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Beyond preference and performance: host plant selection by monarch butterflies, *Danaus plexippus*

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The connection between adult preferences and offspring performance is a long-standing issue in understanding the evolutionary and ecological forces that dictate host associations and specialization in herbivorous insects. Indeed, decisions made by females about where to lay their eggs have direct consequences for fitness and are influenced by interacting factors including offspring performance, defence and competition. Nonetheless, in addition to these attributes of the offspring, a female's choices may be affected by her own prior experience. Here we examined oviposition preference, larval performance and the role of learning in the monarch butterfly, *Danaus plexippus*, which encounters diverse milkweed host species across its broad range and over the course of migration. Monarch females consistently preferred to oviposit on *Asclepias incarnata* subspecies *pulchra*. This plant, however, was associated with poor caterpillar growth, low sequestration of toxins and the highest plant defences (latex and trichomes). We examined flexibility in this apparently maladaptive preference by testing the impact of previous experience and competition on preference. Experience laying on an alternative plant species enhanced preference for that species in contrast to *A. i. pulchra*. In addition, presence of a (competing) conspecific caterpillar on *A. i. pulchra* had a strongly deterrent effect and reversed host plant preferences. Thus, monarch butterflies exhibit preferences contrary to what would be expected based on offspring development and sequestered defences, but their preferences are altered by learning and competition, which may allow butterflies to shift preferences as they encounter diverse milkweeds across the landscape. Learning and perception of threats (i.e. competition or predation) may be critical for most herbivorous insects, which universally experience heterogeneity among their potential host plants.

Keywords: *Asclepias*, competition, learning, milkweed, oviposition, sequestration

Introduction

Across vertebrate and invertebrate taxa, a female's choice of where to lay eggs is amongst the decisions with the most important fitness consequences (Martin and Roper 1988, Penn and Brockmann 1994, Shine and Harlow 1996, Geist and Dauble 1998, Rudolf and Rödel 2005, Munga et al. 2006, Navarte et al. 2013). In herbivorous

insects with limited mobility, the laying site is also the offspring's food source, making the decision especially crucial. Egg-laying choices in herbivorous insects have consequences for offspring growth (Gripenberg et al. 2010), defence (Denno et al. 1990) and competition (Anderson et al. 1996). Behavioral ecologists have hypothesized that herbivorous insects should make adaptive choices, laying eggs on host plants where offspring grow the fastest (Jaenike 1978), or on host plants where offspring are the best defended from predators (Kessler and Baldwin 2001). Nonetheless, herbivore oviposition choices can be flexible depending on female experience (Papaj 1986, Snell-Rood and Papaj 2015) and social context (Corbet 1973, Stamp 1980). We use a variety of approaches to understand how offspring performance, defence, prior experience and competition shape oviposition decisions in monarch butterflies, *Danaus plexippus*, which are long-distance migrants that encounter a wide range of host plants.

The classic hypothesis to explain oviposition decisions in herbivorous insects is the preference–performance hypothesis (PPH), which posits that females should prefer to oviposit on the plant species on which their offspring have the highest performance (Levins and MacArthur 1969, Jaenike 1978, Damman and Feeney 1988, Thompson 1988, Thompson and Pellmyr 1991, Mayhew 1997, Gripenberg et al. 2010). A recent meta-analysis found broad support for the PPH (Gripenberg et al. 2010), but there are many exceptions (Berdegué et al. 1998, Scheirs et al. 2000), which can provide insight into the relative importance of different factors influencing complex egg-laying decisions.

One of the factors that may impact egg-laying choices beyond offspring performance is risk of predation. It has been hypothesized that females may prefer to lay their eggs on host plants, or even parts of host plants (Kessler and Baldwin 2001), that provide their offspring with protection from predators, despite such hosts not providing the greatest growth potential (Thompson 1988, Denno et al. 1990, Ballabeni et al. 2001, Murphy 2004). For some herbivorous insects, natural enemies pose a greater mortality risk than plant factors, especially for later developmental stages (Cornell and Hawkins 2015). Herbivores may reduce predation by consuming toxic plants and sequestering toxins for their own defence (Awmack and Leather 2002, Singer et al. 2004). For example, *Phratora vitellinae* beetle larvae that feed on willow leaves sequester salicylates. Female beetles avoid ovipositing on low salicylate willow species, although larvae have higher growth rates on these species, as the larvae are then poorly defended (Denno et al. 1990). Sequestering insects may therefore be an exception from predicted associations between adult preference and offspring growth in cases where oviposition preference results in highly defended offspring.

Many herbivorous insects encounter resource variation in space and time over the course of their lifetime (Agrawal et al. 2002). Learning is one way to cope with such environmental variation (Shettleworth 1998, Eliassen et al. 2009, Jones and

Agrawal 2017). For butterflies, previous experience ovipositing on particular host plants can increase preference for those plants (Traynier 1984, Papaj 1986, Landolt and Molina 1996, Cunningham et al. 1998). For example, in cabbage whites, *Pieris rapae*, females learn host plant leaf colours through oviposition experience (Snell-Rood and Papaj 2015). Learning of host plants traits is hypothesized to increase search efficiency (Snell-Rood and Papaj 2015) and may be particularly common in species that encounter variation in host plant abundance or quality.

Competition can also change female preferences for oviposition sites. For example, the risk of larval competition for plant resources can flexibly alter female preferences. Many butterflies avoid ovipositing on plants with conspecific eggs (Brantjes 1976, Rausher 1979, Auerbach and Simberloff 1989) or where larvae are present (Corbet 1973, Renwick and Radke 1980, Kessler and Baldwin 2001), presumably due to the risks of competition for leaf tissue or egg cannibalism (Stamp 1980). Indeed, it should be to a female's advantage to flexibly shift host plant preferences in the presence of competition. Accordingly, for egg-laying females, multiple factors including offspring food quality, defence, prior experience and competition likely impact oviposition choices, but these have rarely been studied together.

