1   **Natural selection and maladaptive plasticity in the red-shouldered soapberry bug**

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7

8   **Abstract**

9   Natural selection and phenotypic plasticity can both produce locally differentiated phenotypes,

10   but novel environments or gene combinations can produce plasticity that works in opposition to

11   adaptive change. The red-shouldered soapberry bug (*Jadera haematoloma*) was locally adapted

12   to feed on the seeds of an introduced and a native host plant in Florida in the 1980s. By 2014,

13   local differentiation was lost and replaced by phenotypically similar populations all adapted to

14   the introduced host, likely as a result of gene flow. Here, I quantify the effects of these two host

15   plants on individual performance, natural selection, and phenotypic plasticity. I find that the seed

16   coat and seedpod of the native host have strong negative effects on juvenile survival and adult

17   reproduction compared to the introduced host. I find support for the hypothesis that the seedpod

18   is driving diversifying natural selection on beak length, which was previously locally adapted

19   between hosts. I also find maladaptive plasticity induced by host plant: bugs develop beak

20   lengths that are mismatched with the seedpod size of the host they are reared on. This plasticity

21   may be the result of gene flow; hybrids in the 1990s showed the same pattern of maladaptive

22   plasticity, and plasticity is stronger in the present in areas with high gene flow. Although

23   ongoing natural selection has produced locally adapted genotypes in soapberry bugs,

24   maladaptive plasticity has masked the phenotypic difference between populations in the field.

25

26 **Introduction**

27   The role of plasticity in natural selection and local adaptation is controversial (West-

28 Eberhard 2003; Crispo 2007, 2008). In some cases, plasticity may facilitate an evolutionary

29 response, either by allowing populations to persist and maintain genetic variation in novel

30 habitats or by bringing populations close enough to an adaptive peak for selection to act on

31 underlying genetic variation (Price et al. 2003; Yeh and Price 2004; Lande 2009). In other cases,

32 plasticity inhibits genetic change by masking genetic variation in phenotypes under selection

33 (Sultan and Spencer 2002; Borenstein et al. 2006). Plasticity may also be maladaptive and move

34 phenotypes away from local fitness maxima. Maladaptive plasticity may generate phenotypic

35 differences in nature in the absence of divergent selection (eg, countergradient variation;

36 Conover and Schultz 1995). In systems with divergent natural selection between habitats,

37 maladaptive plasticity may dampen, mask, or even reverse the phenotypic signal of adaptive

38 genetic differentiation in nature.

39   Maladaptive plasticity is expected to arise most often in novel habitats due to a lack of

40 evolutionary history between environmental cues and physiological responses. Individuals may

41 develop traits that would have been adaptive in the historical context, but are not adaptive in the

42 new environment; novel habitats may also expose cryptic genetic variation, producing

43 phenotypes that have never been exposed to selection in any habitat (Ghalambor et al. 2007).

44 This type of maladaptation should be purged by selection relatively quickly. Novel genotypes,

45 like those produced in hybrids or backcrosses between diverged populations coming into

46 secondary contact, have also not experienced selection and should therefore be more likely to

47 harbor maladaptive variation. Unlike in novel environments, however, it may be difficult for

48   selection to purge maladaptive phenotypes in hybrids if opposing selection continues to act on

49   each parental type.

50   Local adaptation to different host plants is common in plant-feeding insects (eg, Bush

51   1969; Carroll and Boyd 1992; Ferrari et al. 2008; Downey and Nice 2011), where host plant

52   defenses are a major driver of natural selection and differentiation (Ehrlich and Raven 1964;

53   Wheat et al. 2007; Toju 2009). Natural selection is often invoked as the source of local

54   adaptation; however, other processes commonly produce local trait differentiation (eg, plasticity,

55   correlated selection, genetic drift). To lend support to the hypothesis that a trait is under

56   divergent selection by an environmental feature that differs between two habitats, we should first

57   answer some basic questions: Does the proposed selective agent have a substantial impact on

58   individual performance? Do some trait values have higher performance than others when

59   challenged by the selective agent, and does the effect of the trait value on performance differ

60   between environmental types? What aspect(s) of performance are influenced by the selective

61   agent and are they important for survival and reproduction in nature?

62   The red-shouldered soapberry bug *Jadera haematoloma* (Hemiptera: Rhopalidae) is a

63   textbook case of rapid local adaptation to a novel environment. Soapberry bugs are native to the

64   southern peninsula and Keys of Florida, where they have evolved with a native balloon vine

65   (*Cardiospermum corindum*). In the 1950s, the Taiwanese golden rain tree (*Koelreuteria elegans*)

66   was widely introduced to the peninsula of Florida and colonized by soapberry bugs. The native

67   *C. corindum* has large, inflated seedpods, hard seeds, high nitrogen content, and asynchronous

68   fruiting throughout the year. The invasive *K. elegans* has flattened seedpods that are open for

69   most of the development of the seed, soft seeds, high lipid content, and a single, synchronous

70   fruiting period each autumn (Umadevi and Daniel 1991; Carroll and Boyd 1992; Carroll et al.

71   1998). By 1988 there was clear evidence of local host-associated adaptation in soapberry bugs.

72   Juveniles had higher survival and shorter development time on their local host than on the

73   alternative host (Carroll et al. 1997, 1998). Differences in dispersal morphology were found

74   between host-associated populations: individuals from *K. elegans* had higher frequencies of non-

75   dispersive morphs than individuals from *C. corindum*, hypothesized to be due to differences in

76   host plant reproductive synchrony (Carroll et al. 2003). Adult feeding morphology was locally

77   adapted: adult females adapted to *C. corindum* had long beaks, while females from populations

78   on *K. elegans* had evolved short beaks (Carroll and Boyd 1992). It was hypothesized that long-

79   beaked females would be more successful at accessing seeds inside of the large pods of *C.*

80   *corindum,* which should increase nutrient acquisition and therefore energy available to invest in

81   reproduction. On *K. elegans*, it was hypothesized that short beaks would be more efficient for

82   feeding through the flat pods, which would increase the speed of nutrient acquisition and give

83   females with short beaks a competitive advantage. Beak length was highly heritable (Dingle et

84   al. 2009) and only weakly affected by developmental host plant in pure host races. In contrast,

85   individuals with mixed ancestry showed a profound, potentially maladaptive influence of

86   developmental host on beak length: female crosses and backcrosses between host races

87   developed longer beaks when raised on the seeds of the small-podded host, *K. elegans*, than on

88   the large-podded *C. corindum* (Carroll et al. 2001). All of these locally adapted phenotypes

89   suggest divergent selection was acting between the two host plants. The direct effects of host

90   plant defenses on performance and the role of the seedpod in natural selection on beak length

91   have remained untested.

92   By 2014, the pattern of local adaptation between hosts had shifted dramatically. Adult

93   morphology, survival, and development time are all converging across populations on both host

94   plants (Cenzer 2016). The phenotypes that now dominate across the state of Florida are those

95   adapted to the non-native host, *K. elegans*. It is likely that this phenotypic pattern is the result of

96   gene flow increasing from populations adapted to *K. elegans* to those on *C. corindum*. The novel

97   host has increased in frequency and the native host has become less common during the last 25

98   years (Carroll and Loye 2006). As gene flow increases, more hybrids should be present in nature,

99   particularly on the native host. Dingle et al 2009 (using bugs collected from the field in 1991)

100   showed that experimentally produced hybrids had higher plasticity than pure host races in

101   response to developmental host. As gene flow increases hybridization in the field, therefore,

102   developmental plasticity induced by each host plant is likely to become more common.

103   In this study, I addressed three primary questions. In order to understand how host plants

104   drive natural selection, we need to know what plant traits have the greatest effects on insect

105   fitness. Therefore, I first asked: 1) How do two physical host plant defenses, the seed coat and

106   the seedpod, influence patterns of juvenile and adult performance on each host plant? I then

107   addressed the key questions: 2) How does the host seedpod influence natural selection on beak

108   length? and 3) How does host plant influence plasticity in morphology?

