Choice of oviposition sites by *Manduca sexta* and its consequences for egg and larval performance

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Abstract

The preference-performance hypothesis predicts that female insects should prefer to lay eggs in locations that enhance offspring performance. This study examines the choices of females regarding where to oviposit within plants, focusing on the hawkmoth *Manduca sexta* L. (Lepidoptera: Sphingidae) and its host *Datura wrightii* Regel (Solanaceae) in the southwestern USA. Smaller *Datura* leaves provide cooler microclimates for eggs (which may lead to faster embryonic development, shortening their exposure to egg predators) and more nitrogen for larvae. In contrast, large leaves reach temperatures that are stressfully high (which slows embryonic development) and provide less nitrogen for larvae. Thus, we would expect females to oviposit on small leaves. To examine whether leaf size influences female preference and offspring performance, we used laboratory and field studies to address the following questions. (1) On what size leaves do females typically oviposit? (2) Does the distribution of eggs in nature differ from that expected by chance? And (3) how does leaf size affect survival or growth of eggs and larvae? We find that oviposition choices of females do not lead to the highest probability of offspring survival. Females lay eggs on larger leaves, likely due to the greater accessibility of those leaves; however, eggs are more likely to hatch on small leaves. Larvae grow faster on large leaves, but larvae are also surprisingly mobile, suggesting that the consequences of oviposition site are minor once eggs have hatched. Larval mobility was seen only in the field, not in the laboratory, emphasizing the importance of field studies for predicting real-world performance. Although females' leaf choices are potentially risky for eggs, the threats of high temperature and predation may vary sufficiently in space and time that there is no consistent selection for strong preferences. Furthermore, the fitness consequences for eggs and larvae largely offset each other and offspring are sufficiently mobile to cope with the conditions where they are laid.

Introduction

A longstanding challenge regarding plant-herbivore interactions has been to determine how females distribute their offspring within and among plants. The preference-performance hypothesis predicts that females should prefer to lay eggs in locations that enhance the performance of their offspring (Jaenike, 1978; Thompson, 1988). Poorly chosen sites may lead to high mortality or slow growth of embryos or hatchlings (Groenteman et al., 2006).

'Optimal' oviposition choice, however, is subject to a number of constraints (Gripenberg et al., 2007a). First, even when site quality has a large effect on individual fitness, it may be difficult for a female to assess that quality (Hopper, 1999; Gripenberg et al., 2007b). Second, site quality may vary in space and time, so that selection does not consistently favor any one behavior (Rausher, 1979; Roslin & Salminen, 2009; Ruhnke et al., 2009). Third, females may have to choose between multiple, conflicting aspects of quality, including the direct effects on their own fitness (Scheirs & De Bruyn, 2002). Consequently, there is no simple coupling between female preference and offspring performance. Indeed, recent reviews conclude that the choices of many insects do (Gripenberg et al., 2010) or do not (Refsnider & Janzen, 2010) maximize offspring

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survival. In addition, results found in one setting may not hold in others. For example, different patterns may emerge in field environments compared with laboratory tests (Teodoro et al., 2009). Thus, it is difficult to make a priori assumptions about a species' preference-performance relationship.

Tests of the preference-performance hypothesis have centered on choices among different species or individuals of host plants, but sites may differ as much within a plant as they do among plants (Duffield & Chapple, 2001; Kessler & Baldwin, 2002; Ruusila et al., 2005; Groenteman et al., 2006; Clark et al., 2011). The suitability of leaves differs based on their location, age, and size. The younger, smaller leaves typically are more tender and contain higher levels of nitrogen, frequently a limiting resource for insects. Indeed, many insect hatchlings prefer young leaves (Raupp & Denno, 1983; Gall, 1987; Coley et al., 2006). Furthermore, as a leaf grows, so does the thickness of its boundary layer: the thin layer of still air adhering to the leaf (Woods, 2010). Thicker boundary layers more strongly resist heat and moisture transfer between the leaf and its surroundings (Nobel, 2005). As a result, microclimates for leaf-dwelling insects can diverge substantially both from ambient macroclimates and from leaf to leaf (Schuepp, 1993; Potter et al., 2009). Small insect stages – e.g., eggs and young larvae – should be particularly affected by leaf size as they live entirely within leaf boundary layers (Woods, 2010).

