

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
	HI_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 S3 – Number of cardenolide samples generated for leaf and wing tissue across each milkweed species.

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. Across all species, leaf and wing tissue contained strongly distinct cardenolide profiles, reinforcing the notion that sequestration involve active processing of leaf cardenolides.

Model Term	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 S5 – 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 S6 – ANOVA results for a linear mixed model directly testing for local adaptation in sequestration ability. As with Table S5, 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 S7 – Coefficient of variation in cardenolide sequestration across each milkweed species. Note that variation is lowest on GOPH and ASCU.

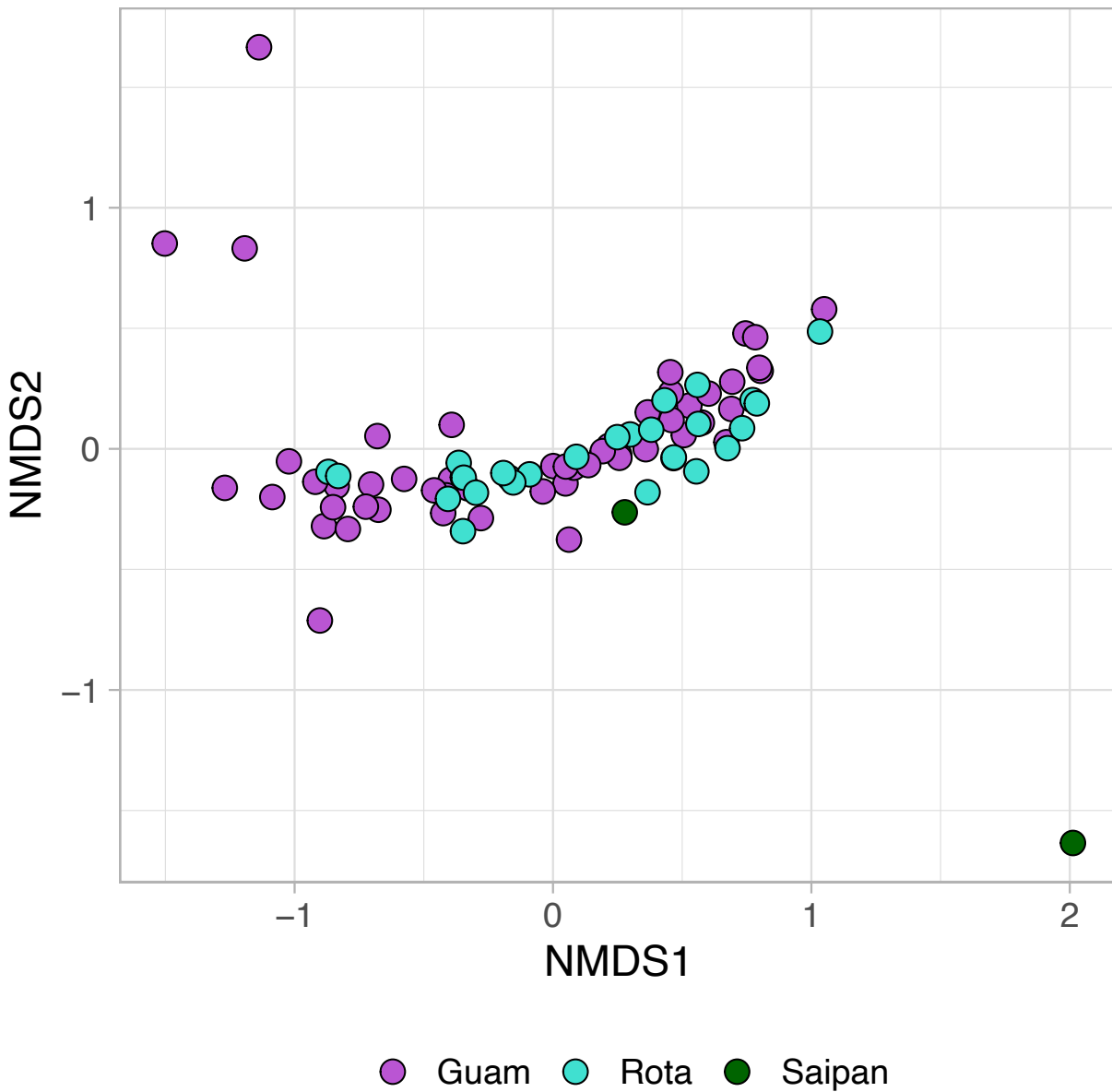


Figure S1 – NMDS plot of wing cardenolides from wild-caught monarchs in the Mariana Islands. Butterflies from Guam (n = 54) and Rota (n = 27) generally had indistinguishable cardenolide profiles, consistent with both populations feeding primary on the numerically dominant host *Asclepias curassavica*. 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).

