Population	Family ID	GOPH	ASCU	AINC	ASFA	ASYR	ASPEC	Total
Australia	AU_1_2017	9	3	0	2	3	0	17
	AU_A1_2018	3	0	1	1	1	2	8
	AU_A10_2018	1	0	1	0	1	0	3
	AU_A11_2018	1	0	0	0	0	0	1
	AU_A12_2018	2	2	1	1	2	2	10
	AU_A13_2018	1	2	1	0	1	2	7
	AU_A14_2018	1	0	1	1	0	1	4
	AU_A2_2018	1	2	2	2	1	1	9
	AU_A5_2018	2	1	1	0	2	0	6
	AU_A8_2018	1	0	1	0	1	0	3
	AU_A9_2018	1	1	0	1	1	0	4
		23	**11**	**9**	**8**	**13**	**8**	**72**

Population	Family ID	GOPH	ASCU	AINC	ASFA	ASYR	ASPEC	Total
California	CA_1017.1B_2018	0	1	0	0	1	0	2
	CA_11_2018	1	1	0	0	0	1	3
	CA_13_2017	1	0	0	1	0	0	2
	CA_13_2018	0	1	1	2	1	2	7
	CA_2_2017	1	1	0	2	1	0	5
	CA_2_2018	2	1	1	1	1	2	8
	CA_23_2018	0	1	1	1	1	1	5
	CA_3_2018	5	2	2	2	1	3	15
	CA_5_2017	1	2	0	1	1	0	5
	CA_605.1_2018	0	1	1	0	1	1	4
	CA_7_2017	1	0	0	0	1	0	2
	CA_7_2018	1	1	0	1	2	1	6
	CA_8_2017	1	1	0	0	2	0	4
	CA_9_2017	1	0	0	1	1	0	3
	CA_946.1_2018	0	0	0	1	0	0	1
	CA_X3_2018	3	1	2	2	1	2	11
		18	**14**	**8**	**15**	**15**	**13**	**83**

Population	Family ID	GOPH	ASCU	AINC	ASFA	ASYR	ASPEC	Total
Eastern North America	ENA_1_2017	4	3	0	1	2	0	10
	ENA_2_2017	2	2	0	2	0	0	6
	ENA_47_2017	1	0	0	1	0	0	2
	ENA_56_2017	1	1	0	1	2	0	5
	ENA_70_2017	1	1	0	0	0	0	2
	ENA_A_2018	1	2	1	1	1	2	8
	ENA_B_2018	2	1	2	1	3	1	10
	ENA_C_2018	1	1	2	1	0	1	6
	ENA_P_2018	2	2	2	1	3	3	13
	ENA_Q_2018	3	1	2	1	2	2	11
	ENA_S_2018	2	1	2	1	0	1	7
		20	**15**	**11**	**9**	**15**	**10**	**80**

Population	Family ID	GOPH	ASCU	AINC	ASFA	ASYR	ASPEC	Total
Guam	GU_10_2018	0	1	0	0	0	0	1
	GU_13_2018	0	1	0	1	0	1	3
	GU_19_2018	2	2	1	1	2	2	10
	GU_21_2018	1	1	0	0	0	0	2

	GU_25_2018	2	1	1	1	1	1	7
	GU_26_2018	2	1	1	1	1	0	6
	GU_30_2018	0	1	0	0	1	0	2
	GU_32_2018	1	1	0	0	1	0	3
	GU_38_2018	2	1	1	1	1	2	8
	GU_40_2018	1	1	1	0	0	1	4
	GU_41_2018	1	1	1	0	1	1	5
	GU_43_2018	3	2	2	1	2	2	12
	GU_901.1B_2018	1	1	1	0	0	0	3
		16	**15**	**9**	**6**	**10**	**10**	**66**
Population	**Family ID**	**GOPH**	**ASCU**	**AINC**	**ASFA**	**ASYR**	**ASPEC**	**Total**
Hawaii	HI_1_2018	3	2	2	2	2	2	13
	HI_10_2017	2	3	0	1	3	0	9
	H1_17_2018	0	1	0	1	1	0	3
	Hi_19_2017	0	1	0	1	1	0	3
	HI_19_2018	2	1	1	1	0	1	6
	HI_2_2018	3	2	2	1	1	3	12
	HI_20_2017	1	0	0	1	0	0	2
	HI_21_2018	2	0	1	1	0	0	4
	HI_22_2017	6	3	0	1	2	0	12
	HI_5_2017	1	1	0	1	0	0	3
	HI_5_2018	0	0	1	0	0	0	1
	HI_944.2_2018	1	0	2	0	0	1	4
		21	**14**	**9**	**11**	**10**	**7**	**72**
Population	**Family ID**	**GOPH**	**ASCU**	**AINC**	**ASFA**	**ASYR**	**ASPEC**	**Total**
Puerto Rico	PR_103_2018	2	1	1	2	1	1	8
	PR_105_2018	2	2	0	0	2	1	7
	PR_107_2018	1	2	2	0	2	2	9
	PR_109_2018	2	0	0	0	1	1	4
	PR_111_2018	2	2	2	2	2	2	12
	PR_112_2018	2	2	1	2	0	1	8
	PR_113_2018	0	1	2	0	1	0	4
	PR_P1_2018	0	1	0	0	0	0	1
	PR_PM2_2018	1	1	0	0	0	0	2
	PR_PM4_2018	2	2	2	2	2	2	12
		14	**14**	**10**	**8**	**11**	**10**	**67**