109   Based on known differences in physical defenses and current and historical patterns of

110   local adaptation, I proposed the following hypotheses: H1) Juvenile mortality is driven by the

111   tough seed coat, which prohibits early feeding and causes starvation. H2) Juveniles reared on the

112   native *C. corindum* will develop shorter beaks than those raised on *K. elegans*, consistent with

113   historical patterns of maladaptive plasticity documented in hybrids between host races; this

114   change may be coupled with plasticity in body size and dispersal morph due to correlations in

115   these three phenotypes. Alternatively, selection may have purged deleterious plasticity in areas

116   where gene flow is high, in which case we should observe neutral or adaptive plasticity. H3) If

117   maladaptive plasticity is being generated by gene flow, I expect a latitudinal gradient such that

118   developmental plasticity is strongest in the south, where gene flow between hosts is more

119   common, and becomes weaker moving north away from the sympatric zone. H4) The presence

120   of the seedpod reduces adult fitness by reducing access to seeds. H5) Adults with longer beaks

121   will access more seeds within closed pods and adults with short beaks will feed more efficiently

122   on seeds in open pods, consistent with divergent natural selection on beak length.

123   To test these hypotheses, I conducted three experiments. The first tested the effect of the

124   rearing host and the seed coat on juvenile survival, development time, and morphology (H1, H2,

125   & H3; Fig. 1a). The second and third experiments tested the effect of the seedpod on adult

126   performance and natural selection on beak length in the lab and field, respectively (H4 & H5;

127   Fig. 1b,c).

128

129   **Methods**

130   *Collection*

131   I collected *J. haematoloma* in April 2014 for the juvenile performance experiment and in

132   December 2013 for the adult performance laboratory experiment from 8 locations in Florida

133   (Fig. 2; coordinates in Table S1). The adult performance field experiment was conducted in April

134   2014 with field-collected individuals from Key Largo. Populations at the northern four sites

135   (Gainesville, Leesburg, Lake Wales, and Ft. Myers) occur on *K. elegans* and the southern two

136   sites (Key Largo and Plantation Key) occur on *C. corindum*. Bugs occur on both host plants in

137   Homestead, the only known location where these two host plants occur in close proximity

138   (<5km). For laboratory experiments, I collected host plant seeds from each site in December

139   2013 and April 2014 and stored them at 4°C until they were used for rearing. I discarded seeds

140   with visible indications of previous feeding and tested all seeds for viability by placing them in

141   water and discarding seeds that floated. I collected from 5-10 individual trees at each *K. elegans*

142   site and 3-15 individual vines at each *C. corindum* site. For the field experiment, fully inflated

143   green pods were collected from the field and stored in gallon Ziploc bags with a paper towel for

144   1-2 days at room temperature before the experiment began. Pods with signs of feeding by other

145   arthropods (especially caterpillars of the silver-banded hairstreak, *Chlorostrymon simaethis*)

146   were discarded. Pods were gently squeezed to locate holes where first instar caterpillars may

147   have entered if no obvious signs of feeding (eg, frass) were located.

148   *Rearing conditions*

149   For laboratory experiments, all rearing was carried out in Sanyo Versatile Environmental

150   Test Chambers at 28°C during the day and 27.5°C at night, 50% relative humidity with a 14:10

151   Light:Dark cycle, following spring climate conditions in the field and those used in Carroll et al

152   1998. Adults collected from the field were housed as mating pairs in vented Petri dishes lined

153   with filter paper and given water in a microcentrifuge tube stoppered with cotton ("water pick")

154   and 3 seeds of their field host plant. Eggs were collected daily until hatching. Nymphs were

155   removed within 12 hours of hatching to reduce egg cannibalism and housed individually in

156   mesh-lidded cups lined with filter paper with a water pick and a seed of their assigned host plant

157   treatment. Additional seeds (a total of 2 for *K. elegans* and 3 for *C. corindum*, for a total seed

158   mass of ~150mg) were added at 7 days of age. This seed mass has been shown in previous

159   experiments to be more than sufficient for individuals to reach adulthood, and is considered *ad*

160   *libitum* (Carroll et al. 1997). Individual containers were rotated daily within mesh boxes (each

161   holding 36 individuals), and boxes were rotated daily within the growth chamber to reduce the

162   effect of specific location. Water, paper and cotton were changed weekly for nymphs and adults.

163   Nymph survival and whether or not they had reached adulthood were assessed daily.

164

165   **Assessing the effects of rearing host and seed coat on juveniles**

166   The first experiment (Fig. 1a) tested the effects of the rearing host species and the seed

167   coat on juvenile survival, development time, and morphology (H1, H2, & H3). For this

168   experiment, I used the second laboratory generation (F2) of juveniles descended from the April

169   2014 field collection. Their parents, the first laboratory generation (F1), were reared using the

170   rearing conditions described above and were mated with individuals from the same population

171   but different families to avoid inbreeding. F2 individuals were reared in a split-brood cross-

172   rearing design: full siblings from each family were randomly assigned to a rearing host (either *C.*

173   *corindum* or *K. elegans*) and a seed treatment (seeds were either intact or had the seed coat

174   cracked) such that all families were represented in each treatment combination. The seed

175   treatment was administered by gently clamping seeds in pliers and tightening the pliers just until

176   a crack formed in the seed coat. Upon reaching adulthood, individuals were frozen and stored at

177   -20°C for morphological analyses. Beak length (the distance from the anterior tip of the tylus to

178   the distal tip of the mouthparts), thorax width (at the widest part of the pronotum), and forewing

179   length (from anterior to distal tip) were measured for all individuals to the nearest 0.02 mm using

180   Mitutoyo digital calipers for. Each individual was identified as either macropterous (long

181   forewings) or brachypterous (truncated forewings)(Carroll et al. 2003).

182

183   ***Assessing the effects of the seedpod on adult performance***

184   *Lab feeding trials*

185   The second experiment (Fig. 1b) I conducted was designed to test the effect of the

186   balloon vine seedpod on adult performance (H4) and natural selection on beak length (H5). For

187   this experiment, I used adult females from the first laboratory (F1) generation descended from

188   individuals collected in the field in December 2013. Individuals were reared following

189   conditions described above, on the same host species their parents were collected from in the

190   field. When individuals reached adulthood, females were isolated with water and no seeds for 2-

191   5 days before feeding trials began to allow their cuticle to harden completely. Males were not

192   used in feeding trials, and were given 1-2 additional seeds within 24 hours after eclosion. Males

193   were not used in the feeding trails because they are substantially smaller than females and never

194   have beaks long enough to reach seeds within seedpods. Furthermore, local adaptation in beak

195   length has not been observed in males, and so there is no reason to suspect differential selection

196   is acting on male beak length (Carroll and Boyd 1992).

197   Seedpods were prepared by opening dried, intact seedpods of either *C. corindum*

198   collected in the Florida Keys or *C. grandiflorum* collected in Davis, California. *C. grandiflorum*

199   is a congener of *C. corindum* with pods of similar shape and size that is commonly fed on by *J.*

200   *haematoloma* in Davis. The average pod radius used in this experiment was 7.60±0.31mm. The

201   mean pod size is within one standard deviation of the mean beak length measured in the field in

202   Florida in December 2013 (7.45±0.10mm), and should therefore encompass the range of pod

203   sizes where soapberry bugs are most likely to feed in nature. The seedpods used in this

204   experiment were smaller than historical *C. corindum* seedpod sizes, which should make the

205   assessment of the fitness costs of the seedpod conservative, since on average more females will

206   be capable of reaching the seeds within the closed seedpods in the experiment than in the average

207   naturally occurring seedpod. Both dry and green seedpods occur in nature; seedpods begin

208   drying when the seed reaches maturity and seeds may remain inside dry pods for several weeks.