Monarch butterflies are specialist herbivores of plants in the genus *Asclepias* (Apocynaceae) from which they sequester toxic cardenolides for their own defence (Brower et al. 1967, Reichstein et al. 1968). Milkweed species vary widely, quantitatively and qualitatively, in the cardenolide compounds that they contain (Seiber et al. 1983). Although there are active mechanisms in sequestration (Nelson 1993), monarch butterflies reared on different host plants usually vary in cardenolide concentrations, and thereby in the emetic effects that they have when eaten by predators (Malcolm 1994). Due to the extraordinary multi-generational annual migratory cycle of monarch butterflies, they encounter a broad variety of milkweeds that vary in multiple traits including cardenolide levels, impacting larval sequestration (Malcolm and Brower 1989). The migration of monarchs dictates that different populations and generations of butterflies encounter different milkweed species, and their caterpillars can grow on most of those species (Agrawal 2017). Research that has examined the preference–performance hypothesis for monarchs, however, has reported conflicting results (Cohen and Brower 1982, Oyeyele and Zalucki 1990, Zalucki et al. 1990, Ladner and Altizer 2005), and larval sequestration has not been considered in these studies.

Monarch butterflies are tractable herbivorous insects, but with migratory behavior and demonstrated learning abilities (Blackiston et al. 2011, Cepero et al. 2015), that make them an ideal system in which to examine multiple factors influencing egg-laying behavior. We use closely related milkweeds to examine five factors likely to influence oviposition preference in monarch butterflies: 1) host plant quality for larval growth, testing the preference–performance hypothesis, 2) host plant quality for larval defence via sequestration, 3) host

plant defences against herbivores including two barriers to feeding, latex and trichomes, 4) prior experience ovipositing on particular host plants, and 5) larval competition, as it is known that monarch caterpillars are negatively impacted by intraspecific density (Brower 1961, Flockhart et al. 2012, Nail et al. 2015).

Material and methods

We used four closely related milkweeds (*A. curassavica*, *A. perennis*, *A. incarnata* ssp. *incarnata* and *A. incarnata* ssp. *pulchra*) that belong to the same clade but vary widely in cardenolide levels (Fishbein et al. 2011). *Asclepias i. pulchra* and *A. i. incarnata* have long been separated as closely related subspecies (Woodson 1954), are phylogenetically distinct (Fishbein et al. 2011), and differ in traits such as leaf shape (Agrawal et al. 2009a, b). *Asclepias curassavica* is a tropical milkweed that has been introduced into the southern US, *A. perennis* is native to Texas and the southeast, *A. i. incarnata* is widespread across central and eastern North America, and *A. i. pulchra* occurs in Texas and the eastern seaboard. The four plant taxa (henceforth species) therefore overlap in Texas and Florida, two regions traversed by migrating monarchs.

Seeds were washed with 5% bleach, scarified and cold stratified in damp petri dishes at 4°C for 10–14 days after which they were placed in an incubator at 28°C for 3–5 days. We planted seedlings in 10 cm diameter pots in potting soil and reared them in a growth chamber at 14 h day:light cycle, 28°C during the day and 26°C at night. We watered plants every other day and fertilized with dilute fertilizer (NPK 21-5-20, 150 ppm) approximately 5, 10 and 33 days after planting. Around 35 days after planting, we potted plants into 15 cm diameter pots and moved them into mesh cages in the field where we continued to fertilize them once a week. Experiments were conducted when plants were about 50 days old (Supplementary material Appendix 1 for more details).

To determine oviposition preference we used monarch females (n = 12) from a combination of three sources (a lab colony = NY, and commercial breeders in FL and MA, USA, Supplementary material Appendix 1 for details). Females were checked for spores of the protozoan parasite *Ophryocystis elektroscirrha* (Oe) by applying a 1-cm^2 piece of clear tape to each side of the abdomen and examining the piece of tape and attached scales for spores under a dissecting scope (Altizer et al. 2000, Mueller and Baum 2014). Oe can affect between 8 and 70% of individuals in wild populations (Altizer et al. 2000), is transmitted via larval consumption of spores that have been scattered by adults on host plant leaves (Bartel et al. 2011), and can affect adult flight behavior (Bradley and Altizer 2005), fitness (Altizer and Oberhauser 1999), and host plant selection (Lefèvre et al. 2010). Monarchs with detectable spores of Oe were immediately frozen and not included in experiments.

To test for preference, monarchs were given a choice in 1-m^3 field cages with one plant from each of the four species

for one hour, and then given a fresh set of plants and allowed to lay for an additional hour. We removed all eggs from plants and analysed the total number of eggs laid over the combined two hours using a Poisson-distributed generalized linear mixed effects model (GLMM) in the lme4 package (Bates et al. 2015) in R. The number of eggs laid on each plant species was the response variable, and plant species, source of monarchs, and interaction between source of monarchs and plant species were included as fixed effects, and individual female was included as a random effect. We determined the significance of a given fixed effect using type II Wald χ^2 tests using the Anova function in the car package (Fox and Weisberg 2011). We conducted planned comparisons of how many eggs were laid on each plant species using simultaneous tests for general linear hypotheses using the glht function in the multcomp package (Hothorn et al. 2008).

