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Linkages between nectaring and oviposition preferences of *Manduca sexta* on two co-blooming *Datura* species in the Sonoran Desert

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Abstract. 1. The oviposition choices of phytophagous insects determine the environment that their offspring will experience, affecting both larval performance and host plant fitness. These choices, however, may be influenced by other activities, such as nectar foraging.

2. In the Sonoran Desert, *Manduca sexta* (Lepidoptera: Sphingidae) oviposits primarily on the perennial herb *Datura wrightii*. It has been reported to oviposit on the smaller-flowered, co-blooming, sympatric annual *Datura discolor* as well. *Datura* is also *M. sexta*'s most important source of nectar in this region. Nectaring and oviposition decisions thus determine *M. sexta*'s reproductive success, as well as the relative benefits (pollination) and costs (herbivory) that each *Datura* species derives from this interaction.

3. The nectaring and oviposition choices of adult *M. sexta* on these congeners were studied to investigate how nectar foraging influences oviposition. Larval performance on the two hosts was then assessed.

4. Nectaring and oviposition were behaviourally linked, with *M. sexta* preferring the perennial *D. wrightii* as both a nectar source and larval host when given a choice between the two species. This preference disappeared, however, when only *D. discolor* bore flowers.

5. In the laboratory, larvae developed at equal rates when fed *D. wrightii* or *D. discolor* leaves, but survival was higher on *D. wrightii* when larvae fed on intact plants.

6. These findings suggest that while female preferences match larval performance in most cases, the link between nectaring and oviposition may at times bias moths to lay eggs on inferior larval hosts.

Key words. Herbivory, *Manduca sexta*, oviposition decisions, pollination, preference–performance hypothesis.

Introduction

In many phytophagous insects, the environment that juveniles experience is determined largely by the oviposition decisions of adult females. Offspring development and survival in turn directly impact female fitness. This relationship informs the preference–performance hypothesis, which predicts that adults will evolve oviposition preferences for hosts and sites associated with high offspring performance (Jaenike, 1978; Thompson, 1988; Gripenberg *et al.*, 2010). Indeed, gravid females are often sensitive to habitat variation and host quality when selecting sites in which to lay eggs, and will discriminate among sites based on differences in food quality and abundance (Awmack & Leather, 2002; Sentis *et al.*, 2012), larval competition (Williams & Gilbert, 1981; Wise & Weinberg, 2002), larval predation risk (Higashiura, 1989; Spencer *et al.*, 2002; Diamond & Kingsolver, 2010), and other environmental factors (Eilers *et al.*, 2013).

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Adult preferences for particular sites or hosts, however, do not always align with the quality of those sites for offspring (Mayhew, 1997; Gripenberg *et al.*, 2010; Refsnider & Janzen, 2010). For example, strong oviposition cues may cause a female to lay eggs even when the host is well defended against herbivory (Stowe, 1998), or even fatal to larvae (Davis & Cipollini, 2014). Furthermore, the sites that will maximise an individual larva's performance and that will maximise the fitness of its mother are not always the same (Scheirs *et al.*, 2000; Rosenheim *et al.*, 2008). For example, expending time and effort to find and assess host plants may trade off against the number of eggs a female is able to lay in her lifetime (Rosenheim *et al.*, 2008; König *et al.*, 2016). Similarly, other adult activities such as mating and feeding may bias females towards nearby resources that could be inferior for offspring development (Courtney, 1981; Scheirs & De Bruyn, 2002; Janz, 2005; Forister *et al.*, 2009).

In the Sonoran Desert (U.S.A.), the primary larval host plant of *Manduca sexta* (Lepidoptera: Sphingidae) is the perennial forb *Datura wrightii* (Solanaceae). *Datura wrightii* flowers are also the principal nectar resource for *M. sexta* (Alarcón *et al.*, 2008), and the moths are *D. wrightii*'s primary pollinators (Bronstein *et al.*, 2007). Thus, *M. sexta* adults act as mutualists of *D. wrightii*, while larvae act as antagonists, often of the same individual plant. From the plant perspective, then, the risk of herbivory is a cost associated with receiving pollination services. Indeed, a single fifth-instar caterpillar can completely defoliate its host (Mcfadden, 1968). From the perspective of the moths, a link between nectaring and oviposition may be maintained if host-searching females use the strong and conspicuous cues presented by the flowers as reliable cues for oviposition sites, as well as indicators of plant quality (Adler & Bronstein, 2004).

