1 **Host plant adaptation during recent global range expansion in the monarch butterfly**

2

3 **Abstract**

4

5 Herbivores that have recently expanded their host plant ranges provide an opportunity to

6 test hypotheses about the evolution of host plant specialization. Here, we take advantage of the

7 contemporary global range expansion of the monarch butterfly (*Danaus plexippus* L.) and

8 conduct a reciprocal rearing experiment involving monarch populations with divergent host plant

9 assemblages. Specifically, we ask the following main questions: (1) Do geographically disparate

10 populations of monarch butterflies show evidence for local adaptation to their host plants? If so,

11 what processes generate this pattern? (2) How is dietary breadth related to performance across

12 multiple host species in monarch populations? (3) Does variation in performance differ across

13 host species? We find evidence for local adaptation in larval growth rate and survival, driven by

14 loss of performance of derived monarch populations reared on ancestral hosts. Migratory North

15 American monarchs, which have comparatively broad host breadth, have higher mean

16 performance than derived non-migratory populations across all host plant species. Monarchs

17 reared on their sympatric host plants show lower performance variation than monarchs reared on

18 allopatric hosts. The pronounced performance loss of derived populations on ancestral hosts

19 highlights the potential importance of non-adaptive processes (especially relaxed selection) in

20 generating patterns of host specialization.

21

22 **Keywords**: Range expansion, local adaptation, herbivory, monarch butterfly, diet breadth

23

Introduction

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 perforance 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 flower thrips (Karban 1989), spider mites (Agrawal 2000, Magalhães *et al.* 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), while 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 and Blanquart *et al.* 2013). Finally, even in cases where local adaptation is identified, many studies do not attempt to distinguish

47 underlying mechanisms generating this pattern. Thus, we still have limited understanding for

48 whether local adaptation to hosts in arthropod herbivores is driven primarily by antagonistic

49 pleitropy (i.e. genotypic tradeoffs) versus conditional neutrality (i.e. genotypes that are beneficial

50 on one host but selectively neutral on other hosts) (but, see Gompert *et al.* 2015, Gompert and

51 Messina 2016).

52   Herbivorous insects that have recently expanded their geographic and/or host plant range

53 provide valuable opportunities to address questions about the evolution of host plant adaptation

54 and specialization (Feder *et al.* 1988, Carroll et al. 2005, Jahner *et al.* 2011, Bean *et al.* 2012,

55 Gompert *et al.* 2015), especially in cases where the introduction history is well-known. In their

56 newly-established range, herbivorous insects may adapt to novel species of host plants not

57 encountered in the ancestral range (Louda *et al.* 1997, Van Klinken and Edwards 2002, Erbilgin

58 *et al.* 2014), a more restricted set of hosts (either novel or ancestral) (Pateman *et al.* 2012), or an

59 expanded range of host species that are ancestral, novel, or a mixture of both (Singer *et al.* 1993,

60 Graves and Shapiro 2002). These scenarios result in different sets of predictions for how

61 adaptation will proceed in the introduced range.

62   In instances where dietary breadth is reduced during range expansion, directional

63 selection may increase performance on the subset of available host plants. This adaptation may

64 come at a cost in performance on absent ancestral hosts, especially if there are strong cross-host

65 performance tradeoffs. Tradeoffs are expected to be more likely if novel hosts are

66 phylogenetically and chemically disparate from ancestral hosts (e.g. Pearse and Hipp 2009,

67 Bertheau *et al.* 2010, Rasmann and Agrawal 2011). By contrast, in situations where range

68 expansion involves no change or an increase in host plant breadth, we do not expect for a

69 reduction in performance on ancestral hosts.

70   When herbivore populations are exposed to many or few hosts, we expect different

71   outcomes of natural selection. Because spatial and temporal heterogeneity act to maintain genetic

72   variance for fitness across environments (Gillespie and Turelli 1989, Kassen 2002, Chakraborty

73   and Fry 2016), we predict that broad dietary breadth should promote maintenance of genetic

74   variation for performance through balancing selection (Joshi and Thompson 1995). Second,

75   dietary generalists might be expected to have larger effective population sizes (Packer *et al.*

76   2005, Li *et al.* 2014), which are also predicted to maintain genetic variation and performance

77   across hosts (Whitlock 1996). Thus, on average, we expect higher mean performance across host

78   species in herbivore populations with broader dietary breadth.

79   Several hypotheses involving host plant adaptation have been put forward based on mean

80   performance across hosts, but we also predict that variance in performance should be lower for

81   demes/populations reared on sympatric compared to allopatric hosts. Demes reared on

82   novel/allopatric hosts could have high variation in performance, possibly due to expression of

83   cryptic genetic variation (Schlichting 2008) or inappropriate expression of canalized responses

84   under novel environments (Van Buskirk and Steiner 2009). For example, the classic experiments

85   of Clausen, Keck, and Hiesey (1940) showed that high elevation clones of *Potentilla glandulosa*

86   grown at sea level had much more variation in stem height and flowering time than clones grown

87   at their elevation of collection, and *Lasthenia fremontii* genotypes showed broader hydrological

88   niche breadths when exposed to novel low-competition environments (Emery and Ackerly

89   2014). However, this hypothesis has not been formally tested (to our knowledge) in plant-

90   herbivore systems.

91   We chose to test these predictions in the monarch butterfly (*Danaus plexippus*, Danaidae:

92   Danainae, L.), which has dramatically expanded its range over the last 180 years (Figure 1).

93   Although best known from its ancestral range in North America, the monarch can now be found

94   in locations around the world, where it forms geographically isolated, generally non-migratory,

95   year-round breeding populations. In most cases, establishment dates for these populations are

96   well-documented, with a wave of out-of-North America expansion taking place over the last

97   ~180 years (Vane-Wright 1993, Zalucki and Clarke 2004). It is thought that the monarch's

98   establishment in these locations is due to human-mediated introduction of host plants. However,

99   the monarch's introduction dates and population genetics are consistent with a natural wave of

100   expansion (Pierce *et al.* 2014a, Zhan *et al.* 2014, Hemstrom and Freedman in prep). In most

101   locations where the monarch has become established, it has highly restricted dietary breadth and

102   often feeds on evolutionarily novel host species (see 'Background').

103   Here, we use the monarch's recent global range expansion to test ideas about host plant

104   adaptation. We address the following three questions about monarch performance (see Figure 2):

105   (1) *Do monarch populations show local adaptation to their host plant assemblages?* (2) *Do*

106   *monarch populations with broader dietary breadth have higher mean performance across hosts?*

107   (3) *Is variation in performance lower in sympatric host plant x monarch population*

108   *combinations?* We test these predictions using a common garden approach by raising monarchs

109   from different populations on a variety of ancestral and novel host plant species.