We examined caterpillar performance by randomly distributing freshly hatched caterpillars (from eggs laid by females in the previous experiment) across plants of all four species in field cages, with one caterpillar per plant (initially n ≥ 29 caterpillars per host plant species). At the seventh day after hatching, caterpillars were collected and subsequently reared individually in the lab on cut leaves of the same host plant species they had been feeding on in the field. Moving caterpillars into individual containers at this stage prevented larger instars from moving between plants. Caterpillars were weighed on the fifth day (n ≥ 17 per host plant species), ninth day (n ≥ 13 per host plant species), and as pupae (n ≥ 9 per host plant species). The adult butterflies were also checked for Oe spores and were then frozen at −80°C for chemical analysis. Caterpillar survival across host plants was analysed using a Fisher's exact test. We used a MANOVA to examine four measures of caterpillar performance: mass at day five, mass at day nine, pupal mass and days to pupation. Independent variables were plant species, maternal source (our lab colony, FL, MA), individual mother, interactions between plant species and monarch source and individual mother, and whether that caterpillar had detectable spores upon emergence. We also analysed differences in caterpillar mass at day five, mass at day nine and growth rate (pupal mass/number of days to pupate) using linear models. We analysed the number of emergent adults with detectable spores of Oe per host plant species using a Fisher's exact test.

To examine leaf cardenolide levels and sequestration in adult butterflies we used high performance liquid chromatography (HPLC) on leaves from each plant species (n = 6 individuals per plant species) and whole adult butterflies reared on each plant species (n = 6 per host plant species, 3 males and 3 females). All samples were frozen at −80°C, then freeze-dried at −40°C and ground in a mill to a fine powder. To extract cardenolides we added 1.6 ml of MeOH to 50 mg aliquots of each of the powdered samples. We then added ~30 FastPrep beads to each sample and we agitated the samples twice for 45 s at a speed of 6.5 m s^{-1} on a homogenizer, followed by centrifugation at 12 000 rpm for 15 min. We pipetted 1 ml of supernatant into 1.4 ml tubes

in 96-well racks and added 20 µg of the internal standard cardenolide digitoxin to each tube. Samples were dried in a centrifugal concentrator at 35°C. To resuspend the samples, we added 200 µl MeOH to each sample and filtered it with a MultiScreen Solvinert 0.45 µm low-binding hydrophobic PTFE plate. We analyzed 15 µl of each sample by HPLC using a Phenomenex Gemini NX 3u C18 110A 150 × 4.6 mm column and with diode array detection. The injections were eluted at a constant flow of 0.7 ml min⁻¹ with a fast gradient of acetonitrile and water as follows: 0–2 min at 16% acetonitrile; 2–25 min from 16% to 70%; 25–30 min from 16% to 70%; and 30–35 min from 70% to 95%, followed by a 10 min reconditioning step at 16%. Peaks were recorded at 218 nm. Cardenolides were identified by their characteristic absorbance shape and maximum between 214 and 222 nm. The concentrations of cardenolides were determined by relative area compared to the internal digitoxin standard. To estimate the total cardenolide concentration we summed all of the peak areas in a sample. We compared cardenolide concentrations sequestered by butterflies across plant species and butterfly sex using a two-way ANOVA and Tukey-corrected multiple comparisons using the glht function in the multcomp package (Hothorn et al. 2008).

To assess other plant defensive factors that could be influencing oviposition preference and larval performance we examined leaf latex and leaf trichome density. Latex measurements were collected from n > 40 individuals of each plant species according to established protocols (Agrawal et al. 2015a, b). We cut the last 3 mm of the tip of the youngest fully expanded leaf and absorbed the exuded latex with a pre-weighed 1 cm diameter filter paper disk. Filter paper disks were weighed upon return to the lab. Leaf trichome density data were from Agrawal and Fishbein (2006), in which leaf trichome densities were quantified on 24 species, including the four used in this study (one leaf disc from n ≥ 6 plants per plant species). Species differences in mass of latex exuded were analysed using a one-way ANOVA and Tukey post hoc tests. We compared counts of trichomes (per cm²) across the four species using a one-way ANOVA. The total cardenolide concentration was compared across leaf samples using a one-way ANOVA with Tukey post hoc tests.

To examine the effect of previous oviposition experience on preference, mated females (n ≥ 20 per treatment) with no prior exposure to milkweed plants were placed individually in 1-m³ cages in the field with plants of either *A. i. pulchra* or *A. i. incarnata* to gain experience ovipositing on one host plant species, or in an empty cage. After 24 h we gave all butterflies two fresh plants, one *A. i. pulchra* and one *A. i. incarnata* (choice test). We counted the number of eggs laid on these two plants with a GLMM with a Poisson distribution. Fixed effects included plant species on which eggs were laid (*A. i. incarnata* or *A. i. pulchra*), the treatment (no previous experience, previous experience with *A. i. incarnata*, and previous experience with *A. i. pulchra*), the interaction between plant species and treatment, the source of butterflies and the interaction between source of butterflies and the plant species. Individual butterfly was included as a random effect.

The final experiment assessed how the presence of a conspecific caterpillar affected female oviposition preference (n ≥ 12 butterflies per treatment). The experiment had three one-hour choice tests (stages) that each female proceeded through sequentially. The numbers of eggs laid were measured for each stage. First, we established baseline preferences for the two plant species (*A. i. incarnata* and *A. i. pulchra*), by presenting females a choice test with one plant from each species (pre-exposure stage) for one hour. Females then immediately proceeded to the exposure stage, in which the two plants were replaced with fresh plants for one hour. One of these plants was being fed upon by a large monarch caterpillar from our colony (4th or 5th instar, Supplementary material Appendix 1 for more details). Finally, these plants were replaced with a third pair of fresh plants with no damage (post-exposure) for one hour. The post-exposure stage was used to test whether the effect of exposure to caterpillar damage on preferences persisted even with different, undamaged, plants. In other words, did the females learn an association between plant species and plant damage that affected later oviposition choices? Separately for the two treatments (caterpillar on *A. i. incarnata* and caterpillar on *A. i. pulchra*), we used linear mixed effect models to analyse the effect of experimental stage and source of monarchs on the proportion of eggs they laid on the plant species we manipulated in that treatment. We included individual butterfly as a random effect.

