

1 Dispersal-behavioral plasticity within an insect-host system  
2 undergoing human-induced rapid environmental change  
3 (HIREC)

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7

## 8 Abstract

9 As environments change, often drastically, due to human activities, dispersal-behavioral plasticity can be-  
10 come a key mediator of metapopulation connectivity and the interactions between an individual and its  
11 altered environment. Our goal was to investigate the traits and ecological processes that affect plastic  
12 dispersal responses within an insect-host system undergoing human-induced rapid evolutionary change  
13 (HIREC). Since the 1960s, populations of the red-shouldered soapberry bug from Florida, USA, originally  
14 feeding on the seeds of *Cardiospermum corindum* on the islands, quickly adapted to feeding on an invasive,  
15 ornamental tree, *Koelreuteria elegans*, on the mainland, which led to host-specific wing polyphenism. Here,  
16 we measured the morphology of >3,500 soapberry bugs field collected from 2013 to 2020 and the flight per-  
17 formance of 378 total soapberry bugs tested in a flight mill during Fall 2019 and Winter 2020. Flight tests  
18 showed females and mainland natives exhibited variable flight responses mediated by mass, while males  
19 were consistent, wing-dependent flyers. However, historical specimens showed annual rises in flightless  
20 morphs for males and dwindling wing-to-body sizes for island natives since 2013. Despite uncertain fu-  
21 ture fitness consequences, plasticity could help predict mobility character and agent dispersal behavior and  
22 ultimately help identify whether recent trends signal adjustment or maladaptation to HIREC.

23 **Keywords:** flight dispersal; phenotypic plasticity; sexual dimorphism; host adaption; morphology;  
24 HIREC

## 1 Introduction

In the past decade, several ecologists have called to action the need to translate adaptive plasticity into explicit science not only to better understand how organisms make decisions in response to their environment but also to redefine behavioral ecology as a discipline [1,2,3,4,5]. The call was ushered in response to a rise in consciousness since the 1990s [6,7,8,9] about humans as an evolutionary force and its irrefutable, accelerating discord with the field's approach to studying animal behavior within the environmental conditions that animals originally evolved in [10]. As shown, human-induced rapid environmental change (HIREC) has stemmed from rapid discontinuities with our environmental and evolutionary past, as direct and indirect results of human activities have been inundating organisms with novel conditions, leading biota to either sink [11,12] or adapt [13,14] but mostly sink [15]. In HIREC, these novel conditions facilitated by humans cause great or significant changes in evolutionary potential, from directional selection to genetic or plastic expression, and they facilitate these changes at speeds faster than organisms' evolutionary past. In many cases, organisms are not doing poorly with HIREC because they cannot respond fast enough but because they show maladaptive behavioral responses, such as those associated with ecological or evolutionary traps [16]. From insects attracted to laying their eggs on artificial surfaces (e.g. oil or roads) resulting in their death [17,18] to 'silent' insect mass extinctions [19], there seems to be an approaching homogeneous species bottleneck, and yet there is a growing consensus that plasticity could act as both a means for organisms to weather and resist HIREC [20] and as a blueprint for humans to reconstruct ethology.

When defined conservatively, plasticity is the expression of multiple phenotypes from one genotype when exposed to different environments [21,22]. Plasticity can affect all levels of ecological organization, but its most pertinent influence has been its link to environmental change and evolutionary response. A meta-analysis comparing the rates of phenotypic change across 68 animal systems suggested that, for systems undergoing HIREC, plasticity contributed up to twice as much to phenotypic change as genetic effects [23]. Additionally, among plants and invertebrates, increases or decreases in plasticity following anthropogenic disturbances were shown to be directed by trait type and taxon [24]. Although assessing the degree in which plasticity is expressed is important, what is largely missing in the HIREC literature are patterns of behavioral plasticity and their trait dependencies within a species [25]. Already, researchers have demonstrated how generalists and diet-expansive species can raise their chances of coping with extinction [26]. Furthermore, it has been shown how behavioral plasticity can be used to help predict the adaptability of a species undergoing habitat degradation due to reductions in spatial complexity [27] or urbanization resulting in spatial fragmentation [28]. Others have demonstrated how behavioral plasticity can be confounded by body size [29] and habitat breadth [30]. In turn, the need to test individual plastic behaviors in response to HIREC has become more prevalent alongside the demand to demonstrate how plastic-behavioral traits link to other, slower-evolving processes, like adaptations in life history or morphology. For insects and their behavioral responses involving diapause or threshold traits like polyphenisms and polymorphisms, anthropogenic environmental sources of variation in transgenerational and maternal effects have been more documented [25,31]. This has opened avenues to study the plasticity of more allusive and intractable forms of behavior like dispersal.

