ELECTRONIC SUPPLEMENTARY MATERIAL

To accompany: Loss of migratory behaviour increases infection risk for a butterfly host (Satterfield, Maerz, and Altizer)

I. Historical occurrences of tropical milkweed and monarch winter-breeding

(a) Herbaria and record searches

We conducted an herbaria search and reviewed historical documents to better understand when the planting of tropical milkweed and monarch winter-breeding became more common in the southern U.S. Through the Index Herbariorium [1] and other web resources, we identified 175 distinct herbaria in the U.S. for which records were accessible online (in addition to the University of Georgia Herbarium), relative to an estimated 800 total herbaria nationwide [2]. We searched for records of *Asclepias curassavica* in the continental U.S. without date restrictions and eliminated duplicate specimen records. We performed a similar search for a native species, butterfly milkweed (*Asclepias tuberosa)*, to observe if any trends in tropical milkweed records were simply attributable to greater collector interest or awareness about milkweeds. Further, to survey distribution and gardening practices concerning tropical milkweed, we searched the Biodiversity Heritage Library (www.biodiversitylibrary.org) for occurrences of "*Asclepias curassavica*" in English-language documents from the U.S. Finally, we surveyed natural history literature on monarchs to understand when winter-breeding was previously documented in the southern U.S.

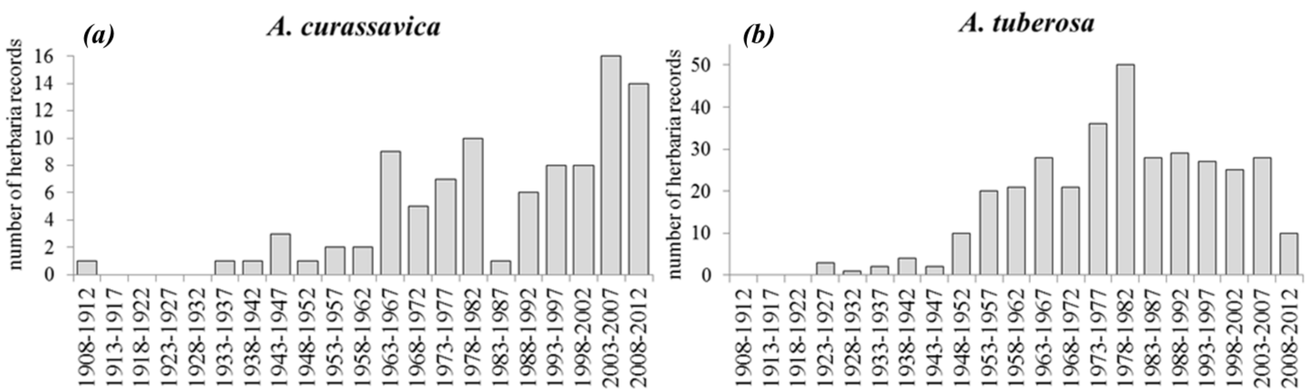
(b) Results

Eleven historical documents from the Biodiversity Heritage Library indicate that tropical milkweed was being planted in American gardens and hot-houses in the 19th century, as early as 1806 [3]. Articles from historical gardening journals and manuals discuss how to plant tropical milkweed (e.g., [4], dated 1898) and describe *A. curassavica* as "often cultivated for ornament in our Southern States" ([5], dated 1901), "worthy of a place in our gardens," (see [6] from 1890), and a "choice flowering annual adapted for sowing on a hot-bed" ([7] from 1841). An additional eight records note the distribution of tropical milkweed. By the dawn of the 20th century, tropical milkweed was described as occurring in the southern U.S. with a limited distribution (e.g., see [8] from 1897, [9] from 1912). In 1954, Woodson [10] noted its occurrence as "occasional ruderals" in southern California, Florida, Louisiana, and Texas.