209   It would have been interesting to compare both green and dry pods in the laboratory experiment,

210   but it was not logistically feasible; therefore, green pods were evaluated separately in the field

211   (described below). In laboratory experiments, the original seeds in experimental pods were

212   removed and discarded. Three seeds of the host species from which individuals were collected in

213   the field (*C. corindum* or *K. elegans*) were glued into the natural position and orientation within

214   each pod with Elmer's glue and allowed to dry for 1-2 hours. For closed pod treatments, the

215   three segments of the pod exterior were then reattached with glue. For open pod treatments, each

216   segment of the pod exterior was lined with glue, placed in the 'closed' position, and then

217   detached again. This was done to ensure that an equal quantity of glue was present on pods of

218   both treatment types to control for possible deterrent effects of glue on feeding. All parts of the

219   pod were retained together and included in the treatment container. Glue was allowed to dry

220   overnight (12-18 hours) before feeding trials were conducted.

221   After their cuticles hardened, females were held in mesh-lidded containers (BugDorms,

222   MegaView Science, Taiwan) with seeds and seedpods of their seedpod treatment and a water

223   pick for feeding observations. When soapberry bugs feed, they drill a hole into the seed using the

224   serrated ends of their beak (modified labium). Once the hole has been created, the second and

225   third segments of the labium bend characteristically and a thread-like stylet is inserted into the

226   seed. For this experiment, we included both drilling behavior and stylet insertion as feeding

227   behavior. Probing the seed without beginning drilling was not considered feeding. It was not

228   possible to see how the beak interacted with the seed inside of closed pods; therefore, feeding

229   was defined for bugs in the closed pod treatment as having the beak fully inserted into the pod

230   and remaining stationary. It is not known whether soapberry bugs can satiate within 8 hours,

231   although individual feeding bouts often last several hours. The amount of time each individual

232   spent in the feeding trial was not a significant predictor of whether or not an individual was

233   observed feeding, suggesting satiation was not reducing feeding activity during the feeding trail.

234   I conducted feeding trials of 5.75-8 hour duration in the lab at ambient humidity and

235   temperature. Latency time before feeding began, feeding activity, and weight change were

236   recorded during the feeding trial. Latency time was determined by visually scanning all

237   individuals each minute for the first 60 minutes of the feeding trial and recording when feeding

238   began. After the first 60 minutes, each individual was assessed for feeding activity (yes or no) at

239   30-minute intervals. Each female was weighed before being introduced to the feeding trial and

240   immediately after the feeding trial was concluded. Weight change was quantified as the percent

241   of the initial body weight gained or lost per hour during the feeding trial. Trials began between

242   8:15 am and 12:06 pm PT and concluded between 3:12pm and 7:16 pm PT between January 28

243   and February 25, 2014, all during daylight or twilight hours when soapberry bugs normally feed.

244   After feeding trials concluded, females were returned to the growth chamber and retained

245   with the seeds in their treatment pod (no additional seeds were added) for the remainder of the

246   experiment. After a week, females were given an opportunity to mate. Mates were assigned from

247   within the same population, but from different mothers, who had emerged at least 48 hours prior

248   to introduction to allow for the complete hardening of the cuticle. If multiple suitable males were

249   available, pairs were generated randomly. A single male was isolated with each female in a clean

250   inverted plastic cup (45mm diameter, 31mm tall) within each female's container for 24 hours to

251   allow mating. The seedpod was excluded from the cup to stop males from feeding on treatment

252   seeds. Once a female started laying eggs after mating, eggs were collected and weighed daily for

253   the next 10 days, or until death. Individuals who did not lay eggs in the first 30 days after

254   eclosion (the estimated adult life expectancy in the field (Carroll 1991)) were counted as not

255   reproducing. At the end of 30 days, or after mortality, all individuals were frozen at -20°C and

256   stored for morphological analyses. Closed seedpods were then opened and each seed was

257   examined for feeding damage. Beak length, thorax width, and wing length were measured for all

258   individuals with Mitutoyo calipers under a dissecting scope to ±0.02mm accuracy.

259   *Field feeding trial*

260   The third experiment (Fig. 2c) was designed to test the effect of the balloon vine seedpod

261   on adult performance (H2) and natural selection on beak length (H3) in the field. The field

262   feeding trial was conducted in Key Largo using adult females collected from the field during a 3-

263   day period from April 6-8, 2014. Instead of dry pods, this experiment used mature green pods of

264   *C. corindum* collected in the field containing naturally occurring seeds. In this experiment, the

265   seedpod treatment was less invasive: for the 'open' treatment, all three segments of the seedpod

266   were opened manually, and for the 'closed' treatment seedpods were left intact as they were

267   found in the field. Individual females were introduced to mesh-lidded BugDorms with a single

268   pod of their treatment group. Trials were conducted outdoors in Key Largo in the shade on April

269   9, 2014. Females were starved by withholding food (but not water) for 24 hours prior to the

270   feeding trial to motivate feeding. Prior adult feeding and individual age were unknown.

271   Individuals were introduced between 8:20 and 8:57 am and removed between 4:10 and 5:22 pm.

272   Latency to feed and time spent feeding were measured for each individual. Beak length, thorax

273   width, and wing length were measured alive for all individuals using Mitutoyo calipers.

274   **Statistical Analyses**

275   All analyses were conducted in R version 3.2.2 ("Fire Safety"). The sets of models

276   evaluated for each response variable are discussed in greater detail below. For each response

277   variable, all models were compared using the Akaike Information Criterion (AIC), a metric that

278   ranks the relative quality of a set of models based on fit and simplicity, with the exception of egg

279   number, for which models were compared using weighted AIC (wAIC). All models with a >5%

280   probability of being the best model out of the set were examined for each response variable.

281   Effects are only reported for factors that were consistent in their effect direction and significance

282   in all examined models unless otherwise stated. The specific test statistics and effect sizes

283   reported in the results section were taken from the model with the highest probability.

284   *Statistical analyses of juvenile traits*

285   For analyses of juvenile traits, the performance cost of the seed coat (H1) was evaluated

286   by including the seed treatment as a fixed factor in the analyses of survival and development

287   time. The developmental effect of each host plant on morphology (H2) was evaluated by

288   including rearing host plant as a fixed factor in the analyses of beak length, body size, and wing

289   morph. Recent work demonstrates that gene flow between host-associated populations is likely

290   very high in the southern part of the range, particularly from the invasive to the native host

291   (Cenzer 2016). Moving north away from where the two plants co-occur, rates of gene flow

292   between the two hosts should decrease. Therefore, I evaluated the effect of gene flow on beak

293   length plasticity (H3) by including the interaction between latitude and rearing host plant in the

294   analyses of beak length. Analyses for each juvenile response variable are discussed in more

295   detail below.

296   For each juvenile response variable (survival, development time, thorax width, beak

297   length, and wing morph), all possible models including the main effects of rearing host (*C.*

298   *corindum* or *K. elegans*), ancestral host (*C. corindum* or *K. elegans*), sex (male or female), and

299   all possible two-way interactions were considered. All models were also analyzed as generalized

300   linear mixed models with the additional random factor of individual population nested within

301   collection host and the random factor of family nested within individual population. For survival,

302   seed treatment (cracked or uncracked) was also included as a main effect and in all possible pair-

303   wise interactions. Sex was excluded from the analyses of survival because it was not possible to

304   sex individuals that died before reaching adulthood. For analyses of beak length, I used the

305   residuals of the linear model log(beak length)~log(thorax width) as the response variable. This

306   controlled for body size and improved both normality and homoscedasticity.

307   Differences in survival between treatments created heavily unequal sample sizes in all

308   morphological traits and development time. Survival in high mortality treatments may have been

309   non-random with respect to the genotypes that successfully reached adulthood, causing a single

310   generation of strong selection. In order to distinguish plasticity from short-term selection, I only

311   evaluated development time and morphological traits for individuals in the cracked seed

312   treatment, which had high survival across host plants. Analyses of beak length revealed the

313   unexpected result that plasticity in response to rearing host was maladaptive (Fig. 5a); this led to

314   the hypothesis (H3) that maladaptive plasticity was the result of gene flow. To test H3, the top

315   six models for beak length were re-analyzed with the added fixed factor of latitude and the

316   latitude*rearing host interaction. I assessed whether or not the addition of latitude and the

317   interaction improved model fit using Chi-squared tests comparing (1) each model with vs.

318   without latitude and (2) each model with latitude vs. with the latitude*rearing host interaction.