While the *M. sexta*–*D. wrightii* relationship is fairly tightly coupled in the south-western U.S.A., *M. sexta* is also known to oviposit on the annual plant *Datura discolor* (Reisenman *et al.*, 2013). This species, a congener but not the sister species of *D. wrightii* (Bye & Sosa, 2013), overlaps in geographical range with *D. wrightii*; they flower at the same time and sometimes in close proximity (G. Smith, unpublished). In addition to being a smaller plant overall, *D. discolor*'s flowers are usually fewer and significantly smaller (6.25 vs. 9.75 cm average corolla flare), less fragrant, and offer half the nectar volume (30.5 vs. 65.1 μl) offered by *D. wrightii* (Raguso *et al.*, 2013; Reisenman *et al.*, 2013). As a result, *D. discolor*'s flowers are of lower quality as a nectar source and are likely to be less conspicuous to moths. Furthermore, independent of floral reward quality, the larger perennial *D. wrightii* and smaller annual *D. discolor* probably differ in their quality as larval resources. For example, consistent with studies in other systems, the faster-growing *D. discolor* may produce more inducible defenses than the slower-growing *D. wrightii* (e.g. Coley, 1987; Stamp, 2003; Endara & Coley, 2011), but may also have higher concentrations of key nutrients (e.g. Foulds, 1993; Han *et al.*, 2005; He *et al.*, 2016). Larger and actively growing plants may also be better hosts due to higher available biomass and the high nutritional quality of growing tissue (Price, 1991).

The larval resource quality of these plant species could potentially either match or mismatch their floral reward quality. This is likely to produce situations in which floral cues may either reinforce or conflict with host quality cues for foraging moths. In this paper, we investigate: (i) whether adult moths prefer *D. wrightii* or *D. discolor* for nectaring and oviposition; (ii) whether perceived floral quality reliably predicts larval host quality; and (iii) how floral cues affect oviposition preferences. We predicted that: (i) the larger-flowered, perennial *D. wrightii* would be preferred by adult moths for nectaring and oviposition; (ii) consistent with the preference–performance hypothesis, the species preferred by adults would be a superior larval host; and (iii) due to the link between oviposition and nectaring, the presence of flowers would bias oviposition decisions when moths are given a choice between host species.

Methods

Moth rearing for adult behavioural experiments

Naïve adult *M. sexta* were obtained from a laboratory colony maintained at the University of Arizona (Levin *et al.*, 2016). Larvae were fed an artificial wheat germ-based diet (see Davidowitz *et al.*, 2003). To stimulate rapid eclosion from the pupa, larvae were reared on a long-day cycle, with LD 17:7 h, at 25 °C and 40–50% RH. After pupation, male and female moths were kept on a diurnal temperature cycle of 26:24 °C day: night until 3 day prior to eclosion, when they were placed in polypropelene screen cages (31 × 31 × 32 cm) under ambient light conditions. In these cages they were given the opportunity to mate for 3 days after eclosion before they were used in experiments.

Plant rearing

Datura wrightii and *D. discolor* plants were grown from seed in the greenhouse. Seeds were obtained in the field from the Santa Rita Experimental Range, Arizona (31–50′00″ N; 110-51′10″ W). Seeds were germinated and grown communally until 1 week after they germinated, at which point they were transferred into 25.4-cm (10 in; *D. wrightii*) or 20.3-cm (8 in; *D. discolor*) pots filled with a 3:2:1 mixture of Sun Gro Sunshine Mix #3 (Agawam, Massachusetts), vermiculite and sand (Bronstein *et al.*, 2007). The greenhouse was kept partially shaded, and maintained between 21 and 35 °C. Plants were watered every other day.