Table S1 – Number of wing cardenolide samples for each maternal family, separated by milkweed species. Families are arranged by source population. Note that for monarchs reared in 2017, only four milkweed species (GOPH, ASCU, ASYR, ASFA) were available. In total, we analyzed cardenolides from 440 individual monarchs. Milkweed species abbreviations are as follows: GOPH = *Gomphocarpus physocarpus*, ASCU = *Asclepias curassavica*, AINC = *Asclepias incarnata*, ASFA = *Asclepias fascicularis*, ASYR = *Asclepias syriaca*, ASPEC = *Asclepias speciosa*.

Species	Leaf Samples	Wing Samples
GOPH	54	112
ASCU	38	84
AINC	20	60
ASFA	20	59
ASYR	32	76
ASPEC	19	60

Table S2 – Number of cardenolide samples generated for leaf and wing tissue across each milkweed species.

Compound	Retention Time (Minutes)	Species	Absorbance Peak (nm)
Aspecioside	1.120	ASYR, ASPEC	219.13
Frugoside	5.933	ASCU, GOPH, AINC, ASFA	220.12
Calotropin	6.660	ASCU, GOPH	218.99
Calactin	7.443	ASCU, GOPH	218.77
Digitoxin (Internal Standard)	10.693	All samples	219.20

Table S3 – Cardenolides present in the current study whose identities could be verified with authentic standards. Frugoside was only recorded from AINC and ASFA in trace amounts and may reflect small amounts sequestered by neonate larvae during their first ~12 hours of development on ASCU cuttings, prior to being transferred onto their focal host plants. All compounds, with the exception of digitoxin, provided by A. Agrawal and C. Duplais.

Species	Sum of Squares	F	p
GOPH	13.27	103.5	<0.001
ASCU	7.72	57.2	<0.001
AINC	3.93	15.1	<0.001
ASFA	4.79	20.1	<0.001
ASYR	7.68	27.0	<0.001
ASPEC	3.19	9.5	<0.001

Table S4 – MANOVA results for milkweed species level comparisons of leaf and wing cardenolide profiles. Each row corresponds to a single species-level comparison of leaf and wing cardenolides. Across all species, leaf and wing tissue contained strongly distinct cardenolide profiles, reinforcing the notion that sequestration involves active processing of leaf cardenolides.

Milkweed Species	Compound	Absolute Amount (mg/g)	% of Total Sequestered	PR Amount (mg/g)	Ratio (PR / Others)
Asclepias curassavica	**Frugoside**	4.190	34.4 %	4.013	0.958
	RT 2.150	1.674	13.8 %	4.249	2.538
	RT 0.830	1.270	10.4 %	2.015	1.587
	Calotropin	1.235	10.1 %	0.689	0.558
	Calactin	0.865	7.1 %	0.968	1.119
	RT 5.100	0.600	4.9 %	0.598	0.997
Gomphocarpus physocarpus	**Frugoside**	1.455	26.9 %	1.887	1.297
	RT 0.830	0.785	14.5 %	1.730	2.204
	RT 6.383	0.579	10.7 %	0.481	0.831
	Calactin	0.564	10.4 %	0.834	1.479
	RT 1.593	0.544	10.0 %	1.064	1.956
	Calotropin	0.487	9.0 %	0.335	0.689
Asclepias syriaca	**Aspecioside**	2.966	48.3 %	0.128	0.043
	RT 1.890	0.879	14.3 %	0.098	0.111
	RT 3.590	0.744	12.1 %	0.357	0.480
	RT 3.380	0.708	11.5 %	0.320	0.452
	RT 2.870	0.144	2.4 %	0.031	0.215
Asclepias speciosa	**Aspecioside**	1.447	44.0 %	0.096	0.066
	RT 3.380	0.592	18.0 %	0.104	0.176
	RT 3.590	0.418	12.7 %	0.037	0.089
	RT 1.890	0.404	12.3 %	0.363	0.899
	RT 2.870	0.070	2.2 %	0.000	0.000

Table S5 – Primary sequestered cardenolide peaks across milkweed species, averaged across all monarch populations. The top six compounds are shown for *A. curassavica* and *G. physocarpus*, and the top five compounds are shown for *A. syriaca* and *A. speciosa*. For compounds whose identities are unknown, retention times are listed. Percent of total sequestered refers to within-species totals. In the second column from the right, absolute sequestered amounts are shown for the Puerto Rican population only. The rightmost column shows the ratio of sequestered cardenolides for Puerto Rican monarchs relative to species-level totals across all populations. Note that the ratio for aspecioside sequestered from A. syriaca is 0.043, corresponding to 23 times lower sequestration of this compound in Puerto Rican monarchs. For graphical depictions of chromatograms, see Figure 1A. Monarchs reared on *A. incarnata* and *A. fascicularis* contained small amounts of frugoside, RT 6.383, and RT 2.150.