The herbaria search showed that records of tropical milkweed have become more common in the southern U.S. in recent years. The search yielded 101 records of tropical milkweed; most records (n=72) were from Florida. Of these, records from south Florida (south of Sarasota, n=43) tended to have earlier collection dates (mean date=1978, range = 1936-2011) than those from north Florida (n=29; mean date = 1995, range=1960-2012). Other records came from Arizona (n=1), California (n=13 with mean date = 1986, range = 1909-2011), Connecticut (n=2), Louisiana (n=4), Mississippi (n=1), Missouri (n=2), South Carolina (n=5), and Utah (n=1). Examining temporal changes in these records across the southern half of the U.S. (n=96, including AZ, CA, LA, MS, SC, and FL), we found that few records existed before 1940 and a modest increase in records occurred over the last 50 years. Notably, a rise in records occurred in the 1960s and 1970s, followed by a sharper rise in the 2000s (Figure S1*a*). A similar temporal trend is observed when excluding south Florida, which was likely the first place in the U.S. to be colonized by tropical milkweed. The herbaria search of the native butterfly milkweed (*A. tuberosa*) yielded 349 records, primarily from South Carolina, Florida, and Alabama. As with tropical milkweed, records of butterfly milkweed were scarce before 1940 and increased in the 1960s. However, record frequency for native butterfly milkweed appears fairly constant in recent decades, with little change in number of specimens per decade since 1970 (Figure S1*b*).

A survey of historical scientific literature indicates that monarchs have used tropical milkweed during the regular breeding season for as long as it has been available, but that breeding behaviours during winter were not observed until later. For example, two early records note monarch caterpillars feeding on tropical milkweed during the spring and summer. The first mention of *A. curassavica* in the U.S. occurred in a Georgia natural history book published in 1797 [11]. Abbot illustrates a monarch caterpillar ("Papilio Archippus") feeding on tropical milkweed in April, presumably in 1797 or before. Another report describes monarch larvae on *A. curassavica* in Missouri in August 1868 [12]. The first mention of winter-breeding in the southern U.S. appeared in 1937 [13], when monarch eggs and larvae were observed in January on *A. curassavica* in Orlando, FL. Four additional records in Texas and Jacksonville, FL note monarch larvae during the winter of 1957-1958 [14]. To our knowledge, no additional records of winter-breeding in the southern U.S (excluding south Florida) appear in the literature until 2010, when 95 sightings of winter-breeding monarchs (larvae, pupae or ovipositing females) were recorded in the southern U.S. between 2002-2010 above 27ºN [15].

Figure S1. Number of herbaria records documented in the southern U.S. between 1908 and 2012, for (a) tropical milkweed, *A. curassavica*, and (b) butterfly milkweed, *A. tuberosa*, based on a search of 176 herbaria. A simple analysis of covariance examining records over time showed significant differences for *A. curassavica* and *A. tuberosa* records.



(c) Conclusions

These searches suggest that, at least to a limited extent, tropical milkweed has long been a part of North American gardens, and monarchs utilized it during the typical breeding season. During the past two decades, modest increases in *A. curassavica* herbaria records and widespread observations of winter-breeding activity on tropical milkweed have occurred. It is important to distinguish sightings of *winter-breeding* monarchs (eggs, larvae, or pupae during Dec-Feb) from sightings of *adult* monarchs found during the winter. Small sub-populations of adult monarchs have been found in the southern U.S. flying or roosting (but not breeding) during the winter months as early as 1875 [16]. These adults overwinter especially along the Florida Gulf coast and to a lesser degree along the southern Atlantic coast. We examined parasite samples from these "coastal overwintering" populations, discussed in the main text.

II. Additional tables and figures

Table S1. Number of monarch field samples and sampling locations by year and source population. Site locations varied over the two years of the study, with some sites sampled both years and other sites sampled during only one year. Summer 2011 through winter 2012 is considered Year 1; summer 2012 through winter 2013 is Year 2. Winter-breeding samples included those collected by investigators at the University of Georgia (N=51 and N=45 for years 1 and 2, respectively) and by volunteer citizen scientists (N = 352 and 219 for years 1 and 2). Samples were assigned to sub-regions nested within each source for the purpose of the statistical analysis (linear mixed model) described in section III below. Our sub-regions were based on NOAA U.S. climate regions [17], however, we note exceptions: (i) a single site in Kansas was grouped into the Ohio Valley, (ii) sites in Manitoba, Canada were grouped into the Upper Midwest, (iii) sites in Southern Ontario comprise an additional sub-region, and (iv) sites in the southeast are separated into coastal and non-coastal sub-regions. U.S. states are abbreviated.