110

111   **Methods**

112

113   *Background*

114   **Timing of range expansion**: Monarchs became established on many Pacific Islands

115   beginning approximately 180 years ago, with the first conclusive records coming from Hawaii in

116 1841 (Vane-Wright 1993). They then moved southwestward across the Pacific in a stepwise

117 fashion, eventually becoming established in Australia in 1871 (Vane-Wright 1993, Zalucki and

118 Clarke 2004). Another population became established in the Mariana Islands (including Guam),

119 with the first records coming from 1887 (pers. obs., British Natural History Museum); the Guam

120 population represents an independent out-of-Hawaii expansion (Hemstrom and Freedman, in

121 prep). Finally, in a separate out-of-North America expansion, monarchs established in the

122 Caribbean, including Puerto Rico (Zhan *et al.* 2014). The timing of the monarch's establishment

123 in Puerto Rico is less certain but likely occurred thousands of years ago (Figure 1). Puerto Rican

124 monarchs are treated as their own subspecies, *D. plexippus portoricensis* (Ackery and Vane-

125 Wright 1984) and are phenotypically distinctive from North American *D. plexippus plexippus*.

126   **Host plant associations in novel range**: Monarchs that colonize novel areas like tropical

127 islands become non-migratory and have access to only one or two species of milkweed host. This

128 is in contrast to the ancestral North American monarch population, whose seasonal migration

129 brings it into contact with more than 100 species of native *Asclepias* (Apocynaceae:

130 Asclepiadoideae) hosts (Woodson 1954), 34 of which have been documented as host plants

131 (Malcolm and Brower 1986). Derived non-migratory monarch populations are often exposed to

132 evolutionarily and chemically novel host plants. For example, the monarch's primary host plants

133 in parts of Australia are *Gomphocarpus physocarpus* and *G. fruticosus* (Oyeyele and Zalucki

134 1990), and the primary hosts in the Hawaiian islands are *G. physocarpus* and *Calotropis*

135 *gigantea* (Pierce *et al.* 2014b) (Figure 1; Table 1). All of these non-*Asclepias* hosts are native to

136 subtropical Africa and India and have only recently become established outside of these areas.

137 Many derived non-migratory monarch populations are also associted with tropical milkweed (*A.*

138   *curassavica* L.); the geographic origin of this species is uncertain but is believed to be in Central

139   or South America (Woodson 1954).

140

141   *Host plant propagation*

142   Seeds of six species of milkweed were collected with permits from around the world

143   between 2015-2018. Milkweed species were chosen in accordance with (1) their prevalence as

144   host plants for monarchs from each region and (2) to maximize representation within the

145   milkweed phylogeny (see Agrawal and Fishbein 2008 and Appendix 1). When possible, seeds

146   were collected by fruit, which ensures full-sib relatedness among fruit-mates due to their

147   pollination biology (Wyatt and Broyles 1994). Seeds were stored either at room temperature or

148   were cold stratified, depending on their germination requirements. In 2017, we used only four

149   milkweed species (*Asclepias fascicularis* [ASFA – Western North America], *Asclepias syriaca*

150   [ASYR – Eastern North America], *Asclepias curassavica* [ASCU – Guam], *Gomphocarpus*

151   *physocarpus* [GOPH – Australia and Hawaii]); in 2018, we grew the same four species as well as

152   two additional species (*Asclepias speciosa* [ASPEC – Western North America], *Asclepias*

153   *incarnata* [AINC – Eastern North America]) (Table S2).

154   Beginning in February (2017) and March (2018) through September, plants were grown

155   from seed and transplanted into 1 gallon plastic pots in UC Soil Mix media, in two greenhouses.

156   Large pots and fertilization produced large plants capable of supporting multiple caterpillars, a

157   situation sometimes encountered in the field (pers. obs.). Plants (N=634 total across years) were

158   grown in a completely randomized design under ambient light (long days) and at 28°C in the

159   same two greenhouses. Approximately one quarter of plants (157/634) were used in multiple

160   feeding trials during the experiment because of limited sample sizes in some species; in these

161   cases, we waited at least three weeks before applying new caterpillars to a plant that had already

162   been used in a feeding trial. Plant ID was included as a random effect in all analyses to account

163   for position and prior feeding effects (see below).

164   Across both years, plants were subject to low levels of common greenhouse pests,

165   particularly flower thrips, green peach aphids, spider mites, and whiteflies. In 2017, oleander

166   aphids (*Aphis nerii*) became established in both greenhouses. To control these aphids, in early

167   June, plants were submerged in a dilute solution of water with castile soap (Dr. Bronners) to

168   dislodge aphids, then promptly rinsed.

169

170   *Monarch butterfly collection*

171   Monarchs from 16 sites were collected with permits as live adult females from their

172   respective locations and transported to Davis, CA in glassine envelopes (Table S3). In some

173   cases, adults could not be collected in sufficient numbers, and so larvae were collected and

174   reared to eclosion instead. In these cases, larvae were collected over a broad spatial (i.e. separate

175   plants and separate sites) and temporal range (i.e. different developmental stages) to minimize

176   the chance of sampling full or half sibs. Monarchs were kept alive as adults in glassine envelopes

177   and fed a 5:1 water : honey mixture daily. For adult butterflies reared from larvae, we used hand-

178   pairing to achieve mating within populations (Clarke and Sheppard 1956), with care taken to

179   minimize the chance of crosses between potential sibs (Mongue *et al*. 2016). Field-collected

180   adult females used for oviposition were sometimes infected with the protozoan parasite

181   *Ophryocystis elektroschirrha* (OE) (Table S3), with infection rates generally reflecting those that

182   occur in natural populations. In 2017, we used butterflies from four populations (eastern North

183   America [ENA], western North America [CA], Hawaii [HI], Australia [AU]); in 2018 we used

184 the same four populations as well as two additional populations (Guam [GU] and Puerto Rico

185 [PR]) (Table S3).

186   Adult females were set up in oviposition cages with *A. curassavica* for ~24 hours to

187 produce eggs that were used in the experiment, and females typically produced 20-100 eggs per

188 24 hours. Eggs were collected at the end of each 24 hour period and transferred to labelled petri

189 dishes with a damp paper towel and a small number of *A. curassavica* leaves. These petri dishes

190 were then stored either in the greenhouse or a lab benchtop, with water added as necessary to

191 prevent leaves from drying out. As soon as the eggs reached the "black head" stage (*Zalucki et*

192 *al.* 2001), petri dishes were checked every 12 hours for emergence, and within 24 hours of

193 hatching, neonates were transferred onto their respective experimental host plants.

194

195   *Experimental design and data collection*

196   We reared caterpillars in a fully factorial design, with all monarch populations reared on

197 all potential host species. When possible, we further stratified this design across individual

198 maternal families. Because of the logistical challenges associated with having all populations

199 developing concurrently, the experiment was carried out over the course of approximately three

200 months in both 2017 and 2018. Since host plants were also continuously growing and potentially

201 changing in condition during this time frame, we reared at least one monarch population over the

202 duration of the experiment in both years in an attempt to account for possible temporal effects

203 (see Figure S1) and also included a model term for plant age in all statistical analyses (see

204 below).

205   As soon as neonates hatched, they were randomly assigned to an individual plant. We

206 placed between 1-5 neonates per plant depending on their availability, though 85% of plants

207 received the full complement of five neonates (see Table S4). Neonates were transferred with a

208 fine paintbrush onto newly expanded leaves at the top of each plant. Plants were then fully

209 enclosed using custom-made polyester Super-Aire™ sleeves (A-Roo LLC), with the bottom end

210 sealed using a metal twist tie and the top sealed with binder clips (see Figure S2). In total, we set

211 up approximately 4,000 neonate caterpillars over the course of the experiment.

212   Neonates were left to grow for eight days and then scored for survival and weighed;

213 almost all larval mortality in monarchs occurs within this window (Zalucki and Malcolm 1999,

214 Zalucki *et al.* 2001). Any surviving larvae that could be found were put into petri dishes and

215 weighed to the nearest milligram. Larvae that could not be found were assumed to have died

216 during their early development; in some cases we found the remains of first instar caterpillars

217 (Figure S2). If plants were large enough, all surviving larvae were returned. If not, we used a

218 random number generator to select which larvae to return to the plant.

219   After caterpillars reached their fifth and final instar, plants were checked daily to capture

220 dates of pupation. Pupae were transferred into individually labeled 16 oz containers; on the day

221 of eclosion, adult butterflies were kept in the container in which they eclosed for 6-8 hours to

222 allow their wings to dry, at which point they were transferred into a glassine envelope. We

223 recorded the mass of these newly eclosed adults and later measured forewing length, width, area,

224 aspect ratio, and roundness, as well as hindwing area (for methods, see Freedman and Dingle

225 2018) and levels of adult cardenolide sequestration (Freedman and Vannette, in prep).

