

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 ± 0.31 mm. 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 ± 0.10 mm), 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 ± 0.02 mm 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 $\log(\text{beak length}) \sim \log(\text{thorax width})$ 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 probability 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 affected 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

572 **Acknowledgements**

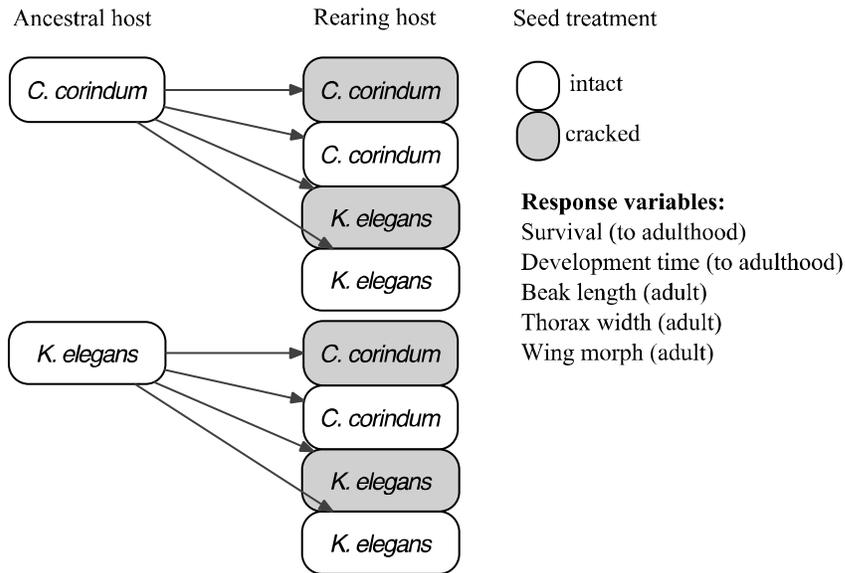
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577 within Florida State Parks.

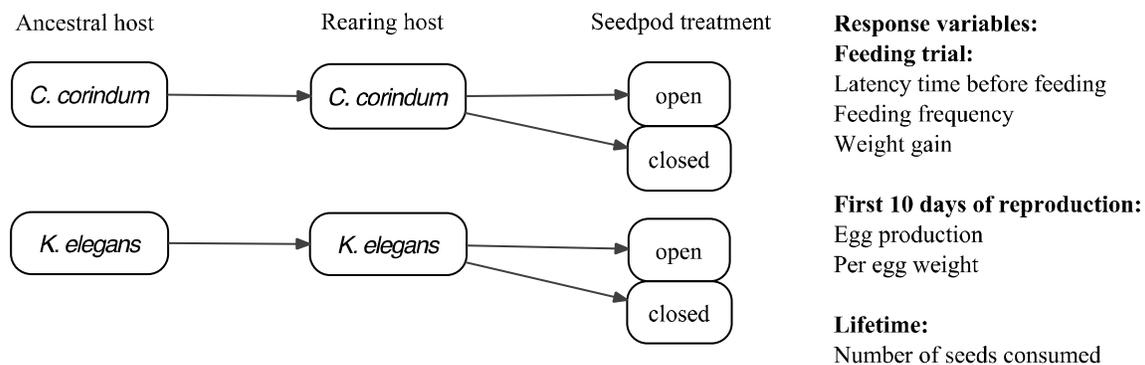
578

579 **Figures:**

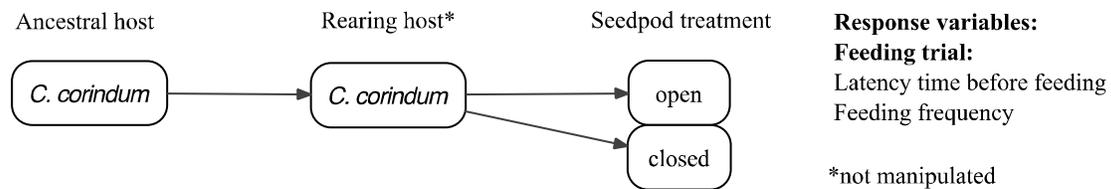
a. Experiment 1: Juvenile performance



b. Experiment 2: Adult performance (lab)



c. Experiment 3: Adult performance (field)