Flight experiments

To determine the preferences of adult moths for nectaring and oviposition on the two *Datura* species, we performed preference assays between July–September 2014 and August–September 2015. Adult moths were removed from the mating cage 3 day after eclosing, and taken to a large empty greenhouse (22 m × 7.5 m × 4 m, hereafter 'flight house') during the first 3 h after sunset, between 19.30 and 22.30 hours. To reduce the effect of ambient light on the behaviour of the moths, the flight house was lit by red light, as *M. sexta* does not have a red receptor (Bennett & Brown, 1985). An evaporative cooling system was used to maintain a temperature of approximately 26 °C and to generate directional air flow along the long axis of the space. Because the flight house holds approximately 660 m^3

of air, and the cooling system circulated air from outside the greenhouse, the flight house was unlikely to be saturated by the volatiles of the four experimental plants. No other plants were kept in the flight house for the duration of these experiments.

At the upwind end of the flight house, two potted individuals of each plant species were placed in a checkered array approximately 2 m apart. The diagonal positions of the different species were randomised each night. Individual plants were selected to approximately match in size. Four plant treatments were used, based on which species was flowering on a given night: both species bearing flowers (hereafter D1 + W1), neither species bearing flowers (D0 + W0), only *D. discolor* bearing flowers (D1 + W0), and only *D. wrightii* bearing flowers (D0 + W1).

Moths were removed from the mesh cages in which they eclosed and placed into release tubes constructed out of wire mesh, where they were allowed to acclimate for at least 15 min. Moths were then released sequentially 10 m downwind of the plant array. Moths were allowed to move, forage, and oviposit freely in the array for a maximum of 10 min or until they were inactive for 3 min (consistent with Riffell *et al.*, 2008), at which point they were captured. There was at least a 5-min interval between capture and release of a subsequent moth to allow pheromones to dissipate. During a moth's bout, we recorded each plant individual with which the moth interacted, as well as the timing and duration of each behaviour. Behaviours were classified as probing or entering flowers ('nectaring'), curling the abdomen and laying eggs ('ovipositing'), and resting on a surface without doing any of these described actions ('landing'). Due to darkness and our inability to closely approach moths without disturbing them, we were unable to definitively identify visually when a moth was drinking nectar or when they actually laid eggs. Thus, our measures were based on behaviours we could visually assess during moth foraging and which demonstrated the moths' intention to nectar or oviposit, i.e. when they entered the flowers or curled their abdomens. We were able to verify oviposition after the fact by examining the plants for eggs after the bout and recording their number and location on the plants. Eggs were not removed between bouts, but the presence of eggs on a plant did not affect oviposition preferences ($N = 64$ moths, linear mixed model $P = 0.76$).

Larval growth experiments

To investigate the developmental consequences of feeding upon *D. wrightii* and *D. discolor*, we conducted no-choice growth assays in June and August 2015. One egg from the laboratory colony 1 day before hatching was randomly placed on a 14-mm leaf disc of either *D. wrightii* or *D. discolor*. The leaf disc and egg were placed on filter paper in 55-mm-diameter Petri dishes ($n = 75$ for each species). Every day, larval mortality as well as the instars of surviving larvae were recorded. Leaf discs were replaced daily, and three drops of water were applied to the filter paper to prevent the larvae and leaf discs from drying out. Larvae were kept under the same temperature and light conditions described earlier.

When a larva reached the third instar, the amount of food provided was increased from one to three leaf discs. One disc was provided between 09.00 and 10.00 hours, and the other two discs between 15.00 and 17.00 hours. Pre-fourth-instar larvae never consumed all available leaf material, indicating that food was probably not limiting. When larvae moulted to the fourth instar, they were transferred to a −20 °C freezer and then placed in a drying oven at 50 °C for 24 h. After drying, larvae were weighed. To prevent any undigested plant material from influencing these measures, larvae were frozen while their guts were still empty after their moult to the fourth instar.