226   In total, we analyzed the following performance metrics: (1) larval survival and (2) mass

227 on day eight, (3) time to pupation, (4) time to eclosion, (5) mass at eclosion, (6) adult wing

228 morphological characteristsics, and (7) adult cardenolide sequestration. Larval survival has clear

229 fitness implications. Mass on day eight, time to pupation, and time to eclosion are all related to

230   development rate and were highly correlated with each other. We expect for natural selection to

231   favor faster development rates, as this shortens the window when larvae are most susceptible to

232   abiotic (heat, cold, rainfall) and biotic (predators, parasitoids, pathogens) sources of mortality.

233   Eclosion mass and adult wing morphology may infuence fecundity and/or dispersal ability, while

234   cardenolide sequestration ability may reflect distastefulness to potential predators.

235

236   *Plant Trait Sampling*

237   We sampled two plant defense traits to see if they could explain variation in performance.

238   We measured latex production and cardenolide concentration, since these have been shown to be

239   important determinants of larval monarch growth and survival (Zalucki and Kitching 1982,

240   Zalucki *et al.* 2001, Agrawal *et al.* 2015). Immediately prior to adding neonate caterpillars, most

241   plants (n = 565 / 634) were sampled for constitutive levels of latex production. We also collected

242   leaf discs to measure constitutive levels of cardenolide production for 163 plants using the

243   methods outlined in Zehnder and Hunter (2007). For full details on latex and cardenolide

244   sampling, see Appendix 2.

245

246   *Statistical analysis*

247   *Question 1: Do monarch populations show local adaptation to their host plant assemblages?*

248   To quantify local adaptation, we use the approach outlined in Blanquart *et al.* (2013),

249   which models the residual variation in performance after accounting for inherent differences

250   among environments (*i.e.* milkweed species) and demes (*i.e.* monarch populations) (see Figure

251   2). Briefly, this approach summarizes local adaptation according to:

$$\Delta_{SA} = \frac{1}{P}\sum_{i=1}^{P}\overline{W}_{i\to i} - \frac{1}{P(P-1)}\sum_{i=1}^{P}\sum_{\substack{j=1 \\ j\neq i}}^{P}\overline{W}_{j\to i}$$

252   where Δ_{SA} captures the difference in performance between all combinations of monarch

253   populations on their sympatric milkweed hosts ($W_{i\grave{e}i}$) versus all combinations of monarch

254   populations on their allopatric milkweed hosts ($W_{j\grave{e}i}$) (Figure S3). We fit linear and generalized

255   linear mixed effects models that included fixed effects for milkweed species and monarch

256   population of origin and a term for sympatric/allopatric status that describes the magnitude of

257   local adaptation. Models were fit using the lme4 package version 1.1.21 (Bates *et al.* 2015) in R

258   version 3.4.4 (R Core Team). In all models, we included the following terms as covariates:

259   greenhouse, plant usage (first or second exposure to caterpillar feeding), plant age, and

260   experiment year. In models using performance information from adult butterflies, we included

261   infection status with the parasite OE and butterfly sex as covariates. In all models, we included

262   random intercepts for plant ID nested within plant maternal family of origin, monarch maternal

263   family, and greenhouse block. For a full summary of statistical models used, see Table S5.

264   For all continuous performance metrics (larval mass at day eight, days to pupation, days

265   to eclosion, mass at eclosion, adult wing morphological measures), we fit models with Gaussian

266   error distributions. In the model using larval mass at day eight as a response variable, we log

267   transformed this measure to account for the non-linear accumulation of larval mass through

268   caterpillar development. For ease of interpretation, we report larval mass results using back-

269   transformed values, which therefore represents the geometric rather than the arithmetic mean.

270   Because time to pupation and time to eclosion were almost perfectly correlated ($R^2 = 0.945$), we

271   only report results for time to eclosion. For larval survival to day eight, we fit a model with a

272   binomial error distribution. All results were summarized using type II analysis of variance in the

273   package 'car' v3.0.2 (Fox and Weisberg 2011). We also calculated marginal means in the

274   package 'emmeans' v1.3.4 (Lenth 2019) and report these throughout the text.

275

Question 2: Do 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, while derived populations have narrow dietary breadth.

283

Question 3: Is variation in performance lower in sympatric host plant x monarch population

combinations?

To analyze variance in performance, we calculated the coefficient of variation (CV = σ^2/μ) in performance for each maternal family x plant genotype combination (n = 717). 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, since 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, since 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 zero values when calculating CVs.

298

Results

299

300

301   *Question 1: Do monarch populations show local adaptation to their host plant assemblages?*

302   For performance metrics associated with larval growth rate and survival, monarchs

303 exhibited a pattern of local adaptation, indicated by significantly higher performance on

304 sympatric compared to allopatric host plants. This effect was detectable, despite substantial

305 variation in performance owing to inherent differences among host plants and monarch

306 populations.

307   Host plant identity was by far the biggest source of variation in larval monarch

308 performance ($\chi^2 = 69.65$, DF $= 5$, p < 0.001). The host plant species in this experiment may

309 encompass up to 40-50 MY of divergence (note: this estimate is uncertain, see Fishbein et al.

310 2011) and employ disparate defense strategies against larval monarchs. For example, our host

311 species differed more than 20-fold in latex production and 50-fold in cardenolide production

312 (Figure 3). In general, larval performance was lowest on milkweed species with high latex

313 production. All populations performed worst on *A. speciosa*, which also had the highest latex

314 production of the species tested. By contrast, monarchs tended to perform well on milkweed

315 species with high cardenolide concentrations (*A. curassavica* and *G. physocarpus*).

316   For larval mass on day eight, monarchs reared on their sympatric hosts ($\bar{x} = 237$ mg)

317 were over 16% larger than monarchs reared on their allopatric hosts ($\bar{x} = 198$ mg) ($\chi^2 = 15.74$,

318 DF $= 1$, p < 0.001). There were strong differences in day eight larval mass among host plants (χ^2

319 $= 69.65$, DF $= 5$, p < 0.001), with a 3-fold mass difference between *Asclepias curassavica* ($\bar{x} =$

320 343 mg) and *A. speciosa* ($\bar{x} = 102$ mg). Monarch population also explained a substantial portion

321 of variation ($\chi^2 = 32.51$, DF = 5, p < 0.001), with the highest mass in monarchs from eastern

322 North America ($\bar{x} = 320$ mg) and lowest mass in monarchs from Puerto Rico ($\bar{x} = 123.7$ mg)

323 (Figure 3a, Table 1). Monarch populations from Australia and Hawaii, which are associated with

324 a novel host (*G. physocarpus*), had no performance advantage on this host relative to populations

325 naïve to this host (Figure 4a). By contrast, monarchs from areas with only derived hosts had

326 sharply reduced performance on their ancestral North American hosts relative to ancestral North

327 American populations (Figure 4a). Thus, the signal of adaptation appears to come from a loss of

328 performance of recently range-expanded populations on ancestral hosts.

329 Survival on sympatric host plants was slightly higher (79.7%) than survival on allopatric

330 hosts (75.7%) ($\chi^2 = 3.98$, DF = 1, p = 0.046). Milkweed species again explained the largest

331 proportion of variation in survival ($\chi^2 = 23.12$, DF = 5, p < 0.001), with highest survival on *A.*

332 *incarnata* (85.3%) and lowest survival on *A. speciosa* (68.8%). Differences in survival among

333 populations were more modest ($\chi^2 = 12.17$, DF = 5, p = 0.033) (Figure 4b).