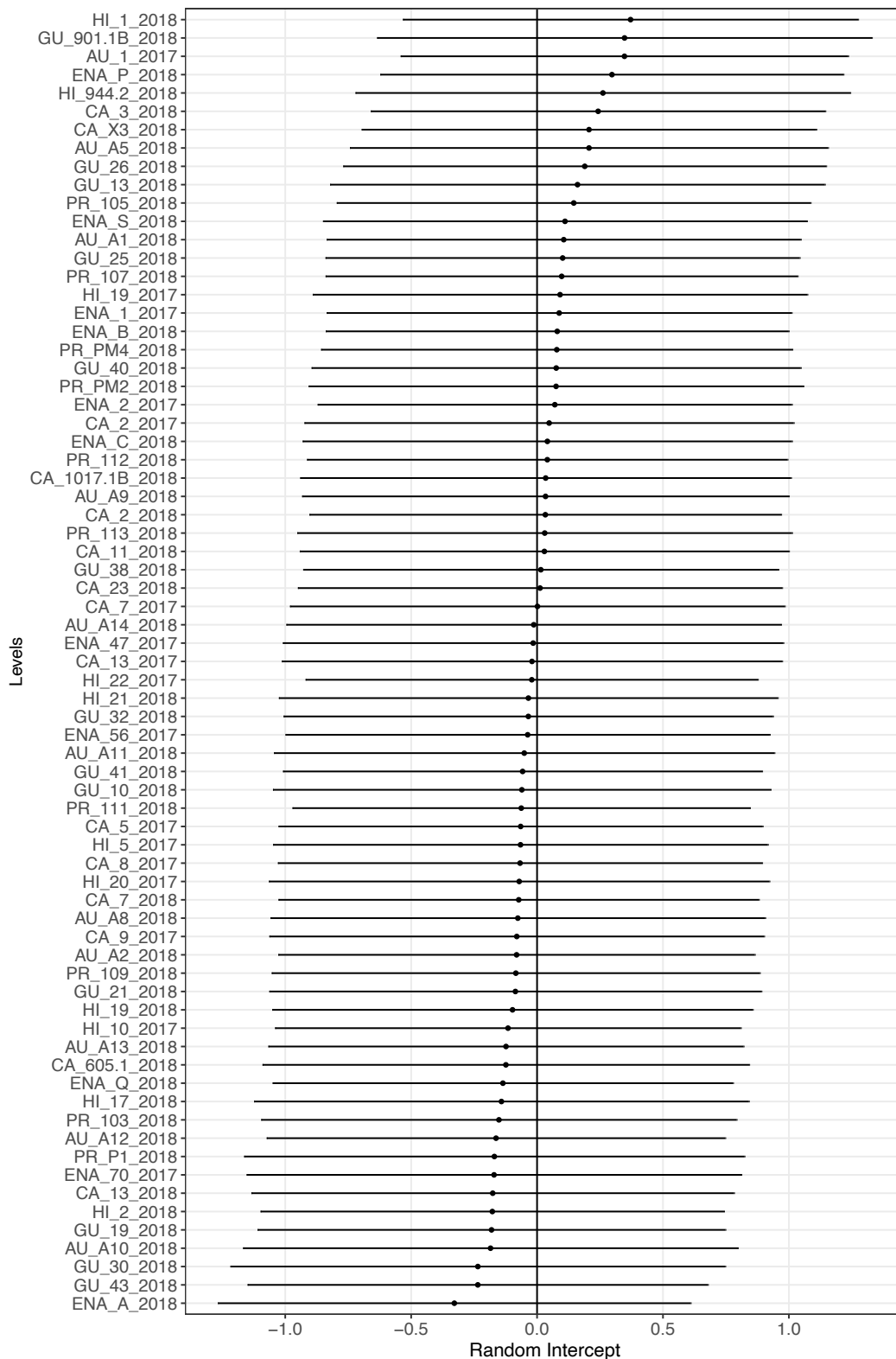


Figure S2 – Distribution of random intercepts for each maternal family. Error bars correspond to ± 1 SD. For context, the overall intercept in the associated model is 5.54, which represents the overall average of sequestered cardenolides on GOPH, ASCU, ASYR, and ASPEC.