source population	sub-regions	states/colonies	milkweed habitat	time periods	total samples	total sites
summer-breeding	Ohio Valley Northeast Southeast Southern Ontario Upper Midwest	OH, IL, IN, TN, KS CT, NH, NJ, NY, PA NC, VA Ontario, Canada IA, MI, MN, WI; Manitoba, Canada	primarily native milkweeds including *A. syriaca*, *A. incarnata*, *A. tuberosa*	year 1 (June 1-Oct. 1, 2011)	1276	54
				year 2 (June 1-Oct. 1, 2012)	1290	52
Mexico overwintering	Michoacán	Sierra Chincua and Cerro Pelón colonies	no milkweed	year 1 (March 5, 2012)	835	1
				year 2 (Feb. 12-15, 2013)	1555	2
winter-breeding	South central Coastal southeast	LA, TX FL, GA, SC	tropical milkweed (*A. curassavica*)	year 1 (Dec. 1-March 1, 2011-2012)	403	23
				year 2 (Dec. 1-March 1, 2012-2013)	264	18
coastal overwintering	Coastal southeast	FL, SC	no milkweed	year 1 (Dec. 1-March 1, 2011-2012)	169	3
				year 2 (Dec. 1-March 1, 2012-2013)	85	3

Table S2. Origins of *OE* isolates in virulence experiment (location, date, monarch sex, and population).

isolate	city/colony	state	date collected	sex	source population
1	Atlanta	GA	10/14/2011	M	Summer-breeding
6	Sylvester	GA	10/24/2011	M	Summer-breeding
7	Hazelton	IA	8/25/2011	M	Summer-breeding
15	Lawrence	KS	9/12/2011	M	Summer-breeding
20	Dugald	Manitoba, Canada	7/23/2011	F	Summer-breeding
21	Pinconning	MI	9/13/2011	M	Summer-breeding
31	Gilbert	MN	8/25/2011	F	Summer-breeding
36	Durham	NC	9/28/2011	F	Summer-breeding
42	Athens	GA	9/12/2011	F	Summer-breeding
46	Katonah	NY	9/5/2011	F	Summer-breeding
47	Willoughby Hills	OH	7/8/2011	F	Summer-breeding
61	Millersburg	PA	9/21/2011	F	Summer-breeding
64	Newtown	PA	10/4/2011	F	Summer-breeding
66	Phoenixville	PA	9/14/2011	M	Summer-breeding
69	Oakridge	TN	8/1/2011	M	Summer-breeding
74	Arlington	TX	10/8/2011	M	Summer-breeding
78	Wisconsin Rapids	WI	6/12/2011	M	Summer-breeding
2	Cerro Pelón	Michoacán, MX	3/5/2012	M	Mexico overwintering
12	Cerro Pelón	Michoacán, MX	3/5/2012	F	Mexico overwintering
13	Cerro Pelón	Michoacán, MX	3/5/2012	M	Mexico overwintering
14	Cerro Pelón	Michoacán, MX	3/5/2012	M	Mexico overwintering
19	Cerro Pelón	Michoacán, MX	3/5/2012	M	Mexico overwintering
23	Cerro Pelón	Michoacán, MX	3/5/2012	M	Mexico overwintering
24	Cerro Pelón	Michoacán, MX	3/5/2012	F	Mexico overwintering
26	Cerro Pelón	Michoacán, MX	3/5/2012	M	Mexico overwintering
34	Cerro Pelón	Michoacán, MX	3/5/2012	F	Mexico overwintering
38	Cerro Pelón	Michoacán, MX	3/5/2012	M	Mexico overwintering
45	Cerro Pelón	Michoacán, MX	3/5/2012	F	Mexico overwintering
48	Cerro Pelón	Michoacán, MX	3/5/2012	F	Mexico overwintering
51	Cerro Pelón	Michoacán, MX	3/5/2012	F	Mexico overwintering
54	Cerro Pelón	Michoacán, MX	3/5/2012	M	Mexico overwintering
55	Cerro Pelón	Michoacán, MX	3/5/2012	F	Mexico overwintering
62	Cerro Pelón	Michoacán, MX	3/5/2012	F	Mexico overwintering
68	Cerro Pelón	Michoacán, MX	3/5/2012	F	Mexico overwintering
72	Cerro Pelón	Michoacán, MX	3/5/2012	F	Mexico overwintering
73	Cerro Pelón	Michoacán, MX	3/5/2012	M	Mexico overwintering
77	Cerro Pelón	Michoacán, MX	3/5/2012	F	Mexico overwintering
4	Savannah	GA	1/5/2012	M	Winter-breeding
8	San Antonio	TX	2/10/2012	M	Winter-breeding
9	Galveston	TX	2/15/2012	M	Winter-breeding
17	Hitchcock	TX	1/5/2012	F	Winter-breeding
25	Kenner	LA	2/19/2012	M	Winter-breeding
32	Useppa Island	FL	2/21/2012	M	Winter-breeding
33	Austin	TX	12/19/2011	F	Winter-breeding
35	Seabrook	TX	2/2/2012	F	Winter-breeding
37	Lakeland	FL	1/22/2012	F	Winter-breeding
41	New Orleans	LA	1/25/2012	F	Winter-breeding
43	Melbourne	FL	2/12/2012	M	Winter-breeding
49	Houston	TX	12/28/2011	F	Winter-breeding
52	League City	TX	1/26/2012	F	Winter-breeding
53	Port St. Joe	FL	12/4/2011	F	Winter-breeding
56	League City	TX	2/14/2012	M	Winter-breeding
58	Orlando	FL	1/7/2012	F	Winter-breeding
63	Hitchcock	TX	12/21/2011	F	Winter-breeding
65	Houston	TX	12/30/2011	M	Winter-breeding
70	Seabrook	TX	12/25/2011	F	Winter-breeding
75	New Orleans	LA	12/15/2011	M	Winter-breeding