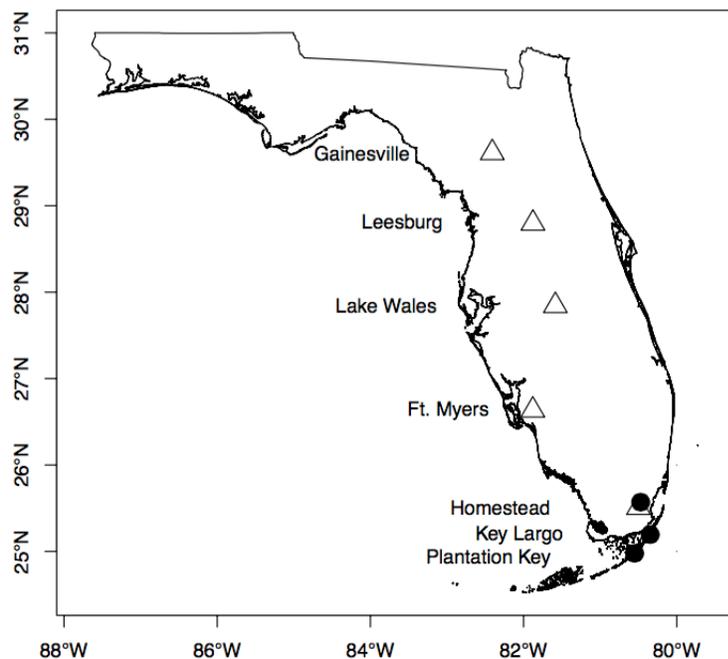
580

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

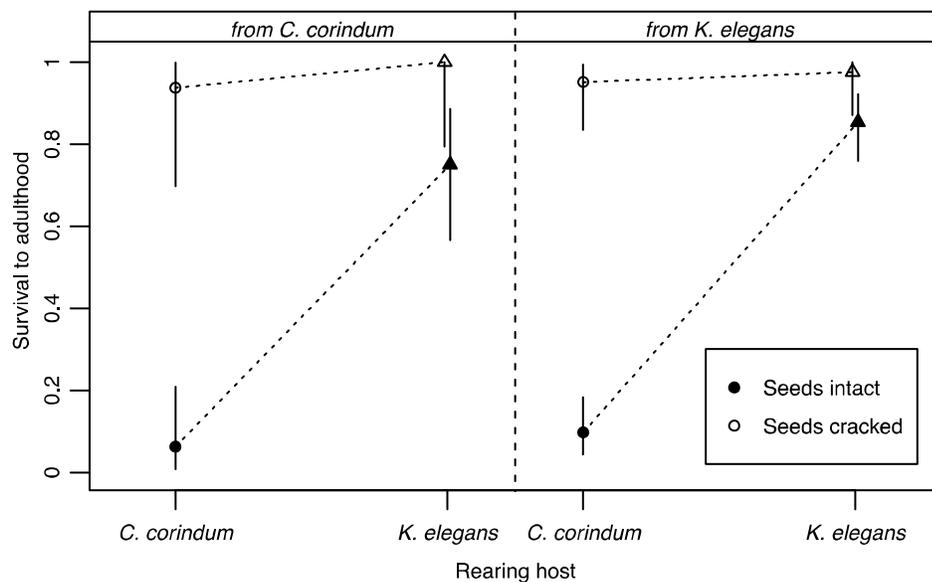
591 Gainesville=GV, Leesburg=LB, Lake Wales=LW, Ft. Myers = FM, Homestead [C.

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

593

594

595



596

597 **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*

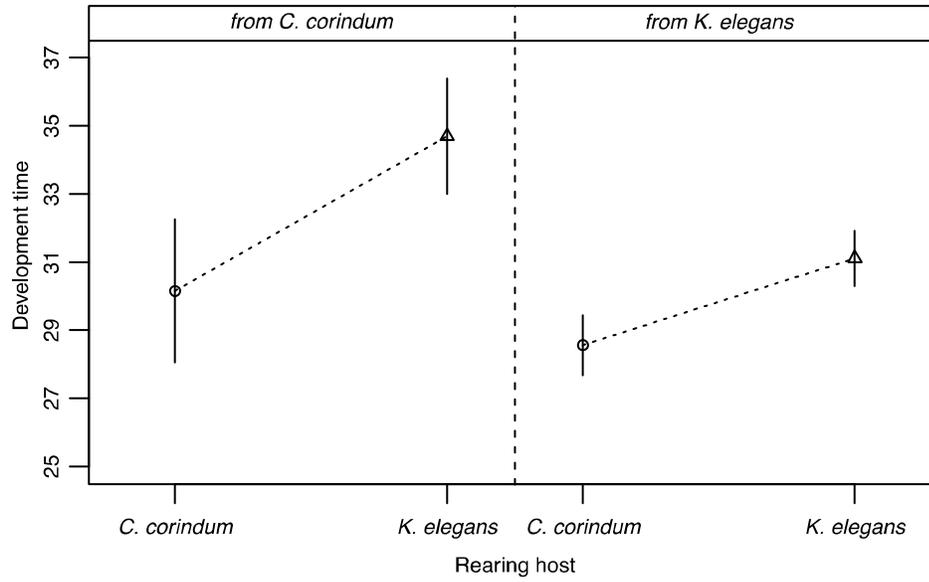
599 (triangles). Points on the left represent bugs whose ancestors were collected on *C. corindum*