Results

We found a strong effect of host plant species on the number of eggs laid by female monarchs ($\chi^2_3 = 18.26$, $p < 0.001$), with monarchs laying ~70% more eggs on *Asclepias i. pulchra* than each of the other host plants ($p < 0.01$; Fig. 1a). There was no significant effect of monarch source, or interaction between monarch source and host plant on the numbers of eggs laid by females.

Caterpillar survival was lowest on *A. i. pulchra* at 22% (9 caterpillars survived to pupation), in contrast to 39% on *A. curassavica* (12 caterpillars survived), 43% on *A. i. incarnata* (15 caterpillars survived) and 42% on *A. perennis* (15 caterpillars survived), but these differences were not statistically significant (Fisher's exact test, $p = 0.17$). Across all host plant species, caterpillars that died did so around the third day after hatching. Host plant did affect caterpillar performance (MANOVA; Wilks $\Lambda = 0.083$, $F = 5.88$, $p < 0.001$). Our multivariate measure of performance was also impacted
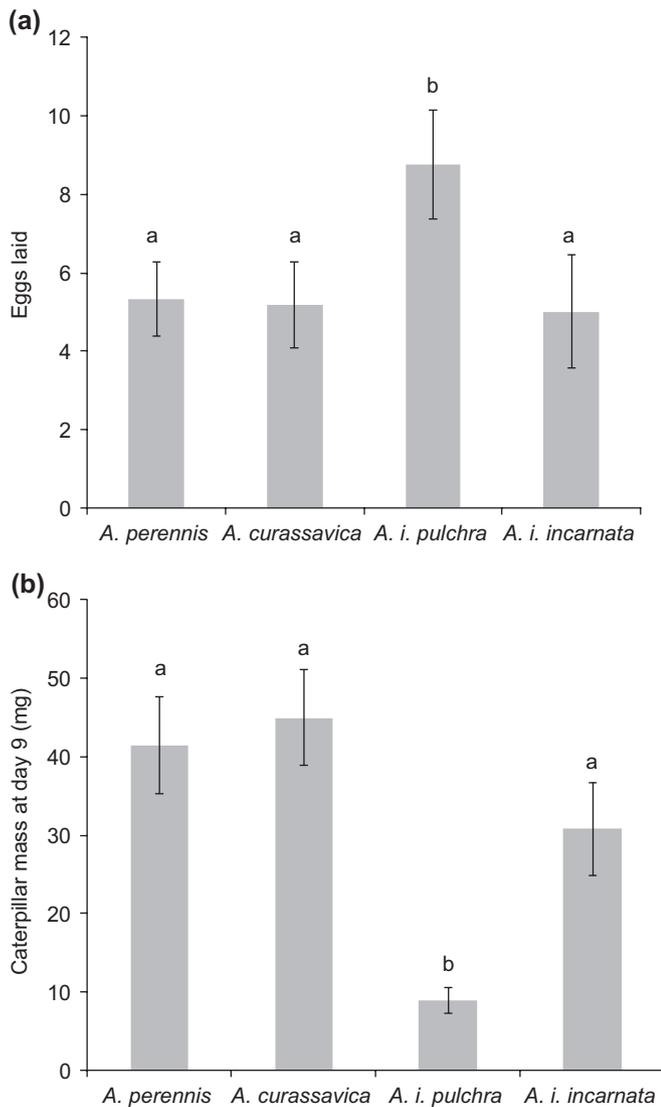
(a)

(b)

Figure 1. (a) Mean (± SE) total numbers of eggs laid by females on each of the four milkweed plant species in the combined two 1-h choice tests. Different letters indicate significant differences from Tukey-corrected multiple comparisons following a poisson-distributed GLMM. (b) Mean (± SE) monarch caterpillar mass on the ninth day after hatching on each of the four milkweed host plant species. Different letters indicate significant differences in Tukey-corrected multiple comparisons between plant species following a linear model.

by monarch source (Wilks $\Lambda = 0.33$, $F = 3.12$, $p = 0.009$) and individual mother (Wilks $\Lambda = 0.098$, $F = 1.77$, $p = 0.027$). Masses at day five and day nine were significantly affected by host plant (day five: $F_{3,56} = 15.83$, $p < 0.0001$; day nine: $F_{3,39} = 10.50$, $p < 0.0001$), day five mass was affected by monarch source ($F_{2,56} = 3.85$, $p = 0.027$) but day nine mass was not affected by monarch source ($F_{2,39} = 0.16$, $p = 0.86$). In contrast, mass at day nine was affected by individual mother ($F_{8,39} = 2.36$, $p = 0.036$), but mass at day five was not ($F_{8,56} = 1.81$, $p = 0.094$). Post hoc tests revealed that caterpillars reared on *A. i. pulchra* were much smaller than caterpillars
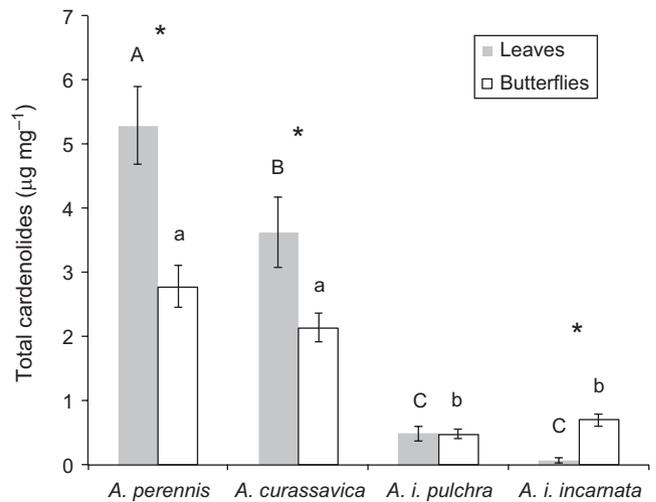