Later instars were too voracious to maintain on leaf discs in the laboratory. Thus, to measure the effect of host diet on growth through the fourth and fifth instars and to pupation, 10 additional third-instar larvae reared on *D. wrightii* and eight larvae reared on *D. discolor* were transferred onto intact plants of the corresponding species in the greenhouse. Larvae were then reared individually in large tubs with either two *D. wrightii* or four *D. discolor* plants to provide roughly equal amounts of leaves. The larvae were allowed to eat leaf material freely, and defoliated plants were replaced with uneaten ones as needed. Every day, the instar of the larvae was recorded until they began wandering behaviour indicative of the start of pupation. Two weeks after wandering started, pupae were unearthed, weighed, and assessed for viability.

Data analysis

Analyses were performed using R version 3.1.0 (R Development Core Team 2014). *t*-tests and χ^2 tests were performed in base R, while linear mixed models (LMMs) were performed using the lme command in the nlme package (Pinheiro *et al.*, 2014). In these models, moth individual was used as a random effect. Tests of nectaring preferences between the flower species were restricted to nights when both species had open flowers, and to moths that visited at least one flower during a bout. For tests comparing the amount of time moths spent nectaring and ovipositing on the two species, the time spent was log-transformed to improve normality. To assess oviposition preferences within each treatment, false discovery rate (FDR)-corrected *post hoc* LMMs were performed, again using moth individual as a random effect. To assess the linkage between nectaring and oviposition, analyses were restricted to moths that both nectared and oviposited at least once during their bout. A contingency table was then constructed for nectaring and oviposition, with each observation consisting of one moth's interaction with one plant. A Pearson's χ^2 test was run on this table. To control for potential individual effects (as each individual supplied one observation for each plant available during its bout), the test was re-run with each individual moth sequentially removed. The results of these tests were qualitatively identical to the test reported below, with every iteration producing a P-value < 0.001.

Results

Flight experiments

Adult moths visited both *D. wrightii* and *D. discolor* in the arrays, and individuals often nectared and oviposited on both

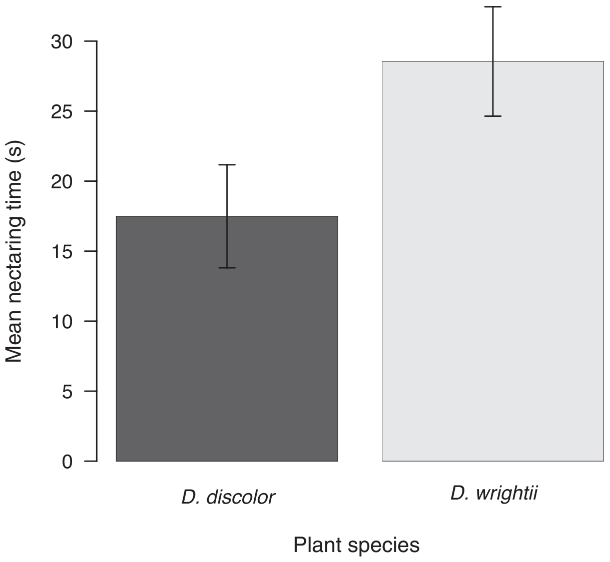

Fig. 1. Time spent (mean ± SE) by moths nectaring at the annual *Datura discolor* and the perennial *Datura wrightii* when both species were flowering. Moths nectared significantly longer on *D. wrightii* than on *D. discolor*.

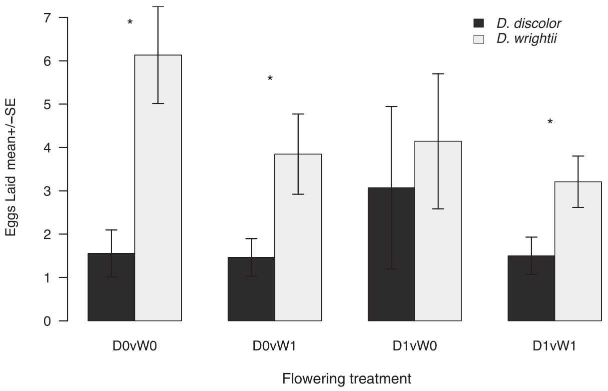

Fig. 2. Number of eggs (mean ± SE) laid by *Manduca sexta* on the annual *Datura discolor* and the perennial *Datura wrightii* under different treatment conditions. Dark bars represent *D. discolor*, while light bars represent *D. wrightii*. The treatments are as follows. D0vW0, neither species bore flowers; D0vW1, only *D. wrightii* bore flowers; D1vW0, only *D. discolor* bore flowers; D1vW1, both species bore flowers. An asterisk (*) indicates a statistically significant difference in the number of eggs laid by moths on the two plant species in that treatment. Moths also had a significant overall preference for *D. wrightii*.

species. When flowers of both species were present, 61% (11/18) of moths that visited a flower during their bout visited at least one flower of each species, and among females that laid eggs, 53% (36/67) laid at least one egg on each species.