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



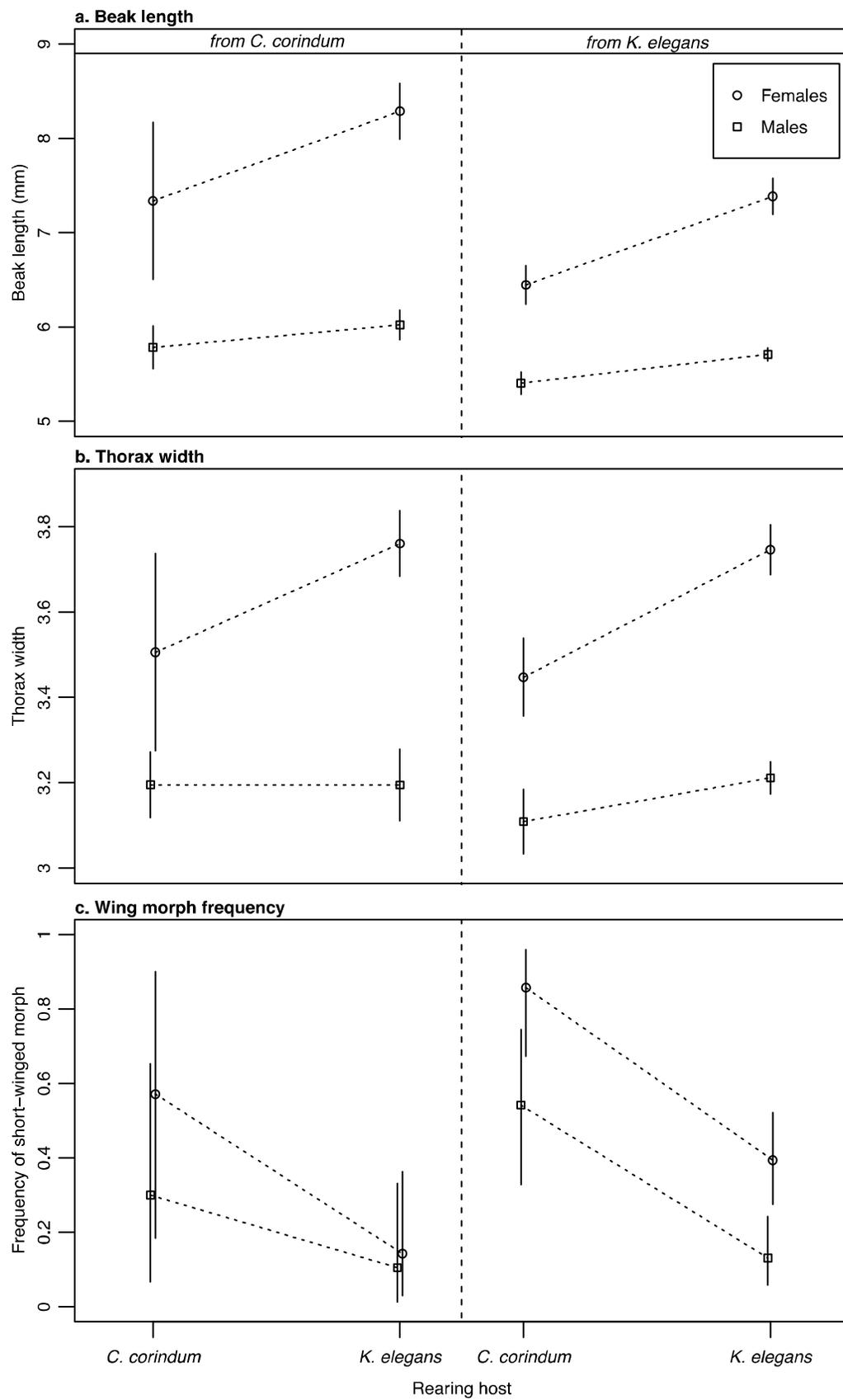
604

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

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

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

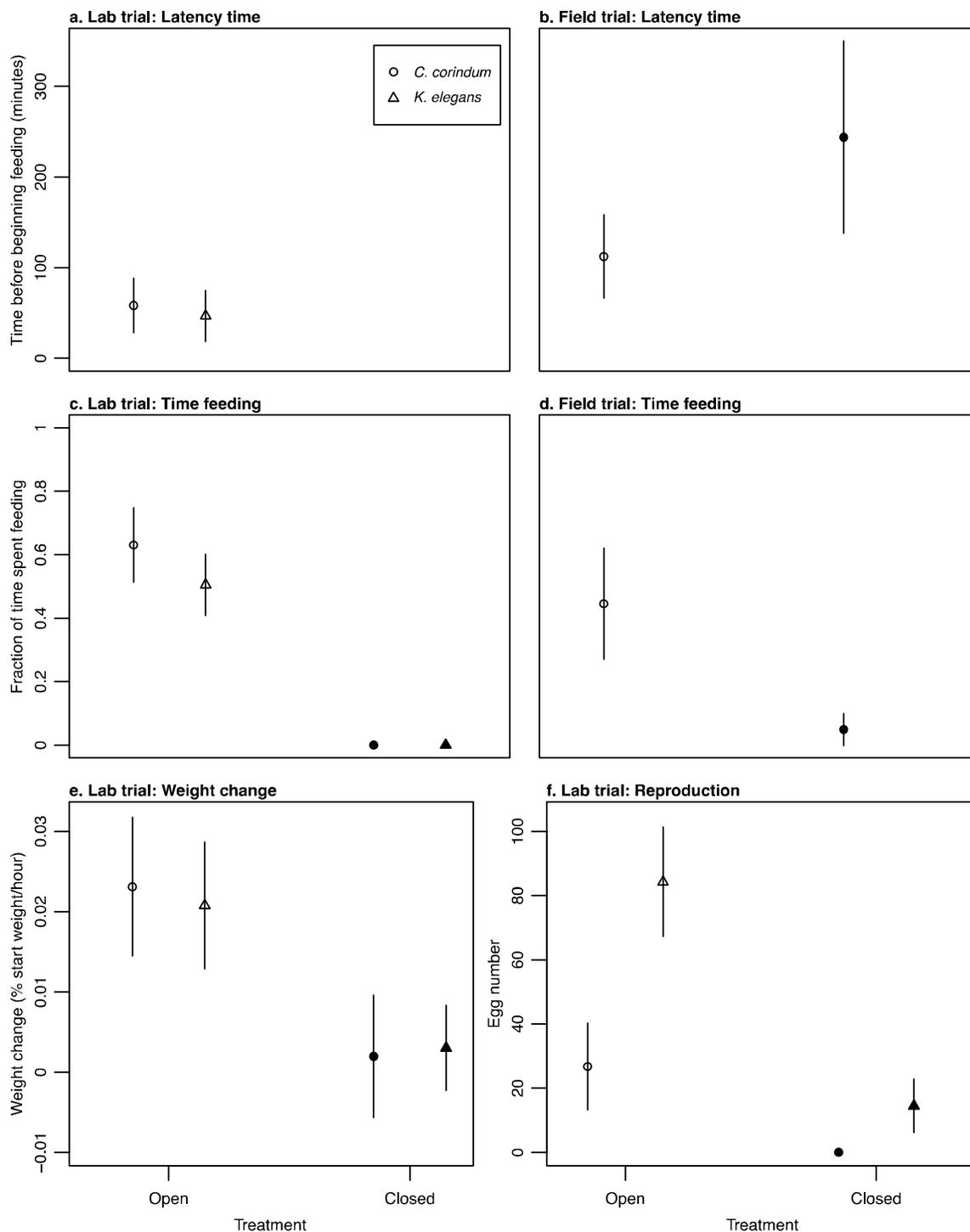
608 the right were collected from *K. elegans*. Error bars represent 95% confidence intervals.



610

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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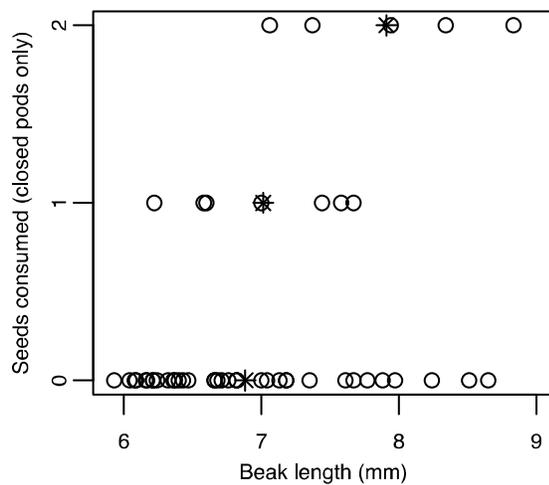
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617

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.



627
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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