

Oviposition preference and larval performance of North American monarch butterflies on four *Asclepias* species

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Abstract

Monarch butterflies, *Danaus plexippus* L. (Lepidoptera: Nymphalidae), occur world-wide and are specialist herbivores of plants in the milkweed family (Asclepiadaceae). In North America, two monarch populations breed east and west of the continental divide in areas populated by different host plant species. To examine the population variation in monarch responses to different *Asclepias* species, we measured oviposition preference and larval performance among captive progeny reared from adult butterflies collected in eastern and western North America. Host plant use was evaluated using two milkweed species widely distributed in eastern North America (*A. incarnata* and *A. syriaca*), and two species common to western North America (*A. fascicularis* and *A. speciosa*). We predicted that exposure to different host plant species in their respective breeding ranges could select for divergent host use traits, so that monarchs should preferentially lay more eggs on, and larvae should perform better on, milkweed species common to their native habitats. Results showed that across all adult female butterflies, oviposition preferences were highest for *A. incarnata* and lowest for *A. fascicularis*, but mean preferences did not differ significantly between eastern and western monarch populations. Larvae from both populations experienced the highest survival and growth rates on *A. incarnata* and *A. fascicularis*, and we again found no significant interactions between monarch source population and milkweed species. Moreover, the average rank order of larval performance did not correspond directly to mean female oviposition preferences, suggesting that additional factors beyond larval performance influence monarch oviposition behavior. Finally, significant family level variation was observed for both preference and performance responses within populations, suggesting an underlying genetic variation or maternal effects governing these traits.

Introduction

Insect traits that govern the use of different host plant species can be subject to selection at a variety of spatial scales, leading to changes in oviposition behavior and larval performance on different plant species (Rausher, 1982; Via, 1986; Jaenike, 1989; Bossart & Scriber, 1995; Etges & Ahrens, 2001; Ballabeni et al., 2003). This is because herbivorous insects frequently encounter a range of host plant species that vary chemically and physically, and individual insects differ in their response to plant traits,

leading to opportunities for adaptive evolution in response to locally abundant plant species (Rausher, 1984; Rossiter, 1987; Malcolm et al., 1989). Indeed, strong host-plant driven selection has been linked with population differentiation and the local adaptation of insects on common host plants, even in the face of high gene flow among subpopulations (Bossart & Scriber, 1995; Alstad, 1998; Futuyma, 1998; Mopper, 1998; Forister, 2004).

Monarch butterflies, *Danaus plexippus* L. (Lepidoptera: Nymphalidae), occur on islands and continents world-wide and feed exclusively on plants in the milkweed family Asclepiadaceae (Ackery & Vane-Wright, 1984; Vane-Wright, 1993). Factors driving the selection of host plants by monarchs, including host plant age, size, and cardenolide content, have been well-studied within single butterfly

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populations (Cohen & Brower, 1982; Malcolm & Brower, 1986; Oyeyele & Zalucki, 1990; Zalucki et al., 1990; Haribal & Renwick, 1998a,b; DiTommaso & Losey, 2003). For example, in field studies focusing on single host plant species, monarch females have been shown to lay the most eggs on individual plants that are taller, closer to flowering, and with intermediate levels of cardenolides (Cohen & Brower, 1982; Malcolm & Brower, 1986; Oyeyele & Zalucki, 1990). Indeed, monarch larvae are known to sequester cardenolides to repel predators, and cardenolide content is thought to be an important evolutionary factor in host choice (Fink & Brower, 1981; Alonso-Mejia & Brower, 1994), although monarchs can also regulate their own cardenolides across a wide range of host plant variation (Cohen & Brower, 1982; Malcolm, 1991). Milkweed species that are more toxic probably provide a better defense for monarch caterpillars and adults, but high cardenolide concentrations, in addition to high latex flow, can slow development and decrease the survival of early instar larvae (Oyeyele & Zalucki, 1990; Zalucki et al., 1990, 2001a,b). Other traits of individual plants, including nitrogen content, other secondary compounds, latex content, and leaf morphology should also affect female host choice and larval feeding behavior (Cohen & Brower, 1982; Oyeyele & Zalucki, 1990; Lavoie & Oberhauser, 2004).

Within North America, monarchs have been recorded as feeding on 27 different plant species in the genus *Asclepias*, and a small number of related genera including *Cynanchum* and *Sarcostemma* (Malcolm & Brower, 1986). However, separate populations in North America and elsewhere encounter only a subset of potential hosts (Ackery & Vane-Wright, 1984), and no single host plant overlaps the entire geographic range of monarchs (Woodson, 1954). Thus, locally abundant *Asclepias* species could generate ecologically significant selection pressures on monarch host use. For example, the geographic variation in sugars used to produce flavonol glycosides suggest that among *Asclepias* species in North America, plants in the east evolved differently from those that occur west of the continental divide (Haribal & Renwick, 1998b).