Although both plant species were visited, moths preferred to nectar at the larger-flowered perennial *D. wrightii*. On nights when both species bore flowers, a *D. wrightii* flower was the first flower visited more frequently than a *D. discolor* flower (22 first visits to *D. wrightii*, 10 to *D. discolor*). Cumulatively, moths spent more time nectaring on *D. wrightii* ($N = 53$ bouts, mean = 28.6 s, SE = 3.9 s) than on *D. discolor* ($N = 39$ bouts, mean = 17.5 s, SE = 3.7 s; log-transformed LMM. $P = 0.006$; Fig.1). This difference was due to a higher average number of visits made to *D. wrightii* flowers (*D. wrightii*, $N = 53$ bouts, mean = 4.32 visits, SE = 0.56 visits; *D. discolor*, $N = 39$ bouts, mean = 2.44 visits, SE = 0.37 visits; LMM, $P = 0.015$), rather than to longer durations of individual visits (LMM, $P = 0.65$).

Females also preferred *D. wrightii* as a host plant. They spent more time ovipositing on *D. wrightii* ($N = 89$ visits, mean = 100.2 s, SE = 10.6 s) than on *D. discolor* ($N = 61$ visits, mean = 74.2 s, SE = 13.3 s; log-transformed LMM, $P = 0.0093$), laying nearly twice as many eggs on *D. wrightii* per bout (*D. wrightii*, $N = 62$ bouts, mean = 6.37 eggs, SE = 0.57 eggs; *D. discolor*, $N = 49$ bouts, mean = 3.61 eggs, SE = 0.59 eggs; LMM $p = 0.0002$). There was a significant interaction between plant species and treatment on the number of eggs laid. More eggs were laid on *D. wrightii* when neither species bore flowers (D0 + W0, FDR-corrected LMM, $P = 0.002$), when both species bore flowers (D1 + W1, FDR-corrected LMM, $P = 0.033$), and when only *D. wrightii* bore flowers (D0 + W1, FDR-corrected LMM, $P = 0.033$). However, equivalent numbers of eggs were laid on both species when only *D. discolor* was flowering (D1 + W0, FDR-corrected LMM, $P = 0.66$; Fig. 2).

First visits were made more frequently to flowering plants than to non-flowering plants (41/57, 72%). There was also a statistically significant association between nectaring and oviposition behaviours for both plant species: a moth was more likely to both nectar and oviposit on the same plant than it was to perform only one of those behaviours (χ^2: *D. wrightii*, $N = 60$ moths, $P = 0.0004$; *D. discolor*, $N = 49$ moths, $P = 0.02$). Despite this strong functional association between nectaring and oviposition behaviours, there was no difference in the number of eggs laid on flowering versus non-flowering plants ($N = 64$ moths, LMM, $P = 0.79$). Nor was nectaring time correlated with either the number of eggs laid ($N = 42$ moths, LMM, $P = 0.73$) or the amount of time spent ovipositing ($N = 42$ moths, LMM, $P = 0.52$).

Larval growth on cut leaves

Survival and growth rate did not differ between *M. sexta* larvae grown on the two species of *Datura* in the laboratory. Larvae did not differ in their probability of surviving to the fourth instar (*t*-test, $P = 0.62$) or their final mass (*t*-test, $P = 0.91$) as a function of which *Datura* species they fed upon. Larvae developing on *D. wrightii* took marginally longer to moult to the second instar (*D. wrightii*, $N = 55$ larvae, mean = 2.76 ± 0.1 days; *D. discolor*, $N = 55$ larvae, mean = 2.51 ± 0.11 days, *t*-test $P = 0.08$). However, there was no difference in the total time taken by larvae to reach the fourth instar, the point at which this experiment ended (*t*-test, $P = 0.32$).