334 Time to eclosion was faster for monarchs reared on sympatric host plants ($\bar{x} = 22.5$ days)

335 compared to monarchs on allopatric hosts ($\bar{x} = 22.8$ days) ($\chi^2 = 4.81$, DF = 1, p = 0.028). As with

336 larval mass on day eight, milkweed species explained the largest proportion of variation in time

337 to eclosion ($\chi^2 = 62.71$, DF = 5, p <0.001), with time to eclosion fastest on *A. curassavica* ($\bar{x} =$

338 21.4 days) and slowest *on A. speciosa* ($\bar{x} = 24.5$ days). Monarch populations also differed in time

339 to eclosion ($\chi^2 = 30.80$, DF = 5, p <0.001), with eclosion occurring fastest for eastern North

340 America ($\bar{x} = 21.5$ days) and slowest for Puerto Rico ($\bar{x} = 23.7$ days) (Figure 4c).

341 We did not find a signature of local adaptation in adult biomass or forewing area, nor

342 strong differences among populations ($\chi^2 = 9.83$, DF = 5, p = 0.080) or milkweed host species

343 ($\chi^2 = 8.98$, DF = 5, p = 0.110). There was also no sympatric/allopatric effect for adult eclosion

344   mass ($\chi^2 = 0.00$, DF = 1, p > 0.9). Adult eclosion mass and wing area were highly correlated

345   (Figure S4), though population-level differences were more pronounced for forewing area than

346   for eclosion mass (Table S5), reflecting migratory status. Our results show that selection from

347   host plants is acting primarily at the early stages, with large effects on early instar survival and

348   also development time.

349

350   *Question 2: Do monarch populations with broader dietary breadth have higher mean*

351   *performance across hosts?*

352   In comparing ancestral and derived populations as groups, we found that ancestral North

353   American populations, which have broad dietary breadth, had significantly higher mean growth

354   rates across all host species ($\chi^2 = 10.45$, DF = 1, p = 0.001). However, we did not find

355   significantly higher overall survival in the ancestral North American populations ($\chi^2 = 1.41$, DF

356   = 5, p = 0.234). Time to eclosion was faster in ancestral populations ($\chi^2 = 10.42$, DF = 1, p =

357   0.001) (Figure 4c), and ancestral populations tended to have higher eclosion mass than derived

358   populations at eclosion ($\chi^2 = 2.78$, DF = 1, p = 0.095) (Figure 4d).

359

360   *Question 3: Is variation in performance lower in sympatric host plant x monarch population*

361   *combinations?*

362   Monarchs reared on their sympatric hosts had less variable mass of day eight than

363   monarchs reared on their allopatric hosts ($\chi^2 = 4.53$, DF = 1, p = 0.033) (Figure 5c). This result

364   did not change if we used randomization tests to equalize the number of maternal families tested

365   per monarch population (Appendix 3). Milkweed species was the strongest predictor of variation

366   in performance ($\chi^2 = 24.77$, DF = 5, p <0.001, Figure 5a). This result was driven by low variation

367   in performance on *A. incarnata* (CV = 0.691) and *A. curassavica* (CV = 0.791) relative to other

368   milkweed species, particularly *A. syriaca* (CV = 0.989). By contrast, monarch populations did

369   not differ significantly in their CV (χ^2 = 7.79, DF = 5, p = 0.168, Figure 5b).

370

371   **Discussion**

372

373   We found evidence for local adaptation to host plant assemblages in monarch butterflies,

374   with better larval performance (growth rate and survival) on sympatric compared to allopatric

375   hosts. This result is consistent with our predictions and suggests that divergent selection

376   pressures across the monarch's global range have resulted in local adaptation to host plant

377   assemblages over contemporary time scales (~1000 generations). Our local adaptation pattern

378   was driven by primarily by poor performance of derived populations reared on their ancestral

379   hosts. By contrast, derived populations had little or no performance advantage over ancestral

380   naïve populatons on the novel host plants with which they now associate. Our data suggest that

381   after ~1000 generations, population-level differences in monarch performance are driven not by

382   directional selection for increased performance on novel hosts, but instead by relaxed selection

383   and decreased performance due to loss of association with ancestral hosts.

384   Our findings are similar to those reported by Gompert *et al.* (2015) and Forister *et al.*

385   (2009) in their studies of novel host plant utilization in *Lycaeides melissa* butterflies: *L. melissa*

386   populations that utilize alfalfa as a novel host resource have a slight performance advantage on

387   alfalfa relative to naïve *L. melissa* populations that feed on the ancestral host (*Astragalus*), but

388   alfalfa-associated populations show a marked decrease in performance when returned to the

389   ancestral *Astragalus* host plant. The loss of performance by derived populations on ancestral

390 hosts suggests that neutral evolutionary processes (e.g. relaxed selection) may be an important

391 component of host plant specialization (Hardy *et al.* 2016), especially under circumstances where

392 derived populations become fully reproductively isolated from ancestral populations. Relaxed

393 selection could drive the breakdown of enzymatic machinery or of behaviors that enable growth

394 and survival on ancestral hosts, such as leaf notching or trichome shaving (Agrawal 2017). Our

395 result is also consistent with the idea of local adaptation being driven by loci with conditionally

396 neutral, rather than antagonistically pleiotropic, effects across hosts (Gompert *et al.* 2015),

397 although formally demonstrating this pattern would require a genome-wide survey of fitness

398 effects across many loci. Further experiments using additional monarch populations and/or

399 populations from other independent out-of-North America expansions are needed to formally test

400 how (1) time since establishment and (2) degree of genetic differentiation might be associated

401 with performance on ancestral hosts.

402   Monarch population identity was a major source of variation in larval performance, with

403 the ancestral North American populations consistently outperforming derived populations,

404 regardless of host plant identity. This result is consistent with our prediction that populations

405 with broader host plant breadth should have higher mean performance across the full range of

406 hosts. However, because of the relatively small number of populations (n = 6) and independent

407 range expansions (n = 2) that we tested, it is difficult to determine exactly why we saw this

408 pattern. For example, host plant breadth is necessarily conflated with migratory status and

409 effective population size in our study: only the large, migratory North American populations

410 have access to diverse assemblages of host plants. This is reflective of a broader issue in studies

411 of herbivore dietary breadth: generalist populations and species often have larger geographic

412 ranges and population sizes (Janz and Nylin 2008, Jahner *et al.* 2011, Slove and Janz 2011).

413   Thus, the higher mean performance across hosts that we observed in the ancestral North

414   American populations could be explained by the maintenance of genetic variation for

415   performance in migratory populations with broader dietary breadth, but it could also be

416   explained by inbreeding depression in derived lineages with smaller population sizes.

417   We did not find evidence for local adaptation in the adult performance metrics (eclosion

418   mass, wing morphology) that we tested. However, we did find modest population-level

419   differences in eclosion mass and strong population-level differences in adult forewing area that

420   correspond to differences in migratory status. Migratory North American populations generally

421   had higher body mass and significantly larger forewings than derived non-migratory populations,

422   consistent with other studies of trait divergence in migratory vs. non-migratory monarchs

423   (Altizer and Davis 2010, Li *et al.* 2016). The absence of a local adaptation effect for these

424   measures could reflect a developmental threshold pattern in monarch larvae, whereby pupation

425   occurs after larvae have reached a predetermined size that is host plant invariant. Futhermore, it

426   is possible that we would have detected local adaptation had we measured other aspects of adult

427   monarch performance, such as longevity or lifetime fecundity (Scheirs *et al.* 2005).