Figure 2. Plot of HPLC estimates of cardenolide concentrations of host plant leaves and adult monarch butterflies reared on those host plants (means ± SE, on a dry mass basis). Asterisks indicate significant differences in total cardenolides between plants and butterflies within a host plant species. Uppercase letters indicate significant differences in cardenolides between host plant species using a one-way ANOVA with Tukey post hoc tests and lowercase letters indicate significant differences in cardenolides between butterflies reared on different host plant species using a two-way ANOVA and Tukey-corrected multiple comparisons.

reared on any other host plant at day five ($p < 0.01$) and day nine ($p < 0.01$; Fig. 1b). In fact, caterpillars on *A. i. pulchra* were four-fold smaller than caterpillars on *A. curassavica* at day nine. These differences in mass likely represent a combination of differences in growth rate and developmental time. Growth rate over the entirety of development was significantly affected by host plant ($F_{3,31} = 4.35$, $p = 0.011$), but not by monarch source ($F_{2,31} = 2.64$, $p = 0.088$) or individual mother ($F_{8,31} = 0.76$, $p = 0.64$). Pupal mass did not differ across the host plant species, but caterpillars reared on *A. i. pulchra* took 2–4 days longer to reach pupation than caterpillars reared on the other host plants. Of the emergent butterflies, 63% of those reared on *A. i. pulchra* had detectable spores of the parasite Oe in contrast to 26% of those reared on *A. perennis*, 33% of those on *A. i. incarnata* and 8.3% of those on *A. curassavica*, but these differences were not statistically significant (Fisher's exact test, $p = 0.087$).

Leaf cardenolide levels varied among host plants ($F_{3,20} = 36.96$, $p < 0.001$), and post hoc tests demonstrated all species were different from each other ($p < 0.05$), with the exception of low cardenolide *A. i. pulchra* and *A. i. incarnata*. The highest cardenolide host plant, *A. perennis*, contained nearly ten-fold more cardenolides than *A. i. pulchra* (Fig. 2). Butterfly cardenolide levels, a measure of sequestration and defence, varied over nine-fold depending on host plant ($F_{3,17} = 35.12$, $p < 0.001$), but were not affected by butterfly sex ($F_{1,17} = 0.36$, $p = 0.56$), or interaction between plant species and sex ($F_{3,17} = 2.63$, $p = 0.083$). Butterflies reared on *A. perennis* and *A. curassavica* had three-fold more ($p < 0.05$)

cardenolides than butterflies raised on *A. i. pulchra* or *A. i. incarnata* (Fig. 2).

Our examination of other plant defence traits likely associated with preference or performance revealed that milkweed species significantly varied in the latex exuded from leaves ($F_{3,188} = 3.919$, $p = 0.0096$), with *A. i. pulchra* exuding over 20% more latex than any of the other plant species (Fig. 3a). The only statistically significant post hoc comparison, however, was that *A. i. pulchra* exuded over two-fold more latex than *A. perennis* (Tukey HSD; adjusted $p = 0.005$). Plants varied up to fifteen-fold in trichome density (hairs per cm²) (ANOVA; $F_{3,21} = 16.59$, $p < 0.001$), with *A. i. pulchra* having over 250% more trichomes than any of the other three species ($p < 0.001$; Fig. 3b).

To assess how previous oviposition experience influences preference, we gave females experience ovipositing on either *A. i. incarnata* or *A. i. pulchra*, or no oviposition experience, and then examined their subsequent oviposition choices.
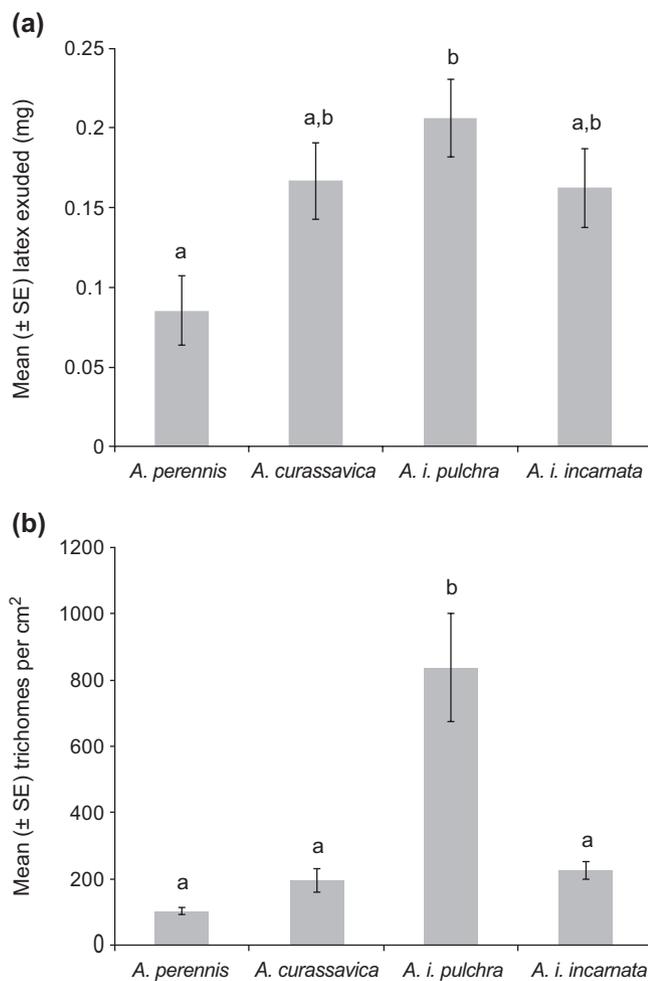
(a)

(b)

Figure 3. (a) Mean ($\pm$ SE) mass of latex exuded by clipped plant leaves for each plant species. Letters indicate significant differences in Tukey HSD tests. (b) Mean ($\pm$ SE) density of trichomes on leaves of each plant species. Letters indicate significant differences in Tukey HSD tests.