Genetically based geographic variation in host plant preference and larval performance has been demonstrated in several other butterfly species. For example, Thompson (1993) identified a genetically based preference among western swallowtail butterflies for host plant species from their source habitat. Eastern tiger swallowtail butterflies (*Papilio glaucus*) sampled along a latitudinal gradient showed greater oviposition preference and larval performance on tree species that were relatively more abundant at their collection sites (Bossart & Scriber, 1995). Among Glanville fritillary butterflies in the Åland Islands, adults preferred to lay eggs on locally abundant host plant

species, and genetic differences in oviposition preference were consistent with field observations of host use (Kuussaari et al., 2000). In studies of the nymphalid butterfly *Polygonia c-album*, Janz (1998) showed that similar genetic mechanisms determined both within- and among-species differences in female oviposition preferences. Taken together, these studies point to genetically based variation in butterfly responses to plant attributes combined with divergent selection driven by differential host plant abundance.

Surprisingly few field or laboratory studies have examined host plant use of adult or larval monarchs across multiple naturally occurring milkweed species. Among a few exceptions, Bartholomew & Yeargan (2002) compared the oviposition behavior of monarchs on *A. syriaca* and *C. larva* in several fields in Kentucky, and found that monarchs preferred *A. syriaca* when both plant species inhabited the same field, but would use either plant in single species stands. Among spring monarchs migrating north through Texas, Calvert (1999) showed that females laid eggs on seven endemic milkweed species, with some species harboring relatively more eggs than others. In South Florida, monarchs preferred the introduced tropical milkweed *A. curassavica* over native *A. incarnata* in mixed stands, possibly because of the higher cardenolide content of the former (Malcolm & Brower, 1986). Another introduced milkweed species (*A. fruticosa*) has been used for field studies of host use by Australian monarchs (e.g., Oyeyele & Zalucki, 1990). However, no published studies have examined species-specific patterns of host use by *D. plexippus* from multiple geographic locations.

In this study we compared oviposition preferences and larval performance of monarch butterflies on four different *Asclepias* species. Our goal was to examine whether monarchs from eastern and western North America showed evidence of local adaptation to host plant species that commonly occur in their native breeding range. We selected four milkweed species that demonstrated a broad distribution within each of the respective populations and for which parts of the chemistry had been previously studied by other authors. We predicted that monarchs should show greater oviposition preference, higher larval survival, and faster development on milkweed species from within their native breeding ranges. By comparison, monarchs feeding on novel hosts plant species could experience an inability to detoxify or digest host foliage, or might not respond to milkweed properties when selecting leaves for oviposition. Secondary goals of this study were to examine genetic variation within populations for host use traits and to determine whether the directionality of larval performance on milkweed species corresponded with mean female oviposition preferences.

Methods

Natural history of the study system

Native and introduced monarch butterflies inhabit islands and continents world-wide (Ackery & Vane-Wright, 1984). In parts of North America, monarchs undergo a spectacular annual migration where summer breeding generations are separated by a long distance movement to overwintering sites (Urquhart & Urquhart, 1978; Brower & Malcolm, 1991; Nagano et al., 1993; Brower, 1995). In the early spring, the same butterflies that winter in Mexico and coastal California, mate and re-colonize parts of their breeding range (Brower & Malcolm, 1991; Van Hook, 1993). Western monarchs migrate towards overwintering sites near the Californian coast whereas eastern monarchs form wintering colonies in the neovolcanic mountains in Central Mexico.

Monarch larvae feed exclusively on plants in the family Asclepiadaceae (Ackery & Vane-Wright, 1984; Malcolm & Brower, 1986; Malcolm, 1991). Milkweed species vary greatly in their leaf chemistry, latex production, size, and leaf morphology (including thickness and hairiness). These traits have been shown to influence larval feeding preferences, cardenolide sequestration for monarch defense, larval growth rates, total food consumption, and larval survival (Brower et al., 1984; Oyeyele & Zalucki, 1990; Zalucki & Malcolm, 1999; Lavoie & Oberhauser, 2004). For example,

both cardenolides and flavonoids are known to be important in the post-alignment oviposition behavior of monarch butterflies, including which appendages are used to assess plant suitability and the actual number of eggs laid per plant (Haribal & Renwick, 1998a,b). Milkweed species vary greatly in both their concentration of cardenolides and the actual glycoside compounds produced (Malcolm et al., 1989; Malcolm, 1991; Haribal & Renwick, 1998b), and larvae have been shown to regulate their own cardenolide sequestration across a range of host plant variation (Cohen & Brower, 1982). Moreover, within North America, the sugars comprising milkweed quercetin flavonoid patterns appear to follow a geographic divide, with *Asclepias* species from the eastern USA and Canada using different sugar components than those from western North America (Haribal & Renwick, 1998b).