428   We predicted that monarchs reared on their sympatric host plants would show reduced

429   variation in performance relative to monarchs reared on allopatric hosts, and we did find this to

430   be the case. This result is consistent with other studies that have found greater performance

431   variation in species transplanted outside of their realized niche (e.g. Emery and Ackerly 2014),

432   although to our knowledge this represents the first such demonstration in a plant-herbivore

433   system. We also found that the coefficient of variation (CV) in performance was significantly

434   different between milkweed species. Species-level differences in CV in performance were driven

435   primarily by low levels of variation in *A. incarnata* and *A. curassavica*. These two species also

436   happened to have the lowest latex production of any of the species tested. By contrast, the

437   species with the highest latex production, *A. speciosa*, also had the highest performance CV.

438   These results suggest that latex production may be important for explaining performance

439   variation among monarch families and accord with previous findings that latex production is the

440   primary driver of larval mortality in monarchs (Zalucki *et al.* 2001).

441   Local adaptation to host plants is a central part of hypotheses about macroevolutionary

442   patterns of specialization and speciation in herbivores (Janz and Nylin 2008). Our results provide

443   an example of local adaptation developing over contemporary time scales in a highly mobile

444   insect herbivore with a well-characterized range expansion history. Furthrmore, we show that

445   local adaptation to host plants in this system does not seem to be strongly driven by cross-host

446   tradeoffs, even in cases where monarchs have colonized a phylogenetically and chemically novel

447   host genus (*Gomphocarpus*). Although the conditions that gave rise to this pattern may be

448   somewhat restrictive due to the isolated nature of our island populations and their host plant

449   assemblages, our results still demonstrate how the early stages of host plant specialization may

450   proceed and highlight the value of collections and well-documented knowledge of range

451   expansion history to understand adaptation.

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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

Table 1 – Summary of monarch populations and host plant species used in experiment. Abbreviations are used in figures for brevity.

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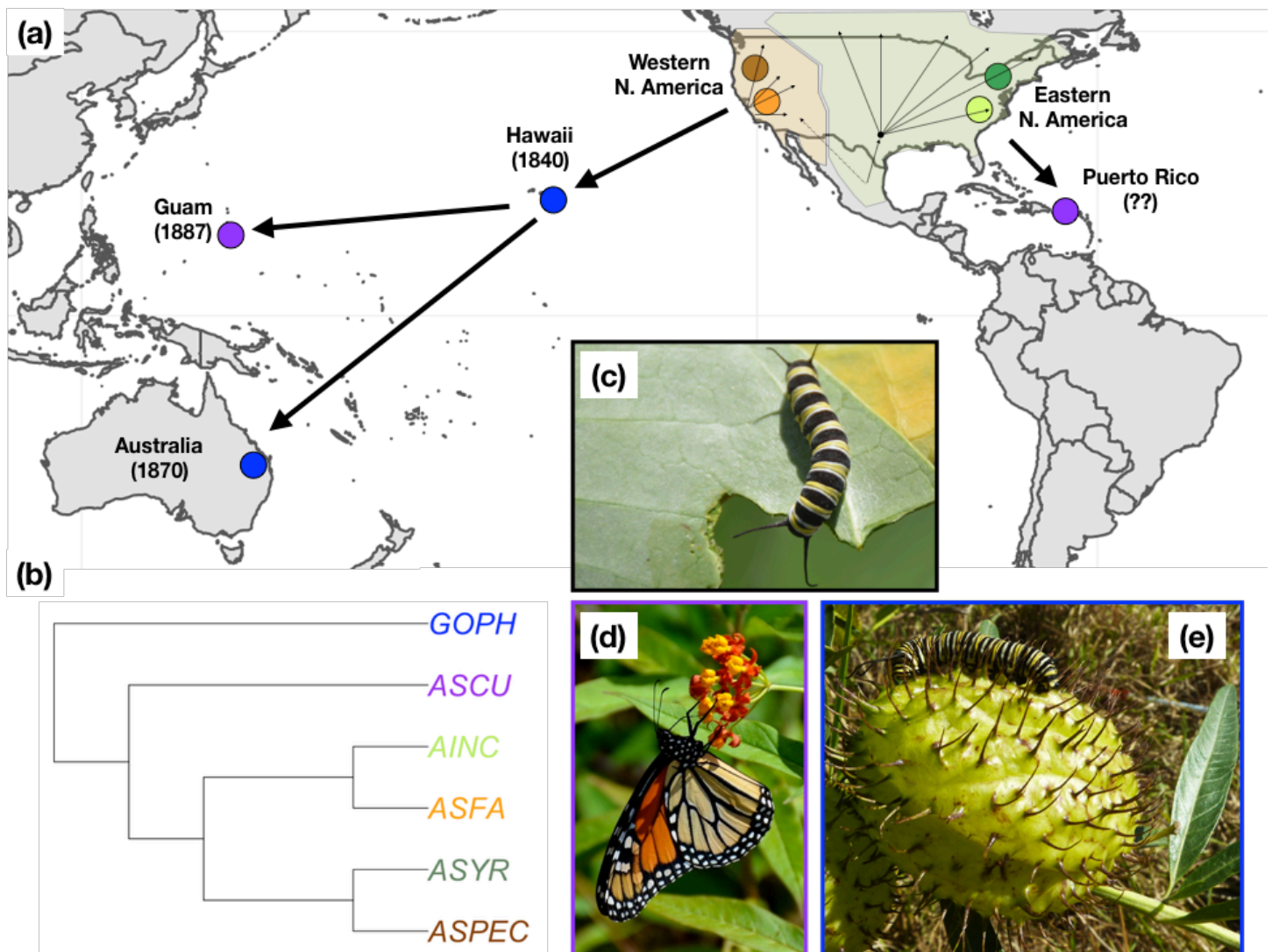
Figure 1 – (a) Host plant associations of monarch butterfly populations around the world. Arrows correspond to routes of establishment for monarchs, with numbers in parantheses showing the earliest records of monarchs from a particular location (Zalucki and Clarke (2004)). Monarchs separately colonized 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 used in this experiment built using sequences provided in Agrawal and Fishbein (2008). (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.