We found an effect of plant species, with more eggs laid on *A. i. pulchra* regardless of treatment ($\chi^2_1 = 284.12$, $p < 0.001$). There was no overall effect of treatment on the number of eggs laid ($\chi^2_2 = 1.32$, $p = 0.52$), but there was an interaction effect between plant species and treatment ($\chi^2_2 = 11.25$, $p = 0.0036$). Butterflies with no previous experience or previous experience with *A. i. pulchra* laid an average of 31% and 34% of their eggs on *A. i. incarnata* respectively. In contrast, butterflies with previous experience with *A. i. incarnata* laid more eggs (44%) on *A. i. incarnata* (Fig. 4). There was no effect of monarch source on the number of eggs laid ($\chi^2_2 = 0.67$, $p = 0.72$), but there was an interaction between monarch source and plant species ($\chi^2_2 = 31.76$, $p < 0.001$), with butterflies from all three sources preferring *A. i. pulchra* to *A. i. incarnata*, but with a weaker preference in butterflies from the FL source than in butterflies from MA or our lab reared butterflies.

In the final experiment we examined how the presence of a conspecific caterpillar impacted oviposition preference over three stages. We analysed butterflies that experienced a caterpillar on *A. i. incarnata* separately from butterflies that experienced a caterpillar on *A. i. pulchra*. For the *A. i. incarnata* butterflies, there was no effect of experiment stage on butterfly behavior ($\chi^2_2 = 1.05$, $p = 0.59$). For the *A. i. pulchra* butterflies, there was an effect of experimental stage ($\chi^2_2 = 10.76$, $p = 0.0046$), with butterflies laying a lower proportion of eggs on *A. i. pulchra* when there was a caterpillar present on *A. i. pulchra* than in the pre-exposure ($p = 0.041$) or post-exposure hours ($p = 0.0052$), but there was no difference between pre- and post-exposure. In the pre-exposure stage, butterflies in both treatments laid ~40% of their eggs on *A. i. incarnata* and 60% on *A. i. pulchra* (Fig. 5). When there was a caterpillar present on one of the host plants, butterflies laid
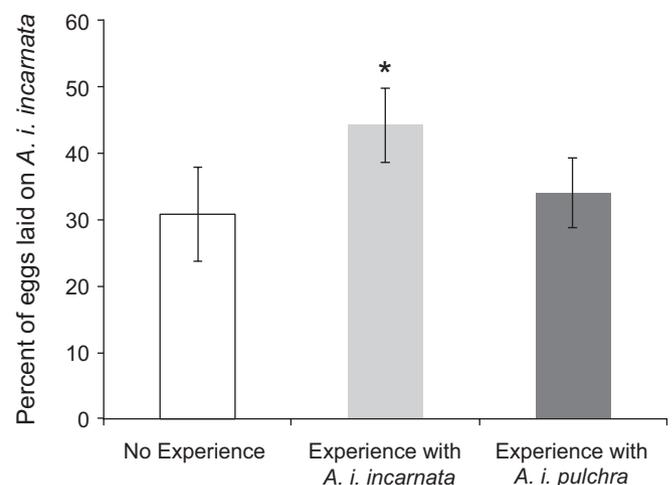
Figure 4. Mean ($\pm$ SE) percent of eggs laid on *A. i. incarnata* in comparison to *A. i. pulchra* in choice tests. When butterflies had no previous oviposition experience, versus experience ovipositing on *A. i. incarnata* or experience ovipositing on *A. i. pulchra* for 24 h. The asterisk indicates a significant interaction between plant species and experience treatment in a GLMM with a poisson distribution.
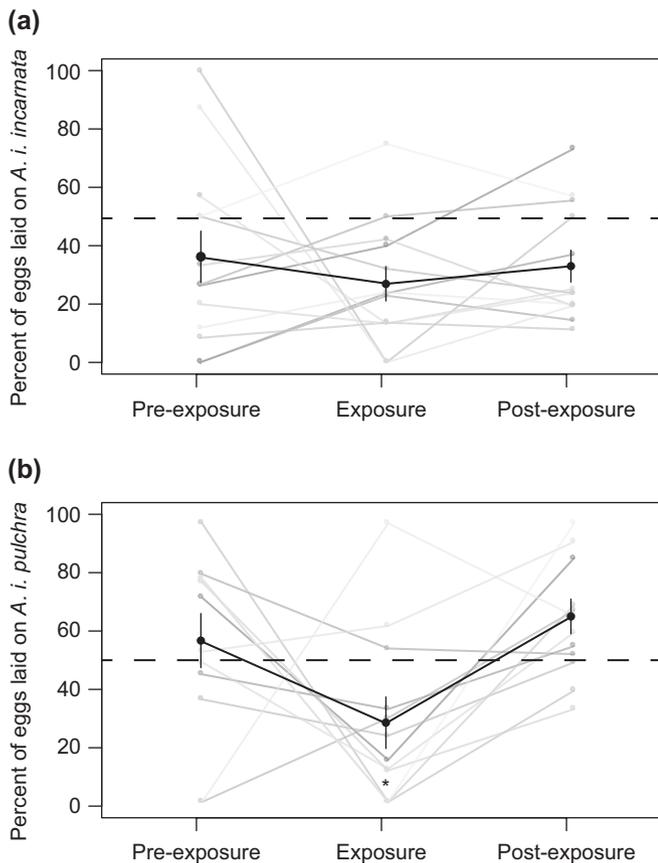
(a)