319   Latitude and the interaction were included post-hoc; therefore, the results of these comparisons

320   are discussed here in the context of hypothesis development rather than hypothesis testing, and

321   latitude was not added to the analyses of any other traits.

322   Survival and wing morph were modeled using a binomial error distribution, while

323   development time, thorax width, and beak length had Gaussian error distributions. Five outliers,

324   identified using the boxplot function in R, were excluded from the analyses of development time.

325   The results were qualitatively the same if 3 of these outliers (45, 46, and 48 days) were included

326   (mean±95% CI development time was 31.17±0.83 days); however, the analyses were not robust

327   to the inclusion of two extreme outliers (72 and 73 days). These outliers were likely caused by

328   individuals exhausting their food supply prior to reaching a large enough size to molt to

329   adulthood, and are probably not informative for assessing any of the effects intended to be

330   studied in this experiment. Normality of residuals was tested using Shapiro-Wilk tests and

331   homoscedasticity was assessed using studentized Breusch-Pagan tests (in the R package lmtest).

332   *Statistical analyses of adult traits*

333   For analyses of adult traits, the performance cost of the seedpod (H4) was evaluated by

334   including the seedpod treatment as a fixed factor in the analyses of each response variable.

335   Natural selection on beak length (H5) was evaluated in four ways: (1) the effect of beak length in

336   lab trials on the response variables latency time and time spent feeding in the open pod

337   treatment; (2) the effect of beak length in lab trials on the number of seeds fed on in closed pods;

338   (3) the effect of the interaction between beak length and seedpod treatment on latency time and

339   feeding activity in field trials; and (4) the effect of the interaction between beak length and

340   seedpod treatment on egg production in lab trials. Analyses for each response variable are

341   discussed in more detail below.

342   In lab feeding trials, 0/48 individuals were observed feeding through closed pods while

343   58/63 individuals were observed feeding on open pods; the effect of the seedpod treatment on

344   latency to feed and feeding activity in the lab were considered to pass the intraocular trauma test

345   and therefore did not undergo any formal analyses. I analyzed latency to feed and feeding

346   activity in the lab on open pods only. For both of these response variables, I analyzed all possible

347   generalized linear models including the main effects of host plant, beak length, and body size

348   and all possible pairwise interactions. Family (nested within host plant) and individual (nested

349   within family) were included as random factors in generalized linear mixed models. Latency

350   time had a negative binomial error distribution and feeding activity (whether an individual was

351   observed feeding at each observation time) had a binomial error distribution.

352   In field trials, I analyzed the effect of pod treatment, beak length, and body size on

353   latency time and feeding activity. For both response variables, I tested all generalized linear

354   models including pod treatment, beak length, body size, and all possible pairwise interactions.

355   Field trials were conducted in Key Largo with naturally occurring pods; therefore only one host

356   plant (*C. corindum*) was used. Generalized linear mixed models including individual number as a

357   random factor were also analyzed for field trials. I analyzed field latency time with a Gaussian

358   error distribution and field feeding activity with a binomial error distribution.

359   Weight change, egg production, egg weight, and the number of seeds consumed were

360   only measured in the laboratory experiment. To explore what factors effected weight change, I

361   analyzed all possible models including host, seedpod treatment, beak length, and all possible

362   two-way interactions with a Gaussian error distribution. The effect of seedpod and host plant on

363   individual egg weight was analyzed by comparing all possible models including treatment, host

364   plant, and body size main effects and body size*host plant and body size*treatment interactions

365   using a Gaussian error distribution. I treated whether or not each seed had been fed on as a

366   binomial response variable and analyzed generalized linear models looking at the fixed effects of

367   host plant, beak length, and the beak length*host plant interactions as well as the random effects

368   of individual identity and family.

369   I analyzed egg production using Markov Chain Monte Carlo (MCMC) simulations in the

370   'map2stan' function in the 'rethinking' package in R (McElreath 2016). No individuals in the

371   closed pod treatment on the host *C. corindum* produced any eggs. Generalized linear models

372   cannot handle a treatment combination with no variance; MCMC was the best modeling

373   approach for dealing with this problem. I approximated a negative binomial distribution for egg

374   number using a gamma Poisson; the negative binomial distribution is commonly used for

375   reproduction data because it is the distribution that results from many independent trials (in this

376   case, females) where each trial is a series of yes/no events (in this case, whether or not an egg

377   was produced at a given time point). Models were run for each main effect alone and for all

378   additive and pairwise interactions. The model including all three main effects plus the

379   treatment*beak length interaction was run to test for the effect of divergent natural selection on

380   beak length by seedpod treatment with a host effect. All models were compared using weighted

381   AIC using the 'compare' function in the rethinking package. One model held 100% of the

382   weight; therefore results are reported only for this model.

383

384   **Results**

385   ***Assessing the effects of rearing host and seed coat on juveniles***

386   *Cracking the seed coat increases juvenile survival (H1)*

387   The cracked seed treatment was associated with a dramatic increase in survival

388   probability on both *C. corindum* (0.079 vs 0.94) and *K. elegans* (0.83 vs 0.98)(z-value=7.70,

389   p<0.001) (Fig. 3). This was in addition to the clear difference in survival between the two rearing

390   host plants (z-value=9.43, p<0.001) which was also observed in earlier experiments (Cenzer

391   2016). The effect of the cracked seed treatment increasing survival was stronger for individuals

392   reared on *C. corindum* (z-value=-2.23, p=0.026). Nymph mortality was heavily skewed towards

393   very young individuals, such that 94% of nymph mortality occurred in the first 7 days after

394   hatching (Fig. S1).

395   *Rearing host has strong plastic effects on morphology & development time (H2 & H3)*

396   There was a significant effect of rearing host on development time, such that individuals

397   reared on *K. elegans* developed more slowly than individuals reared on *C. corindum* (t-

398   value=3.35, p<0.001, df=98) (Fig. 4). Historically, development time was faster for populations

399   from *K. elegans*; however, although the trend in this experiment was in that direction, it was not

400   significant.

401   Individuals descended from populations on *K. elegans* had shorter beaks than those from

402   *C. corindum* (t-value=-5.22, p<0.001, df=105)(Fig. 5a) as predicted by historical patterns of local

403   adaptation (Carroll and Boyd 1992). The effect of rearing host on beak length was significant

404   and in the opposite direction: individuals reared on *K. elegans* had longer beaks than those reared

405   on *C. corindum* (t-value=2.79, p=0.006, df=105). Males had shorter beaks than females,

406   consistent with known sexual dimorphism (t-value=-3.64, p<0.001, df=105). Adding the

407   interaction between latitude and rearing host plant significantly improved the fit of all tested

408   models. The effect of latitude alone was not significant (t=-0.36), but there was a significant

409   interaction between latitude and rearing host plant. The effect of rearing host plant on beak

410   length became weaker moving north away from where the two plants co-occur (t=-2.51,

411   p=0.014, df=103).

412   Individuals reared on *K. elegans* had significantly larger thorax widths than individuals

413   reared on *C. corindum* (t-value=4.08, p<0.001, df=106) (Fig. 5b). Males had consistently smaller

414   thorax widths than females (t-value=-5.93, p<0.001, df=106). There was a significant interaction

415   such that males raised on *K. elegans* were smaller than predicted by the main effects alone (t-

416   value=-2.21, p=0.03, df=106).

417   Being from a population collected from *K. elegans* increased the probability of having

418   short forewings (z-value=-2.29, p=0.022). In contrast, being reared on *K. elegans* decreased the

419   probability of having short forewings (z-value=4.51, p<0.001). Being male also decreased the

420   probably of having short forewings (z-value=3.11, p=0.002)(Fig. 5c).

421   ***Assessing the effects of the seedpod on adult performance***

422   *The host seedpod decreases adult performance (H4)*

423   In field trials, 13 of the 15 individuals in the open treatment were observed feeding, while

424   4 of the 15 in the closed treatment were observed feeding (z=2.45, p=0.014). Individuals on the

425   closed treatment took longer on average to begin feeding, although this effect was not

426   statistically significant (Fig. 6b). Individuals in the closed pod treatment spent significantly less

427   time feeding than individuals in the open treatment (z-value=3.63, p<0.001)(Fig. 6d). Results for

428   beak length are discussed in the next section.