Larval growth on live plants

Larvae reared on live plants in the greenhouse performed better on *D. wrightii* than on *D. discolor*. All 10 of the larvae reared on *D. wrightii* plants survived to pupation. In contrast, larvae reared on *D. discolor* experienced higher mortality, with three out of eight dying as fifth instars before pupation (χ^2, $P = 0.034$). On *D. wrightii,* the durations of the third instar (mean = 5.1 days, SE = 0.23 days) and fifth instar (mean = 2.83 days, SE = 0.4 days) were significantly shorter than on *D. discolor* (third instar: mean = 7.63 days, SE = 0.92 days, *t*-test, $P = 0.029$; fifth instar: mean = 6 days, SE = 1.05 days, $P = 0.036$). Of the five *D. wrightii*-reared larvae that were tracked through pupation, three (60%) produced viable pupae, whereas none of the five larvae reared on *D. discolor* that attempted to pupate was successful. Thus, pupal survivorship differed significantly between the two host species (χ^2, $P = 0.038$).

Discussion

Oviposition decisions of female insects are critical to the success of their offspring. *Manduca sexta* is a narrow specialist, ovipositing on only one to two host species, almost exclusively Solanaceae, in a given habitat; its nectar plants are extremely limited as well (Alarcón *et al.*, 2008). In the desert in the south-western U.S.A., *M. sexta* uses the co-blooming perennial *Datura wrightii* and the annual *D. discolor* as both nectar and oviposition resources, and in the field can choose between these species when they co-occur. In this study, we predicted that adult moths would preferentially nectar at the larger, more rewarding flowers of *D. wrightii*, and that the presence of these flowers would lead moths to oviposit on the same plants. We also predicted that larval performance would match adult preference, but that the presence of flowers on the available plants would modify oviposition preferences.

Adult preferences and larval performance

Consistent with these predictions, we found evidence that adult females have a strong preference to both nectar and oviposit on the larger-flowered perennial *D. wrightii*. This overall preference was consistent with larval performance. While the differences in host quality did not become apparent until later in development, all larvae reared on live *D. wrightii* plants survived to pupation, and most pupated successfully, whereas none pupated successfully on the annual *D. discolor*. This result, though based on a small sample size, strongly suggests that *D. wrightii* is a superior larval host to *D. discolor*. As such, our results are consistent with the preference–performance hypothesis (Gripenberg *et al.*, 2010), as female oviposition preference is biased towards the superior larval host.

Although most moths showed a preference towards *D. wrightii*, many nectared at both species and spent at least some time ovipositing on the annual *D. discolor*. Hence, *D. discolor* was apparently perceived as a suitable larval host, despite the larval mortality we observed on this species. When the only flowers in the array were *D. discolor*, however, the moths' preference for *D. wrightii* disappeared. This is consistent with our prediction that nectaring behaviour would influence *M. sexta*'s oviposition decisions, in this case biasing decisions towards an otherwise disfavoured, lower-quality host. This bias may be driven by the strong link between nectaring and oviposition behaviour, a phenomenon reported in *M. sexta* as early as 1850 (Glover, 1856 *in* Karban, 1997). Given this link, when only *D. discolor* flowers are present, its floral cues may increase either the apparency or perceived host quality of the annual plant relative to the flowerless *D. wrightii*. It is worth noting that while the presence of flowers on *D. discolor* eliminated the preference for *D. wrightii*, it did not reverse that preference: rather, *D. discolor* flowers only improved the apparent quality of *D. discolor* as an oviposition substrate, to the point where it became as attractive as *D. wrightii*.

