Adult pollen diet essential for egg maturation by a solitary *Osmia* bee

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A R T I C L E   I N F O

A B S T R A C T

Reproduction is a nutritionally costly activity for many insects, as their eggs are rich in lipids and proteins. That cost seems especially acute for non-social bees, which for their size, lay enormous eggs. All adult female bees visit flowers, most of them to collect pollen and nectar, or sometimes oils, to feed their progeny. For adult bees, the need for pollen feeding has only been detailed for the honey bee, *Apis mellifera*. To experimentally test for the reproductive value of adult pollen feeding by a non-social bee, *Osmia californica* (Hymenoptera: Apiformes: Megachilidae), young female bees plus males were released into large glasshouse cages provided with either a male-fertile sunflower cultivar or a pollen-less one. Females regularly visited and drank nectar from flowers of both cultivars. Abundant orange pollen was seen regularly in guts of females confined with the male-fertile sunflowers, indicative of active pollen ingestion. All females' terminal oocytes (next egg to be laid) were small at emergence. Oocytes of females confined with the pollen-less sunflowers remained small, despite frequent nectaring and exposure to other floral stimuli. In contrast, the basal oocytes of female *O. californica* with access to pollen had swelled to full size within ten days following emergence, enabling them to lay eggs in provided nest tubes. Adult females of this solitary bee required dietary pollen to reproduce; nitrogen stores acquired as larvae were inadequate. Early and regular pollen feeding in part paces the onset and maximum tempo of solitary bees' lifetime reproductive output.

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1. Introduction

1.1. Pollen feeding by adult insects

Many adult insects visit flowers for food (Proctor et al., 1996). Most visitors drink nectar, whose sugars often fuel their immediate energetic needs, particularly for flight (Heinrich, 1975). Pollen feeding by diverse adult insects is far less commonly documented than nectar drinking (reviewed in Roulston and Cane, 2000), but from several case studies, pollen feeding by some insects improves their longevity and reproductive output (reviewed in Wäckers et al., 2007). For example, gravid female flower flies (Syrphidae) with a growing oocyte added pollen to their nectar diet, presumably because they needed the pulse of dietary protein to synthesize yolk proteins (Haslett, 1989). Oocytes remained small in flies denied dietary pollen (Schneider, 1969). Adult female heliconiid butterflies extract essential amino acids from collected pollen, which ends up in their eggs (O'Brien et al., 2003). Dietary pollen extended their longevity and fecundity, which has also been shown for adult female seed beetles (Leroi, 1978) and thrips (Kirk, 1985). Like most butterflies and moths, only the adults of these various insects fed from flowers.

Bees are the quintessential floral foragers, both for self-maintenance and for acquiring food for their offspring. Nectar sugars power bees' flight (Nachtigall et al., 1989). Females of most bee species also blend collected nectar into larval diets (Michener, 2007). Pollen constitutes a substantial fraction of the larval bee's diet too (e.g., 33% for *Megachile rotundata*) (Cane et al., 2011). In contrast, pollen feeding by adult bees is rarely reported and poorly understood.

1.2. Pollen feeding by adult bees

Bees descended evolutionarily from wasps, switching from animal tissues to pollen for their dietary proteins (Michener, 2007). Pollen is the sole source of dietary protein for larvae of all solitary bees and most social ones (Michener, 2007; Roulston and Cane, 2000). Adult female bees collect pollen from flowers, usually transporting it externally in hairy scopal brushes for deposition at the nest (Thorp, 2000). We know that foraging females consume as well as transport nectar while foraging, but what about pollen?

Most non-parasitic species of bees provide each offspring with an individual food cache of pollen moistened with regurgitated nectar (or in some cases, floral oils). However, most insights into pollen needs of adult bees come solely from studies of the European honey bee, *Apis mellifera* L. Young honey bee workers

("nurse bees") gorge on pollen to produce nutritious glandular secretions fed to young larvae and queens (Crailsheim et al., 1992; Dietz, 1975). Older workers shift to foraging, drastically curtailing pollen consumption as a part of their caste-specific behavioral ontogeny (Hrassnigg and Crailsheim, 1998; Szolderits and Crailsheim, 1993).

These traits of honey bees contrast sharply with those of most bee species, which are solitary, not social. For example, worker honey bees are sterile, whereas every female solitary bee is fertile and bears the nutritional cost of maturing eggs. Unlike honey bees, very few species of solitary bee are known to augment larval provisions with glandular secretions. For these and other reasons, the role that pollen feeding plays in reproduction by adult solitary bees cannot be extrapolated from the shifting, caste-specific pollen feeding needs of sterile worker honey bees. Instead, dietary pollen needs of adult solitary bees must be understood in terms of their own nutritional fluxes and reproductive outlays.

Both highly social bees like the honey bee (Michener, 1974), as well as cleptoparasitic or "cuckoo" bees (Alexander and Rozen, 1987), can mature many smaller oocytes simultaneously, reflecting their need to lay many eggs continually (honey bee) or episodically (cleptoparasites). In contrast, nesting female solitary bees lay relatively few eggs in their lifetimes, but those eggs are enormous for an insect of their size (Iwata and Sakagami, 1966). Solitary bees generally lay only 1–2 eggs daily (Neff, 2008), even with unlimited bloom and long foraging days in an ideal greenhouse environment (Bohart and Cross, 1955; Maeta and Kitamura, 2005). The cavity-nesting mason bee Osmia lignaria averaged 1.8 nest cells completed per day in a greenhouse stocked with excess bloom (Tepedino and Torchio, 1982). Hence, the slow pace of egg maturation, not extrinsic conditions, seems to cap their daily oviposition rate (Neff, 2008), with lifetime reproduction simply reflecting longevity. The yolk lipoproteins (vitellogenins) invested in their eggs must come from a dietary nitrogen source, which for bees is pollen. Conceivably, their vitellogenins could be synthesized from pollen proteins held over from the larval stage or else gained during adult pollen feeding.

