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Host plant adaptation during contemporary range expansion in the monarch butterfly

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Herbivores that have recently expanded their host plant ranges provide opportunities to test hypotheses about the evolution of host plant specialization. Here, we take advantage of the contemporary global range expansion of the monarch butterfly (*Danaus plexippus*) and conduct a reciprocal rearing experiment involving monarch populations with divergent host plant assemblages. Specifically, we ask the following questions: (1) Do geographically disparate populations of monarch butterflies show evidence for local adaptation to their host plants? If so, what processes contribute to this pattern? (2) How is dietary breadth related to performance across multiple host species in monarch populations? (3) Does the coefficient of variation in performance vary across sympatric versus allopatric hosts? We find evidence for local adaptation in larval growth rate and survival based on sympatric/allopatric contrasts. Migratory North American monarchs, which have comparatively broad host breadth, have higher mean performance than derived nonmigratory populations across all host plant species. Monarchs reared on their sympatric host plants show lower coefficient of variation in performance than monarchs reared on allopatric hosts. We focus our discussion on possible mechanisms contributing to local adaptation to novel host plants and potential explanations for the reduction in performance that we observed in derived monarch populations.

KEY WORDS: Diet breadth, herbivory, local adaptation, monarch butterfly, range expansion.

The vast majority of plant-feeding arthropod species exhibit narrow and highly specialized diets (Forister et al. 2015). Verbal and mathematical arguments often explain this restriction of dietary breadth in the context of cross-host performance tradeoffs (i.e., “the jack of all trades is the master of none” hypothesis) (Rausher 1984; Futuyma and Moreno 1988; Joshi and Thompson 1995). However, there is mixed emprical evidence to support this pattern. For instance, positive cross-host performance relationships—in which genotypes conferring a performance advantage on one host are postively associated with performance on another host—may be more common than performance tradeoffs (Futuyma and Philippi 1987; Fry 1996; Agosta and Klemens 2009; Rasmann and Agrawal 2011; García-Robledo and Horvtiz 2012; Forister et al. 2012).

Performance tradeoffs are also central to hypotheses about local adaptation in populations of arthropod herbivores (Karban 1989; Via 1991; Agrawal 2000). Numerous studies have found evidence for local adaptation in plant–arthropod interactions, and in some cases local adaptation has evolved over as few as three generations (Karban 1989). However, many of these studies focus on relatively sedentary species such as wingless thrips (Karban 1989), spider mites (Agrawal 2000; Magalhães et al. 2007, 2009), scale insects (Hanks and Denno 1994), or aphids (Via 1991). Definitions of local adaptation also differ between studies: some interpret GxE interactions broadly as evidence for local adaptation (Cogni and Futuyma 2009), whereas others use more specific criteria (e.g., “home vs. away,” “local vs. foreign,” or “sympatric vs. allopatric” definitions of local adaptation) (see Kawecki and Ebert 2004; Blanquart et al. 2013). Finally, even in cases where local adaptation is identified, many studies do not attempt to distinguish underlying mechanisms generating this pattern. Thus, we still have limited understanding for whether local adaptation to host plants in arthropod herbivores is driven primarily by genotypic tradeoffs across hosts (i.e., antagonistic pleiotropy) versus genotypes that are beneficial on one host but selectively neutral on other hosts (i.e., conditional neutrality) (but see Gompert et al. 2015; Gompert and Messina 2016).

Herbivorous insects that have recently expanded their geographic and/or host plant range provide valuable opportunities to address questions about the evolution of host plant adaptation and specialization (Feder et al. 1988; Carroll et al. 2005; Jahner et al. 2011; Bean et al. 2012; Gompert et al. 2015), especially in cases where the introduction history is well known. In their newly established range, herbivorous insects may adapt to novel species of host plants not encountered in the ancestral range (Louda et al. 1997; Van Klinken and Edwards 2002; Erbilgin et al. 2014), a more restricted set of hosts (either novel or ancestral) (Pateman et al. 2012), or an expanded range of host species that are ancestral, novel, or a mixture of both (Singer et al. 1993; Graves and Shapiro 2003). These scenarios result in different sets of predictions for how adaptation will proceed.

In instances where dietary breadth is reduced during range expansion, directional selection may increase performance on the subset of available host plants. This adaptation may come at a cost in performance on absent ancestral hosts, especially if there are strong cross-host performance tradeoffs (antagonistic pleiotropy). Tradeoffs are expected to be more likely if novel hosts are phylogenetically and chemically disparate from ancestral hosts (e.g., Pearse and Hipp 2009; Bertheau et al. 2010; Rasmann and Agrawal 2011). Even in the absence of strong cross-host tradeoffs, herbivores that become specialized on novel hosts can show decreased performance on ancestral hosts (Grosman et al. 2015; Gompert et al. 2015). Potential reasons for this reduction in performance on ancestral hosts can include (1) stochastic accumulation of mutations that are neutral on the novel host but compromise performance on ancestral hosts (Kawecki 1994; Kawecki 1997), (2) genetic drift driving loss of alleles that were beneficial on ancestral hosts but are selectively neutral on novel hosts (i.e., relaxed selection), (3) ongoing co-evolution between ancestral hosts and herbivores that increases ancestral host plant resistance (Bergelson et al. 2001).

By contrast, in instances where herbivores maintain broad dietary breath, we predict that balancing selection should maintain additive genetic variation for fitness across hosts (Joshi and Thompson 1995; Gloss et al. 2016). This prediction relies on the assumption that genotypes show cross-host tradeoffs and is a specific expectation of the more general pattern that spatial and temporal heterogeneity act to maintain genetic variation for fitness across environments (Gillespie and Turelli 1989; Kassen 2002; Bergland et al. 2014; Chakraborty and Fry 2016). We also expect dietary generalists to have larger effective population sizes (Packer et al. 2005; Li et al. 2014), which should counteract the effects of genetic drift and help to maintain genetic variation for performance across hosts (Whitlock 1996). Thus, on average, we expect higher mean performance across host species in herbivore populations with broader dietary breadth. However, in practice, when we observe poor performance by a relatively specialized herbivore on alternative host plants, it is difficult to disentangle the relative contributions of selective processes associated with dietary specialization versus demographic processes associated with the colonization of a new geographic region where the novel host plant occurs.

Several hypotheses involving host plant adaptation have been put forward based on mean performance across hosts, but we also predict that variance in performance should be lower for demes/populations reared on sympatric compared to allopatric hosts. Here, we are explicitly interested in the coefficient of variation in performance, which is independent of mean performance and can be a proxy for additive genetic variation (Houle 1992). Demes reared on novel/allopatric hosts could have high variation in performance, possibly due to expression of cryptic genetic variation (Schlichting 2008) or inappropriate expression of canalized responses under novel environments (Van Buskirk and Steiner 2009). For example, the classic experiments of Clausen et al. (1940) showed that high elevation clones of *Potentilla glandulosa* grown at sea level had much more variation in stem height and flowering time than clones grown at their elevation of collection, and *Lasthenia fremontii* genotypes showed broader hydrological niche breadths when exposed to novel low-competition environments (Emery and Ackerly 2014). This hypothesis is rarely evaluated in plant-herbivore systems, although some studies have found support for increased performance variation in herbivores on novel hosts in artificial selection experiments (e.g., Kawecki 1995, Magalhães et al. 2007).