(b)

Figure 5. Effect of the presence of a monarch caterpillar and corresponding plant damage on monarch female oviposition preference. Gray lines are the percent of eggs laid on the manipulated host plant by individual females over the three stages. The black line indicates the mean (± SE) for all females in that treatment. The dashed line highlights 50%, or no preference. (a) Treatment in which females were exposed to a large caterpillar on *A. i. incarnata*, (b) treatment in which females were exposed to a caterpillar on *A. i. pulchra*. Females were first given a pre-exposure stage to determine baseline preference for the two plant species. Next females received an exposure stage when a large conspecific caterpillar was placed on one of the host plant species. In the post-exposure stage, immediately after, females were given two fresh plants, one of each species. The asterisk indicates a significant effect of experimental stage on proportion of eggs laid from a linear mixed-effect model.

~30% of their eggs on the plant with the caterpillar present and 70% of their eggs on the alternative plant regardless of plant species. This results in a complete reversal of the previously observed oviposition preference for *A. i. pulchra* over *A. i. incarnata*. There was no effect of monarch source on the distribution of eggs for either treatment (*A. i. incarnata*: $\chi^2_1 = 0.0047$, p = 0.95; *A. i. pulchra*: $\chi^2_1 = 0.37$, p = 0.54).

Discussion

For many species, a female's decision about where to lay eggs can affect her offspring's rate of development (Penn and Brockmann 1994), phenotype (Shine and Harlow 1996)

and risk of predation (Martin and Roper 1988). It has been proposed that oviposition site preferences should be genetically determined, and adaptive such that females lay their eggs in sites where their offspring perform the best (the preference–performance hypothesis) (Jaenike 1978). Many females, however, due to long lives or high mobility, encounter variable environmental conditions and in these circumstances we might expect female oviposton preferences to be flexible depending on current and local conditions (Jones and Agrawal 2017). We found that for monarch butterflies, females exhibited oviposition preferences that were not ideal for offspring growth rate, or sequestration of defensive compounds, but these preferences were flexible depending on females' own previous experience and the presence of conspecific competitors. This result has potential to be nearly universal, and only with subsequent tests of multiple factors impacting preference and performance can we arrive at a more predictive framework for ovipositional choices.

Across multiple different experiments, monarchs showed an oviposition preference for *Asclepias i. pulchra*, even over its sister subspecies *A. i. incarnata*. On *A. i. pulchra*, caterpillars showed the slowest growth and lowest amount of sequestered defensive toxins as adults. Experience ovpositing on *A. i. incarnata* modified this preference. However, the only factor that reversed female preference was the presence of conspecific caterpillars, supporting the important role of caterpillar competition and cannibalism indicated by previous studies (Brower 1961, Flockhart et al. 2012, Nail et al. 2015).

Plant defence and sequestration

Our first set of experiments tested the classic preference–performance hypothesis (PPH). The preference for *A. i. pulchra*, on which caterpillars were ~25% the size of caterpillars on the other host plants on their ninth day, rejects the PPH. The reduced caterpillar performance that we observed is clearly not explained by toxicity from cardenolides, as higher cardenolide host plants had higher caterpillar growth. Previous research indicates that overall, cardenolides may negatively impact monarchs, but this appears to depend on the condition of caterpillars and the specific cardenolides consumed (Seiber et al. 1980, Zalucki et al. 2001, Rasmann et al. 2009, Agrawal 2017). In contrast, latex exudation and leaf trichome densities are much more consistently associated with plant resistance to monarchs (Hulley 1988, Agrawal et al. 2012, 2015a, b, Robertson et al. 2015). In the current experiment, *A. i. pulchra* exuded the most latex across the four *Asclepias* and had the highest densities of trichomes. We observed caterpillars on *A. i. pulchra* (and only *A. i. pulchra*) shaving trichomes before beginning to consume the leaf, a behavior that likely slows consumption and thereby development, especially of early instars.

Butterflies reared on the four host plants were more similar in cardenolide concentrations than the host plant leaves themselves, but they did vary concordantly with leaf cardenolide levels. It has been proposed that monarchs sequester the most efficiently from milkweeds with intermediate levels

of cardenolides (Malcolm and Brower 1989), which has been suggested as an explanation for the oviposition preference for plants with intermediate cardenolide levels found in other studies (Zalucki et al. 1990). If intermediate cardenolide concentrations were the driving factor behind monarch preference or performance, however, we might expect the highest preference and performance on *A. curassavica* (Zalucki and Kitching 1982), which was not the case. Overall, on *A. i. pulchra* monarchs sequester the lowest concentration of cardenolides, therefore the host plant preferred by females has both the lowest caterpillar growth rate and generated the most poorly defended adults.

The lack of association between preference, performance and sequestration in our study is counterintuitive. One possible explanation could be differences in rates of predation on eggs or small caterpillars on different host plants. We examined caterpillar performance in cages in the field within which caterpillars were protected from predators. In pipevine swallowtail butterflies, *Battus philenor*, females lay eggs on plants with dense trichomes. Lacewing larvae are predators on small caterpillars, but on plants with dense trichomes lacewings' search and capture of caterpillars is reduced (Fordyce and Agrawal 2001). Plants with dense trichomes may actually be preferred by ovipositing females because although they may have an associated cost in larval growth, the trichomes could physically defend offspring from predators. It is worthy of investigation whether eggs or small caterpillars on *A. i. pulchra* experience less predation in the field than on other host plant species. If this were the case, trichome density could be an explanation not only for slow caterpillar growth on this host plant, but also a potential explanation for female preference.