Predictor	Sum of Squares	R^2	F	DF	p
Monarch population	3.42	0.033	4.77	5	<0.001
Milkweed species	51.32	0.494	119.49	3	<0.001
Monarch population x milkweed species	6.12	0.059	2.85	15	<0.001
Sex	0.29	0.003	2.01	1	0.066
Residual Error	42.66	0.420			

Table S6 – MANOVA results showing variation explained by milkweed species, monarch population, their interaction, and butterfly sex in the composition of sequestered cardenolides. Compared to quantitative variation in the concentration of sequestered cardenolides (see Table S7), the interaction between monarch population x milkweed species interaction term explained relatively little variation, suggesting that GxE interactions primarily involve variation in the total amount of cardenolide sequestered.

Predictor	χ^2	DF	p
Monarch population	6.91	5	0.227
Milkweed species	61.55	3	<0.001
Monarch population x milkweed species	77.56	15	<0.001
Sex	2.85	1	0.094

Table S7 – ANOVA results for a linear mixed model comparing total sequestered cardenolide concentrations. Here, the primary term of interest is the interaction between monarch population and milkweed species, which reflects GxE interactions for sequestration ability.

Model Term	χ^2	DF	p
Monarch population	9.44	5	0.093
Milkweed species	79.41	3	<0.001
Sympatric / allopatric status	0.16	1	0.687
Sex	1.34	1	0.247

Table S8 – ANOVA results for a linear mixed model directly testing for local adaptation in sequestration ability. As with Table S7, the response variable is total sequestered cardenolides in monarch wings. The primary term of interest is the sympatric/allopatric contrast, which describes the magnitude of performance difference between monarchs reared on sympatric versus allopatric host plants.

Species	Mean Cardenolide Concentration	Standard Deviation	Coefficient of Variation
GOPH	5.42	2.00	0.371
ASCU	12.17	4.83	0.397
AINC	0.45	0.45	1.002
ASFA	0.31	0.24	0.759
ASYR	6.14	4.18	0.681
ASPEC	3.29	3.12	0.949

Table S9 – Coefficient of variation in cardenolide sequestration across each milkweed species. Note that variation is lowest on GOPH and ASCU.

Monarch Population	Milkweed Species	Marginal Mean	SE	df	Lower CL	Upper CL	Group
AU	GOPH	5.242	0.795	115.159	3.667	6.816	A
CA	GOPH	4.819	0.885	188.960	3.074	6.564	A
ENA	GOPH	5.352	0.785	153.641	3.802	6.902	A
GU	GOPH	4.721	0.886	180.993	2.973	6.469	A
HI	GOPH	4.991	0.794	100.655	3.416	6.566	A
PR	GOPH	7.692	1.008	167.648	5.702	9.682	A
AU	ASCU	14.611	1.151	54.918	12.304	16.917	A
CA	ASCU	12.574	1.089	43.170	10.378	14.770	AB
ENA	ASCU	13.079	1.021	42.408	11.018	15.139	A
GU	**ASCU**	**8.886**	**0.941**	**36.731**	**6.978**	**10.793**	**B**
HI	ASCU	11.013	1.052	39.936	8.887	13.140	AB
PR	ASCU	14.963	1.026	41.599	12.892	17.033	A
AU	ASYR	7.164	0.957	150.575	5.274	9.054	A
CA	ASYR	5.250	0.905	107.173	3.456	7.045	A
ENA	ASYR	6.199	0.986	99.274	4.244	8.155	A
GU	ASYR	6.618	1.036	177.648	4.574	8.663	A
HI	ASYR	7.372	1.274	108.765	4.846	9.897	A
PR	**ASYR**	**1.056**	**1.040**	**153.891**	**-0.999**	**3.111**	**B**
AU	ASPEC	3.017	1.388	33.903	0.196	5.837	A
CA	ASPEC	4.656	1.133	23.151	2.313	6.999	A
ENA	ASPEC	1.910	1.348	29.558	-0.844	4.665	A
GU	ASPEC	3.626	1.289	33.878	1.005	6.246	A
HI	ASPEC	4.955	1.481	63.087	1.994	7.915	A
PR	ASPEC	0.896	1.282	34.380	-1.709	3.500	A

Table S10 – Estimated marginal means for total sequestered cardenolide concentration for each monarch population x milkweed species. Group level differences were considered significantly different if they had non-overlapping 95% confidence intervals. Combinations of primary interest are shown in bold.