We tested these predictions in the monarch butterfly (*Danaus plexippus*, Danaidae: Danainae, L.), which has dramatically expanded its geographic range over the last 180 years (Fig. 1). Although best known from its ancestral range in North America, the monarch can now be found in locations around the world, where it forms geographically isolated, generally nonmigratory, year-round breeding populations. In most cases, establishment dates for these populations are well documented, with a wave of out-of-North America expansion taking place over the last ~180 years (Vane-Wright 1993; Zalucki and Clarke 2004). The monarch's introduction dates and population genetics are consistent with a natural wave of expansion (Pierce et al. 2014a; Zhan et al. 2014). The timing of this expansion likely coincided with human introduction of milkweed host plants, some of which are evolutionarily novel host species for monarchs (see "Background" section).

Here, we use the monarch's recent global range expansion to understand patterns of host plant adaptation. We use the sympatric/allopatric definition of local adaptation, because its

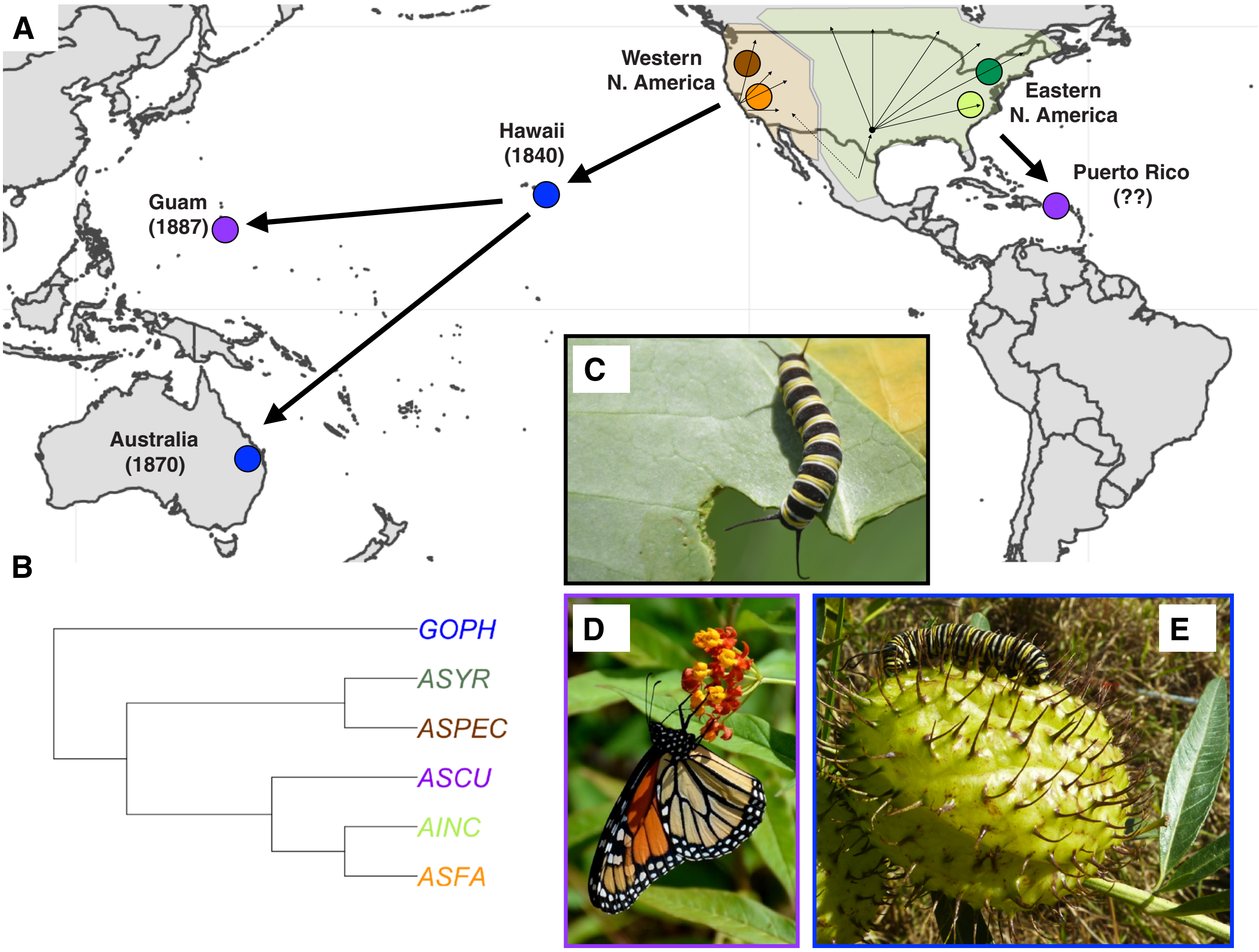

Figure 1. (A) Host plant associations of monarch butterfly populations around the world. Arrows correspond to routes of establishment for monarchs, with numbers in parantheses showing the earliest records of monarchs from a particular location (Zalucki and Clarke 2004). Monarchs separately colonized the Pacific and the Caribbean from North America (Zhan et al. 2014), with establishment in Puerto Rico likely occuring longer ago than for Pacific populations. Colored dots correspond to primary host plant associations for each monarch population. Note that we only display the species used in this experiment and that host plant associations are more extensive than those shown here (see Table S1). (B) Cladogram of relationships among milkweed species pruned from the phylogeny provided in Agrawal and Fishbein (2008) (also see Supporting Information Appendix 1). (C) Monarch caterpillar feeding on *C. procera* in Puerto Rico. (D) Adult monarch necatring on *A. curassavica* in Guam. (E) Monarch caterpillar feeding on fruit of *G. physocarpus* in Queensland, Australia.

measurement is independent of variation in quality of host plants or herbivore demes (Blanquart et al. 2013). We make the following predictions about monarch performance (see Table 1): (1) Monarchs will show evidence for local adaptation, with higher performance in sympatric compared to allopatric combinations. (2) North American monarch populations, which have broader dietary breadth, will have higher mean performance across host plants than monarch populations with more specialized diets. (3) The coefficient of variation in performance will be lower in sympatric than in allopatric combinations. We test these predictions using a common garden approach by raising monarchs from different populations on a variety of ancestral and novel host plant species.

Methods

BACKGROUND

Timing of range expansion

Monarchs became established on many Pacific Islands beginning approximately 180 years ago, with the first conclusive records coming from Hawaii in 1841 (Vane-Wright 1993). They then moved southwestward across the Pacific in a stepwise fashion, eventually becoming established in Australia in 1871 (Vane-Wright 1993; Zalucki and Clarke 2004). Another population became established in the Mariana Islands (including Guam), with the first records coming from 1887 (pers. obs., British Natural History Museum); the Guam population represents an

Table 1. General predictions pertaining to local adaptation and evolution of specialization, and specific predictions for our study.