Nectar resources biasing the oviposition decisions of phytophagous insects have previously been reported for a number of Lepidoptera. For example, the presence of non-host floral resources can influence both the patches adult females visit for several nymphalids (*Euphydryas chalcedona* and *Vanessa cardui*; Murphy *et al.*, 1984; Janz, 2005) and papilionids (*Papilio glaucus* and *Parnassius apollo*; Grossmueller & Lederhouse, 1987; Brommer & Fred, 1999), and the plants they oviposit on within those patches (Karban, 1997; Janz, 2005). Flower presence and nectar volume have also been shown to be strong predictors of oviposition preference among individuals of host species that offer nectar, including *M. sexta* and *V. cardui* (Adler & Bronstein, 2004; Janz, 2005; Janz *et al.*, 2005; Kessler *et al.*, 2010; Kessler, 2012). Only rarely, however, have flowers been shown to bias oviposition decisions among different host species (Forister *et al.*, 2009; although see also Janz, 2005). Forister *et al.* (2009) found that *Lycaeides melissa* (Lycaenidae) larvae performed better on the native *Astragulus canadensis* than on introduced *Medicago sativa* (alfalfa), with native host-reared larvae eclosing as adults three times larger than larvae reared on alfalfa. Consistent with this superior performance, *L. melissa* had a strong preference for the native *A. canadensis* over introduced *M. sativa* when neither bore flowers. Similar to our results, however, this preference disappeared when *M. sativa* bore flowers (Forister *et al.*, 2009).

Beyond these well-studied examples, oviposition biases driven by floral cues could potentially be acting in a wide range of other species as well. Many lepidopterans use the same plant species as both nectar and oviposition resources (Altermatt & Pearse, 2011), and these species appear to preferentially use larval hosts as nectar sources. Indeed, this bias to oviposit on nectar plants may drive the observation that phytophagous lepidopterans with functional adult mouthparts have narrower host ranges than those lacking them (Altermatt & Pearse, 2011).

In addition to food quality and floral bias, several other factors may influence oviposition preferences in this system. First, the larger size of *D. wrightii* plants may have increased the moths' preference for this species. The plant vigour hypothesis (Price, 1991) proposes that, because large and vigorously growing plants are likely to have more available biomass and higher nutritional quality, they should be preferred as oviposition sites. Oviposition preferences consistent with this hypothesis have been found in a variety of insect species (e.g. Heisswolf *et al.*, 2005; reviewed in Cornelissen *et al.*, 2008). Furthermore, as a single *M. sexta* larva can defoliate even a large *D. wrightii* plant before pupation (Mcfadden, 1968), a larger host plant may reduce the probability that a larva needs to seek out a second host, which could lead to costly starvation periods (Boggs & Freeman, 2005), exposure to predators, and/or failure to find another host. *Manduca sexta* larvae are fairly mobile within host plants (Potter *et al.*, 2012), but are rarely observed moving among hosts. Although we attempted to control for average plant size among our experimental plants, *D. wrightii* individuals tended to be taller and had much larger leaves than *D. discolor* individuals. Independent of overall plant size, floral reward size may also contribute to the observed preference: *M. sexta* has previously been shown to lay more eggs on *D. wrightii* individuals with higher nectar volumes (Adler & Bronstein, 2004). Given that *D. wrightii* has higher expected nectar volumes than *D. discolor*, the observed moth oviposition preferences in the present study are consistent with this previous finding. Higher nectar volumes, and therefore presumably slower nectar depletion rates, also probably contributed to the higher number of floral visits made to *D. wrightii*.

Second, spreading eggs among hosts is the typical mode of oviposition for many insects, including sphingid moths such as *M. sexta* (Janzen, 1984; Hopper, 1999; Reisenman *et al.*, 2013; Hufnagel *et al.*, 2017), and is suggested to reduce larval competition and spread risk. This tendency may explain why some eggs were laid on *D. discolor* even when *D. wrightii* flowers were present. The tendency to spread eggs may also explain why we found a link between nectaring and oviposition behaviours, but no relationship between nectaring time and eggs laid. If females are likely to spread eggs within a patch once they find it, nectaring behaviour can release oviposition but not dictate egg number decisions. An experiment that better simulates the spatial scale and variable plant ratios seen in the field would be necessary to test how moths choose to spread eggs. Alternatively, oviposition on *D. discolor* may simply have been mistakes. Although the chemical profiles of *D. wrightii* and *D. discolor* flowers are different (Reisenman *et al.*, 2013), the close relatedness of *D. discolor* and *D. wrightii* may make it difficult for adults to distinguish between the chemical profiles on the leaves, as has been seen in other species (e.g. Davis & Cipollini, 2014). Closer analyses of the leaf chemical profiles are needed to test this possibility for these two species. Because approximately one-third of all eggs laid in this experiment were laid on *D. discolor*, however, we find this possibility unlikely.