Figure S2. Proportion of monarchs heavily infected with *OE* during 2011-2013, aggregated at the state or sub-state level. Pie charts indicate prevalence (black shading) at winter-breeding sites (yellow), summer-breeding sites (blue), and Mexico overwintering sites (green). Coastal overwintering sites are not shown. Each pie chart represents multiple sites grouped by proximity for easier visual display. In the U.S., sample size per pie chart ranges from 8 to 380 samples.

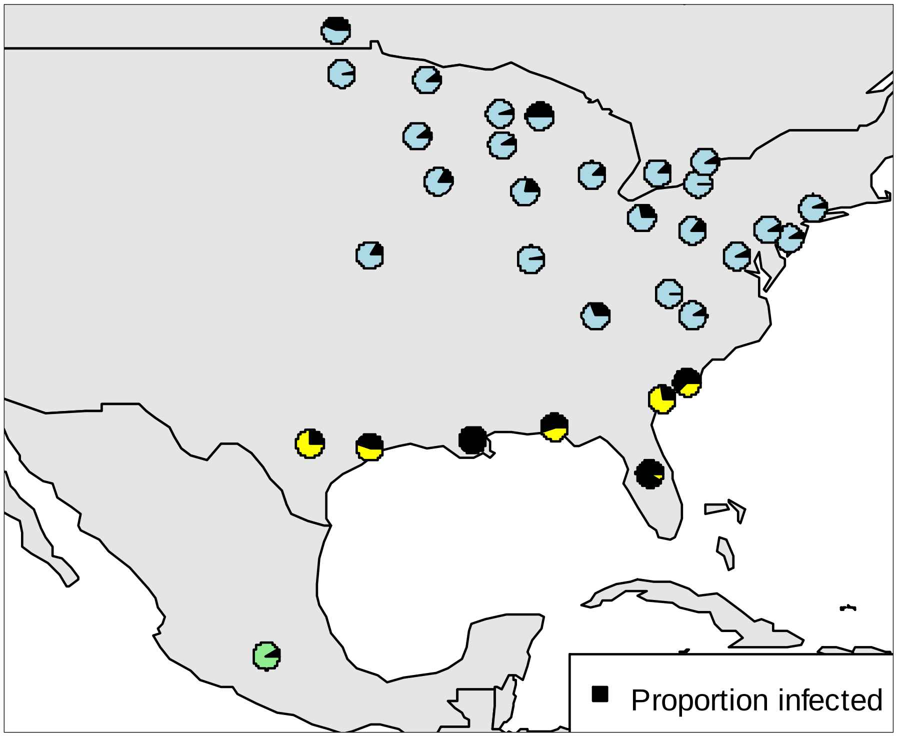


Figure S3. Infection prevalence across all monarchs captured as adults or as larvae/pupae. Monarchs collected as larvae/pupae were more likely to be infected than those collected as adults. This difference could have arisen because monarchs collected as larvae were reared to adulthood by volunteers and tested for *OE* soon after eclosion – before fitness costs of parasitism have been fully borne out. Monarchs caught as wild adults were presumably older, tested days to weeks after eclosion. Thus, this difference in infection likely reflects the parasite-induced mortality that adult monarchs experience. Error bars represent standard error.