General prediction	Specific prediction	Statistical evidence that would support prediction
1. Herbivorous insects will be locally adapted to their host plants.	Monarch populations around the world will have higher mean performance on sympatric milkweed species than on allopatric milkweed species.	Higher mean performance in sympatric versus allopatric combinations
2. Generalist genotypes will have higher mean performance across environments than specialist genotypes.	Ancestral North American monarch populations, which have relatively broad host plant breadth, will have higher mean performance across hosts than derived monarch populations, which have narrow host plant breadth.	Higher mean performance in ancestral North American populations than in derived populations
3. Organisms transplanted outside of their realized niche will have higher variation in fitness than organisms within their realized niche.	Monarch populations reared on allopatric host plants will have higher variation in performance than monarchs reared on sympatric hosts.	Lower coefficient of variation in performance in sympatric versus allopatric combinations

independent out-of-Hawaii expansion (M. Freedman, unpubl. data). Finally, in a separate out-of-North America expansion, monarchs established in the Caribbean, including Puerto Rico (Zhan et al. 2014). The timing of the monarch's establishment in Puerto Rico is less certain but likely occurred thousands of years ago (Fig. 1). Puerto Rican monarchs are treated as their own subspecies, *D. plexippus portoricensis* (Ackery and Vane-Wright 1984) and are phenotypically distinctive from North American and Pacific island *D. plexippus plexippus*.

The population genetic and demographic consequences of this range expansion are not well understood at the level of individual monarch populations, but some information from previous studies is available for some populations. Zhan et al. (2014) provide estimates of genome-wide heterozygosity for populations from North America, Hawaii, Australia, and Puerto Rico, although these estimates are generally based on only three individuals per population. Likewise, Pierce et al. (2014a) report allelic richness based on a set of 11 microsatellite markers for a larger sample of individuals from the same populations. Both studies suggest reduced population genetic diversity in expansion populations attributable to serial stepwise dispersal, with approximately a twofold reduction in genome-wide heterozygosity in nonmigratory Pacific and Caribbean populations relative to the ancestral North American population. Zhan et al. (2014) also used demographic simulations to estimate effective population sizes (*Ne*) for pooled samples from expansion populations. These results suggest a very large ancestral North American *Ne* ($\sim 2 \times 10^6$), with somewhat reduced but still large *Ne* in the Pacific (2×10^5–2×10^6) and a smaller *Ne* for the expansion containing the Puerto Rico population (1×10^5–4×10^5). Neither Zhan et al. (2014) nor Pierce et al. (2014a) included samples from Guam.

Host plant associations in novel range

Monarchs that colonize novel areas like tropical islands become nonmigratory and typically have access to only one or two species of milkweed host. This is in contrast to the ancestral North American monarch population, whose seasonal migration brings it into contact with more than 100 species of native *Asclepias* (Apocynaceae: Asclepiadoideae) hosts (Woodson 1954), at least 34 of which have been documented as suitable host plants (Malcolm and Brower 1986). Derived nonmigratory monarch populations are often exposed to evolutionarily and chemically novel host plants. For example, the monarch's primary host plants in parts of Australia are *Gomphocarpus physocarpus* and *Gomphocarpus fruticosus* (Oyeyele and Zalucki 1990), and the primary hosts in the Hawaiian islands are *G. physocarpus* and *Calotropis gigantea* (Pierce et al. 2014b) (Fig. 1; Table 2). All of these non-*Asclepias* hosts are native to subtropical Africa and India and have only recently become established outside of these areas. Many derived nonmigratory monarch populations are also associated with tropical milkweed (*Asclepias curassavica* L.); the geographic origin of this species is uncertain but is believed to be in Central or South America (Woodson 1954).

HOST PLANT PROPAGATION

Seeds of six species of milkweed were collected with permits from around the world between 2015 and 2018. Milkweed species were chosen in accordance with (1) their prevalence as host plants for monarchs from each region and (2) to maximize representation within the milkweed phylogeny (see Agrawal and Fishbein 2008; Supporting Information Appendix 1). When possible, seeds were collected by fruit, which ensures full-sib relatedness among fruit-mates due to their pollination biology (Wyatt and Broyles 1994). Seeds were stored either at room temperature or were cold

Table 2. Summary of monarch populations and host plant species used in experiment. Abbreviations are used in figures for brevity.

Monarch population	Population abbreviation	Sympatric milkweed species used in experiment	Milkweed abbreviation
Eastern North America	ENA	*Asclepias syriaca*	ASYR
		Asclepias incarnata	AINC
Western North America	CA	*Asclepias speciosa*	ASPEC
		Asclepias fascicularis	ASFA
Hawaii	HI	*Gomphocarpus physocarpus*	GOPH
Guam	GU	*Asclepias curassavica*	ASCU
Australia	AU	*Gomphocarpus physocarpus*	GOPH
Puerto Rico	PR	*Asclepias curassavica*	ASCU

stratified, depending on their germination requirements. In 2017, we used only four milkweed species (*Asclepias fascicularis* [ASFA—Western North America], *Asclepias syriaca* [ASYR—Eastern North America], *A. curassavica* [ASCU—Guam], *Gomphocarpus physocarpus* [GOPH—Australia and Hawaii]); in 2018, we grew the same four species as well as two additional species (*Asclepias speciosa* [ASPEC—Western North America], *Asclepias incarnata* [AINC—Eastern North America]) (Table S2).

Beginning in February (2017) and March (2018) through September, plants were grown from seed and transplanted into 1 gallon plastic pots in UC Soil Mix media, in two greenhouses. Large pots and fertilization produced large plants capable of supporting multiple caterpillars, a situation sometimes encountered in the field (pers. obs.). Plants ($N = 634$ total across years) were grown in a completely randomized design under ambient light (long days) and at 28°C in the same two greenhouses. Approximately one quarter of plants (157/634) were used in multiple feeding trials during the experiment because of limited sample sizes in some species; in these cases, we waited at least three weeks before applying new caterpillars to a plant that had already been used in a feeding trial. Plant ID was included as a random effect in all analyses to account for position and prior feeding effects (see below).

Across both years, plants were subject to low levels of common greenhouse pests, particularly flower thrips, green peach aphids, spider mites, and whiteflies. In 2017, oleander aphids (*Aphis nerii*) became established in both greenhouses. To control these aphids, in early June, plants were submerged in a dilute solution of water with castile soap (Dr. Bronners) to dislodge aphids, and then promptly rinsed.

MONARCH BUTTERFLY COLLECTION

Monarchs from 16 sites were collected with permits as live adult females from their respective locations and transported to Davis, CA in glassine envelopes (Table S3). In some cases, adults could not be collected in sufficient numbers, and so larvae were collected and reared to eclosion instead. In these cases, larvae were collected over a broad spatial (i.e., separate plants and separate sites) and temporal range (i.e., different developmental stages) to minimize the chance of sampling full or half sibs. Monarchs were kept alive as adults in glassine envelopes and fed a 5:1 water: honey mixture daily. For adult butterflies reared from larvae, we used hand-pairing to achieve mating within populations (Clarke and Sheppard 1956), with care taken to minimize the chance of crosses between potential sibs (Mongue et al. 2016). Field-collected adult females used for oviposition were sometimes infected with the protozoan parasite *Ophryocystis elektroschirrha* (OE) (Table S3), with infection rates generally reflecting those that occur in naturally migratory and nonmigratory populations (Altizer et al. 2000). In 2017, we used butterflies from four populations (eastern North America [ENA], western North America [CA], Hawaii [HI], Australia [AU]); in 2018, we used the same four populations as well as two additional populations (Guam [GU] and Puerto Rico [PR]) (Table S3).

Adult females were set up in oviposition cages with *A. curassavica* for ~24 hours to produce eggs that were used in the experiment, and females typically produced 20–100 eggs per 24 hours. Eggs were collected at the end of each 24 hour period and transferred to labeled petri dishes with a damp paper towel and a small number of *A. curassavica* leaves. These petri dishes were then stored either in the greenhouse or a lab benchtop, with water added as necessary to prevent leaves from drying out. As soon as the eggs reached the "black head" stage (Zalucki et al. 2001), petri dishes were checked every 12 hours for emergence, and within 24 hours of hatching, neonates were transferred onto their respective experimental host plants.