Here, we examine whether leaf size influences where females choose to lay their eggs, and in turn whether leaf size affects the performance of eggs and hatchling larvae, in the hawkmoth, *Manduca sexta* L. (Lepidoptera: Sphingidae). We focus on *Datura wrightii* Regel (Solanaceae), a broad-leaved desert perennial found commonly in disturbed soil, and the primary host plant of *M. sexta* in the southwestern USA. *Manduca sexta* is a model species for studying oviposition: a rich history of studies describe females' between-plant preferences, host learning, cues required for oviposition (olfactory, tactile, and visual), and the underlying neural mechanisms that control oviposition behavior (e.g., Garman & Jewett, 1920; Yamamoto et al., 1969; Tichenor et al., 1981; Abrell et al., 2005; Reisenman et al., 2009, 2010). However, the causes and consequences of their oviposition choice within a plant remain largely unknown.

We addressed three questions, using a combination of laboratory and field studies. (1) On what size leaves do females typically oviposit? (2) Does the distribution of eggs in nature differ from that expected by chance? Herein, we test three null hypotheses of leaf choice. Leaves of a particular size class may receive more eggs, strictly by chance, if they are more abundant (hypothesis 1), have larger total surface area (hypothesis 2), or are located on the periphery of a plant (hypothesis 3). Finally, (3) How does leaf size affect survival and growth of eggs and larvae? We expected larvae to feed within 1–2 cm of their oviposition site, as is commonly reported for this species (McFadden, 1968; Nelson, 1996; Woods & Singer, 2001), but we also examined whether larvae can move to a different leaf after hatching. We predicted that females would prefer to lay eggs on smaller leaves, and that eggs and larvae would perform better on those leaves. Smaller *D. wrightii* leaves contain higher levels of nitrogen (G Davidowitz, unpubl.) and provide cooler microclimates (Potter et al., 2009). Larger *D. wrightii* leaves, in contrast, are 3 °C warmer on average during the daytime, and can reach temperatures that are stressfully high for eggs (Potter et al., 2009). High leaf temperatures result in smaller hatchlings, lower hatching success, and prolonged egg development (hatchlings are ca. 10% smaller and egg development is ca. 10–20 h longer; Potter et al., 2009, 2011). Prolonged development constitutes substantial risk; in *M. sexta*, typically 20–45%, but up to 70% in some areas, of mortality stems from egg predators and parasitoids (Mira & Bernays, 2002).

Materials and methods

Study system

Manduca sexta L. occurs throughout much of the western hemisphere, but larvae use a narrow range of host plants: females lay eggs exclusively on plants in the family Solanaceae (Garman & Jewett, 1920; Yamamoto & Fraenkel, 1960), and on one known genus of Martyniaceae (Mechaber & Hildebrand, 2000).

Adults are active primarily from July to September. Females attach eggs (ca. 1.5 mm diameter) singly to the lower surfaces of host leaves (Garman & Jewett, 1920). Eggs hatch after ca. 4–5 days, and larvae typically develop through five instars (Kingsolver, 2007). Young larvae tether themselves to a leaf using silk, and first instars are commonly reported to feed within 1–2 cm of their oviposition site (McFadden, 1968; Nelson, 1996; Woods & Singer, 2001). Typically, there are two generations per year, and individuals stay below ground as pupae during the winter.

In southeastern Arizona, temperatures may exceed the survival threshold for *M. sexta* eggs, particularly under large leaves (Potter et al., 2009). Eggs are primarily attacked by ants, parasitoid wasps, and a variety of sucking predators (especially hemipterans). Larvae are commonly killed by spiders, mantids, wasps, hemipterans, and, in later instars, birds and parasitoids (Mira & Bernays, 2002).