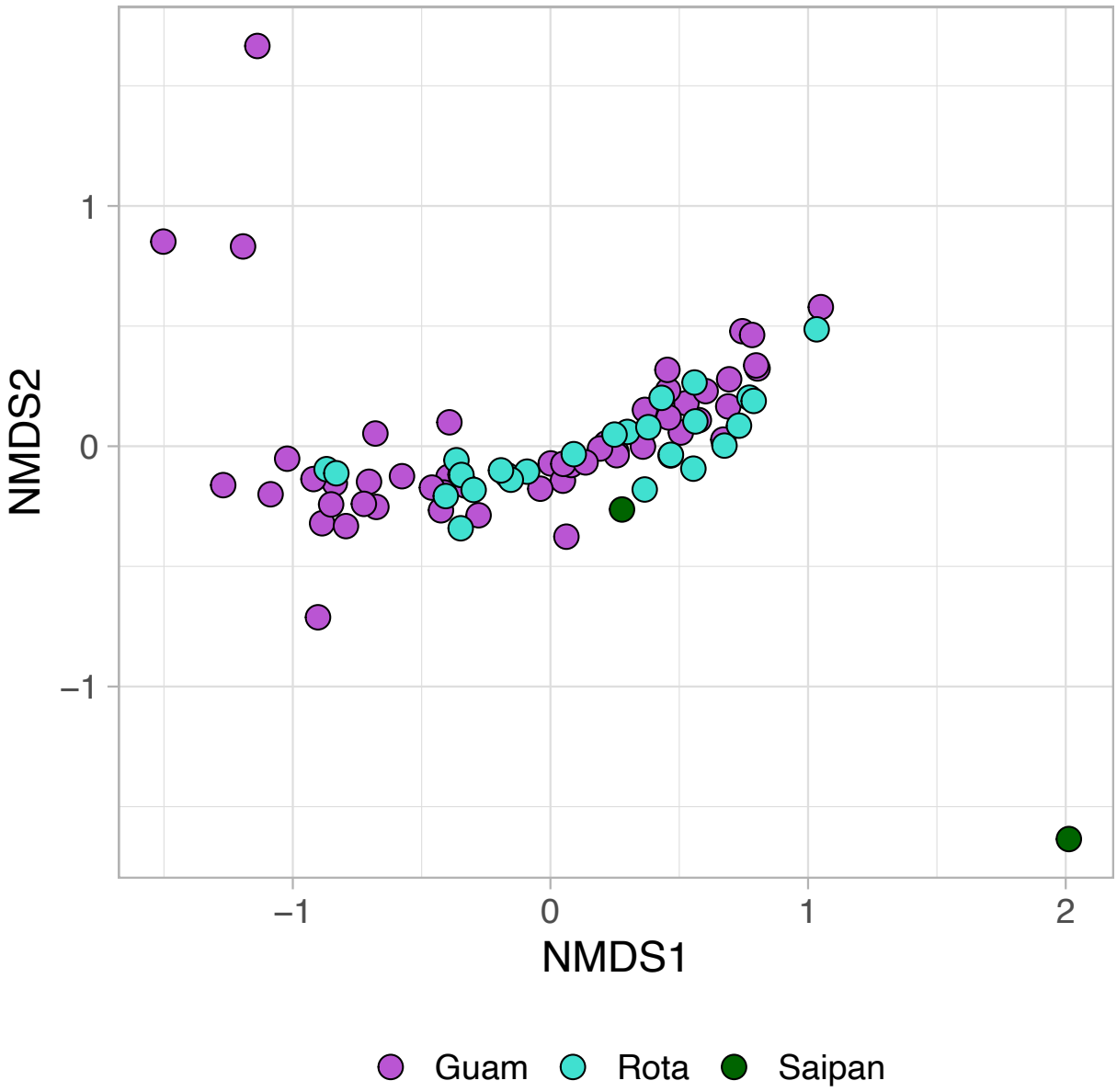

Figure S1 – NMDS plot of wing cardenolides from wild-caught monarchs in the Mariana Islands. Butterflies from Guam (n = 54) and Rota (n = 27) generally had indistinguishable cardenolide profiles, consistent with both populations feeding primary on the numerically dominant host *Asclepias curassavica*. Monarchs from Saipan (n = 2) included one wild-caught individual with a cardenolide fingerprint consistent with developing on A. curassavica, as well as one monarch collected on the day of its emergence on an ornamental *Calotropis gigantea* plant (point in lower right).