EXPERIMENTAL DESIGN AND DATA COLLECTION

We reared caterpillars in a fully factorial design, with all monarch populations reared on all potential host species. When possible, we further stratified this design across individual maternal families. Because of the logistical challenges associated with having all populations developing concurrently, the experiment was carried out over the course of approximately three months in both 2017 and 2018. Because host plants were also continuously growing and potentially changing in condition during this time frame, we reared at least one monarch population over the duration of the experiment in both years in an attempt to account for possible temporal effects (see Fig. S1) and also included a model term for plant age in all statistical analyses (see below).

As soon as neonates hatched, they were randomly assigned to an individual plant. We placed between one and five neonates per plant depending on their availability, although 85% of plants received the full complement of five neonates (see Table S4). Neonates were transferred with a fine paintbrush onto newly expanded leaves at the top of each plant. Plants were then fully enclosed using custom-made polyester Super-Aire^{TM} sleeves (A-Roo LLC), with the bottom end sealed using a metal twist tie and the top sealed with binder clips (see Fig. S2). In total, we set up approximately 4000 neonate caterpillars over the course of the experiment.

Neonates were left to grow for eight days and then scored for survival and weighed; almost all larval mortality in monarchs occurs within this window (Zalucki and Malcolm 1999; Zalucki et al. 2001). Any surviving larvae that could be found were put into petri dishes and weighed to the nearest milligram. Larvae that could not be found were assumed to have died during their early development; in some cases, we found the remains of first instar caterpillars (Fig. S2). If plants were large enough, all surviving larvae were returned. If not, we used a random number generator to select which larvae to return to the plant.

After caterpillars reached their fifth and final instar, plants were checked daily to capture dates of pupation. Pupae were transferred into individually labeled 16 oz containers; on the day of eclosion, adult butterflies were kept in the container in which they eclosed for 6–8 hours to allow their wings to dry, at which point they were transferred into a glassine envelope. We recorded the mass of these newly eclosed adults and later measured forewing length, width, area, aspect ratio, and roundness, as well as hindwing area (for methods, see Freedman and Dingle 2018) and levels of adult cardenolide sequestration.

In total, we analyzed the following performance metrics: (1) larval survival and (2) mass on day eight, (3) time to pupation, (4) time to eclosion, (5) mass at eclosion, (6) adult wing morphological characteristics, and (7) adult cardenolide sequestration, although we focus only on metrics 1–5 in this article. Larval survival has clear fitness implications. Mass on day eight, time to pupation, and time to eclosion are all related to development rate and were highly correlated with each other. We expect for natural selection to favor faster development rates, as this shortens the window when larvae are most susceptible to abiotic (heat, cold, and rainfall) and biotic (predators, parasitoids, and pathogens) sources of mortality. Eclosion mass is expected to be related to lifetime fecundity in monarchs (Oberhauser 1997), although we did not directly assess this.

PLANT TRAIT SAMPLING

We sampled two plant defense traits to see if they could explain variation in performance. We measured latex production and cardenolide concentration, because these have been shown to be important determinants of larval monarch growth and survival (Zalucki and Kitching 1982; Zalucki et al. 2001; Agrawal et al. 2015). Immediately prior to adding neonate caterpillars, most plants ($n = 565/634$) were sampled for constitutive levels of latex production. We also collected leaf discs to measure constitutive levels of cardenolide production for 200 plants using the methods outlined in Zehnder and Hunter (2007). For full details on latex and cardenolide sampling, see Supporting Information Appendix 2.

STATISTICAL ANALYSIS

Prediction 1: Monarchs have better performance on sympatric compared to allopatric hosts

To quantify local adaptation, we use the sympatric/allopatric approach outlined in Blanquart et al. (2013). A sympatric combination refers to larvae of populations grown on host plants naturally encountered at the local site; conversely, any monarch population on nonfamiliar hosts is treated as allopatric (Fig. S3). Briefly, this approach models the residual variation remaining after accounting for inherent differences in quality between host plant species and monarch populations, with the difference in mean performance on sympatric hosts versus allopatric hosts signifying the magnitude of the local adaptation effect. Here, a significant positive effect of sympatric status suggests that monarch populations exhibit a pattern of local adaptation to their host plants. Using sympatric/allopatric contrasts has the advantage of being independent of inherent variation in performance observed in specific environments (host plants) by specific demes (monarch populations) and also in providing a single measure of the degree to which genotypic composition fits local environmental conditions (Blanquart et al. 2013).

We fit linear and generalized linear mixed effects models that included fixed effects for milkweed species and monarch population of origin and a term for sympatric/allopatric status. Models were fit using the lme4 package version 1.1.21 (Bates et al. 2015) in R version 3.4.4 (R Core Team 2018). In all models, we included the following terms as covariates: greenhouse, plant usage (first or second exposure to caterpillar feeding), plant age, and experiment year. In models using performance information from adult butterflies, we further included infection status with the parasite OE and butterfly sex as covariates. In all models, we included random intercepts for plant ID nested within plant maternal family of origin, monarch maternal family, and greenhouse block. For a full summary of statistical models used, see Table S5. In brief, models assessing local adaptation were written according to:

$$\text{response} \sim \text{milkweed spp.} + \text{mon. pop.} + \text{sym. vs. allo.}$$
$$+ \text{covariates} + \text{random effects}$$

For all continuous performance metrics (larval mass at day eight, days to pupation, days to eclosion, mass at eclosion, and adult wing morphological measures), we fit models with Gaussian error distributions. In the model using larval mass at day eight as a response variable, we natural log transformed this measure to account for the nonlinear accumulation of larval mass through caterpillar development. For ease of interpretation, we report larval mass results using back-transformed values, which therefore represent the geometic rather than the arithmetic mean. Because time to pupation and time to eclosion were almost perfectly correlated ($R^2 = 0.945$), we only report results for time to eclosion. For larval survival to day eight, we fit a model with a binomial error distribution. All results were summarized using type II analysis of variance in the package "car" version 3.0.2 (Fox and Weisberg 2011). We also calculated marginal means in the package "emmeans" version 1.3.4 (Lenth 2019) and report these throughout the text. Pairwise comparisons between milkweed species and monarch populations were made using posthoc TukeyHSD tests with the glht function in the package "multcomp" version 1.4.7 (Hothorn et al. 2008).

Prediction 2: Monarch populations with broader dietary breadth have higher mean performance across hosts

To answer this question, we used exactly the same model structure as above. However, instead of treating each monarch population separately, we grouped populations according to whether they are ancestral (eastern and western North America) or derived (Hawaii, Guam, Australia, Puerto Rico). Ancestral populations have broad dietary breadth, whereas derived populations have narrow dietary breadth. These models were of the form:

$$\text{response} \sim \text{milkweed spp.} + \text{ancestral vs. derived}$$
$$+ \text{sympatric vs. allopatric} + \text{covariates} + \text{random effects}$$

We found an effect of ancestral versus derived status for monarch populations (see "Results" section). Because derived populations have restricted host plant breadth, one expectation could be that selection would optimize performance on their local sympatric host, but at a cost to performance on all other hosts. To test this hypothesis, we conducted an additional analysis that specifically assessed the interaction between the ancestral versus derived status of monarch populations and the sympatric/allopatric status of their host plants. Here, a significant interaction between ancestral/derived status of monarch populations and sympatric/allopatric status would suggest that the magnitude of local adaptation differs between ancestral and derived monarch populations. In this analysis, we treated milkweed species and monarch population as random effects.