Female oviposition

To determine whether females choose leaves of particular sizes, we conducted semi-weekly field surveys of *M. sexta* eggs on naturally occurring *D. wrightii* plants around Tucson, AZ. In total, 26 plants were surveyed between 8 July and 20 August, 2006. On each plant, we measured the length of all leaves (for plants larger than 1 m in diameter, we measured all leaves on one representative branch, starting from the base of the plant) and the length of any leaves with eggs. In *D. wrightii*, leaf length and area are strongly correlated (area = $0.43 \times$ length$^{2.05}$; n = 159 leaves; R^2 = 0.97; G Davidowitz, unpubl.). Leaf lengths were measured to the nearest mm and binned to the nearest 1 cm to fulfill χ^2 test requirements.

The first null hypothesis (females oviposit randomly with respect to leaf size) predicts that the size classes of leaves with and without eggs are the same. We analyzed this using a χ^2 test. However, there are two other reasonable null hypotheses for the distribution of eggs. Certain size classes of leaves may receive more eggs even if they are not actively chosen by females, if they have larger total surface area across the plant (= hypothesis 2), or are located on the periphery of a plant (= hypothesis 3). To test hypothesis 2, we converted leaf length into leaf area (see formula above), and regressed the number of eggs on leaves of length x vs. the total surface area represented by leaves of length x. Data were $\log(x + 1)$ transformed to include incidence of zero eggs. If egg deposition is based solely on the total area of leaf size classes (i.e., if more eggs are laid on the leaf size classes with more cumulative area), then the slope of the regression of [$\log(x + 1)$] leaves with eggs on [$\log(x + 1)$] leaf area should equal 1. If the slope of the regression does not equal 1, then eggs are not deposited solely based on the total area of leaf size classes. To test hypothesis 3, we quantified the accessibility of leaves. A blindfolded assistant probed 100 times at each of five plants, and we recorded the length of every leaf he first touched (hereafter termed 'randomly selected leaves'). Probes followed previous observations of moth behavior as closely as possible; for example, more eggs are laid on the top half of the plant than the lower half (Garman & Jewett, 1920; Potter, 2010). Females also oviposit on exterior leaves of a plant; they contact a leaf with their front tarsi while hovering, and curl their abdomen to oviposit underneath (K Potter, pers. obs.; Sparks & Cheatham, 1970). Frequency distributions of leaf sizes for leaves with eggs and randomly selected leaves were compared using a χ^2 test. If the distribution of randomly selected leaves is not different from the distribution of leaves with eggs, then eggs are laid on leaf sizes in proportion to the probability of encountering those leaves as the moth approaches the plant.

Field experiment: survival of eggs and larvae

During August 2008, we conducted field experiments to determine whether leaf size influences the performance of eggs and larvae. Using UV lights, we collected adult females of *M. sexta* from the Santa Rita Experimental Range (SRER; 31°48′N, 110°51′W), ca. 45 km south of Tucson, AZ. Moths were placed in an outdoor $2 \times 2 \times 2$ m flight cage with flowering *D. wrightii*. Eggs laid by these females were collected each morning. We cut each egg out of the leaf on which it was laid so that each egg was still attached to a small patch of leaf. These leaf patches (n = 240) were promptly glued onto leaves of 16 *D. wrightii* plants growing in a 55×80 m plot at the University of Arizona's Campus Agricultural Center (Figure 1), using Elmer's white glue^{TM} (Elmer's Products, Westerville, OH, USA), which we have found to have no effect on egg survival. We inspected focal plants daily and removed any wild eggs and larvae.

We glued the leaf patches with eggs onto leaves representing three leaf size classes (small, 3–5 cm long; medium, 7–9 cm; or large, 11–14 cm). These classes correspond to the lower, middle, and upper ranges of leaf sizes onto which eggs are deposited in nature (Figure 2, dark bars). Eggs were checked every 24 h through hatching, and then until hatchlings completed their first molt. We recorded egg fate as hatched, sucked by hemipteran predators, gone (most likely taken by ants or other predators), or not hatched. All non-hatched eggs were subsequently inspected under a microscope to determine whether they were parasitized, infertile, or contained a dead embryo. Our classification of egg fates is similar to that used by Mira & Bernays (2002). We followed the fates
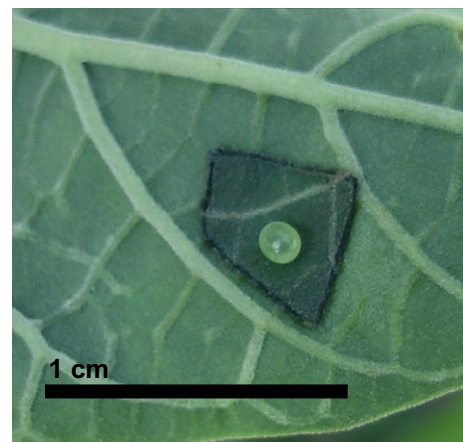