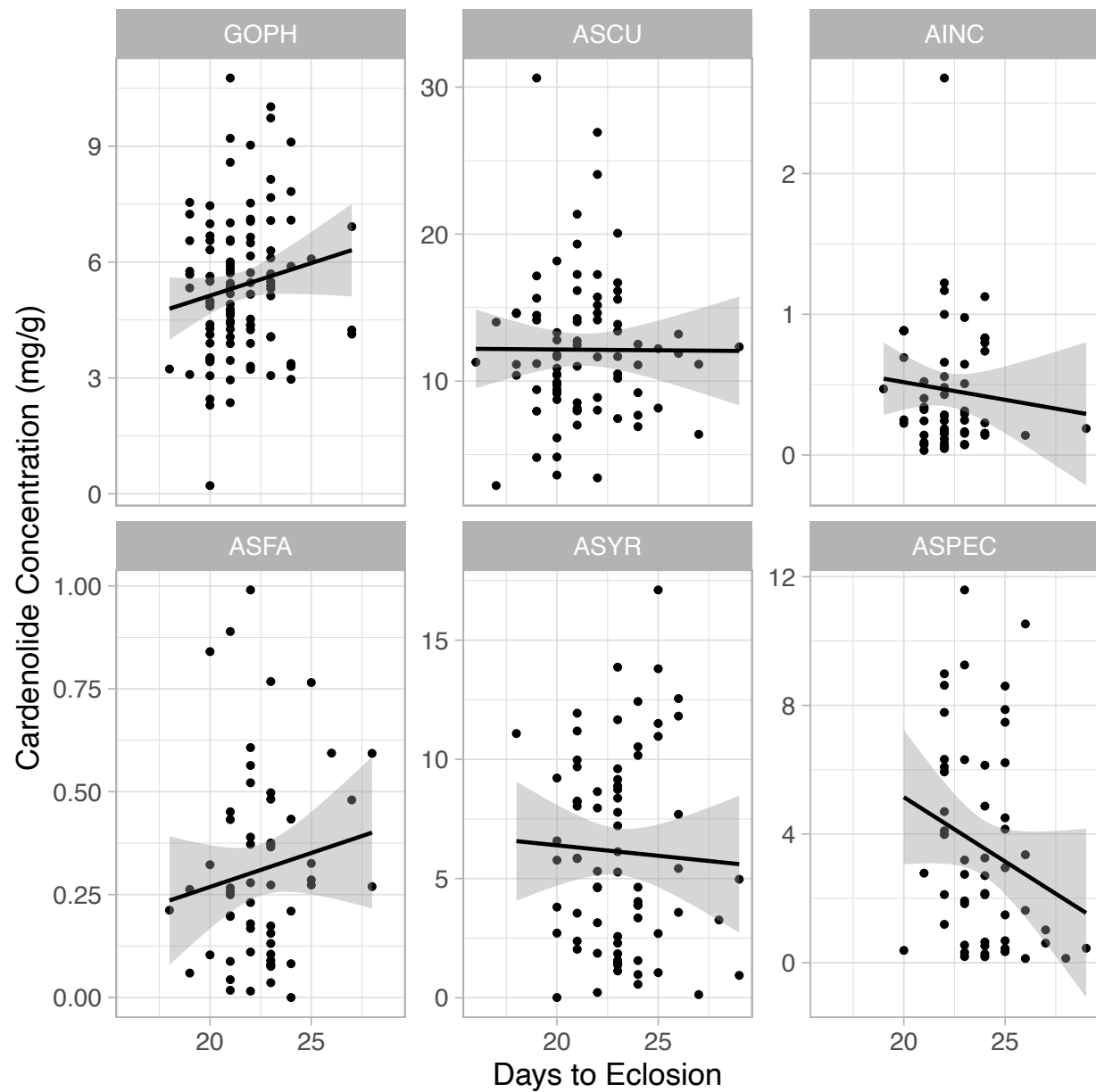


Figure S3 – There was no overall correlation between development time (measured as days from egg hatching until eclosion) and the overall quantity of cardenolide sequestered ($t = 0.198$, $p = 0.844$).