Beyond preference and performance

Another aspect that has the potential to influence caterpillar performance on *A. i. pulchra* is parasite infection. More butterflies reared on *A. i. pulchra* were found to have spores of the protozoan parasite Oe upon emergence than any other host plant, although this difference was not statistically significant. There is conflicting data on whether Oe impacts monarch caterpillar development, with some studies finding negative effects of parasite load on likelihood of emergence as adults (de Roode et al. 2008a), and others finding no effect of infection on caterpillar mortality or development time (Altizer and Oberhauser 1999, de Roode et al. 2008b). Previous research has shown that butterflies reared on *A. i. incarnata* have higher parasite loads than butterflies reared on *A. curassavica*, and have suggested that the higher cardenolide concentrations in *A. curassavica* have a medicative effect reducing parasite loads (de Roode et al. 2008b). The proportion of infected females in our study was almost double for *A. i. pulchra* in comparison to *A. i. incarnata*, although these are both low cardenolide host plants, and *A. perennis* produced more than double the proportion of infected individuals than *A. curassavica* although it has higher cardenolide levels. Regardless of the mechanism, if our trend towards increased

proportion of parasitized individuals when reared on *A. i. pulchra* is repeatable, then this could have further negative consequences for monarchs, as infection is associated with reduced adult lifespan (de Roode et al. 2006) and impaired flight behaviour (Bradley and Altizer 2005), factors that are likely to impact migration.

There is general support for the PPH in the literature (Gripenberg et al. 2010), although many studies, like ours, have demonstrated a disconnect between preference and performance (Rausher 1979, Valladares and Lawton 1991, Underwood 1994, Fritz et al. 2000, Faria and Fernandes 2001). The reasons why females make these apparently maladaptive egg-laying choices are often unclear, although in some systems there is evidence that females are making choices that may increase their own lifetime fitness at the cost of particular offspring, termed 'optimal bad motherhood' (Mayhew 2001). Another possibility is that there is no adaptive explanation for these egg-laying choices, perhaps because a particular plant species has traits that make it a 'sensory trap' for females (Christy 1995, Schaefer and Ruxton 2009). Possible sensory traps might include large leaves, or oviposition stimulants which in monarchs include quercetin glycosides (Haribal and Renwick 1996). Previous research has shown medium levels of quercetin glycosides in *A. i. pulchra*, higher than *A. curassavica* but equivalent to *A. i. incarnata* (Agrawal et al. 2009c), indicating these stimulants are likely not the full explanation for monarch preference. The relative rarity of *A. i. pulchra* in nature compared to more abundant milkweeds such as *A. syriaca* may prevent monarchs from evolving an aversion to this host plant. Further investigation of the causes and consequences of monarch female preference for *A. i. pulchra* would continue to make an important contribution to the preference performance debate.

Experience and perception influence preference

Multiple studies have shown learning from oviposition experience in Lepidoptera (Traynier 1984, Papaj 1986, Landolt and Molina 1996, Cunningham et al. 1999, Dell'Aglio et al. 2016). We demonstrate that monarch butterflies are positively influenced by oviposition experience, especially when that experience is with less preferred plant species. These results are similar to those with herbivorous spider mites, *Tetranychus urticae*, showing that mites never rejected the host plant to which they were adapted, but exposure could increase preference for novel hosts (Agrawal et al. 2002). Learning from oviposition experience may enable females to search for host plants more efficiently, especially when there is enough environmental variation in host plant availability that flexibility in preference is beneficial (Jones and Agrawal 2017). Monarchs migrate over vast distances where they encounter a wide range of milkweed species and communities. They could therefore be expected to benefit from learning, and learning has in fact been demonstrated for monarchs in a number of nectar foraging tasks (Rodrigues et al. 2010, Blackiston et al. 2011, Cepero et al. 2015). Our results indicate that monarchs may also be learning when making

oviposition decisions. Host plant preferences may therefore shift with experience, and such dynamic changes may be especially important over the course migration when species encounter multiple hosts.

We found a strong negative effect of the presence of a conspecific caterpillar, to the extent that it could reverse the preference for *A. i. pulchra* over *A. i. incarnata*. This change in host plant preferences may be an attempt to avoid egg cannibalism. Changes in egg laying behaviour due to predation are common in other taxa such as birds (Eggers et al. 2006) and fish (Lehtonen et al. 2013). Another possibility is that avoidance of a conspecific caterpillar in monarchs reduces the future offspring's competition for food as small larvae are restricted to the host plant on which they were laid. The mechanism by which the presence of caterpillars influences oviposition preferences remains unclear. Monarch caterpillar damage to a milkweed plant results in release of plant volatiles (Wason et al. 2013), which could influence oviposition choices of females (Kessler and Baldwin 2001). Future experiments could fruitfully tease apart the signals, both social and plant-produced, that are affecting this response to the presence of conspecifics.

Monarchs migrate over huge distances where they encounter species and communities of milkweeds that vary broadly in traits including cardenolides, latex and trichomes. These plant traits have important consequences for offspring growth and defensive chemistry of adults. Some of these milkweed species, such as *A. i. pulchra*, may be sensory traps on which caterpillars have poor development and low sequestration, generating poorly defended adults. An alternative explanation is that *A. i. pulchra* confers some other advantage such as protection of eggs from predators by trichomes. In order to understand egg-laying decisions of females it is necessary to examine them from a community perspective. The complete reversal of plant preferences we observed in the presence of a large conspecific caterpillar points to a strong role of predation and competition in decision-making.

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