Milkweed plants

For both oviposition and larval performance experiments we used four milkweed species common to North America: *Asclepias fascicularis*, *A. speciosa*, *A. incarnata*, and *A. syriaca*. The first two species occur in western North America and the latter two are endemic to eastern North America, and all four species encompass a broad area within the breeding range of eastern or western monarchs (Figure 1). In western North America, *A. fascicularis* (narrow-leaved milkweed)

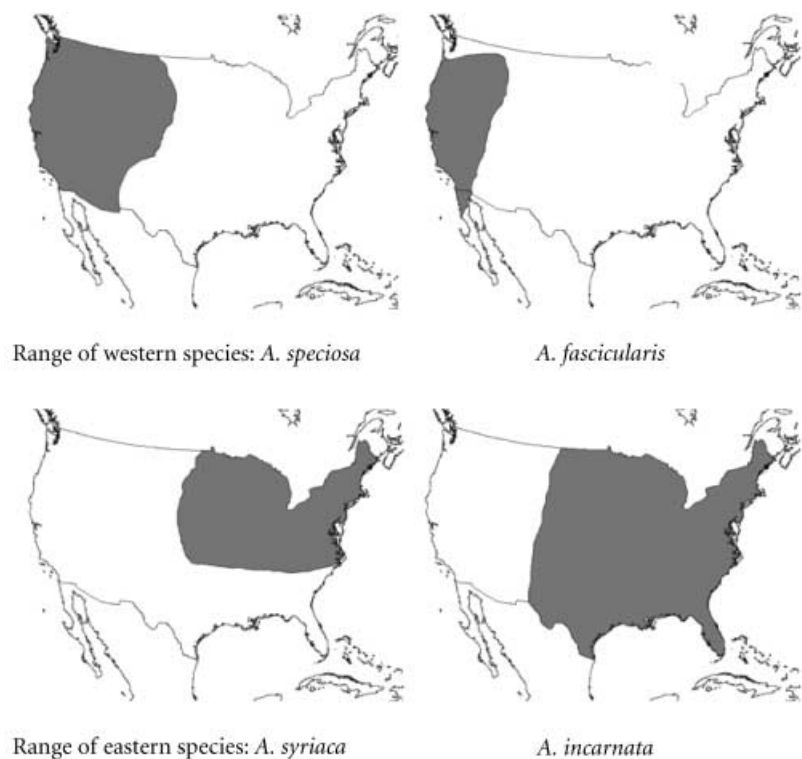


Figure 1 Historical geographic ranges of the four milkweed (*Asclepias*) species used in this investigation, in the USA (from Woodson, 1954).

occurs on roadsides, hills and valleys, typically in moist soil or near irrigated fields across a range of states including Idaho, Utah, and Nevada, to the Pacific Coast (Woodson, 1954). Similarly, *A. speciosa* (showy milkweed) prefers moist, sandy soils and prairie habitats and occurs across the western plains states to the Pacific Coast. Both *A. speciosa* and *A. fascicularis* can co-occur in the same habitats, including irrigated fields and roadside ditches, and are not generally temporally isolated (Woodson, 1954). In eastern North America, *A. syriaca* (common milkweed) is the most abundant milkweed species, inhabiting the Great Plains and Atlantic coast states and growing in prairies, pastures, roadsides, and disturbed habitats. A second eastern species, *A. incarnata* (swamp milkweed) has a similarly wide geographic range but is typically restricted to wetter soils, including flood plains, swamps, and low-lying areas in fields (Figure 1). Although *A. incarnata* is less common than *A. syriaca* and restricted to moist habitats, these plants show a similar seasonal phenology and can be found growing in adjacent habitats (Woodson, 1954).

In this study, we did not quantify plant leaf chemistry, but relied on previously published studies of within- and among-species variation. All four plant species are known to have moderate (*A. syriaca*, *A. speciosa*) or low (*A. incarnata*, *A. fascicularis*) cardenolide concentrations based on previously published assays (Malcolm, 1991). We selected species that differed in leaf morphology: *A. syriaca* (east) and *A. speciosa* (west) plants produce broad, thick, hairy leaves, whereas *A. incarnata* (east) and *A. fascicularis* (west) produce narrower, thin, smooth leaves. Seeds were obtained from R. Gendron of Butterfly Encounters (Dublin, CA, USA), a source specializing in large numbers of naturally occurring milkweed species (S. Altizer, pers. comm.). Approximately 100 plants of each species were grown in 10 cm pots in a greenhouse with an approximate temperature range of 21–29 °C. Plants were watered daily and fertilized weekly with Miracle Gro® (Scotts Miracle-Gro Company, Marysville, OH, USA). Plants were sprayed every second week with insecticidal soap and rinsed thoroughly with water to limit pest abundance. Milkweed stalks were checked for pests before being selected for oviposition and larval performance experiments, and damaged stalks were discarded. All plants used in the experiments described below were 4–5 months old, had been grown under a long photoperiod (L14:D10) and at constant warm temperatures, were between 30 and 60 cm in height, and none had yet flowered, set seed, or showed signs of senescence prior to use.

Butterfly sources

Adult *Danaus plexippus* butterflies were collected from eastern and western migratory populations in North America during fall migration (east) or overwintering (west). In the eastern

population, 148 live monarchs were collected from five locations between August and October 2001: Atlanta, GA; Huntsville, AL; Cape May, NJ; St. Paul, MN; and Ithaca, NY. Migration in this population begins during mid to late August and extends through mid to late October (Brower, 1995). Therefore, most monarchs collected between August and October can be assumed to be part of the migratory generation that travels to Mexico and overwinters until the following March. Western monarchs ($n = 45$) were collected in February 2002 from two overwintering sites near Halcyon and Oceano, CA (and we assumed these included butterflies that had migrated from multiple breeding sites the previous fall). Thus, wild monarchs from both populations were collected as part of the same annual generation. Monarchs were examined for parasites as described in Altizer (2001) and uninfected butterflies were held in a controlled environment chamber at 12 °C and fed 20% honey water solution every 10 days.