Figure 1 Technique for gluing *Manduca sexta* eggs: each egg was cut out of the leaf on which it was laid so that it was still attached to a small square of leaf. These squares were attached to leaves of *Datura wrightii* plants in the field using Elmer's glue.
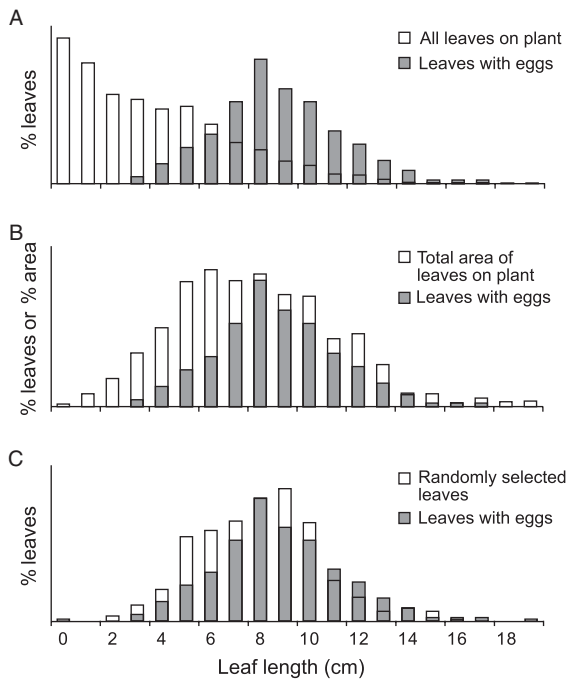
Figure 2 Distribution of *Manduca sexta* eggs on *Datura wrightii* leaves in southeastern Arizona, USA (n = 26 plants, 3354 leaves, 193 eggs). (A) Distribution of the lengths of all leaves on plants compared with those leaves that have eggs. (B) Same data, but presented such that each bin is the percentage of total leaf area that is represented by leaves of that size. For example, although there are many small leaves, they represent only a tiny fraction of available leaf area. Females oviposit on larger leaves than predicted by available leaf area alone. (C) Distribution of the lengths of randomly selected leaves compared with those leaves that have eggs. These distributions do not differ significantly.

of hatched larvae through their first molt, recording both survival and movement between leaves.

Laboratory experiment: larval growth on leaves of different size

In August 2007, we reared field-collected *M. sexta* on potted *D. wrightii* plants to determine how leaf size affects larval growth. We collected 80 eggs from wild *D. wrightii* and glued them onto individual leaves (3–18 cm long) of potted *D. wrightii* (2–3 leaves of different sizes on each of 35 plants). Eggs hatched on the potted plants, and larvae were checked every 2–3 h from hatching until the first molt. We recorded the time of each larva's hatching, size of its initial leaf, number of times it switched to a different leaf, time until the first molt, and mass at the first molt. Experiments were conducted under a L16:D8 photoperiod, at 32 °C. We also tested whether patterns of larval movement were similar to those observed in the field experiment.