429   In lab trials, there was a clear and unambiguous effect of the seedpod on both latency

430   time and time spent feeding (Fig. 6a & 6c). Individuals on *K. elegans* began feeding faster than

431   individuals on *C. corindum* (z-value=-2.75, p=0.006)(Fig.6a) and individuals with larger bodies

432   took longer to begin feeding overall (z-value=2.34, p=0.019). For feeding activity, individuals on

433   *K. elegans* were observed feeding less frequently than those on *C. corindum* (z-value=-2.05,

434   p=0.04)(Fig. 6c). Individuals in the open pod treatment gained more weight per hour than

435   individuals in the closed pod treatment (t-value=4.757, p<0.001, df=92)(Fig. 6e). There was no

436   significant effect of seedpod treatment on individual egg weight (t-value=-1.16, p=0.25). Larger

437   females produced larger individual eggs (t-value=2.68, p=0.01, df=58). Females from *C.*

438   *corindum* laid individual eggs 12% larger than those from *K. elegans* (t-value=-5.34, p<0.001,

439   df=58) and had a stronger relationship between thorax width and egg size (t-value=2.87,

440   p=0.006).

441   For egg production, the model including host species, seedpod treatment, and the

442   interaction outperformed all other models (weight=1). Females laid more eggs in the open pod

443   treatment than in the closed pod treatment (effect estimate 89% quantile: 6.22, 57.06)(Fig. 6f)

444   and females on *K. elegans* laid more eggs than females on *C. corindum* (effect estimate 89%

445   quantile: 5.59, 56.36). The interaction between pod treatment and host plant indicated that the

446   positive effect of open pods on egg production was stronger on *C. corindum* (89% quantile: -

447   54.80, -4.05); this is likely due to the fact that any increase from zero eggs would be

448   proportionally larger than an increase from some eggs.

449   *Beak length influences performance on open and closed seedpods (H5)*

450   Data on latency time in both the lab and field and the number of seeds consumed in the

451   lab support the hypothesis that the host seedpod contributes to divergent natural selection on

452   beak length. In the field, individuals with shorter beaks began feeding more quickly than

453   individuals with longer beaks in the open treatment (t-value=2.64, p=0.023, df=11)(Fig. S2).

454   There was also a significant interaction between thorax width and treatment, such that larger

455   bodied individuals in the open treatment began feeding more quickly than small-bodied

456   individuals (t-value=-2.864, p=0.015, df=11). There was no significant effect of beak length or

457   the beak length*treatment interaction on total time spent feeding in the field.

458   In the lab, individuals with longer beaks took longer to begin feeding when pods were

459   open (z-value=1.98, p=0.048, df=44), consistent with results in the field. Unlike field results,

460   larger individuals in the lab took longer to begin feeding (z-value=2.338, p=0.02). The combined

461   effects of beak length and body size in the lab were less than additive; eg, the interaction

462   between body size and beak length had a significant negative effect on latency time (z-value=-

463   2.05, p=0.041). Individuals feeding on *K. elegans* showed a stronger effect of beak length on

464   increasing latency time than those on *C. corindum* (z-value=2.56, p=0.011)(Fig. S2). Of the 48

465   individuals in the closed pod treatment in the lab, 12 individuals created damage on one or more

466   seeds within the pod. There was a strong positive effect of beak length on the number of seeds

467   damaged by an individual (z-value=2.32, p=0.02, df=118)(Fig. 7).

468

469   **Discussion**

470   There are five main results of this work. First, the tough seed coat drives host-associated

471   patterns of juvenile mortality in soapberry bugs. Second, the seedpod severely inhibits adult

472   performance through feeding success, weight gain, and reproduction. Third, I found support for

473   the hypothesis that the seedpod exerts divergent natural selection pressure on beak length: long

474   beaks improve feeding success on the closed pods of the native *C. corindum* and short beaks

475   improve feeding efficiency on the open pods of the introduced *K. elegans*. Fourth, developmental

476   plasticity was maladaptive, causing females that were reared on each host plant to develop beaks

477   mismatched to the pod size of their rearing host. Finally, plasticity was strongest where the two

478   host plants occur in close proximity, consistent with maladaptive plasticity emerging as a result

479   of ongoing gene flow between host plants. Taken together, these results indicate that the native

480   host plant, *C. corindum*, has strong negative effects on juvenile fitness via the seed coat, strong

481   negative effects on adult performance via the seedpod, and counteracts natural selection via

482   maladaptive plasticity on adult morphology. This suggests that the native host has become a

483   'fitness valley' for soapberry bugs, resulting in much lower performance than the invasive *K.*

484   *elegans*. Gene flow appears to be contributing to maladaptive plasticity in beak length that masks

485   the genetic differences between host-associated populations in the field.

486   Juvenile survival, which was locally adapted between *K. elegans* and *C. corindum* in

487   1988, was dramatically reduced in subsequent decades, likely as a result of asymmetric gene

488   flow from populations adapted to the invasive host to populations on the native host (Cenzer

489   2016). The proximate reason for juvenile mortality on the native host, and to a lesser extent on

490   the introduced host, is demonstrated here to be the seed coat. Given the early mortality that

491   occurs for individuals raised on intact seeds, this is probably the result of newly hatched

492   individuals being unable to penetrate the seed and subsequently starving to death. It could also be

493   that chemical defenses within the seed's exterior, rather than or in addition to the physical

494   barrier, are responsible for early mortality. This opens up further lines of inquiry in this system:

495   Has the thickness or toughness of the seed coat on the native host changed during the last three

496   decades? What aspects of nymphal morphology or physiology allow some individuals to deal

497   with the tough seed coat while others do not? It is possible that this plant defense also exerts

498   selection on beak length, but in first instars rather than in adults, which may be pleiotropically

499   affected by the same factors that influence adult beak morphology.

500   The second plant defense that is clearly important for driving fitness in the field on the

501   native host is the seedpod. The presence of a closed seedpod severely inhibited adult female

502   performance: it negatively effected time to begin feeding, time spent feeding, weight gain, seed

503   access, and egg production. The fitness costs of the seedpod make it a potentially potent driver of

504   natural selection. Performance for short-beaked females in the field may not be as grim as

505   measured in this study, however. Females that are incapable of feeding through closed seedpods

506   in the field may instead compete with males, juveniles, and other short-beaked females for access

507   to seeds after the pods dehisce. All life stages must also compete with other native seed predators

508   on *C. corindum*, most of which feed prior to the opening of the seedpod (eg, the silver-banded

509   hairstreak [*Chlorostrymon simaethis*], Carroll and Loye 2006). The seeds of the introduced host

510   have not been adopted as a food source by any other species, so competition on this host is

511   entirely intraspecific.

512   The pattern of beak length evolving to match local seedpod size has been observed in

513   multiple populations and species of soapberry bugs across hosts and continents (Carroll and

514   Loye 1987; Carroll and Boyd 1992; Carroll and Loye 2012). These patterns of local adaptation

515   are highly suggestive of divergent selection being exerted by seedpod size on beak length.

516   Carroll and Boyd (1992) suggested that individuals with longer beaks have a competitive

517   advantage by being able to access seeds in large pods, while individuals with shorter beaks have

518   an advantage of greater feeding efficiency on flattened pods. In this study, I found support for

519   both of these hypotheses, although I did not find evidence that they scale up to measurable

520   differences in reproduction. It may be that the advantages of increased feeding success and

521   efficiency for fitness are relatively subtle or that they are only accrued in the presence of

522   competition, which was not included in this study.