Measuring oviposition preference

The monarchs used in oviposition preference experiments were the offspring of wild-captured adults from western North America, and grand-offspring of wild adults from eastern North America. Captive monarchs were reared from egg to adulthood using a breeding design that eliminated the possibility of full-sib matings and maximized the contribution of initial founders to each generation (with more than 120 butterflies per generation per population). To counter the potential influence of natal milkweed species on oviposition preferences, larvae were reared on potted tropical milkweed species from our greenhouse (*A. curassavica* from the neotropics and *A. fruticosa* from Africa), as these species were not used in the experiments, are not native to either population's range, and produce high cardenolide concentrations relative to the North American species we were using (Malcolm, 1991). Both *A. curassavica* and *A. fruticosa* produce relatively smooth (non-hairy) leaves.

In June 2002, females were mated with non-sibling males from their same population, and female abdomens were gently palpated to check for the presence of spermatophores (Van Hook, 1999). Twenty-four oviposition cages (0.6 m³ PVC pipe frames covered with mosquito netting) were placed in a greenhouse at Emory University under the same temperature and light conditions as described above. Each test cage contained four bottled stalks, one for each milkweed species. Host plant stalks were cut to approximately the same height. Each bottle was placed near a corner of the square-shaped cage and no leaves touched its netting. The bottle positions were rotated daily to control for positional effects, and wilted stalks were replaced. Caged monarchs were fed ad libitum from sponges soaked in 20% honey-water solution, and

the sponges were rinsed and fresh solution was added daily. Between use by different females, cages and materials were sterilized for 30 min in a 25% bleach solution. Mated females remained in the oviposition cages for up to 5 days or until they laid 100 eggs. The total number of eggs deposited on each host plant stalk was counted, and only females that laid 50 or more eggs were included in the analysis. To account for differences in oviposition caused by variations in the size or mass of milkweed stalks, we weighed the stalks to the nearest 0.001 g using an electronic balance. Because past work on other butterfly species has shown that preferences for individual stalks within each plant species could confound among-species preference patterns (Singer et al., 2002), and that seasonal changes in plant chemistry can further affect oviposition choice (Haribal & Feeny, 2003), we attempted to minimize this variation by selecting plant stalks of approximately the same age, phenological stage, condition, and size – both within and among each host plant species.

Larval survival and growth rate

In a second experiment, the performance of larvae from each population was evaluated on each of the four host plant species. Eggs were obtained from eight western and eight eastern females that had not been used in preference tests. As before, monarchs used as parents were reared on tropical milkweed (*A. curassavica* and *A. fruticosa*), and eggs were collected from individual females in oviposition cages in the greenhouse. We divided 40 eggs from each female equally among four separate 16 cm diameter sterile Petri dishes. Each dish contained moist filter paper and leaves from one of the four milkweed species (*A. fascicularis*, *A. speciosa*, *A. incarnata*, or *A. syriaca*). Larvae were reared in a laboratory maintained at 23 °C. When all eggs had hatched, first instar larvae in each dish were weighed collectively to the nearest milligram. After larvae reached late second instar, they were weighed again and transferred to 300 ml plastic drinking cups with transparent lids, where they were reared individually to fifth instar and weighed a final time. Fresh milkweed cuttings were added daily, and old cuttings and frass were removed. We recorded larval deaths and the number of days required for larvae to develop from first to fifth instar.

Data analysis

Oviposition preferences were calculated in three ways: (1) the proportion of eggs laid on each host, (2) the number of eggs laid per stalk, and (3) the number of eggs laid per g plant material. Proportions (measure 1) were arcsine-square-root transformed and counts of eggs (measures 2 and 3) were square-root transformed prior to analysis to normalize the error variance. A repeated measures analysis

of variance (SPSS, 2001) was used to test for the effects of source population, milkweed species, and family level effects on the number and proportion of eggs laid on each milkweed stalk. For these analyses, individual monarchs were treated as subjects, and milkweed species was treated as a within-subject effect. Both population origin and family were treated as among-subject factors, where family refers to whether or not females were from the same full-sib group [Model: preference = host plant + population + family(population) + host plant*population + host plant*family(population)]. The factor of greatest interest was the interaction between monarch source population and host plant species, which would indicate whether females from eastern vs. western North America preferred different milkweed species.

To evaluate larval performance, survival was determined as whether or not the larvae reached: (a) late second, or (b) fifth instar. Logistic regression was then used to test the effects of source population and milkweed species on larval survival (Model: survival = population + host plant + population*host plant). We followed other researchers in estimating larval performance as the daily growth rate from hatching to final mass (Bossart & Scriber, 1995; Zalucki & Malcolm, 1999). Thus, larval growth rate was calculated as $[\text{Mass}_{\text{final}} - \text{Mass}_{\text{initial}}]/D$, where $\text{Mass}_{\text{final}}$ is the mass at fifth instar, $\text{Mass}_{\text{initial}}$ is initial larval mass, and D is duration in days from first to fifth instar. Finally, analysis of variance was used to examine the effects of source population, host plant species, and female nested within population on larval performance (ANOVA Model: growth rate = population + host plant + population*host plant + family(population) + family(population)*host plant). In all analyses, family was treated as a random effect nested within population origin.

