hosts both oligolectic and polylectic species of bees (Schlindwein et al. 2005), oligolectic females showed constancy to species differing in pollen

**Table 2** Some representative plant genera with both hybridizing species and pollinator guilds with abundant oligolectic bees

| Hybridizing plant genus | Plant family | Hybridization reference for genus | Genus of prevalent oligolege | Oligolecty reference |
|---|---|---|---|---|
| *Balsamorhiza* | Asteraceae | Ownbey and Weber (1943) | *Osmia* | Cripps and Rust (1989) |
| *Clarkia* | Onagraceae | Hauber and Bloom (1983) | *Diadasia* | MacSwain et al. (1973) |
| *Cucurbita* | Cucurbitaceae | Kirkpatrick and Wilson (1988) | *Peponapis* | Hurd et al. (1971) |
| *Helianthus* | Asteraceae | Rieseberg et al. (2003) | *Melissodes* | Parker (1981) |
| *Larrea* | Zygophyllaceae | Hunziker et al. (1977) | *Anthidium* | Minckley et al. (2000) |
| *Opuntia* | Cactaceae | Benson (1982) | *Diadasia* | Grant and Hurd (1979) |
| *Penstemon* | Plantaginaceae | Wolfe et al. (1998) | *Osmia* | Tepedino et al. (2006) |
| *Phacelia* | Hydrophyllaceae | Heckard (1960) | *Dufourea* | Schlindwein et al. (2005) |
| *Salix* | Salicaceae | Argus (1974) | *Andrena* | Robertson (1929) |
| *Sphaeralcea* | Malvaceae | Tate et al. (2005) | *Diadasia* | Sipes and Tepedino (2005) |
| *Vaccinium* | Ericaceae | Ritzinger and Lyrene (1998) | *Habropoda* | Cane and Payne (1988) |

color (Torchio et al. 1967), and some *Phacelia* species naturally hybridize (Heckard 1960). Additional possibilities represent diverse plant families, life history traits, growth forms, seasonalities, and habitat associations (Table 2). Their floral traits range widely, from the pale-gray catkins of woody willows to compound flowerheads of sunflowers to the massive yellow flowers of vining gourds. Some of these plant genera are quite ubiquitous and common within their geographic ranges, traits favoring the "predictable plethora" explanation for their oligolecty (Wcislo and Cane 1996). Others have unusual floral traits that largely restrict their rewards and sometimes their pollination to oligolectic bees (e.g., Alves dos Santos 2002). The extent of hybridization among such bee-pollinated flowering species is often poorly known, however, and sometimes hidden in botanical taxonomic monographs. Even for species of *Balsamorhiza*, whose putative hybrid individuals present visually striking intermediates that I readily found in Utah, Idaho, and Nevada, no subsequent genetic proof exists for their true hybrid status. Nonetheless, experiments across such divergent lineages and diverse trait sets promise robust generalizations.

Oligolectic bees have been generally overlooked with regard to their foraging constancy, especially in manipulative host choice experiments, to judge by their absence from comprehensive discussions (e.g. Chittka et al. 1999). The few examples reveal some oligoleges' abilities to sometimes discern among congeneric floral hosts, akin to both *Osmia* species' preference for *B. macrophylla* flowers reported here. Palynological evidence for oligolege's foraging fidelities is rarely useful when studying the pollen of closely-related species, as the pollens of closely-related species are often indistinguishable (Erdtman 1969). In one exceptional case, however, larval provision masses of the oligolectic bee, *Dufourea mulleri*, were either purple or yellow, the respective pollen colors of its two local *Phacelia* hosts (Torchio et al. 1967). That a given nest had both purple- and yellow-colored provision masses, but each mass was of a pure color, reveals two foraging attributes of the mother bees, namely, foragers showed pollen constancy on a given day, but shifting foraging fidelities among congeneric flowers over sequential days. Experimental reciprocal placement of bouquets of flowers amid another species' inflorescences was advocated early in such studies (Clements and Long 1923) as a means of testing bees' powers of host discrimination and foraging fidelities. Females of the solitary bee *Centris atripes* foraging at palo verde (*Cercidium*) remained constant to flowers of one or another palo verde species when challenged with mixed bouquets placed amid the branches of a flowering individual (Jones 1978). The bees may have been responding to the two palo verdes' differing patterns of ultraviolet petal markings. The author claimed *C. atripes* to be an oligolege of palo verde, but it seems to be polylectic (Hurd 1979). Strong species-specific differences in floral odor can apparently guide foraging by the oligolectic solitary bee *Habropoda laboriosa*. In mixed bouquets of two species of *Gelsemium* (Loganiaceae), females strongly preferred flowers of *G. sempervirens*, but showed only weak constancy to either species (Pascarella 2007). These studies illustrate what little we know about the extent and limits of foraging constancy by oligolectic bees and their abilities to recognize, discern, and respond to differing visual and olfactory cues of their closely related floral hosts.

Care must be taken with design if future experiments with floral constancy by oligolectic bees in other systems (Table 2) are to yield biologically meaningful interpretations, comparisons, and generalizations. Particular concerns include the following:

1. The species of bee must be identified authoritatively and vouchered, a seemingly obvious but too often disregarded maxim.

2. The sexes of foragers must be discerned, with a focus on females. The pollination efficacies of males are typically inferior to that of oligolectic females (e.g., Parker 1981). Furthermore, males need to visit (and so pollinate) far fewer flowers than females, as males are merely satisfying their personal energetic needs and not provisioning nests.
3. Oligolectic bees are taxonomic floral specialists for pollen, not nectar, so females should be foraging for pollen at their pollen host(s); any mere nectar visits should be separately tallied. Because mature females only forage for pollen to provision their progeny, nesting females therefore are required for experimental purposes.
4. Randomized arrays are useful so that estimates of constancy are not confounded by nearest neighbor effects that inevitably accompany experiments with large bouquets or natural populations. Null probabilities for host switching may be unknowable if bees forage in variably clumped or patchy arrays of host flowers. Proof of the efficacy of randomized arrays is illustrated here by the identical likelihoods of intra- and interspecific moves between *Balsamorhiza* flowers by pollen-foraging *Osmia* (Fig. 2).

Attention to these standards will assure replicate studies amenable to unambiguous interpretation and comparison.

Clearly, speciation among *Balsamorhiza* has not entailed any meaningful divergence in floral traits from even an oligolectic bee's perspective. In these cases, oligolectic pollinators may prefer one species over another (e.g., *B. macrophylla*), but their preference confers no ethological mating barrier. Instead, their lack of constancy contributes to host introgression and hybridization. At the other extreme, divergence in a few key genetic loci is known to alter floral color and morphologies so dramatically as to shift a plant species to an entirely different class of animal pollinators (Bradshaw and Schemske 2003). Contrasting flower color morphs, such as these, sometimes elicit constancy from polylectic bees such as *Bombus* (Jones and Reithel 2001), but in other cases they do not (Leebens-Mack and Milligan 1998). A pair of outcrossing, introgressing species of *Clarkia* likewise differ in floral hue (Hauber and Bloom 1983), but they attract mostly oligolectic bees (MacSwain et al. 1973) whose response to this color difference remains untested. Array experiments with this and comparable systems (Table 2) whose flowers show moderate divergences in floral cues should reveal if and when oligolectic and polylectic bees ever differ in their relative contributions to hybridization, introgression, or reproductive isolation of their common floral host(s).

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