Prediction 3: Variation in performance is lower in sympatric host plant × monarch population combinations

To analyze variance in performance, we calculated the coefficient of variation ($\text{CV} = \sigma^2/\mu$) in performance for each maternal family × plant genotype combination ($n = 717$). Measuring CV at the level of individual plants provides us with a measure of how variable larval performance is within a given maternal family on a single host plant. Here, any plants with only a single neonate added ($n = 5$) or with no surviving larvae at day eight ($n = 24$) were excluded from analysis, because no standard deviation of performance could be calculated for these plants. We then treated the remaining 688 plant-level coefficients of variation as response variables and used the same statistical framework as described above to determine how host plant species, monarch population, and sympatric/allopatric status affected variation in performance.

Here, we focus only on larval mass at day eight as a metric for measuring variation in performance, because this was the only continuous measure for which we had sufficient sample sizes to measure plant-level CVs. In contrast to analyses of mean larval performance, for which we separately analyzed larval mass and larval survival, here we assigned larvae that did not survive a mass of 0 and included these 0 values when calculating CVs. We note that including zero values increases estimates of plant-level CV. Ideally, we would have been able to record the mass of non-surviving larvae at the time of their death, but since most larval monarch mortality occurs in the first 24–72 hours when caterpillar mass is <10 mg, assigning these individuals a mass of 0 should have a relatively small effect on estimates of CV. To test whether the results of this analysis were sensitive to the number of maternal families included, we conducted randomization tests that enforced an equal number of families in each population (Supporting Information Appendix 3). Pairwise comparisons between milkweed species and monarch populations reflect Tukey's HSD tests.

Results

In this section, we first present the main effects of milkweed host plant identity and monarch population identity on performance. We then present the results for our specific predictions.

MAIN EFFECTS OF HOST PLANT SPECIES AND MONARCH POPULATION STATUS

Host plant identity was by far the biggest source of variation in larval monarch performance ($\chi^2 = 69.65$, DF = 5, $P < 0.001$) (Fig. S4), with a threefold mass difference between *A. curassavica* ($\bar{x}$ = 343 mg) and *A. speciosa* ($\bar{x}$ = 102 mg) (Fig. 3A, panel i; Fig. S4A). Milkweed species also explained the largest proportion of variation in survival ($\chi^2 = 23.12$, DF = 5, $P < 0.001$), with highest survival on *A. incarnata* (85.3%) and lowest survival on *A. speciosa* (68.8%) (Fig. S4C). As with larval mass on day eight, milkweed species explained the largest proportion of variation in time to eclosion ($\chi^2 = 62.71$, DF = 5, $P < 0.001$), with time to eclosion fastest on *A. curassavica* ($\bar{x} = 21.4$ days) and slowest on *A. speciosa* ($\bar{x} = 24.5$ days) (Fig. 3C, panel i; Fig. S4E). Milkweed host species was not a strong predictor of eclosion mass ($\chi^2 = 8.98$, DF = 5, $P = 0.110$) (Fig. S4H).

The host plant species in this experiment may encompass up to 40–50 MY of divergence (note: this estimate is uncertain, see Fishbein et al. 2011) and employ disparate defense strategies against larval monarchs. For example, our host species differed more than 20-fold in latex production and 50-fold in cardenolide production (Fig. 2). In general, larval performance was lowest on milkweed species with high latex production. All populations performed worst on *A. speciosa*, which also had the highest latex production of the species tested. In contrast, monarchs tended to perform well on milkweed species with high cardenolide concentrations (*A. curassavica* and *G. physocarpus*) (Fig. 2).

Monarch population also explained a substantial portion of variation in day eight larval mass ($\chi^2 = 32.51$, DF = 5, $P < 0.001$), with the highest mass in monarchs from eastern North America ($\bar{x}$ = 320 mg) and lowest mass in monarchs from Puerto Rico ($\bar{x} = 123.7$ mg) (Fig. 3a, Fig. S4B). Differences in survival among populations were more modest ($\chi^2 = 12.17$, DF = 5, $P = 0.033$) (Fig. 3B, panel i; Fig. S4D). Monarch populations also differed in time to eclosion ($\chi^2 = 30.80$, DF = 5, $P < 0.001$), with eclosion occurring fastest for eastern North America ($\bar{x} = 21.5$ days) and slowest for Puerto Rico ($\bar{x} = 23.7$ days) (Fig. 3C, panel i; Fig. S4f). We did not find strong differences among populations in eclosion mass ($\chi^2 = 9.83$, DF = 5, $P = 0.080$) (Fig. S4g).

Prediction 1: Monarchs have better performance on sympatric compared to allopatric hosts

For performance metrics associated with larval growth rate and survival, monarchs exhibited a pattern of local adaptation, indicated by significantly higher performance on sympatric compared to allopatric host plants (Fig. 3A–D, panel ii). This effect was detectable despite substantial variation in performance owing to inherent differences among host plants and monarch populations (Fig. S4).

For larval mass on day eight, monarchs reared on their sympatric hosts ($\bar{x}$ = 237 mg) were over 16% larger than monarchs reared on their allopatric hosts ($\bar{x}$ = 198 mg) ($\chi^2 = 15.74$, DF = 1, $P < 0.001$) (Fig. 3A, panel ii). Monarch populations from Australia and Hawaii, which are associated with an evolutionarily novel host (*G. physocarpus*), had no absolute performance advantage on this host relative to populations naïve to this host (Fig. 3A, panel I). Survival on sympatric host plants was also higher (79.7%) than survival on allopatric hosts (75.7%) ($\chi^2 = 3.98$, DF = 1, $P = 0.046$) (Fig. 3B, panel ii). Time to eclosion was faster for monarchs reared on sympatric host plants ($\bar{x} = 22.5$ days) compared to monarchs on allopatric hosts ($\bar{x} = 22.8$ days) ($\chi^2 = 4.81$, DF = 1, $P = 0.028$) (Fig. 3C, panel ii). We did not find a signature of local adaptation for eclosion mass ($\chi^2 = 0.01$, DF = 1, $P > 0.9$; Fig. 3D, panel ii). In summary, early larval growth rate, survivorship, and development time were all better on sympatric hosts, whereas adult biomass showed no difference between sympatric and allopatric hosts.

Prediction 2: Ancestral North American monarchs will show higher mean performance across host species than derived populations

In comparing ancestral and derived populations as groups, we found that ancestral North American populations, which have broad dietary breadth, had significantly higher mean growth rates across all host species ($\chi^2 = 10.45$, DF = 1, $P = 0.001$) (Fig. 3A, panel iii). However, we did not find significantly higher overall survival in the ancestral North American populations ($\chi^2 = 1.41$, DF = 5, $P = 0.234$) (Fig. 3B, panel iii). Time to eclosion was faster in ancestral populations ($\chi^2 = 10.42$, DF = 1, $P = 0.001$) (Fig. 3C, panel iii), and ancestral populations tended to have higher eclosion mass than derived populations at eclosion ($\chi^2 = 2.78$, DF = 1, $P = 0.095$) (Fig. 3D, panel iii).