Results

Oviposition preference

Oviposition preference results were obtained from 21 eastern and 25 western females from eight separate family lines within each population. The mean (\pm SE) number of eggs laid by individual eastern females was 170 ± 21 , and by western females was 227 ± 16 . In both eastern and western populations, females responded strongly to host plant species: on average, nearly half of all eggs were laid on *A. incarnata*, and very few eggs were laid on *A. fascicularis* by females from either source population (Figure 2). The rank order preference and mean number of eggs laid on each milkweed species was similar for monarchs from both eastern and western North America: *A. incarnata* received the greatest total number and percentage of eggs (52% of eggs laid by eastern females and 44% by western females).

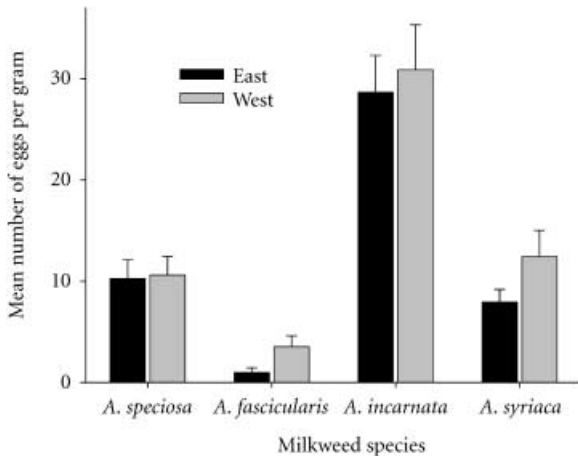


Figure 2 Comparison of oviposition preferences of eastern and western monarchs (*Danaus plexippus*) on four host plant (*Asclepias*) species. Preference here is shown as the number of eggs laid per g of plant material averaged across all females within each population. Bars show mean + SE.

Both *A. syriaca* and *A. speciosa* received intermediate percentages of eggs (for *A. syriaca*, 23% of eggs from eastern and 24% of eggs from western monarchs; for *A. speciosa*, 23% of eggs for eastern and 25% of eggs for western monarchs). Finally, the mean percentage of eggs laid on *A. fascicularis* was just over 1% for eastern females and 4% for western females.

Results from repeated measures analysis of variance were qualitatively similar for all three measures of oviposition preference – the number of eggs per stalk (square-root transformed), number of eggs per g of plant material (square-root transformed), and the proportion of eggs laid per stalk (arcsine square-root transformed). For all three measures, the effect of milkweed species on oviposition behavior was highly significant (e.g., Table 1; Figure 2). However, the population*host plant species interaction was not significant, indicating that the overall preferences of females did not differ significantly at the population level (Table 1). Thus, although western females laid slightly more eggs on *A. fascicularis* than eastern females, and eastern females showed a slightly higher preference for *A. incarnata* than western females (Figure 2), these minor differences were not significant at the 0.05 level.

Within each population, individual females and groups of siblings varied in their response to different milkweed species. Among different eastern families, monarchs uniformly rejected *A. fascicularis* and showed an intermediate preference for *A. speciosa*, but varied in their preferences for *A. syriaca* and *A. incarnata* (Figure 3A). For example, females from one eastern family showed the highest rank-order preference for *A. syriaca*, and the remaining families

showed intermediate (second- or third- order) preference for this species. Among western families, a high variation was observed for preferences towards *A. speciosa* (Figure 3B), with two families showing greatest preference for this species. In addition, the rejection of *A. fascicularis* was not as extreme among western monarchs; fewer western females laid zero eggs on this species relative to eastern females. This interaction between host plant species and family, nested within population, was nearly significant at the 0.05 level for preference measures based on both counts of eggs and eggs per g (Table 1a), but not when preference was measured as the proportion of eggs per stalk (Table 1b).

Larval performance

Monarch survival to late second instar was high (> 91%) across all host plant treatments and populations. Because no predictors were significant in the logistic regression analysis of early survival, the results of this analysis are not reported. There was a highly significant effect of milkweed species on survival to fifth instar (Table 2, Figure 4A). Monarch survival from first to fifth instar was highest on *A. incarnata* (over 85% of larvae survived) and similarly high on both *A. fascicularis* (82%) and *A. speciosa* (79%). Survival was lowest on *A. syriaca* (only 67% of larvae reached fifth instar) and this effect of host plant was statistically significant (Table 2). Eastern monarchs had slightly higher survival to fifth instar than western monarchs, and this effect was marginally significant (Table 2). Moreover, larvae

Table 1 Repeated measures ANOVA results for effects of host plant species (*Asclepias speciosa*, *A. fascicularis*, *A. syriaca*, and *A. incarnata*) and butterfly (*Danaus plexippus*) population origin on oviposition preference measured as: (a) the number of eggs per gram of milkweed (square-root transformed), and (b) the proportion of eggs per stalk (arcsine square-root transformed). Individual females were treated as subjects, and host plant species was treated as a within-subject effect. Tests for among-subject effects alone are indicated by †