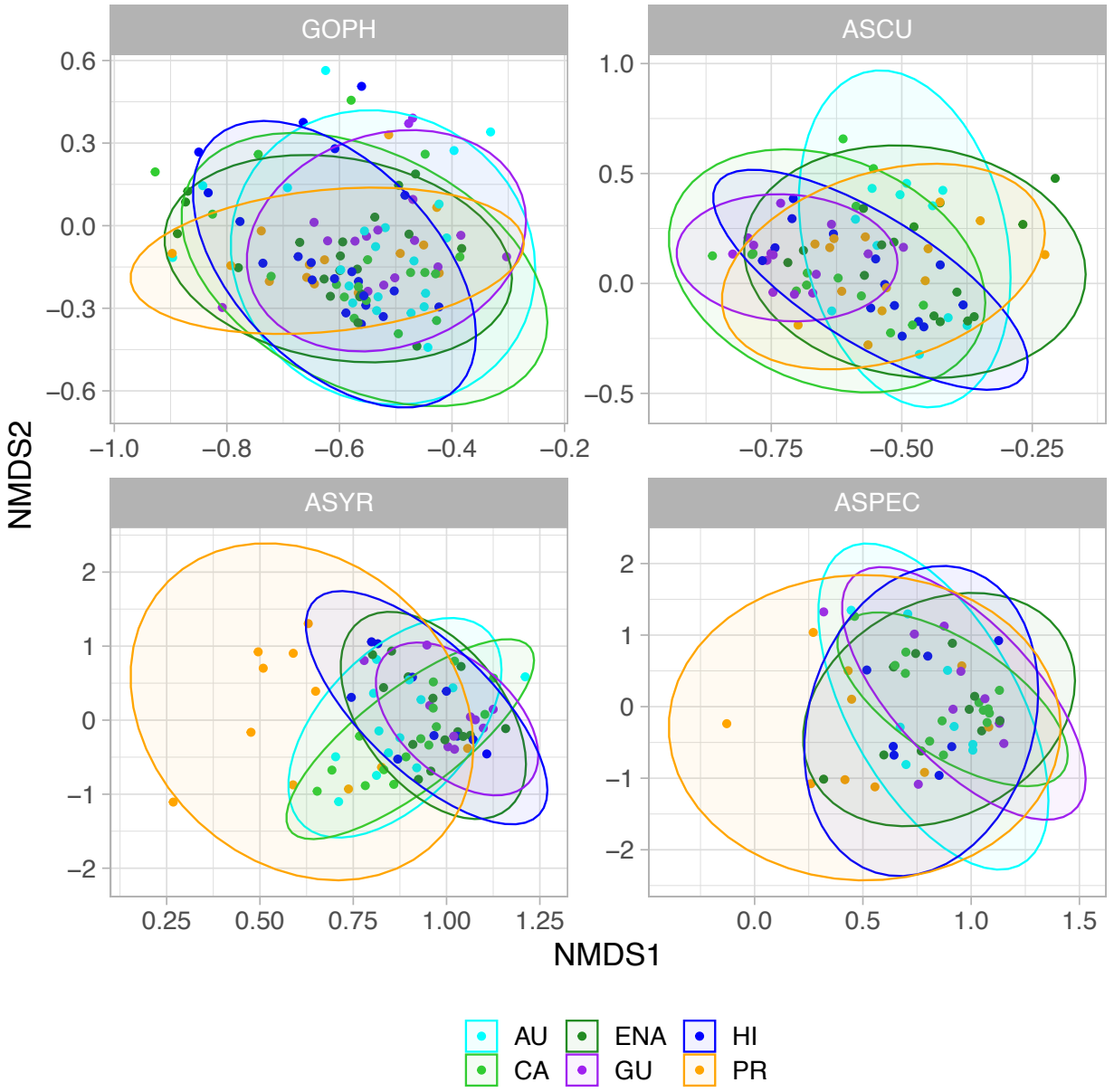

Figure S2 – Multivariate disparity in sequestered cardenolide profiles, shown separately for each milkweed species. Results shown are based on a single overall dissimilarity matrix but are faceted by milkweed species. Ellipses correspond to the 95% confidence profiles and were generated using the stat_ellipse function. All populations appear to have generally similar overall multivariate sequestration profiles, with the potential exceptions of Puerto Rican monarchs reared on *A. speciosa* and especially on *A. syriaca*.