Evidence for a general pollen-eating habit of adult solitary bees was first published by Taniguchi (1956), who dissected a broad diversity of female solitary bees caught at flowers. He found that their crops and midguts often contained visibly abundant pollen. More recent research (Cane et al., 2016) has shown that females of a gregarious solitary bee, Nomia melanderi Ckll., eat several large daily pollen meals throughout their adult lives, suggesting but not proving that adult solitary bees must consume pollen to reproduce. Several laboratory experiments, to be interpreted in detail later in the discussion, provide clues about the relationship between hormones, adult diets of solitary bees, and vitellogenesis. In brief, they showed that bees limited to sugar water or honey failed to grow oocytes (Richards, 1994), but with topical application of JH, vitellogenesis and yolk deposition were initiated (Bell, 1973; Sihag, 1986). Conceivably, a young female's perception of floral cues (e.g. scent, touch, taste), rather than ingestion of pollen, might stimulate JH production and so vitellogenesis, mobilizing lipids and proteins accumulated by the feeding larva and retained during diapause (as moths and butterflies seem typically to do) (reviewed in O'Brien et al., 2003). Alternatively, full maturation of even the first basal oocyte may require pollen proteins ingested as an adult bee, because larval stores are too meager for the vitellogenins needed to provision solitary bees' large eggs.

To compare these two scenarios, newly emerged females of a solitary cavity-nesting Osmia bee were placed in large matching greenhouse flight cages stocked with identical nesting blocks and similar-looking cultivars of sunflowers (Helianthus annuus L.). The bee used, Osmia californica Cresson, specializes on the Asteraceae for pollen (Rust, 1974), especially the tribe Heliantheae, to which sunflower belongs. One cage held a normal, pollen-bearing cultivar, the other had plants of a similar-looking male-sterile cultivar that sheds no pollen, thereby making it possible to isolate pollen consumption from the other stimuli and rewards that foraging bees experience at flowers. Marked newly-emerged female bees of known ages were flown in both cages, along with enough males for mating. Females were sacrificed at different ages and dissected to measure the dimensions of their basal (or terminal) oocyte and score pollen consumption. Thus, yolk deposition and oocyte growth in an individual could be inferred by dissecting females foraging at flowers that either provided a complete diet or one that lacked only pollen but presented the same nectar, scents and other floral stimuli. I hypothesized that adult female bees denied dietary pollen would fail to enlarge their first (or possibly second) basal oocyte, consistent with dissected females regularly seen to contain pollen.

2. Methods

2.1. Experimental design—plants and bees

Two dwarfed commercial sunflower (H. annuus) cultivars were used: 1) 'ProCut Yellow Lite F1′, a pollen-less variety, and; 2) 'Zebulon', a sunflower that produces and sheds pollen (Johnny's Selected Seeds, Winslow, Maine). Flowering of both cultivars is determinant, each individual producing a single, comparably large flowerhead (capitulum) with yellow petals around a yellow-green disk. Forty 3-gallon pots received standard potting soil and seed of one cultivar to grow in our heated greenhouse.

The nesting population of O. californica came from a local population being propagated outdoors on their native floral host, Balsamorhiza sagittata (Pursh) Nutt. Osmia bees pupate in late summer, wintering as dormant adults in their natal cocoons at 4 °C. Following brief incubation, they emerged from 28-April to 6-May. Emerged females were briefly chilled to paint-mark their thoracic dorsa to indicate that day's cohort (four cohorts in all). Females were then released into one or the other walk-in screen cage (7 × 7 × 2 m) with its array of 20 potted blooming sunflowers. Three males were added, enough to see regular courtship rejections (these bees briefly mate only once), but not so many as to overly harass females. Both cages also had a 48-hole drilled wooden nesting block with paper straw liners, using dimensions recommended by Torchio (1989) and used annually by the author for trap-nesting this species. The captive bee populations were watched daily for nectar drinking (extended proboscis), active pollen collection (abdominal drumming of disc flowers), activity around the nesting blocks, and survival.

Bees of known ages from each cage were periodically sacrificed and frozen for dissection. In 2013–14, bees caged with 'Zebulon' were taken without regard to their daily nesting and oviposition schedules. Their basal oocytes ranged widely in size, possibly because some dissected bees might have just laid their daily egg. Therefore, in 2015, we watched (by otoscope) each older female's progress in caching a provision mass. Only when a female was within 1–2 h of oviposition was she captured and frozen for dissection, reasoning that their basal oocytes always would be nearing full size. Females confined to the male-sterile cultivar were sacrificed at comparable hours of the day. Additionally, 15 females from the outdoor stock population were taken returning to their nesting block at noon and dissected for oocyte dimensions. They served as positive controls for pollen feeding and the range of basal oocyte sizes among unmanipulated wild bees.

2.2. Basal oocyte measures and scoring gut pollen content

Dimensions of basal oocytes were measured for 63 freshly thawed bees dissected under insect saline solution. Abdominal