Source	SS	d.f.	F	P
(a)				
Host plant	277.23	3	42.28	0.000
Host*population	6.62	3	1.01	0.393
Host*family (population)	133.32	42	1.54	0.058
Population †	7.75	1	5.99	0.020
Family (population) †	45.77	14	2.53	0.016
(b)				
Host plant	6.53	3	33.7	0.000
Host*population	0.19	3	0.97	0.411
Host*family (population)	3.53	42	1.30	0.150
Population †	0.01	1	0.89	0.350
Family (population) †	0.06	14	0.92	0.545

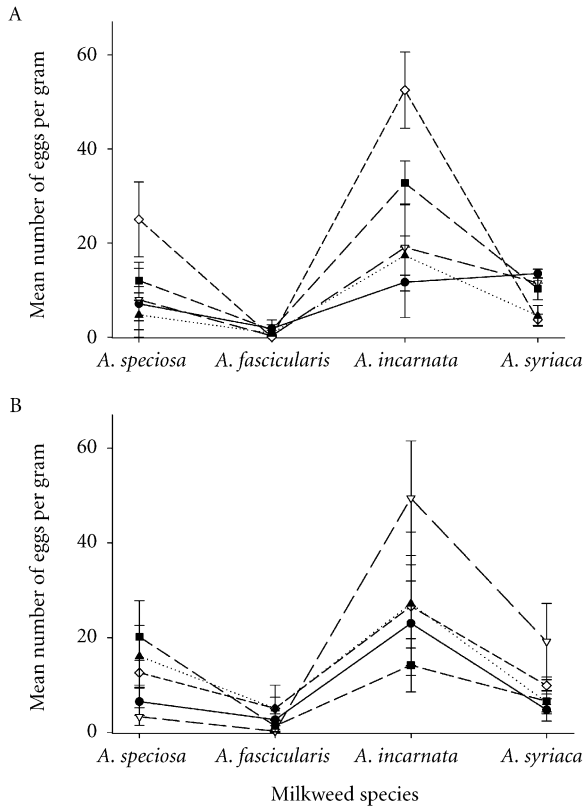


Figure 3 Oviposition preference of female *Danaus plexippus* from: (A) five eastern, and (B) five western families for each milkweed (*Asclepias*) species, measured by the average proportion of eggs laid by females in each family. Each line represents means from a separate family represented by 3–6 full siblings. Bars show mean \pm SE.

from eastern and western monarch populations responded similarly to each of the four host plant species (Figure 4A), and the population by host plant interaction was not significant (Table 2).

For larval growth measured as a change in mass from first to fifth instar (divided by days), monarchs grew fastest on both *A. incarnata* and *A. fascicularis*, and slowest on both *A. syriaca* and *A. speciosa* (Figure 4B). This effect of host plant species was highly significant (Table 3). Monarchs from eastern and western North America showed similar average growth rates in response to host plant species, and statistical analyses showed no significant main effects or interactions involving monarch source population (Table 3). However, as with oviposition preference, we observed significant effects of family (nested within population) and the family by host plant species interaction (Table 3), suggesting that groups of siblings within each population differed in their response to host plant species. For example, within the eastern population, mean growth

rate was highest on *A. incarnata*, but progeny from two of the eight family lines grew the fastest on *A. syriaca*. Similarly, among western families, only four of the eight family lines showed fastest growth on *A. incarnata*, and two showed fastest growth on *A. fascicularis*.

Discussion

Like other herbivorous insect species, monarch butterflies demonstrated a high degree of variation in host use traits as both larvae and adults, and discriminated among multiple *Asclepias* species. Monarchs derived from both eastern and western populations showed the greatest mean oviposition preference for *A. incarnata* relative to other host plant species tested, and females from both populations laid very few eggs on *A. fascicularis*. Larvae from both populations had significantly poorer survival to fifth instar when reared on *A. syriaca*, and larval growth rates (measured as mean weight increase per day) were relatively slower on both *A. speciosa* and *A. syriaca*. However, although host plant influenced monarch oviposition and larval performance, our results did not provide general support for the local adaptation to hosts found within each butterfly population's breeding range. Moreover, larval performance and mean oviposition preferences did not perfectly correspond, in part because monarchs laid very few eggs on one host plant (*A. fascicularis*), which is associated with high larval survival and growth rates.

In many geographic regions, monarchs encounter a variety of host plant species with different physical and chemical characteristics (Zalucki, 1986; Malcolm et al., 1989). Among milkweed traits that influence monarch oviposition and larval performance, cardenolide concentrations have been highlighted as an important factor in many studies (Brower et al., 1972; Zalucki et al., 1990; Malcolm, 1991; Haribal & Renwick, 1998a). Even though monarch larvae sequester cardenolides for defense against predators (Malcolm et al., 1989), adult females have been shown to reject high cardenolide hosts (Oyeyele & Zalucki, 1990;

Table 2 Logistic regression results for effects of host plant species (*Asclepias speciosa*, *A. fascicularis*, *A. syriaca*, and *A. incarnata*), butterfly (*Danaus plexippus*) source population, and family nested within population on larval survival to fifth instar. Relative to the null model, the full model was highly significant (likelihood ratio $\chi^2 = 37.3$, d.f. = 21, $P = 0.017$)