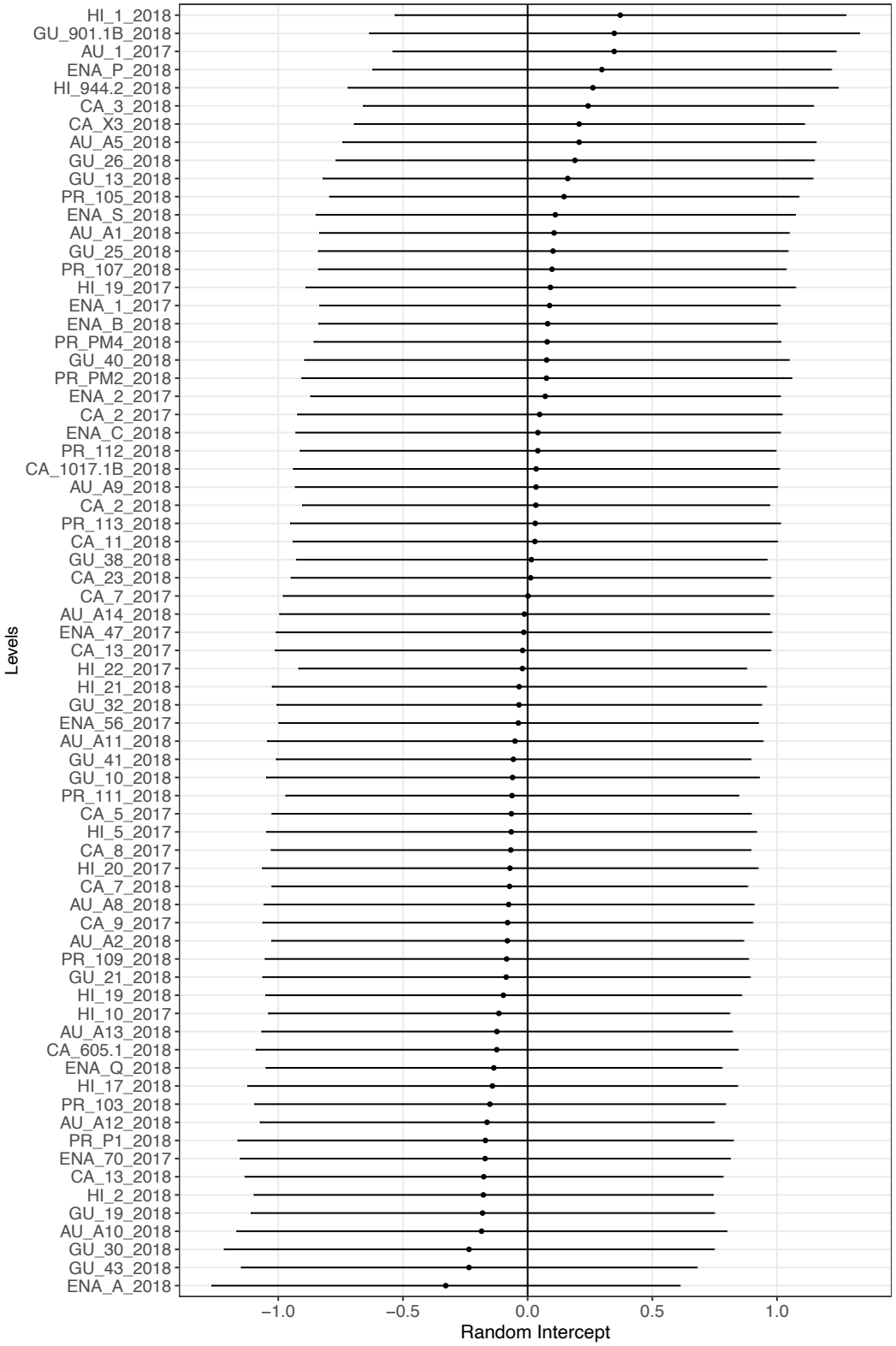

Figure S3 – Distribution of random intercepts for each maternal family. Error bars correspond to ±1 SD For context, the overall intercept in the associated model is 5.54, which represents the overall average of sequestered cardenolides on GOPH, ASCU, ASYR, and ASPEC.