523   Rearing host plant had a strong plastic effect on beak length, body size, and wing

524   morphology. On both hosts, females developed beak lengths that were mismatched with local

525   pod size: females reared on *C. corindum* developed short beaks and females reared on *K. elegans*

526   developed long beaks. This pattern of maladaptive plasticity was not observed for beak length in

527   host races studied in the 1990s; however, it was observed in crosses *between* host races (Carroll

528   et al. 2001). Since individuals with mixed ancestry expressed maladaptive plasticity in the 1990s,

529   increasing gene flow could have increased maladaptive plasticity by creating hybrids and

530   backcrosses in nature. More spatially separated populations should have lower rates of gene

531   flow; therefore, we should expect that hybrids and backcrosses are more common in areas where

532   the two hosts are close together. Consistent with this expectation, I found that developmental

533   plasticity was strongest in the south where the ranges of the two host plants overlap and became

534   weaker moving north away from the native host. The spatial pattern of beak length plasticity and

535   the potential role of gene flow in generating that plasticity both warrant further study.

536   It is also possible that correlated selection on beak length has changed over the past

537   several decades if selection pressure on wing morph or development time has changed.

538   Developing on the introduced *K. elegans* caused individuals in this study to develop more

539   slowly, achieve larger overall body sizes, and produce more dispersive morphs than developing

540   on *C. corindum*. There is a genetic correlation between wing morphology and beak length

541   (Dingle et al. 2009) and I documented a strong correlation between beak length and development

542   time in this study (Fig. S4). If selection on wing morph plasticity or development time has

543   changed since 1988, beak length plasticity could have evolved as a result of correlated selection.

544   Understanding how selection is acting on dispersal morphology and development time in nature,

545   and how that influences correlated selection on beak length, merits further investigation.

546   Maladaptive plasticity itself may exacerbate gene flow, as individuals dispersing from

547   their natal host to the alternative host will have the advantage of a beak length that better

548   matches local pod size. For example, soapberry bugs dispersing from *K. elegans* to *C. corindum*

549   will have longer beaks because of their developmental environment and will therefore be more

550   capable of accessing seeds in the large pods of *C. corindum* even though they are genetically

551 'shorter-beaked'. Dispersing females from *K. elegans* will have the further advantage of

552 increased reproductive output. However, their offspring will then be at a severe disadvantage

553 when they develop on *C. corindum*, as their beaks will be shorter both genetically and plastically.

554 The plastic effects of host plant on dispersal morphology should result in increased dispersal

555 from *K. elegans* to *C. corindum* and reduced dispersal in the other direction, exacerbating gene

556 flow that already appears to be asymmetric due to differences in host plant abundance.

557   Intuitively, maladaptive plasticity should be most common in scenarios where selection

558 has not had an opportunity to act. Maladaptive plasticity has been observed in cases where

559 organisms encounter novel environments that either expose cryptic variation or create a

560 mismatch between perceived cues and the quality that cue is associated with in ancestral habitats

561 (Schlaepfer et al. 2002; Ghalambor et al. 2007; Carroll 2008; Hale and Swearer 2016).

562 Maladaptation may also arise without environmental change if the genetic combinations exposed

563 to selection are themselves novel. This process is typified in hybrid zones, when haplotypes that

564 have been diverging in isolation are combined following secondary contact to produce novel,

565 usually detrimental, phenotypes in hybrids (Burke and Arnold 2001). In soapberry bugs, we see

566 evidence of increased gene flow from populations on *K. elegans* to populations on *C. corindum*

567 (Cenzer 2016). Although natural selection on beak length is ongoing in soapberry bugs, it

568 appears to be unable to overcome the homogenizing effects of gene flow and plasticity. Although

569 genetic differences in beak length remain between host-associated populations, they are entirely

570 masked in the field by the opposing effect of maladaptive plasticity.

571

Acknowledgements
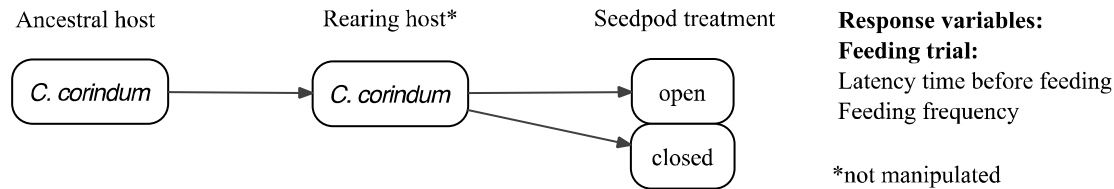
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579   **Figures:**

a. Experiment 1: Juvenile performance
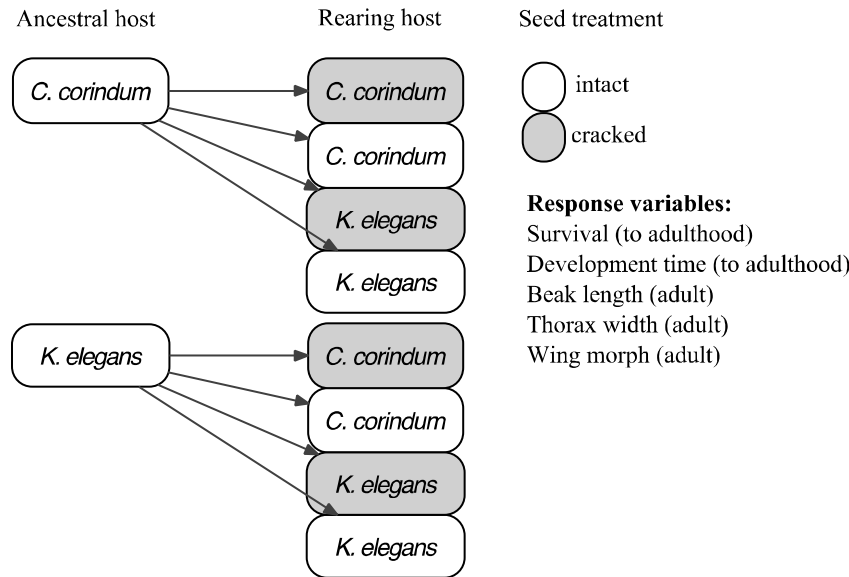
b. Experiment 2: Adult performance (lab)
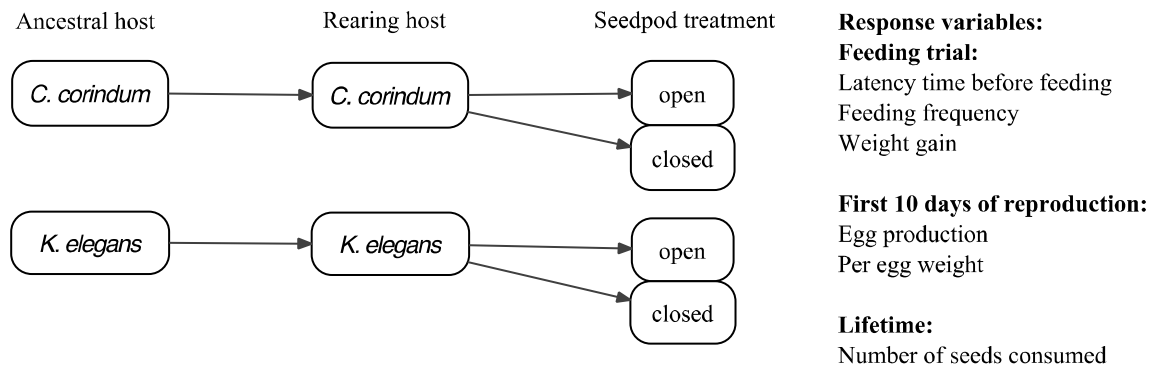
**c. Experiment 3: Adult performance (field)**

580

581   **Figure 1.** Experimental design and response variables for each experiment. a. Experiment 1

582   testing the effect of rearing host and the seed coat on survival, development time, and adult

583   morphology; b. Experiment 2 testing the effect of the seedpod on adult feeding and reproduction

584   in the lab on both hosts; c. Experiment 3 testing the effect of the seedpod on adult feeding in the

585   field on *C. corindum*.