There was no significant interaction between ancestral/derived monarch populations grown on sympatric/allopatric host combinations. Derived populations performed 18.7% better on sympatric compared to allopatric hosts, whereas ancestral populations had a 13.0% advantage on their sympatric hosts. This interaction was not statistically significant ($\chi^2 = 0.36$, DF = 1, $P = 0.542$).

Prediction 3: Coefficient of variation in performance will be lower in sympatric combinations

Monarchs reared on their sympatric hosts had less variable mass on day eight than monarchs reared on their allopatric hosts ($\chi^2 = 4.53$, DF = 1, $P = 0.033$) (Fig. 4C). This result did not change if we used randomization tests to equalize the number of maternal families tested per monarch population (Supporting Information Appendix 3). Milkweed species was the strongest predictor of variation in performance ($\chi^2 = 24.77$, DF = 5, $P < 0.001$,

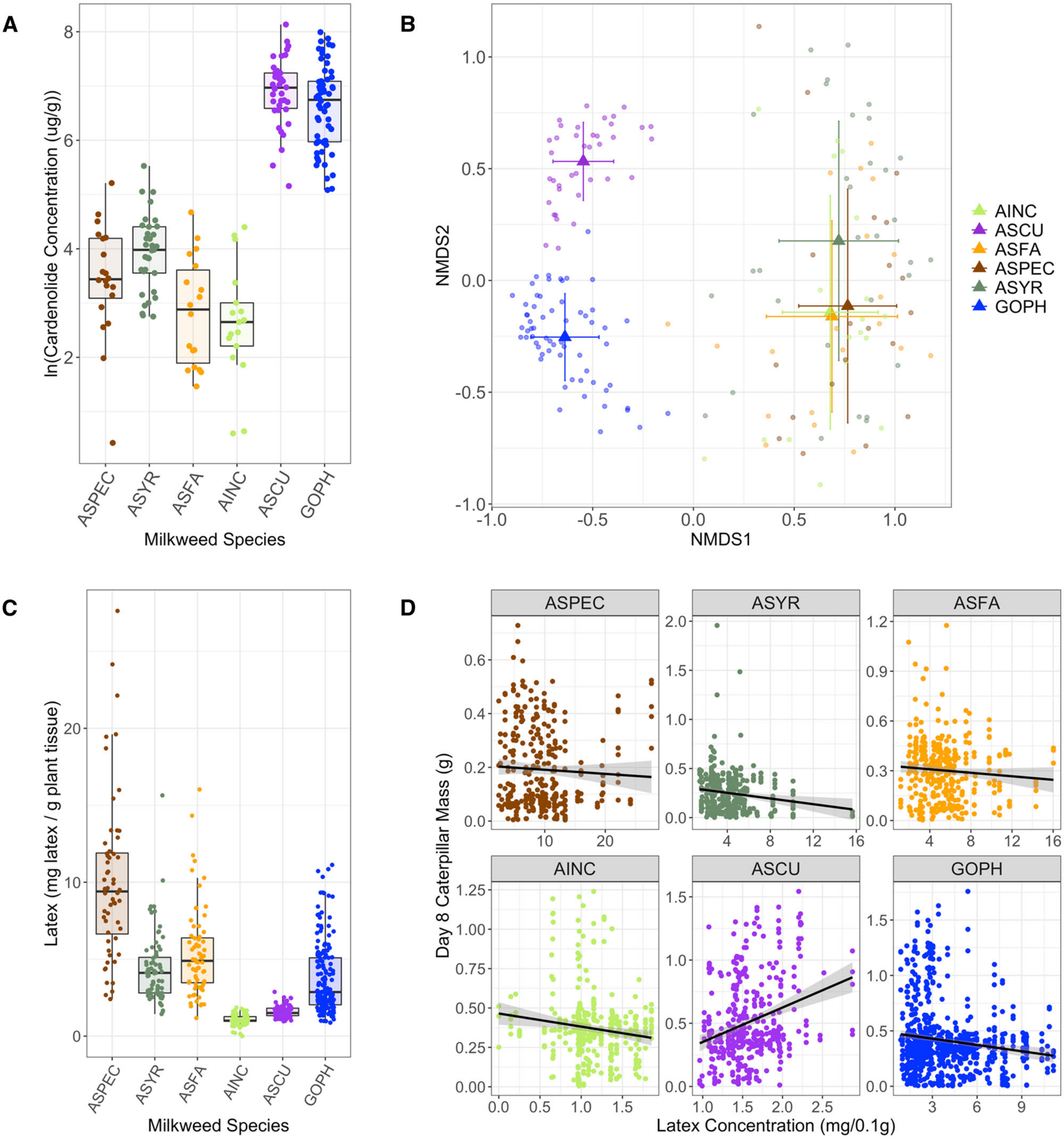

Figure 2. (a) Cardenolide concentrations for each of the six species tested. Note that concentrations are expressed as log(μg cardenolide/g dry leaf tissue). Mean concentrations ranged from as low as 0.23 mg/g in *A. incarnata* to as high as 12.12 mg/g in *A. curassavica*. (b) Nonmetric multidimensional scaling (NMDS) showing variation in the composition of cardenolides across milkweed species. (c) Latex production for each milkweed species, expressed in terms of milligrams of latex per gram of dry leaf tissue. (d) Regressions of day eight caterpillar mass on latex production across milkweed species. Note that both the *y*-axis and the *x*-axis differs for each species.

Fig. 4A). This result was driven by low variation in performance on *A. incarnata* (CV = 0.691) and *A. curassavica* (CV = 0.791) relative to other milkweed species, particularly *A. syriaca* (CV = 0.989). In contrast, monarch populations did not differ significantly in their CV ($\chi^2 = 7.79$, DF = 5, $P = 0.168$, Fig. 4B).

Discussion

Our first prediction was that monarch populations would show a pattern of local adaptation to their host plant assemblages around the world. We found evidence for local adaptation, with better larval performance (growth rate and survival) on sympatric