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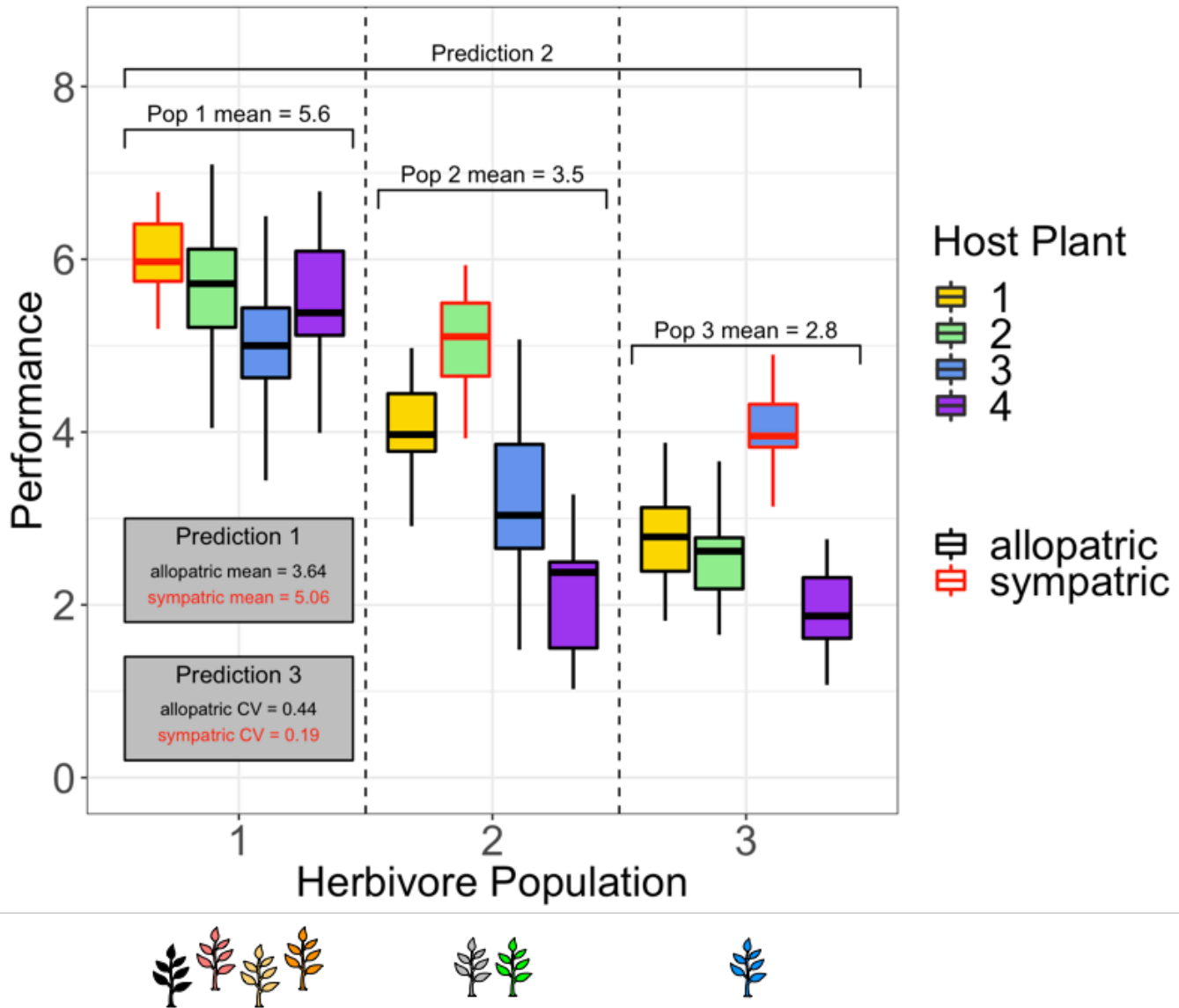
Figure 2 – Predictions for our experiment using a hypothetical example with three herbivore populations and four host plant species. **Prediction 1**: Monarchs will show a pattern of local adaptation to host plants, with sympatric combinations of monarch population and host plant (red outlines) having higher performance than allopatric combinations (black outlines). **Prediction 2**: Monarch population 1, which has the broadest dietary breadth, should also have the highest mean performance across all host plants. **Prediction 3**: Monarchs on their sympatric host plants should show lower variation in performance than monarchs on allopatric host plants.

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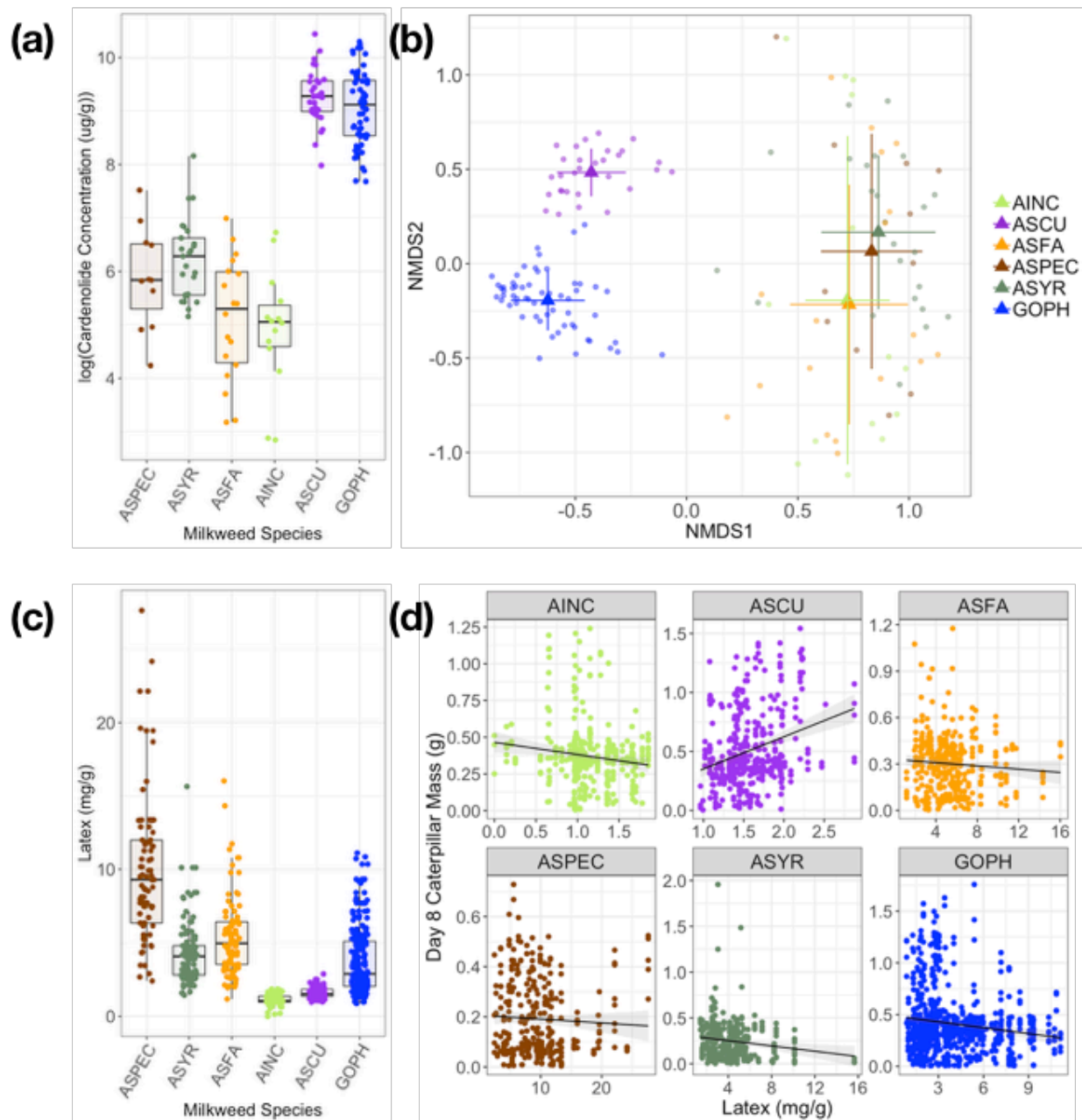
Figure 3 – (a) Cardenolide concentrations for each of the six species tested. Note that concentrations are expressed as log(μg cardenolide / g dry leaf tissue). Mean concentrations ranged from as low as 0.23 mg/g in *A. incarnata* to as high as 12.12 mg/g in *A. curassavica*. (b) Nonmetric multidimensional 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 the x-axis differs for each species.