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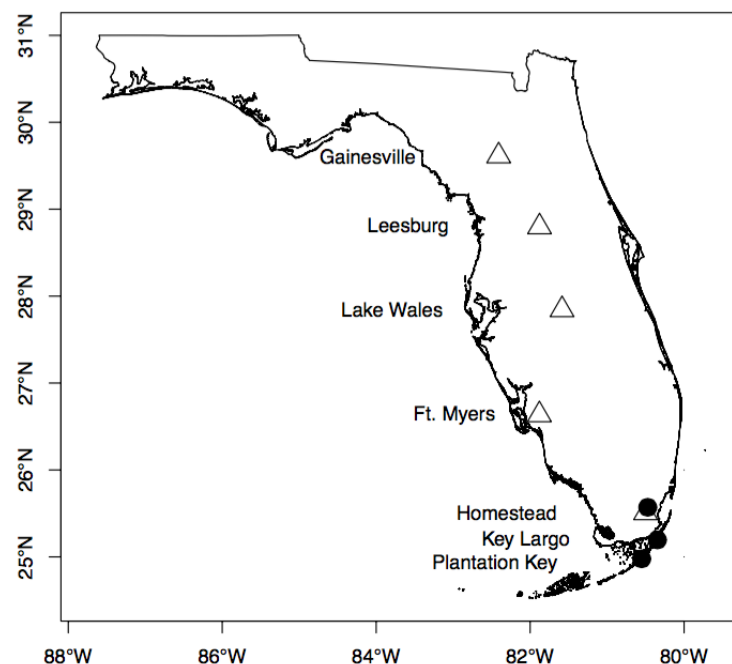
589   **Figure 2.** Collection sites on the introduced host, *K. elegans* (white triangles) and the native

590   host, *C. corindum* (black circles) with location names. Sites are abbreviated in text as follows:

591   Gainesville=GV, Leesburg=LB, Lake Wales=LW, Ft. Myers = FM, Homestead [*C.*

592   *corindum*]=HS1, Homestead [*K. elegans*] = HS2, Key Largo=KL, Plantation Key=PK.

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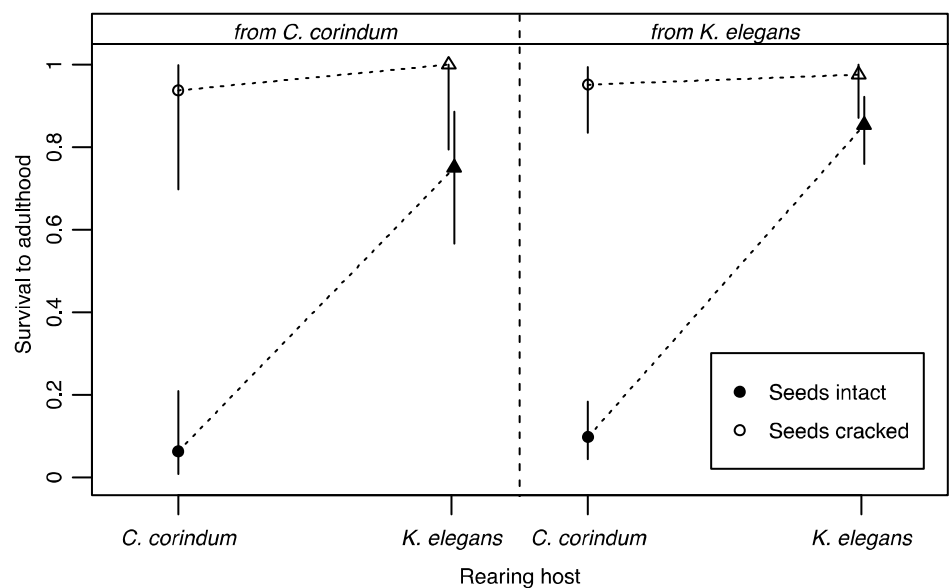
Figure 3. Soapberry bug juvenile survival on seeds with an intact (black) or a cracked (white)

598   seed coats when raised on the native host, *C. corindum* (circles), or the invasive host, *K.elegans*

599   (triangles). Points on the left represent bugs whose ancestors were collected on *C.corindum*

600   while points to the right were collected from *K.elegans*. Error bars represent the 95% binomial

601   confidence interval using the Pearson-Clopper method; points are jittered slightly for error bar

602   visualization.

603

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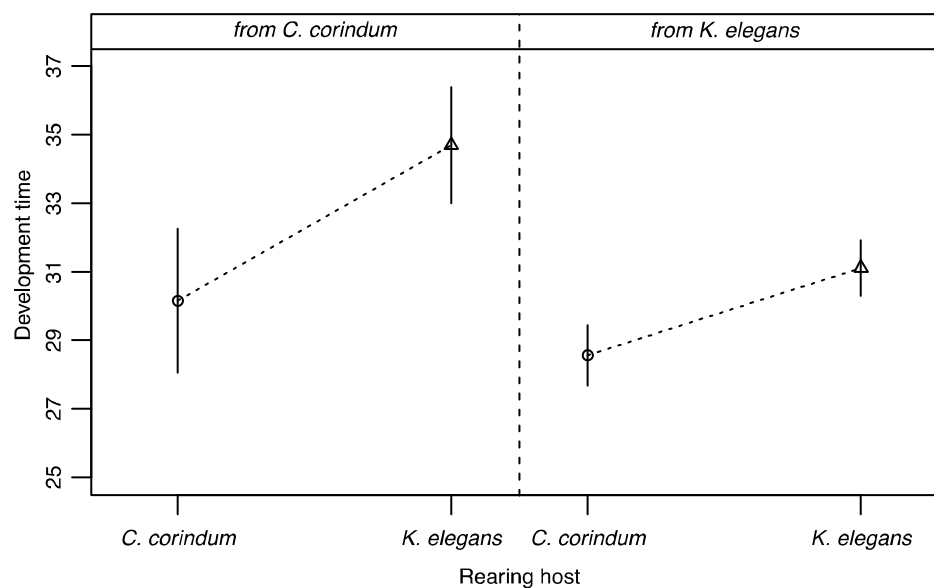
Figure 4. Soapberry bug juvenile development time on seeds with cracked seed coats when

raised on the native host, *C. corindum* (circles), or the invasive host, *K. elegans* (triangles).

Points on the left represent bugs whose ancestors were collected on *C. corindum* while points to

the right were collected from *K. elegans*. Error bars represent 95% confidence intervals.