Results

Female oviposition

We measured the lengths of 3354 leaves on 26 *D. wrightii* plants, of which 193 had eggs. The distribution of leaf lengths was strongly left-skewed (Figure 2A, light bars; range 0.1–19.4 cm). Although small leaves were the most abundant, eggs were primarily found on medium and large leaves (Figure 2A, dark bars; mean length = 9.1 cm; range 3.0–17.8 cm) (χ^2 = 540.4, d.f. = 19, P<0.0001). Thus, we rejected hypothesis 1. The distribution of eggs was still significantly shifted toward larger leaves after we corrected for available surface area represented by leaves of each size class (Figure 2B). The 95% confidence interval (CI) of the slope of the regression of number of leaves with eggs on total leaf area (of each size class) did not include 1.0 [log(eggs + 1) = −0.06 + 1.33 × log(area + 1); 95% CI = 1.10–1.56, R^2 = 0.78, P<0.0001; n = 41]. Therefore, we also rejected hypothesis 2, the null hypothesis that oviposition sites are distributed randomly with respect to available area of leaves of a given size. Rather, females lay eggs on larger leaves more often than predicted by either their abundance or their surface area. To account for plant architecture (hypothesis 3), we compared the distribution of randomly selected leaves with the distribution of eggs (Figure 2C); these distributions were not significantly different (χ^2 = 24.5, d.f. = 17, P = 0.11). The greater accessibility of large leaves may, therefore, lead to their more frequent use by ovipositing females.

Field experiment: survival of eggs and larvae

On several occasions, after heavy rain, eggs and their original square of leaf were missing. We removed these from analysis, reducing the total sample size to 195 eggs. Forty-seven percent of eggs hatched, consistent with observations of *M. sexta* eggs in other locations (Mira & Bernays, 2002). The most frequent category of mortality was 'missing egg', which presumably corresponded to eggs taken away by predators (Figure 3); we observed many egg predation attempts in the field. No eggs were parasitized at this site. The specific cause of mortality did not depend on leaf size (χ^2 = 9.2, d.f. = 6, P = 0.17), but overall hatching success was higher on small leaves (χ^2 = 7.9, d.f. = 2, P = 0.02). Approximately 15% of individuals (28 of 195) survived to their first larval molt.

Laboratory experiment: larval growth on leaves of different size

Approximately 20% of larvae (15 of 80) switched leaves during the first instar (see below). To determine how leaf size affects growth rate, we analyzed only larvae that stayed on their natal leaf. For those larvae, consumption of larger leaves was associated with faster growth. Larvae on larger
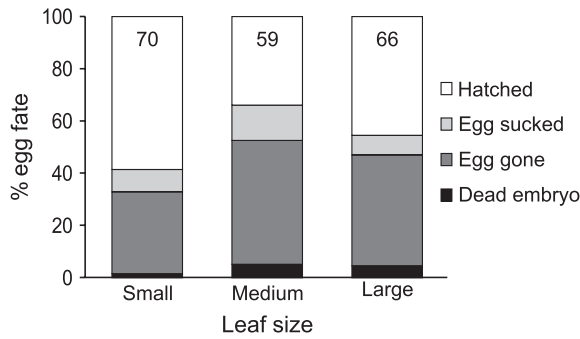
Figure 3 Fates of *Manduca sexta* eggs on *Datura wrightii* leaves in Tucson, AZ, USA (n = 195 eggs; sample size for each category is written on the bar). Eggs were glued onto small (3–5 cm long), medium (7–9 cm), or large (11–14 cm) leaves.

leaves were heavier at their first molt ($R^2 = 0.20$, P<0.001, n = 65; Figure 4A) and molted sooner ($R^2 = 0.11$, P<0.01, n = 65; Figure 4B). The two relationships would likely have been tighter, except that (1) we checked larvae only intermittently, and (2) egg temperature varied before collection, which also affects first instar growth rate (Potter et al., 2011).

Field vs. laboratory comparison: larval movement

Patterns of larval movement differed strongly depending on whether larvae (in both cases, derived from wild adults) were kept in the laboratory or studied in the field: 100% of larvae feeding in the field switched leaves during their first instar vs. only 20% in the laboratory. To compare larval movement in the two experiments, we restricted the laboratory data to the leaf size classes that we used in the field (field: n = 28, laboratory: n = 45; Figure 5A). This analysis showed that larvae not only were more likely to move from their initial leaf in the field, but they also switched leaves more frequently (field: mean no. moves ± SE = 2.3 ± 0.1, n = 28; laboratory: 1.2 ± 0.1, n = 12; t = 3.69, d.f. = 22, P<0.001; Figure 5B). In both laboratory and

field, larvae switched before their first leaf was fully consumed.