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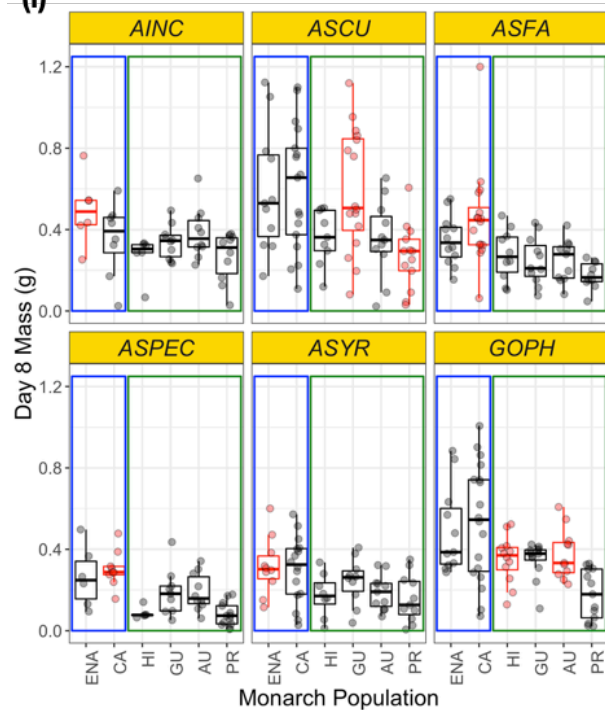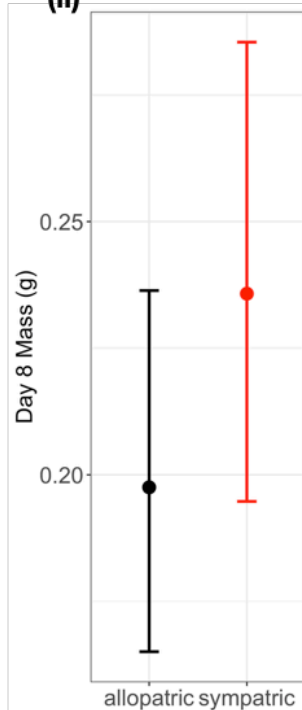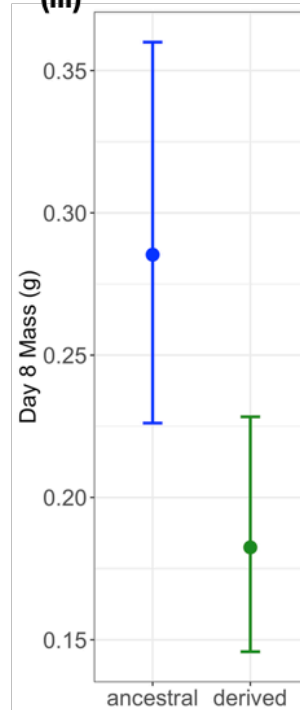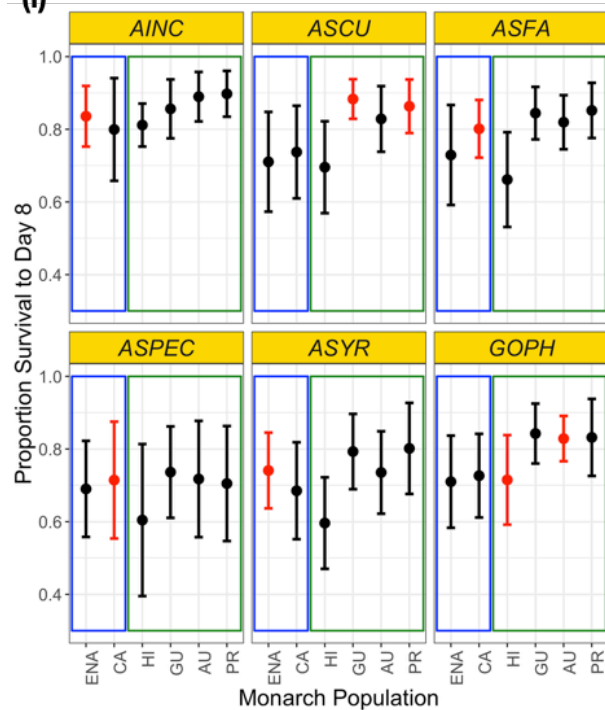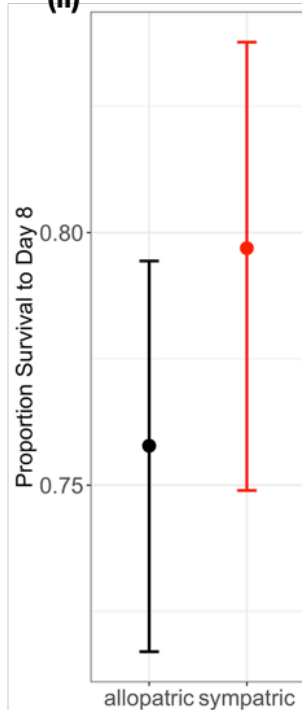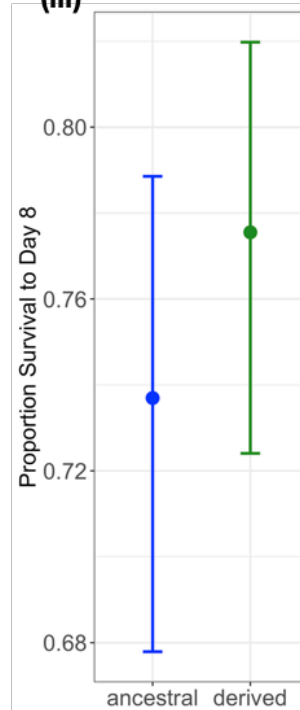
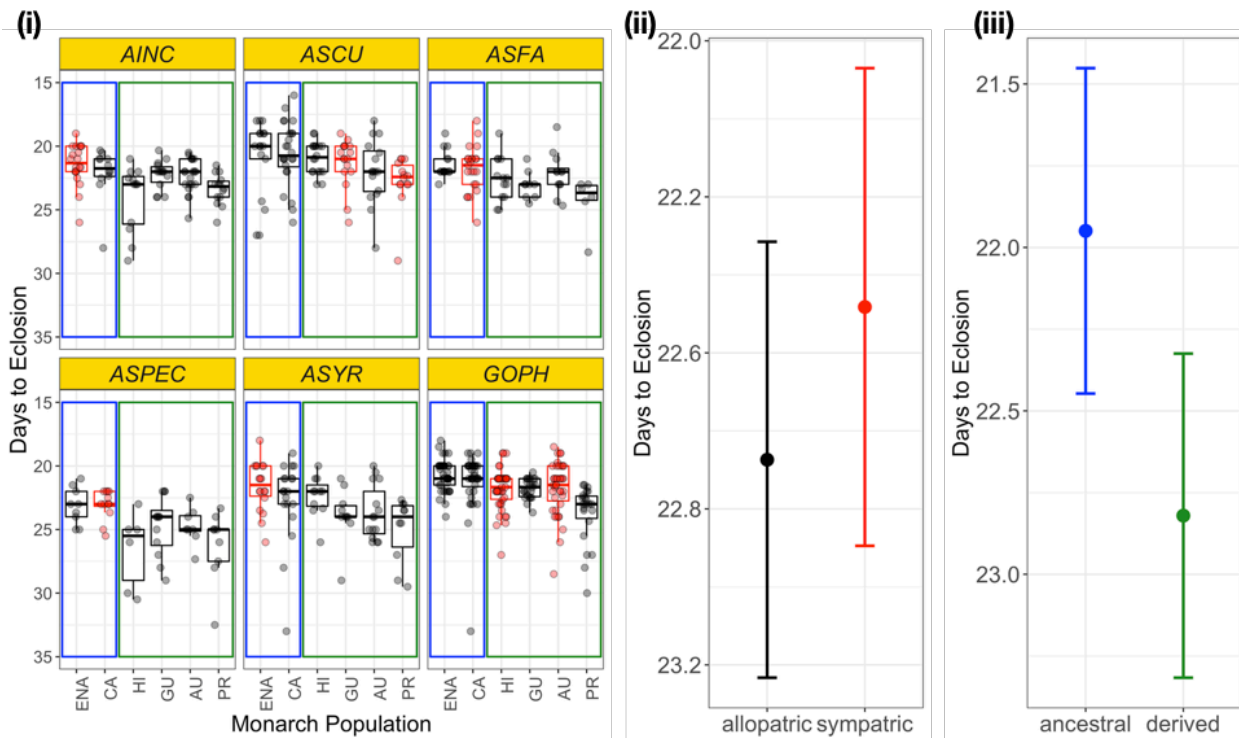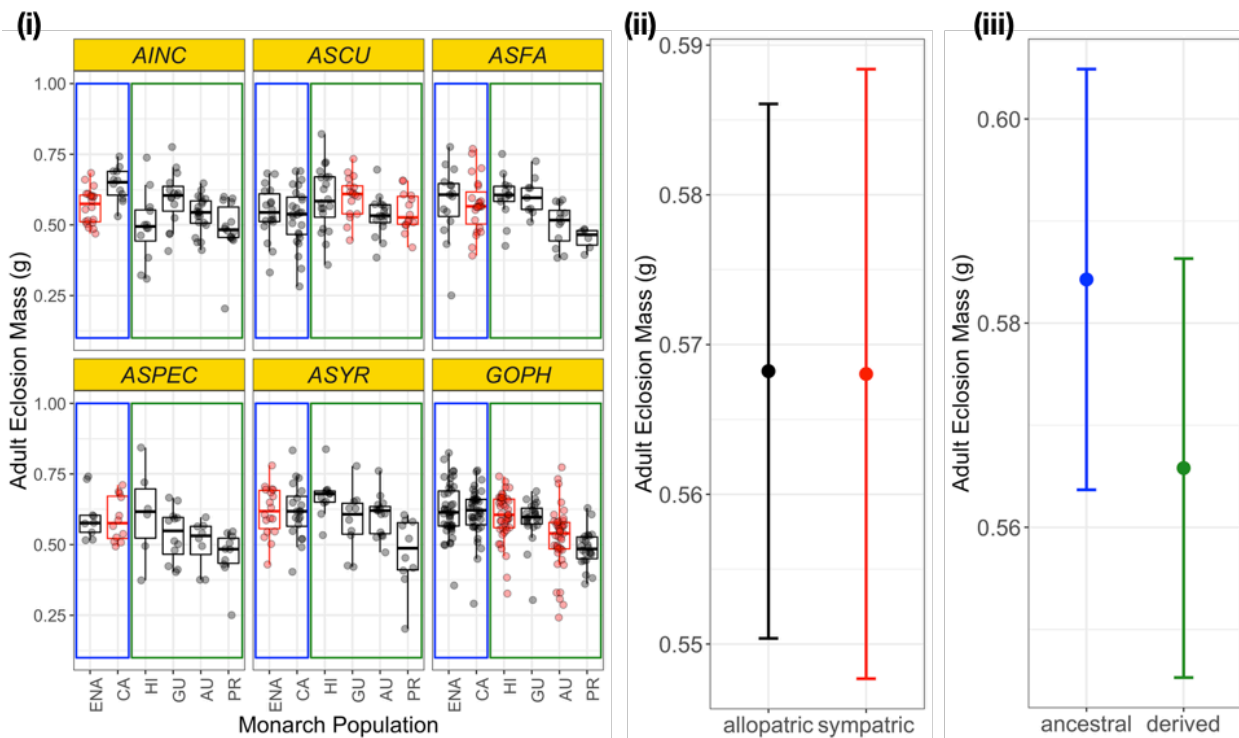**Figure 4** – Summary of performance metrics, separated by (a) larval mass on day eight (b) larval survival on day eight (c) the number of days to eclosion and (d) mass at eclosion. In each figure, the left panel shows raw data for all monarch population x milkweed species combinations, with points corresponding