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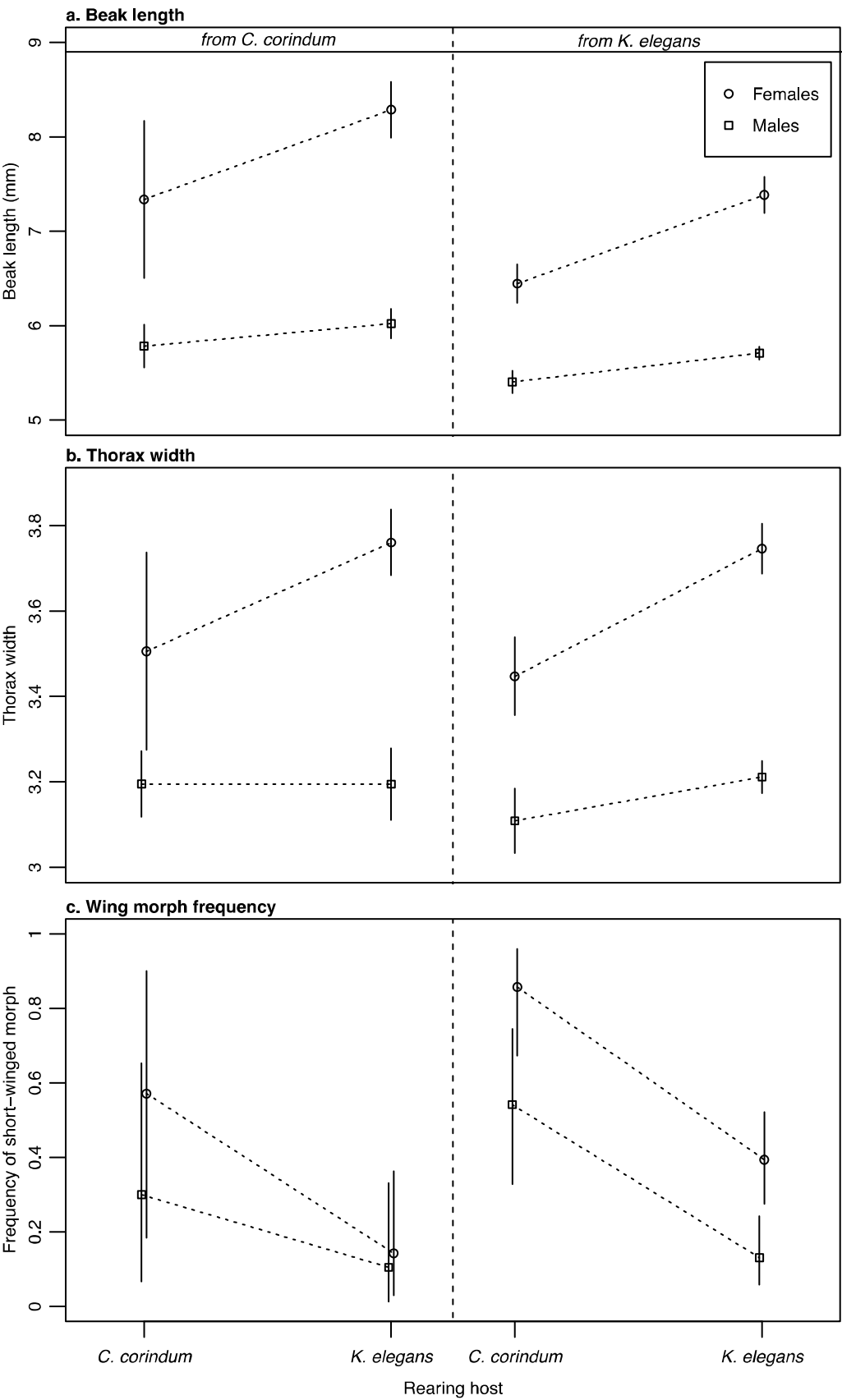
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611 **Figure 5.** Adult morphology for females (circles) and males (squares) for bugs with ancestry

612 from *C. corindum* (left panels) and *K. elegans* (right panels) when reared on *C. corindum* or *K.*

613 *elegans* (x-axis, Rearing host). a. Beak length (mm). b. Thorax width (mm). c. Frequency of

614 short-winged (brachypterous) wing morphs.

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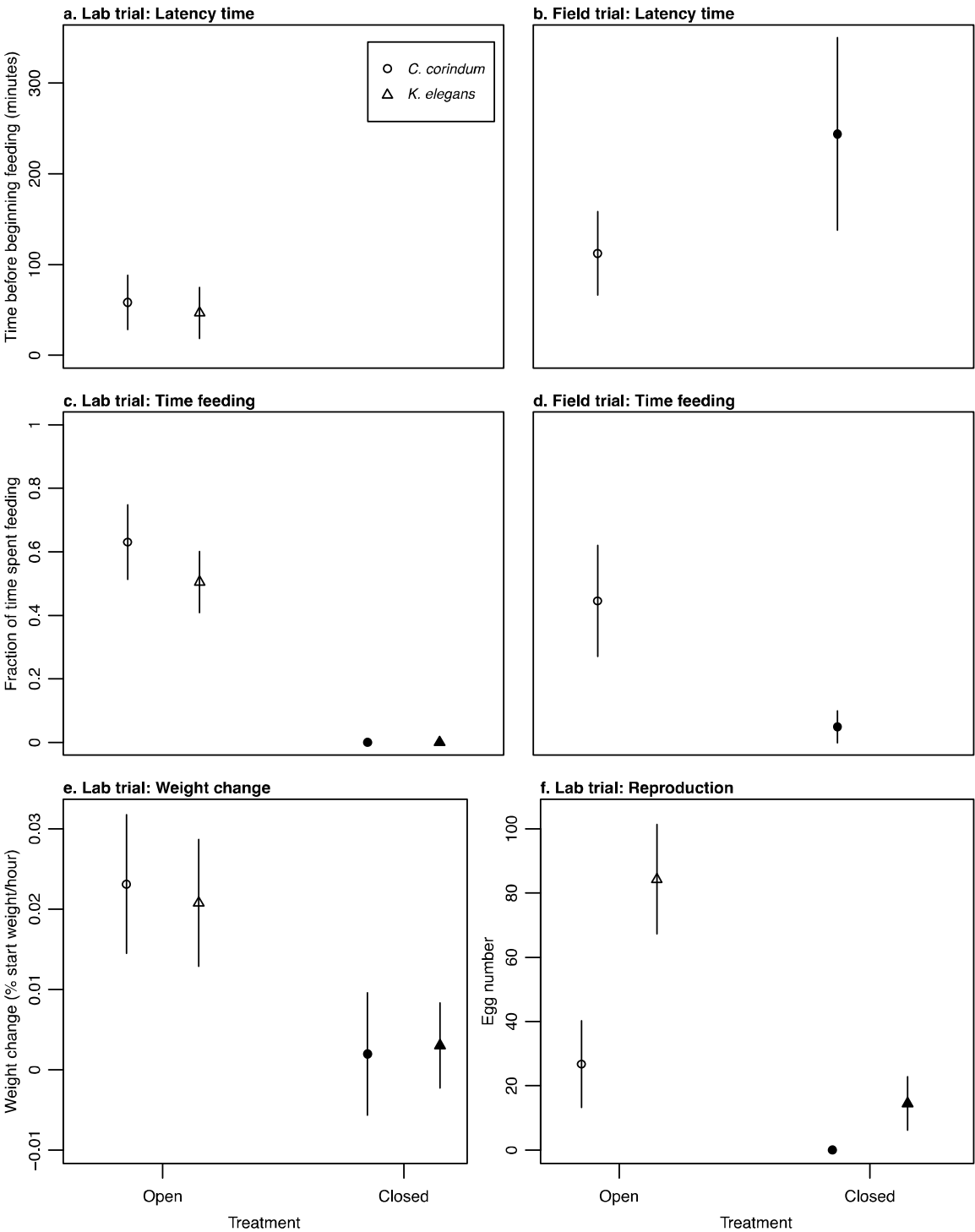
618 **Figure 6.** Performance metrics for open (white) and closed (black) pod treatments on *C.*

619 *corindum* (circles) and *K. elegans* (triangles) in the lab (a, c, e & f) and field (b & d). a. Latency

620   time before beginning feeding in lab trials. No individuals successfully fed through closed pods,

621   so there is no latency time for those treatment groups. b. Latency time before beginning feeding

622   in field trials; measures only collected on *C. corindum* due to the absence of naturally occurring

623   closed pods on *K. elegans*. c. Fraction of time spent feeding in lab trials. d. Fraction of time spent

624   feeding in the field trial on *C. corindum* only. e. Weight change as percent of initial body mass

625   gained per hour in lab feeding trials. f. Number of eggs produced in first 10 days of reproduction

626   in lab trials.

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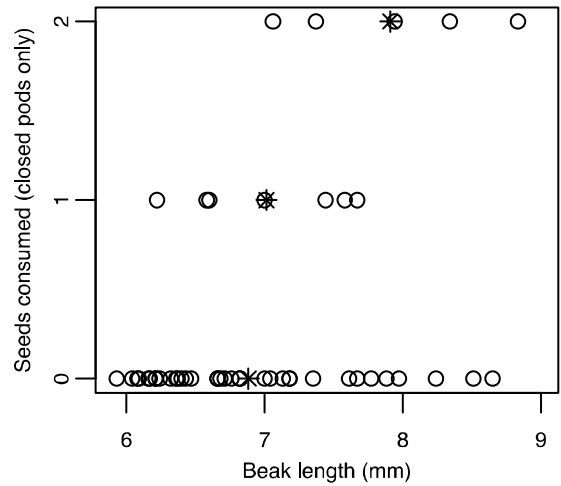
628   **Figure 7.** Number of seeds consumed in closed pods in lab feeding trials as predicted by

629   individual beak length. Stars represent mean beak lengths that consumed 0, 1 or 2 seeds.

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