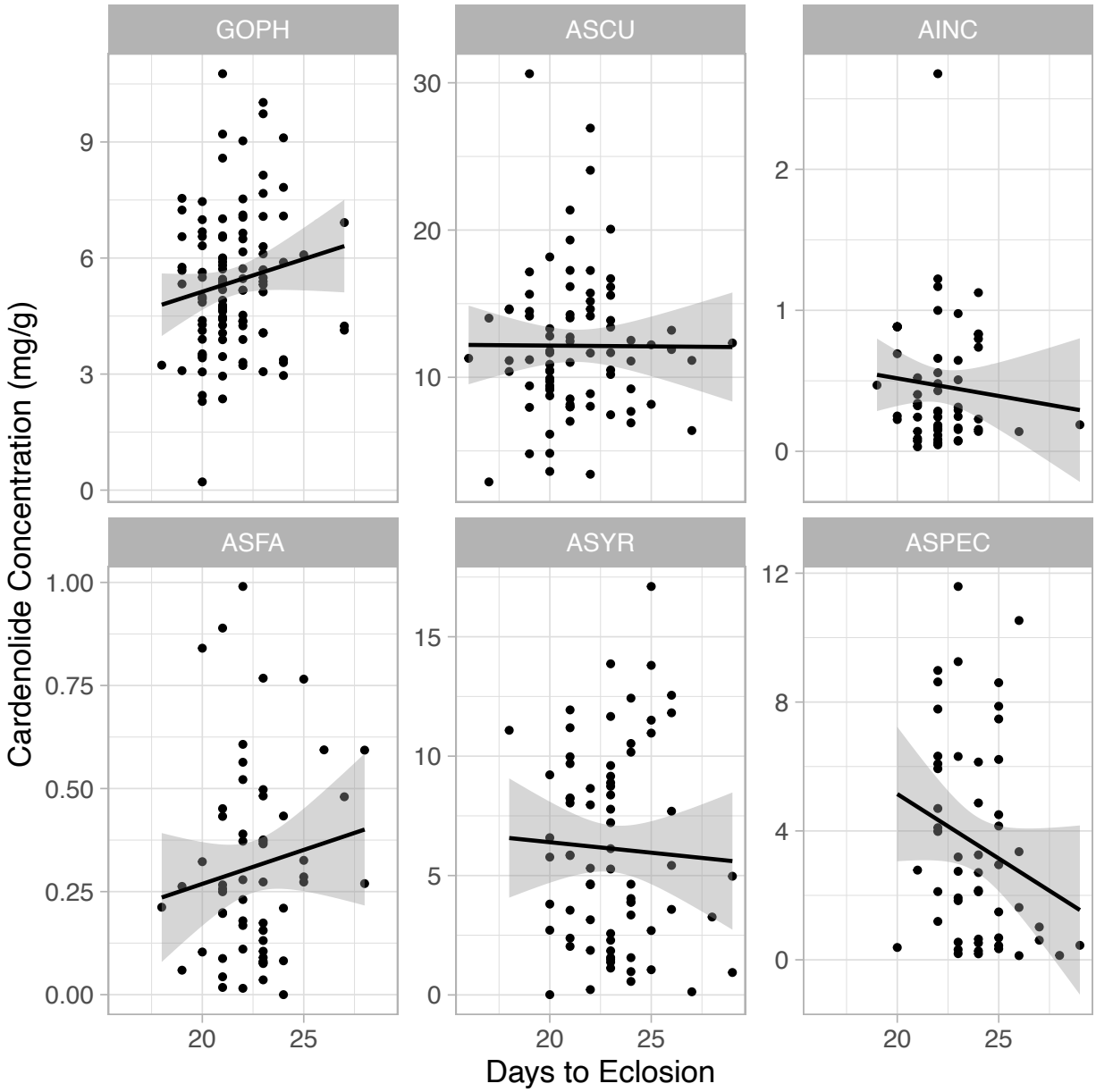

Figure S4 – There was no overall correlation between development time (measured as days from egg hatching until eclosion) and the overall quantity of cardenolide sequestered (t = 0.198, p = 0.844).