to mean for single maternal families. The center panel shows the average sympatric/allopatric effect, while 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.
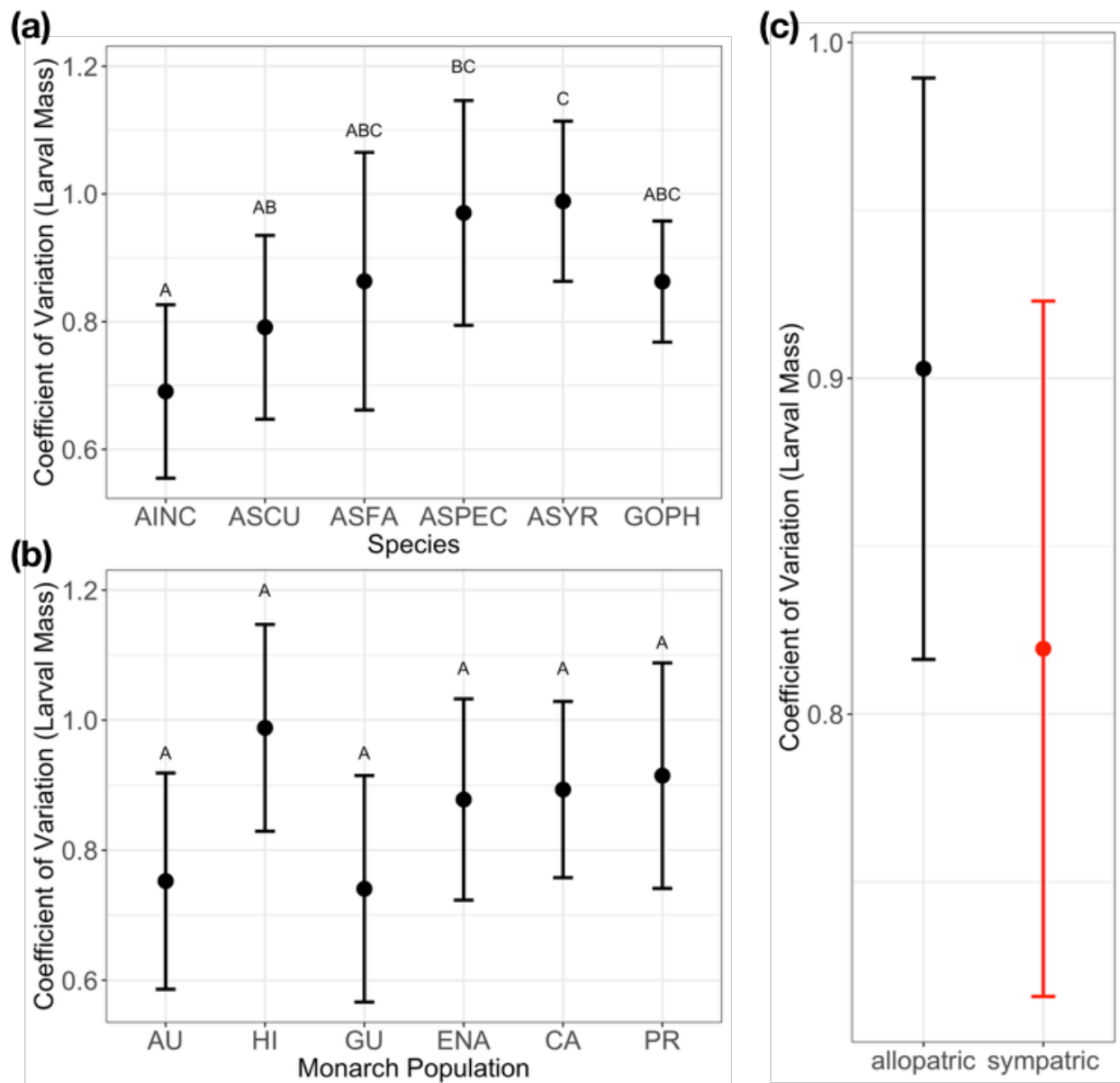
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Figure 5 – Coefficients of variation in monarch performance across (a) milkweed hosts (b) monarch populations and (c) sympatric versus allopatric combinations. Letters correspond to significant differences after correction for multiple comparisons. Monarchs reared on sympatric host plants had significantly lower variation in performance than monarchs reared on allopatric hosts.

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