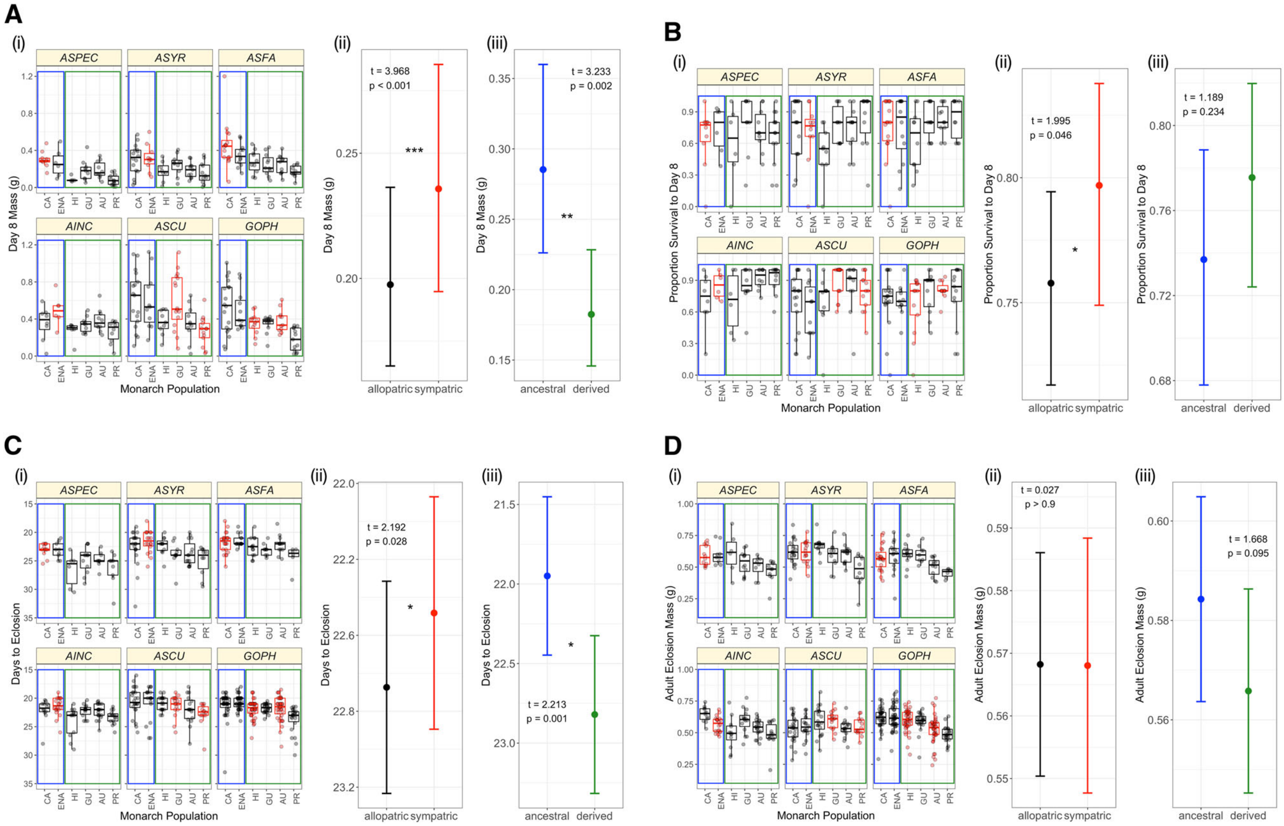

Figure 3. Summary of performance metrics separated by (A) larval mass on day eight, (B) larval survival on day eight, (C) the number of days to eclosion, and (D) mass at eclosion. In each figure, the left panel shows raw data for all monarch population × milkweed species combinations, with points corresponding to mean values for single maternal families. Boxplot color in panel (I) reflects whether combinations are sympatric (red) or allopatric (black). The center panel shows the average sympatric/allopatric effect, whereas the right panel shows the effect of coming from an ancestral (ENA, CA) versus derived (HI, GU, AU, PR) monarch population. Note that the axis for (C) is reversed so that fewer days to eclosion corresponds to higher performance. Panels (ii) and (iii) also show *t* values and associated *P* values for each allopatric/sympatric and ancestral/derived comparison.

compared to allopatric hosts. This result suggests that divergent selection pressures across the monarch's global range have resulted in local adaptation to host plant assemblages over contemporary time scales (~1000 generations). Our results highlight the utility of using the sympatric/allopatric approach for measuring local adaptation, especially for large, fully reciprocal study designs (Blanquart et al. 2013). Standard, more stringent metrics of local adaptation (home vs. away, local vs. foreign) would provide relatively low statistical power for this dataset, owing to the substantial inherent variation in quality among host plants and monarch populations.

Showing that sympatric performance is greater than allopatric performance provides evidence for a pattern of local adaptation, but does not explain the mechanisms underlying the process of local adaptation. One hypothesis for the prevalence of local adaptation in plant–herbivore interactions is cross-host performance tradeoffs (Rausher 1984; Futuyma and Moreno 1988). Although it is possible that genotypic tradeoffs contributed to the overall pattern of local adaptation, we found only modest evidence for such tradeoffs in this system. For example, monarch populations that specialize on evolutionarily novel hosts (monarchs from Hawaii and Australia on *G. physocarpus*) did not have a performance advantage over other naïve monarch populations on this host. Instead, our data revealed a pattern of positive cross-host relationships, with some genotypes consistently performing better than others across hosts. The performance rank order among monarch pouplations remained almost entirely constant across milkweed host species. This pattern is consistent with previous studies that have shown an inherent advantage of particular genotypes across environments (Fry 1996). Strongly contrasting reaction norms across environments are often considered diagnostic for showing local adaptation, but our data show that a local adaptation pattern can also emerge as long as sympatric combinations have a consistent advantage across contexts.

An alternative to the tradeoff hypothesis for the pattern of local adaptation we observed is decreased performance by

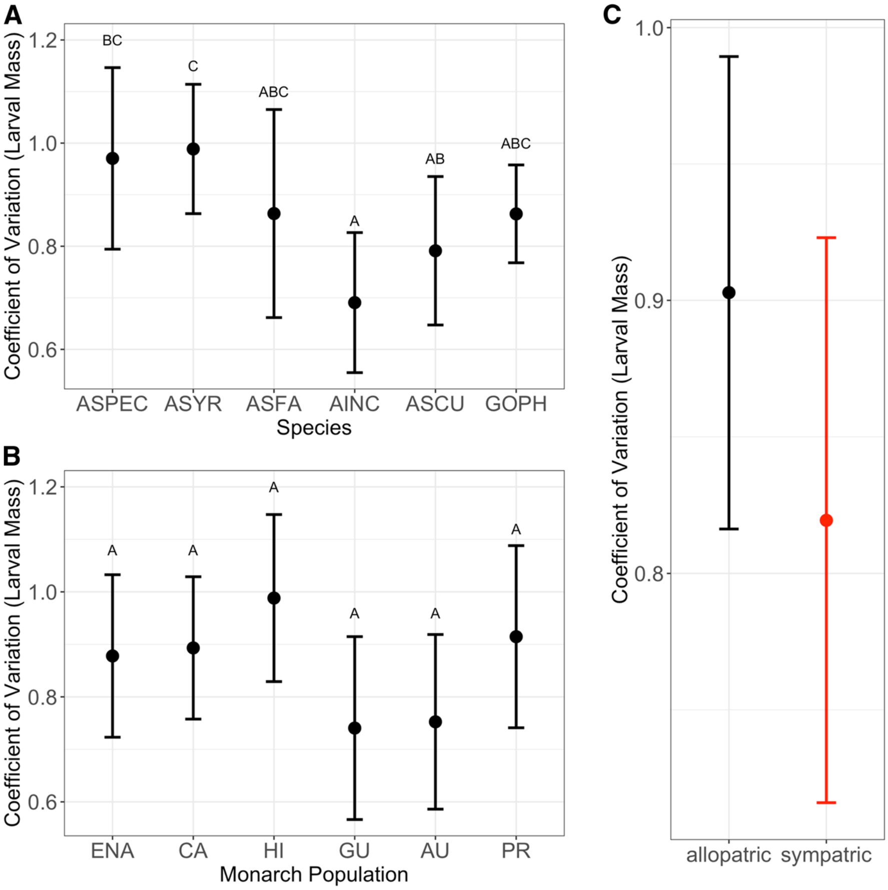

Figure 4. **Coefficients of variation in monarch performance across (A) milkweed hosts, (B) monarch populations, and (C) sympatric versus allopatric combinations. Letters correspond to significant differences after correction for multiple comparisons. Monarchs reared on sympatric host plants had significantly lower variation in performance than monarchs reared on allopatric hosts.**

derived populations on ancestral hosts (e.g., Grosman et al. 2015; Gompert et al. 2015), which could be attributed to either mutation accumulation, genetic drift driving the loss of variants associated with fitness on ancestral hosts, or ongoing coevolution that increases resistance in ancestral hosts. We did find that derived monarch populations showed slightly stronger reductions in larval mass on allopatric hosts (−18.3%) than did ancestral populations (−13.0%), although this pattern was not statistically significant. This pattern presents us with somewhat of a quandary for explaining the mechanism underlying local adaptation, as we are unable to attribute the observed sympatric/allopatric effect to either tradeoffs or loss of performance on ancestral hosts. Formally demonstrating whether the loci underlying local adaptation in this system are antagonistically pleiotropic or conditionally neutral would require a large-scale genome-wide survey of variants correlated with performance (Tiffin and Ross-Ibarra 2014; Gompert et al. 2015).