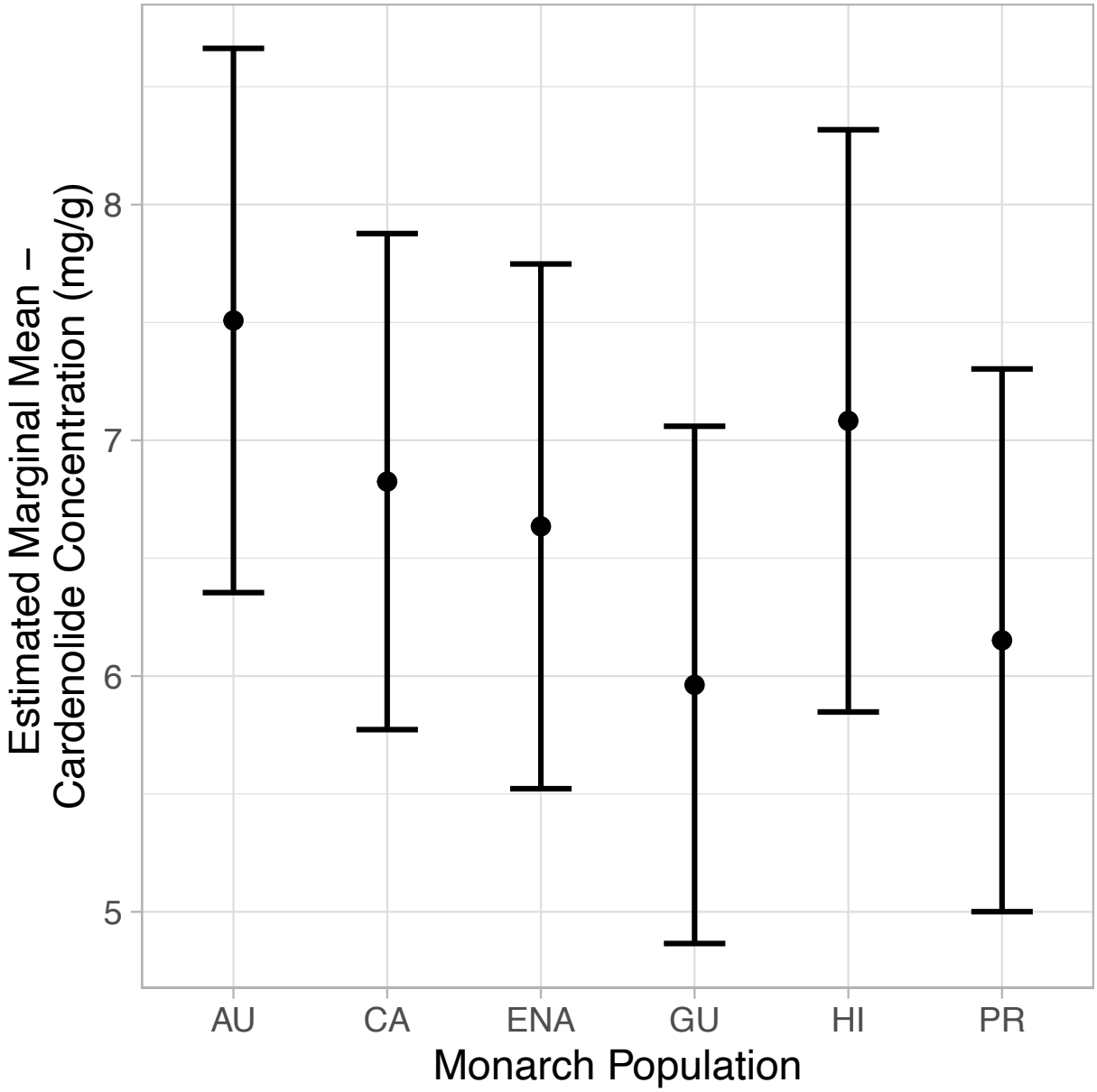

Figure S5 – Estimated marginal means showing wing cardenolide concentration, averaged over all four milkweed species of primary interest and shown by population. Monarch population was not a significant predictor of overall sequestration across all hosts (see Tables S5 and S6), and no pairwise comparisons between populations were significant after correcting for multiple comparisons. Error bars correspond to 95% confidence intervals.

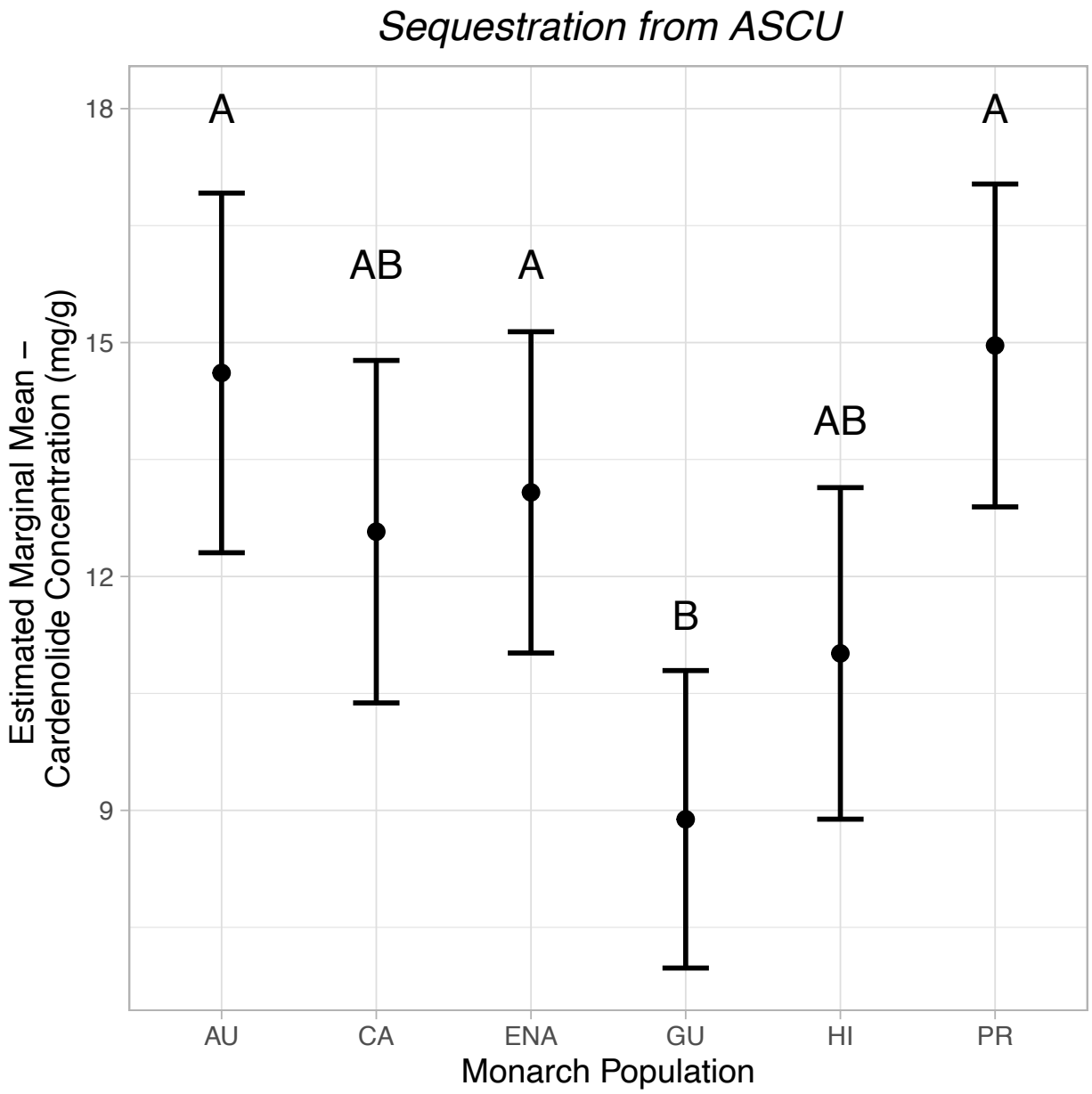

Figure S6 – Estimated marginal means showing wing cardenolide concentration for only monarchs reared on *Asclepias curassavica*. Monarchs from Guam sequestered significantly lower concentrations from ASCU—their sympatric host plant—than populations from Australia, Eastern North America, and Puerto Rico. Error bars correspond to 95% confidence intervals. Results also reported in Figure 4A and Table S10.