It is also striking that larval development on the two species only differed when larvae were grown on intact plants. The observed pattern of low mortality on cut leaves but high mortality on live leaves may be due to high levels of induced defenses, as has been predicted for and found in other short-lived plants (Stamp, 2003; Endara & Coley, 2011). While several *Datura* species, including *D. wrightii*, produce proteinase inhibitors and other defensive compounds in response to methyl jasmonate signalling (Hare & Walling, 2006), induced defences have not been investigated in *D. discolor*. Alternatively, nutritional quality differences between the species may magnify over development or only affect development during the last two instars, when *M. sexta* larvae increase their body mass by 90% (Davidowitz & Nijhout, 2004). Investigating plant responses to herbivory and its effect on larval maturation rate and survival offers interesting avenues for future research.

Plant consequences

Because of the link between oviposition and nectaring for *M. sexta*, *D. wrightii* and *D. discolor* attract their most damaging herbivore (Bronstein *et al.*, 2009) when they attract pollinators. Flexibility in terms of the number of eggs that moths lay after visiting those flowers, however, opens up the possibility that plants could attract pollinator visits while receiving relatively few eggs. With only 1 year in which to reproduce, *D. discolor* may experience higher fitness costs of herbivory damage than its perennial congener, and may display traits to reduce the number of visits it receives. Assuming that *M. sexta* is the primary pollinator of *D. discolor* as well as *D. wrightii*, having smaller and less rewarding flowers than *D. wrightii* may allow *D. discolor* to occasionally export and receive pollen while reducing its apparency, attractiveness, and perceived host quality to the moths that do visit. Indeed, while both species are self-compatible, *D. discolor* has a much higher natural selfing rate (J. L. Bronstein, unpublished), and has the ability to self-pollinate in the bud (i.e. it exhibits cleistogamy; G. Smith, pers. obs.). This ability could further reduce visitation by potential herbivores.

The fact that herbivorous *M. sexta* larvae mature into pollinators of the two *Datura* species means that the congeners could also influence each other's fitness through their shared partner when they co-occur. In this case, as *D. discolor* flowers are attractive to the moths, but *D. discolor* leaves are apparently perceived as poorer larval food, *D. discolor* may gain some of the reproductive benefits from associating with *M. sexta* while paying relatively few of the costs. *Datura wrightii*, on the other hand, would be paying the majority of the herbivory costs while potentially receiving heterospecific pollen and losing pollination services to competing *D. discolor* flowers. It is worth noting, however, that the flowering season of *D. discolor* is nested within the longer flowering period of *D. wrightii* (J. L. Bronstein, unpublished), and therefore *D. wrightii* would not always pay these costs. Field observations of *M. sexta* foraging where these species co-occur would help to determine the degree to which these species compete for pollinator visits in nature.

Conclusions

The preference–performance hypothesis predicts that females will prefer to lay eggs on hosts that favour larval survivorship (Gripenberg *et al.*, 2010), but many factors beyond host quality have the potential to affect adult preference. Consistent with this hypothesis, we found that *M. sexta* adults preferentially nectar and oviposit on *D. wrightii*, on whose leaves *M. sexta* larvae have higher survival during the late stages of their development. Moth preferences, however, disappeared when *D. discolor* was the only species flowering, despite the fact that no larvae in our small experiment successfully completed development on this host.

This result may be due to the strong link between nectaring and oviposition behaviour biasing female host choices. A growing number of Lepidoptera species have been shown to both nectar and oviposit on the same plant species (Altermatt & Pearse, 2011). Thus, future work investigating the mechanisms by which this bias occurs is critical to understanding the conditions under which adult preference and larval performance do not align.

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