Effect	d.f.	Wald χ^2	P
Population	1	3.98	0.046
Host plant	3	18.35	0.004
Population*host	14	0.62	0.893

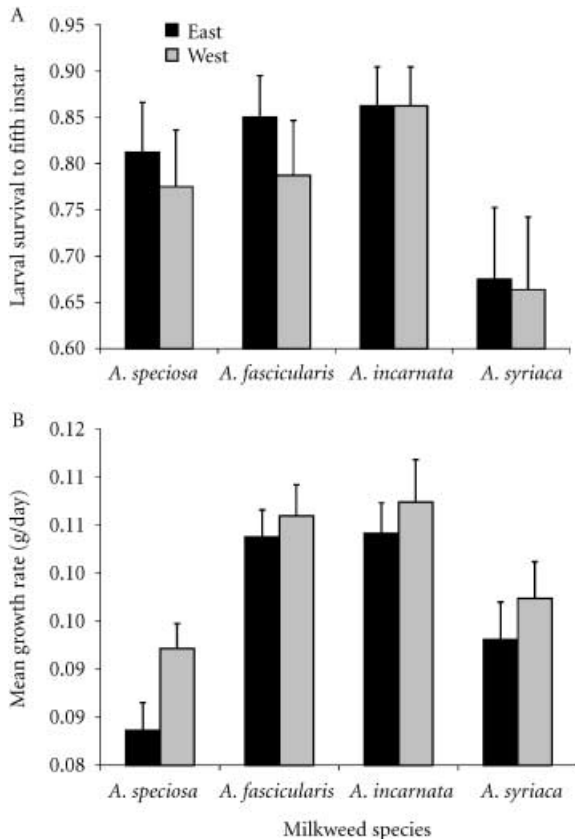


Figure 4 Larval performance in relation to host plant (*Asclepias*) species and monarch (*Danaus plexippus*) source population. (A) Comparison of survival to fifth instar for eastern and western larvae on each milkweed species. (B) Growth rates measured as daily change in mass from first to fifth instar for eastern and western monarchs on each milkweed species. Each population's average represents 6–10 full siblings from each of eight different family groups. Bars show mean \pm SE.

Zalucki et al., 1990; Haribal & Renwick, 1998a), perhaps because high cardenolides levels have been linked with low larval survival and slower development rates (Erickson, 1973; Zalucki et al., 2001a). However, in this study both the most and least preferred milkweed species (*A. incarnata* and *A. fascicularis*) have been reported with the lowest cardenolide concentrations among all milkweed species examined to date, indicating that monarch females did not reject milkweed based solely on cardenolide levels.

Latex production could also influence monarch host use traits (Malcolm & Zalucki, 1996). For example, monarch larvae have been observed cutting trenches across leaves to limit latex flow (Zalucki et al., 2001b), and latex has been demonstrated to cause mortality and to reduce growth rates among early instar larvae. Among the eastern milkweed species used in the experiment, published ranges of

latex volume production for *A. syriaca* (3.8–598 μ l) are much higher than for *A. incarnata* (0.20–50 μ l) (Zalucki & Malcolm, 1999). Although milkweed stalks used in oviposition and larval performance tests were cuttings with presumably less latex flow than intact stalks, the higher latex content of both *A. syriaca* and *A. speciosa* could partly account for the relatively low oviposition and larval growth rates observed on these two host species.

Leaf morphology, including thickness and size, should also influence host choice and larval performance. Both *A. incarnata* and *A. fascicularis* have smooth, thin leaves relative to the tough, hairy leaves of *A. speciosa* and *A. syriaca*, and larval survival and growth rates were higher on the two smooth-leaved host species. Moreover, although larval survival and growth rates were high on narrow-leaved milkweed (*A. fascicularis*), monarch females laid surprisingly few eggs on this species. Visual observations of ovipositing females indicated that single stalks and leaves of *A. fascicularis* bent under the weight of monarch females, and the strength of stalks and size of leaves might represent an important factor limiting monarch oviposition. In wild populations, monarchs might encounter larger stalks of *A. fascicularis*, so that presenting females with single stalks in our greenhouse experiments could have artificially reduced the number of eggs laid on this host. Apart from these factors, *A. fascicularis* has been shown to express similar cardenolide concentrations as its preferred eastern counterpart, *A. incarnata* (Malcolm, 1991).

It is important to note that both plant species (*A. curassavica* and *A. fruticosa*) used to propagate monarchs prior to experiments produce relatively narrow, smooth (non-hairy) leaves, similar to *A. incarnata* and *A. fascicularis*, but have been reported to produce higher cardenolide concentrations than any of the milkweed species used in experimental tests (Malcolm, 1991). Because monarchs were reared

Table 3 Mixed-model analysis of variance for effects of host plant species (*Asclepias speciosa*, *A. fascicularis*, *A. syriaca*, and *A. incarnata*), butterfly (*Danaus plexippus*) source population, and family nested within population on larval growth rate (measured as daily change in mass from first through fifth instar). The F-test denominator for the effect of population was mean square_{family(population)}; for effects of host plant and population*host plant, the F-test denominator used was mean square_{host \times family}

Source	S.S.	d.f.	F	P
Host plant	0.023	3	6.07	0.002
Population	0.001	1	0.26	0.620
Family (population)	0.080	14	4.35	0.000
Host*population	0.001	3	0.29	0.836
Host*family (population)	0.054	42	2.59	0.000
Error	0.212	426		

for only one or two generations on these two species in our laboratory prior to experimental tests, there would have been limited opportunity for laboratory adaptation to these two host plant species in a way that would fundamentally change the results of this study. However, we cannot rule out the possibility that larval experience with a natal host plant influenced the ovipositional preference of resulting females (e.g., reviewed in Davis & Stamps, 2004).