Discussion

The preference-performance hypothesis predicts that insects will prefer to lay eggs in sites that enhance the performance of their offspring (Jaenike, 1978; Thompson, 1988). Here, we report a mismatch between female choice and offspring performance: females of *M. sexta* tend to lay eggs on larger, more accessible *D. wrightii* leaves, but eggs are more likely to hatch on smaller leaves. In contrast to egg performance, larvae grow faster on large leaves. However, larvae are also surprisingly mobile, suggesting that the consequences of oviposition site are minor, once eggs have hatched. Behavior of larvae in the field differed from their behavior in the lab, and their mobility contrasts strongly with what has been previously described for *M. sexta*.

Why did *M. sexta* eggs survive better on smaller leaves? Two primary sources of *M. sexta* egg mortality are temperature and predation, both of which are affected by leaf size. In particular, smaller leaves may provide more suitable microclimates for eggs. In a previous study, large leaves at this site were ca. 3 °C warmer than small leaves during the day, reaching temperatures that were stressfully high for eggs (Potter et al., 2009, 2011). At those large-leaf temperatures, eggs develop slower, and are therefore exposed to predators for longer.

After hatching, larvae grew faster when they consumed larger leaves. This is surprising, because larger *D. wrightii* leaves contain less nitrogen (G Davidowitz, unpubl.), and larvae of *M. sexta* grow faster on high-nitrogen artificial diets (Woods, 1999). However, herein larvae feeding on large leaves reached the second instar ca. 18 h earlier, and were ca. 30% heavier, than larvae feeding on small leaves. It is unlikely that temperature caused this pattern; in the laboratory, microclimates on large and small leaves were
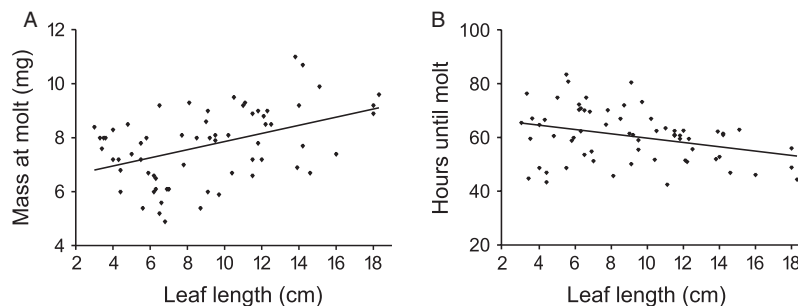

Figure 4 Effect of leaf size on growth of first-instar *Manduca sexta*. Larvae that consumed a larger *Datura wrightii* leaf (A) were heavier by their first molt (y = 0.15x + 6.35, P<0.001), and (B) molted sooner (y = −0.80x + 67.7, P<0.01).
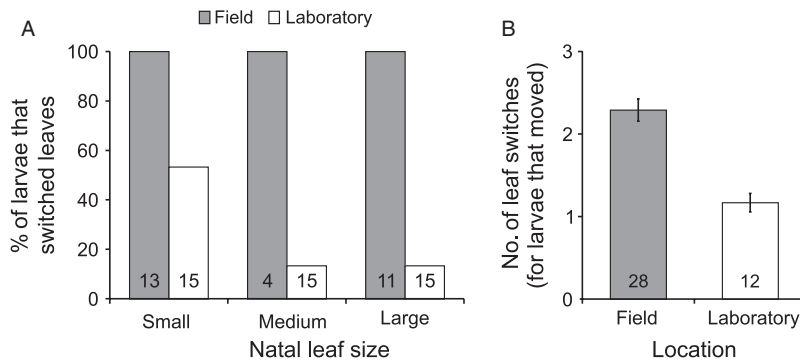
Figure 5 Behaviour of *Manduca sexta* larvae when reared in the field vs. in the laboratory. Sample sizes are on each bar. (A) Field-reared larvae were more likely to switch leaves during their first instar. In the laboratory, larvae were more likely to switch leaves if they hatched on a small leaf. (B) Field-reared larvae switched between leaves more frequently (P<0.001). In every case, larvae switched before their first leaf was fully consumed.

similar. Furthermore, if large leaves were warmer, as they are in the field, larvae should be smaller, not larger (Atkinson, 1994; Davidowitz et al., 2004). Presumably, other age-related leaf changes caused this growth pattern: e.g., smaller leaves may contain higher levels of defensive compounds, as occurs in other solanaceous plants (Eby et al., 1938; Brewer & Hiner, 1950; but see Chaudhuri, 1954). Leaves of *D. wrightii* contain high concentrations of constitutive tropane alkaloids, as well as a suite of other defenses that are induced rapidly in response to herbivory (e.g., polyphenol oxidase and proteinase inhibitors; Hare & Walling, 2006). Whether these chemical defenses are more concentrated, or are induced more rapidly, in smaller *D. wrightii* leaves remains unknown.