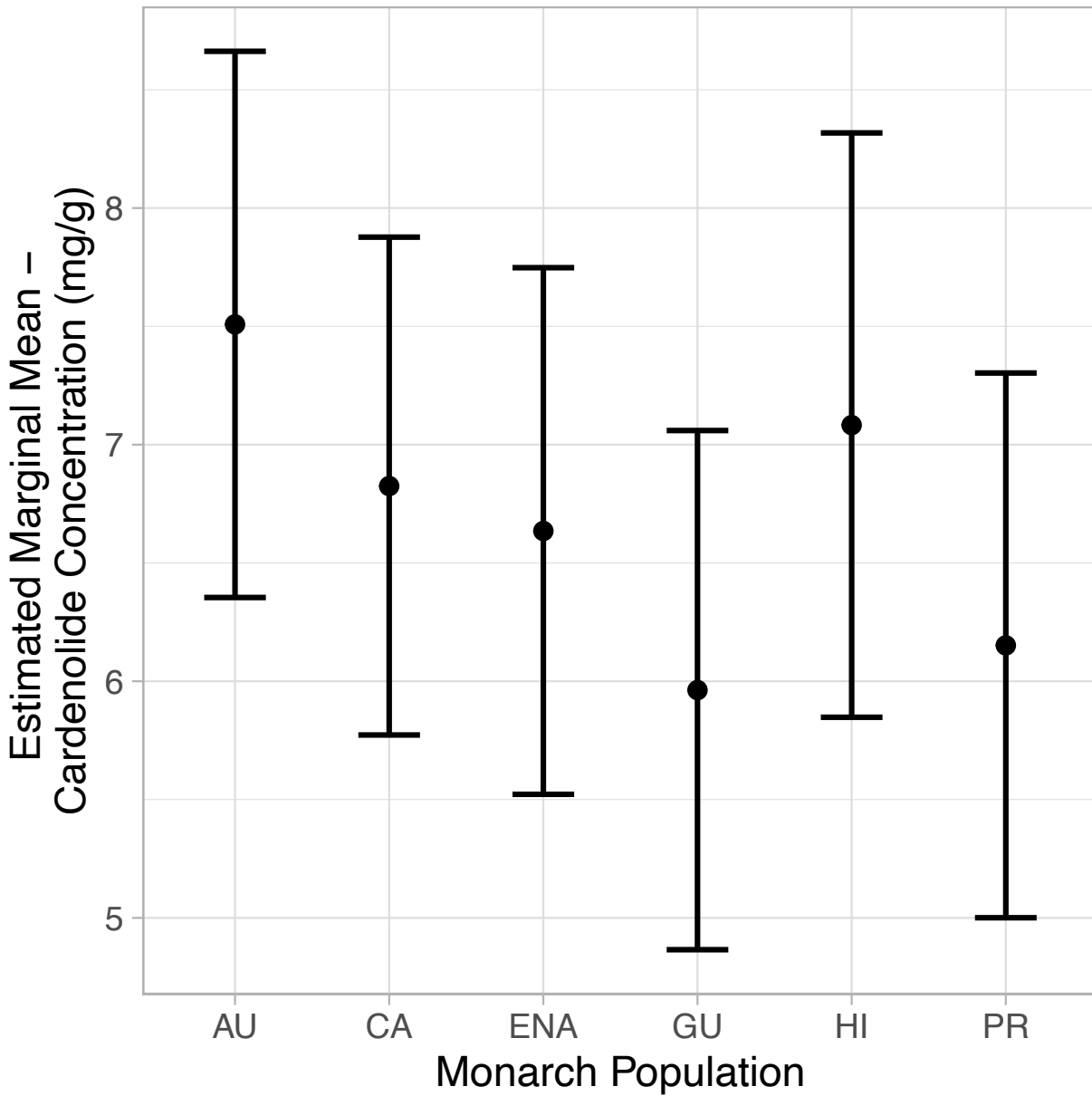


Figure S4 – Estimated marginal means showing wing cardenolide concentration, averaged over all four milkweed species of primary interest and shown by population. Monarch population was not a significant predictor of overall sequestration across all hosts (see Tables S5 and S6), and no pairwise comparisons between populations were significant after correcting for multiple comparisons. Error bars correspond to 95% confidence intervals.