Many studies of herbivorous insects have reported variation in the strength of oviposition preference and larval development on different host species, including studies of Glanville fritillaries (Kuussaari et al., 2000), swallowtail butterflies (Bossart & Scriber, 1995; Wehling & Thompson, 1997), hairstreaks (Forister, 2004), and herbivorous beetles (Ueno et al., 2001; Ballabeni et al., 2003). Many of these studies have also demonstrated genetically based geographic variation in host use traits in a direction consistent with local adaptation for regionally abundant host plant species. This response to host-plant mediated selection should depend on several factors, including the geographic distribution of host plants and insect populations, the strength of selection relative to gene flow among insect populations, and the underlying genetic basis for host use traits.

Monarchs from eastern and western populations in North America showed similar host use traits, indicating that monarch preference and larval performance were not significantly higher on milkweed species native to their respective breeding ranges. The absence of significant interactions between monarch source populations and milkweed origins might result from a relatively recent establishment of these two populations, so that monarchs east and west of the Rocky Mountains might not have been separated long enough to accumulate genetic differences or adapt to local milkweed species. Indeed, mitochondrial DNA markers showed that geographically separated monarch populations in eastern and western North America were genetically similar to monarchs in the West Indies (Brower & Boyce, 1991; Brower & Jeansonne, 2004), providing evidence for a historical population bottleneck or recent geographic radiation. Results from another study of variation at several allozyme loci in eastern and western monarch populations similarly suggested a high gene flow or limited separation period (Shephard et al., 2002).

Although high gene flow between eastern and western monarchs could explain their similarity in host use traits, multiple studies have suggested that phenotypic shifts in other species can be quite rapid, even in the face of substantial inter-population dispersal (Reznick et al., 1997; Palumbi, 2001). Therefore, a more likely explanation for our failure to measure population differences in monarch host use traits could be an absence of selective pressures

for monarchs to specialize on locally abundant milkweed species. In fact, because of the monarchs' migratory behavior in eastern and western North America, each population (and possibly individual females) encounters several common milkweed species during a single breeding season (Malcolm & Brower, 1989). Because each milkweed species presents a unique profile of cardenolide compounds (Malcolm, 1991), flavonol glycosides (Haribal & Renwick, 1998b), leaf morphology and latex flow, a diversity of milkweed species within the geographic range of each population could eliminate any advantage conferred by specialization on any single host plant species. In the wild, monarchs lay eggs singly on the underside of leaves towards the tops of plants (Zalucki & Kitching, 1982), and monarch larvae have been observed on 27 *Asclepias* species in North America (Malcolm & Brower, 1986). Indeed, Chew (1975) noted that insect species that lay eggs singly and visit many different host species might be slow to evolve consistent preferences among hosts, and the ability to use multiple species should be advantageous in the face of temporal and spatial variation in host species occurrence.

In both of our oviposition and larval performance experiments, estimated family means for female preferences and larval development were highly varied, and in several cases the rank order of family means deviated substantially from the population-wide means. Thus, although monarchs are specialized milkweed herbivores, individual and family level responses to different milkweed species were highly variable. In both populations, the preference and performance of siblings on different host species was similar, and we observed a nearly significant effect of family on oviposition patterns and a highly significant interaction between family and host species on larval development. The similar host use traits of related monarchs and differences among family means could reflect a strong underlying genetic basis, or influences of maternal effects.

Although monarchs appear morphologically similar throughout most of their range, populations do not exist in uniform environments. Selection might operate on traits affecting migratory ability, performance on different host plant species, thermal tolerance, and resistance to natural enemies (Brower et al., 1995). In fact, for decades, scientists have hypothesized that monarchs from different regions were genetically distinct, yet almost no empirical studies have addressed this issue directly (but see Brower & Boyce, 1991; Altizer, 2001; Shephard et al., 2002; Brower & Jeansonne, 2004). In North America, the paucity of records of monarchs in the Great Basin or the Northern Rockies suggests that mixing between the eastern and western migratory populations occurs rarely, if at all (but see Pyle, 1999). In this study, we observed no significant differences

in oviposition behavior or larval performance between eastern and western monarchs, suggesting that even though monarchs east and west of the continental divide encounter only a subset of potential hosts within North America, they have not diverged in their responses to the four host species tested in this study. On the other hand, we observed a high degree of heterogeneity in host use traits within each population, and significant family level effects suggested an underlying genetic basis. This final observation is relevant to understanding the genetic composition and evolutionary potential of monarch populations, an issue that will become increasingly important for future conservation efforts in light of increasing threats that monarchs face from habitat loss and environmental change at their breeding and overwintering sites (Oberhauser et al., 2001; Brower et al., 2002; Oberhauser & Peterson, 2003).

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