Our second prediction was that ancestral North American monarch populations would have higher mean performance across hosts, due to the reduced host plant breadth/increased specialization of derived populations. Monarch population identity was indeed a major source of variation in larval performance, with the ancestral North American populations consistently outperforming derived populations, regardless of host plant identity. Although this result matches our predictions, attributing this pattern exclusively to reduced host breadth in derived populations is difficult. First, host plant breadth is conflated with migratory status in this system: the ancestral North American populations migrate across the entire North American continent, whereas the derived populations, with perhaps the exception of Australia (James 1993), do not show evidence for long-distance migration. This pattern is reflective of a broader issue in studies of herbivore dietary breadth: generalist populations and species often have larger geographic ranges and population sizes (Janz and Nylin 2008; Jahner et al. 2011; Slove and Janz 2011). Second, all of our derived populations likely have reduced effective population sizes (Zhan et al. 2014) that render them more susceptible to the effects of both inbreeding depression and loss of additive genetic variation through drift. Thus, the reduced performance across hosts in derived populations with narrow host breadth could reflect a general loss of vigor driven by demographic processes, rather than a cost of specialization/narrow host breadth. The Puerto Rican monarch population potentially highlights the importance of historical demographic processes in driving performance variation: this population had by far the lowest overall mean performance across hosts, and it is also the population with the longest history of isolation.

One way to disentangle the contributions of dietary specialization versus demographic processes in driving patterns of performance variation in this system would be to survey the individual fitness effects associated with variable loci across the genome (Gompert et al. 2015; Gloss et al. 2016; Gompert and Messina 2016). This approach could shed light on whether alleles that show evidence for being maintained by balancing selection in generalist populations are indeed lost in specialist populations.

In contrast to larval performance metrics, we did not find evidence for local adaptation in eclosion mass. The absence of a local adaptation effect for this measure could reflect a developmental threshold pattern in monarch larvae, whereby pupation occurs after larvae have reached a predetermined size that is host plant invariant. The lack of difference in biomass is illustrative of a broader issue in studies of local adaptation, including those involving plant–herbivore systems: it is exceedingly difficult to measure every component of fitness across life history stages, particularly traits like lifetime fecundity (e.g., Scheirs et al. 2005). However, we do know that most monarch mortality occurs in the first 24–72 hours of larval development, when plant latex, cardenolides, and trichomes are most effective as defenses (Zalucki and Malcolm 1999; Zalucki et al. 2001).

We predicted that monarchs reared on their sympatric host plants would show reduced variation in performance relative to monarchs reared on allopatric hosts, and we did find this to be the case. This result is consistent with other studies that have found greater performance variation in herbivores reared on nonfamiliar hosts (Kawecki 1995; Magalhães et al. 2007) and potentially reflects a loss of genetic variation due to directional selection for optimized performance on commonly encountered host plants. We also found that the CV in performance was significantly different among milkweed species. Species-level differences in CV in performance were driven primarily by low levels of variation in *A. incarnata* and *A. curassavica*. These two species also happened to have the lowest latex production of any of the species tested. In contrast, the species with the highest latex production, *A. speciosa*, also resulted in the greatest performance CV. These results suggest that latex production may be important for explaining performance variation among monarch families, a result consistent with other studies showing that latex is the primary driver of larval mortality in monarchs (Zalucki et al. 2001).

Local adaptation to host plants is a central part of hypotheses about macroevolutionary patterns of specialization and speciation in herbivores (Janz and Nylin 2008). Our results provide an example of local adaptation developing over contemporary time scales in a highly mobile insect herbivore with a well-characterized range expansion history. Although the conditions that gave rise to this pattern may be somewhat restrictive due to the isolated nature of our island populations and their host plant assemblages, our results still demonstrate how the early stages of host plant specialization may proceed and highlight the value of collections and well-documented knowledge of range expansion history to understand adaptation.

AUTHOR CONTRIBUTIONS

MGF, SRR, and SYS designed the experiment. MGF and CJ performed the research and collected data. MGF analyzed the data. All authors contributed to writing and editing.

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Seeds for all milkweed plants grown during this experiment were collected and imported under USDA permit number P37-15-00580. Monarchs collected in the United States and its territories were imported under USDA permit P526P-18-02061, and monarchs collected in Australia were imported under USDA permit P526P-17-02696. Monarchs from Puerto Rico were collected under permit number O-VS-PVS15-SJ-00953-25082017 from the Departamento de Recursos Naturales y Ambientales. We thank the following people for assistance with collection and shipment of monarchs: Kelsey Fisher, Leslie Decker, Jaap de Roode, Ayse Tenger-Trolander, Louie Yang, Will Haines, Jacinta Zalucki, Riccardo Papa, Elizabeth Evans, and Yadira Ortiz Ruiz. We especially thank Rachel Vannette for assistance with conducting milkweed chemical measurements. The following people provided milkweed seeds used in this experiment: Jessica Aguilar, Leslie Decker, Amanda Meier, and Anurag Agrawal. We thank the following people for assistance with various aspects of the experiment: Asia Jones, Sue-Ling Choquette, Evan Jordan, Cielle Brown, Anika Hamilton, Moria Robinson, Nick Pardikes, Kyle Christie, and especially Jessica Aguilar. Funding was provided by an Explorer Grant from the National Geographic Society, the Society for the Study of Evolution, the NSF GRFP, the NSF EAPSI program (Award #1614052), and the UC Davis Center for Population Biology. The authors declare no conflicts of interest pertaining to this work.

DATA ARCHIVING

All data and code are archived using Github: https://github.com/micahfreedman and Dryad: https://doi.org/10.25338/B8BS5D.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Histograms showing distribution of when caterpillars were reared over the duration of the experiment.
Figure S2. Photos showing experimental setup.
Figure S3. Visual representation of experimental design.
Figure S4. Estimated marginal means of monarch performance averaged across milkweed species and monarch population for each of the four primary response variables.
Table S1. Monarch populations and milkweed species used in the current study, along with notes on other available host plant species for each monarch population.
Table S2. Summary of milkweed host plants used in experiment.
Table S3. Summary of monarch maternal families used in experiment.
Table S4. Breakdown of number of neonates added to each individual plant.
Table S5. Model averaged means for parameters of interest for (a) larval mass at day eight, (b) larval survival to day eight, (c) number of days to eclosion, (d) mass at eclosion, and (e) adult forewing area.
Table S6. Analyses for models associated with Prediction 2.
Table S7. Marginal means for models associated with Prediction 3.
Appendix 1. Phylogenetic reconstruction
Appendix 2. Methods for plant trait sampling
Appendix 3. Randomization tests