Sequestration from ASCU

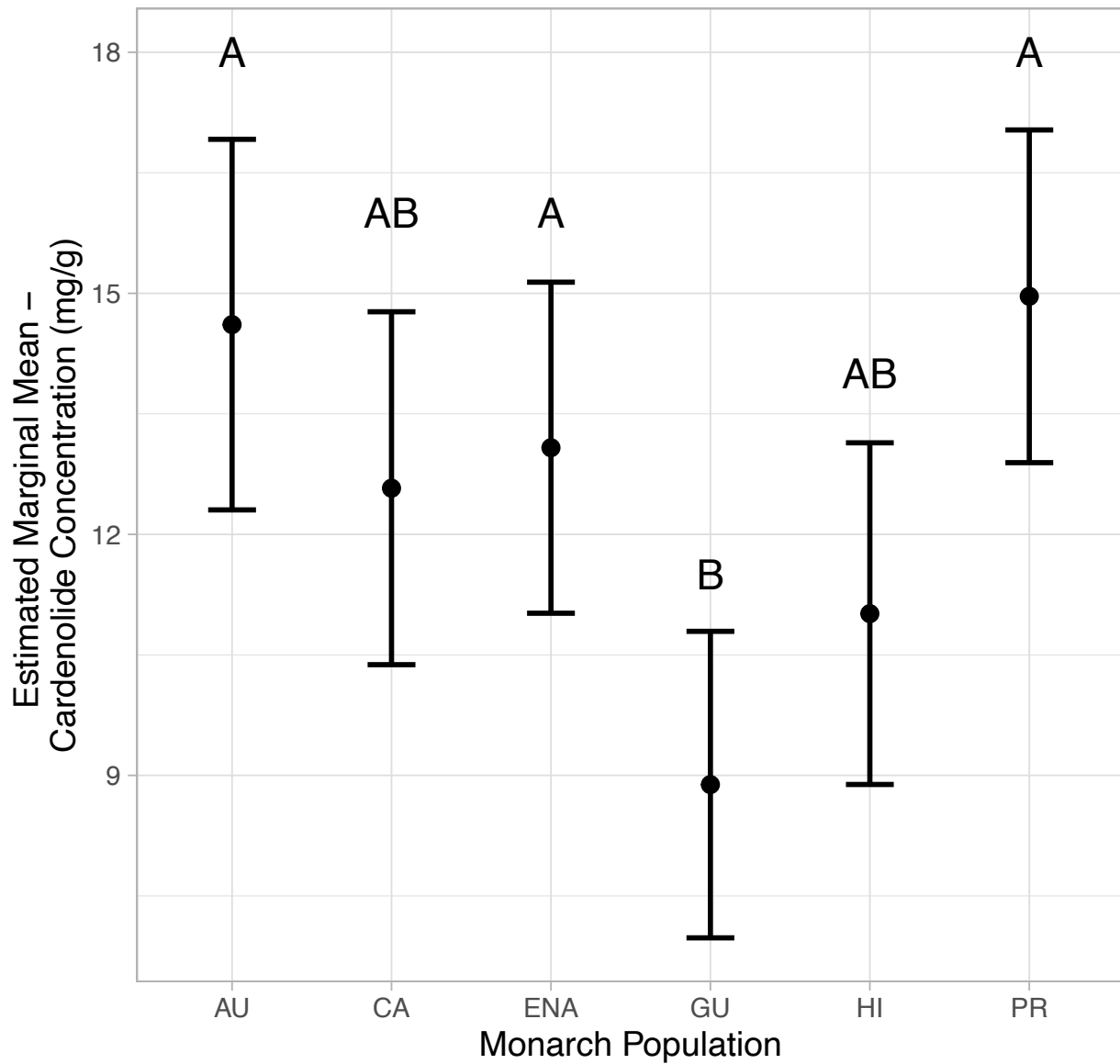


Figure S5 – Estimated marginal means showing wing cardenolide concentration for only monarchs reared on *Asclepias curassavica*. Monarchs from Guam sequestered significantly lower concentrations from ASCU—their sympatric host plant—than populations from Australia, Eastern North America, and Puerto Rico. Error bars correspond to 95% confidence intervals.