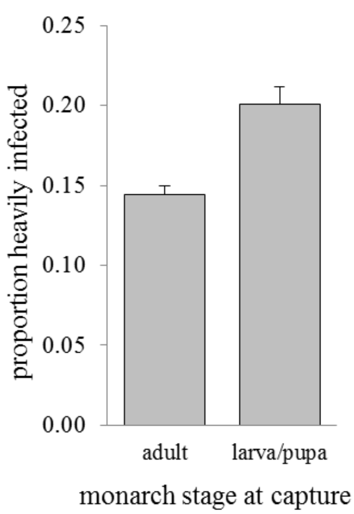


Table S3. Model-averaged parameter estimates for generalized linear mixed model for infection status among all monarchs. For each predictor variable, the table shows relative importance Σ (sum of AIC weights for all top models that included the predictor), regression coefficient b, standard errors, z-values and p-values. Model-averaged parameters are based on top models for which ΔAIC<10. Component models are in table S4.

predictor	relative importance, Σ	level	regression coefficient, b	standard error	z value	p value
source (*reference*: winter-breeding sites)	1.00	Mexico overwintering	-3.32	1.17	2.85	0.0044**
		coastal overwintering	-1.68	0.40	4.18	<0.0001***
		summer-breeding	-3.54	0.44	8.10	<<0.0001***
collection stage (*reference*: larva)	1.00	adult	-0.79	0.21	3.80	0.0001***
year (*reference*: year 1)	0.96	year 2	0.44	0.15	2.91	0.0036**
sex (*reference*: female)	0.69	male	0.22	0.12	1.91	0.0566 NS

Table S4. Component models for model-averaged generalized linear mixed model for infectious status among monarchs. For each component model, the table shows factors included, log-likelihood value, ΔAICc relative to full model, AICc weight, log-likelihood, deviance, marginal R^2 (variance explained by fixed factors) and conditional R^2 (variance explained by both fixed and random factors). For all models, site was treated as a random factor nested within source.

	fixed factors				**Δ AICc**	**AICc weight**	**log-likelihood**	**deviance**	**marginal R^2**	**conditional R^2**
	source	**collection stage**	**year**	**sex**						
model 16	yes	yes	yes	yes	0.00	0.662	-2082.3	4164.5	0.128	0.377
model 14	yes	yes	yes		1.61	0.296	-2084.1	4168.1	0.126	0.375
model 8	yes	yes		yes	6.32	0.028	-2086.4	4172.8	0.121	0.382
model 6	yes	yes			8.14	0.011	-2088.3	4176.7	0.120	0.380

III. Analysis of infection prevalence with a linear mixed model

(a) Methods

We used a linear mixed model in package *nlme* in R v.3.0.3 to examine effects of source, year, and a source-year interaction on infection prevalence. We assigned each site to one of eight sub-regions based on climate (as detailed in table S1). Sub-region was treated as a random effect nested within source. The purpose of aggregating sites was to reduce spatial correlation among prevalence data and determine whether source remained an important explanatory factor. Prevalence per site per year was arcsine-square-root-transformed to normalize variance, and sites with fewer than 8 samples were excluded from this analysis. Because coastal overwintering sites overlap spatially with some winter-breeding sites, coastal overwintering sites are excluded from this analysis to allow sub-region to be fully nested within source (i.e., coastal southeast sub-region nested within only the winter-breeding source population).

(b) Results and conclusions

Large differences in prevalence attributed to source were significant in the linear mixed model ($F_{2,5}$=18.50, p=0.005). Infection prevalence was several times higher among non-migratory (winter-breeding) monarchs sampled in the southern U.S. compared to migratory monarchs sampled at Mexico overwintering sites (t_5=3.12, p=0.03) or in the summer-breeding range (t_5=6.10, p=0.0017). Prevalence estimates for migratory monarchs from summer-breeding sites compared to Mexico overwintering sites were not statistically different. Infection prevalence during the second year of the study was marginally higher than in the first year in the linear mixed model ($F_{1,83}$=4.12, p=0.046).

Results from this linear mixed model, in which sites were aggregated in sub-regions, are congruent with those found using individual-level infection status data (reported in the main text), indicating that significant differences in prevalence persist among sources even when spatial correlation among sites is removed.

IV. References cited in the Electronic Supplementary Material

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