If eggs are more likely to hatch on small leaves, why do females not oviposit there? One possibility is that sources of egg mortality vary sufficiently in space and time (e.g., Mira & Bernays, 2002) that there is no consistent selection favoring this behavior. Even when host quality has a large effect on individual fitness, if it is difficult for a female to predict the quality of a particular site, spreading eggs widely may be the best strategy to maximize fitness (Hopper, 1999; Gripenberg et al., 2007b). Furthermore, females generally are limited by one of two things: the number of eggs they can produce, or the time available for finding suitable sites for them. Females primarily subject to egg limitation should, in theory, discriminate more readily among oviposition sites than those primarily subject to time limitation (Doak et al., 2006). Females of *M. sexta* probably are more constrained by time than by total production of eggs, consistent with our lack of evidence for 'active' leaf choice. Females lay hundreds of eggs over ca. 6 nights (Garman & Jewett, 1920; Sasaki & Riddiford, 1984), and must fly long distances between host plants; furthermore, only ca. 0.5% of eggs survive to adulthood

(Mira & Bernays, 2002). Spending extra time evaluating oviposition sites within a plant may therefore be of little benefit.

Although we found in the laboratory that leaf size affects larval growth, in the field, this effect may be weaker, because hatchlings often move among leaves within a plant. Indeed, larvae were surprisingly mobile. This finding contrasts strongly with what has been previously described for *M. sexta* (Garman & Jewett, 1920; McFadden, 1968; Nelson, 1996). In the field, every larva switched leaves, and most moved multiple times. In contrast, in the laboratory, only ca. 20% of larvae switched leaves. Why larvae should exhibit different patterns of movement in the laboratory and the field remains unknown. We suggest four possibilities. (1) Leaf physiology may differ between field and potted plants; e.g., potted plants may induce weaker defenses in response to herbivory. (2) Plants in the wild may, because of prior herbivory, have had higher levels of induced defenses (Hare & Walling, 2006), prompting field larvae to switch leaves more frequently; our potted plants had no prior exposure to insects. (3) Larvae may move to thermoregulate. Temperature in the laboratory was constant, whereas microclimates around field plants varied as functions of the full suite of environmental variables. Late-instar *M. sexta* larvae thermoregulate by moving between the exterior and interior of a plant (Casey, 1976). (4) Larvae may move in response to predators. Interestingly, many larvae moved to a new leaf just before molting. As molting larvae are defenseless, leaf-switching might help them avoid predators that use visual or volatile cues from their feeding site.

In conclusion, although *M. sexta* females primarily oviposit on large leaves, our results suggest that large leaves are not chosen via an 'active' search process, nor do they confer the highest probability of offspring sur-

vival. Particularly in warm locations, the oviposition choices made by females can be detrimental to their eggs. Our study is not alone in finding a mismatch between female preference and offspring performance. No consensus has yet arisen with regard to the extent to which female oviposition choices reflect adaptation (e.g., Thompson & Pellmyr, 1991; Ladner & Altizer, 2005; Schoonhoven et al., 2005; Craig & Itami, 2008). The ultimate (and mechanistic) reasons for a female's choice seem likely to vary both among and within taxa (Refsnider & Janzen, 2010), as well as at different levels of plant choice. In addition, although female preference typically is predicted to evolve to match offspring performance, natural selection instead may drive adaptation of offspring traits – such as mobility – that allow them to cope with the conditions where they are laid. Here, movement patterns found in the laboratory did not hold for the field, emphasizing the importance of field studies for predicting real-world performance.

Acknowledgements

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