However, plastic expressions of various acts of dispersal can be challenging to study by behavioral ecologists, despite dispersal occurring ubiquitously across many biological systems. Dispersal ability, the potential for movement by animals between locations [32], is a fundamental trait for determining geographic range and gene flow within and between populations [24] and it can be dampened [33,34] or induced by persistent deleterious changes in habitat conditions, particularly for philopatric organisms [35]. Individuals also display various dispersal characteristics, such as endurance, periodicity, and speed, and these traits have been linked to morphological traits like sex [36], body size [37,38], and wing size [39] as well as trade-offs with reproduction [40]. However, capturing movement, especially plastic behaviors in movement, requires reliable tracking methods or machinery, which has led to an explosion of flight mill contraptions in the past six decades for insect flight dispersal quantification [41,42,43]. For ecologists that have taken the approach to empirically capture dispersal, the advantage of studying dispersal-behavioral plasticity arises not only when we consider why or how a species disperses but also ask how fixed or plas-

75 tic acts of dispersal have evolved over time. For species undergoing HIREC, we can further assess how  
76 variability in dispersal can facilitate adaptive or maladaptive strategies in response to HIREC and identify  
77 who the main contributing dispersal agents in a population are. Especially considering that acts of disper-  
78 sal can be directly initiated by individuals, measuring agent dispersal behavior, as opposed to population  
79 genetics, can reveal mobility characteristics that have not necessarily been genetically assimilated yet [44].  
80 This could ultimately better help us understand how environmentally-induced phenotypic variation links  
81 to a population's evolutionary history or guides evolutionary trajectories of adaptation or maladaptation in  
82 response to HIREC. Thus, evidenced patterns of dispersal-behavioral plasticity can help us better infer the  
83 future connectivity and survival of populations.

84 In this paper, we examined how, under an insect-host system undergoing HIREC, plasticity in dis-  
85 persal behavior could help predict mobility character and agent behavior as it depends on an organism's  
86 evolutionary history. As such, we studied the soapberry bug collected from Florida and its host plants  
87 (*Cardiospermum corindum*, native; *Koeleruteria elegans*, invasive) as a promising system for assessing the  
88 connections between HIREC and dispersal-behavioral plasticity. As observed from field collections, the  
89 soapberry bug is a seed-eating and sexually dimorphic insect that has adapted to an invasive, ornamen-  
90 tal tree within the past half-century [45,46]. This rapid evolutionary change has led to host-specific wing  
91 polyphenism [47,48]; adults will either develop long forewings with flight muscle ("long-wing" volant  
92 morph), long forewings without flight muscle or with histolyzed flight muscle ("cryptic" flightless morph),  
93 or a truncated wing without flight muscle ("short-wing" flightless morph). These flightless morphs have  
94 been found at higher frequencies on *K. elegans*, a host plant that synchronously produces ample volumes of  
95 seeds starting in the Fall season, compared to *C. corindum* that variably produces fewer seeds throughout  
96 the year [47]. Furthermore, each host is spatially separated between the mainland (invasive) and the islands  
97 (native) of Florida, which peripherally introduces questions about range-edge dynamics [49,50]. With such  
98 spatial diversity, including observed latitudinal clines in beak length [51], ocean barriers, and mainland  
99 urbanization, we considered how populations deeper in the mainland as opposed to those at the sympatric  
100 zone (i.e. the geographic area where two host plants overlap) or in the islands were dispersing, and how  
101 plastic flight behaviors could inform slower-evolving traits like wing morphology and wing morph fre-  
102 quencies. Furthermore, considering the interconnected evolution of sexual dimorphism within the species  
103 [52], we assessed how sexual asymmetry in morphology leads to sex-biased dispersal [53,54] and how its  
104 ecological consequences will depend on our ability to determine its prevalence and plastic variation in nat-  
105 ural populations. Thus, these patterns of plasticity in a behavior as morphological, mechanistic, metabolic,  
106 and spatial like insect flight movement [55,56,57,58,59] can also provide insights into how behavioral traits  
107 are confounded with weight, sex, or local geographic variation.

108 In our study, we asked which traits and ecological processes affect plastic dispersal responses and which  
109 agents in a population are driving those responses? We also asked whether plasticity could help predict  
110 mobility character and frame how insects are coping or not coping with HIREC? In turn, we measured the  
111 morphology of > 3,500 soapberry bugs field collected from 2013 to 2020 and recorded the flight behavior of  
112 378 total soapberry bugs using a flight mill during Fall 2019 and Winter 2020. We found novel sex-specific  
113 and host-specific plastic flight responses, repeatedly mediated by mass changes. Flight tests showed high  
114 variability in flight response between trials for females and a latitudinal cline in flight response for main-  
115 land natives from *K. elegans*, suggesting trade-offs among flight potential, reproduction, and host plant.  
116 Conversely, males and island natives from *C. corindum* were consistent, homogeneous flyers; males were  
117 2.5x more likely to fly than females and were 1.3x more likely to be long-winged. However, historical  
118 specimens showed annual rises in flightless morphs for males since 2013. Soapberry bugs collected from  
119 *C. corindum* since 2013 also showed dwindling wing-to-body sizes annually. These dependencies on sex,  
120 host plant, and mass suggest that a species ability to cope well with future environmental variation will  
121 depend on differing or biased dispersal agent responses in a population. Furthermore, our findings on the  
122 plastic effects of latitudinal distance from the sympatric zone, provide insight into how degrees of insular-  
123 ity or confluence direct whether a trait exhibits a more fixed or variable effect on dispersal abilities. Little  
124 is known about the traits and spatio-temporal processes that affect plastic dispersal responses, but we of-  
125 fer a complex dispersal behavioral system that exhibits sex-specific and host-specific plastic responses as

126 well as potential maladaptive consequences. As we learn more about the human influences that have in-  
127 undated environmental changes at scales larger and frequencies faster than recent pre-human phenomena  
128 have, we will come to better understand and predict the eco-evolutionary behaviors and plastic responses  
129 engendered by HIREC.

## 130 **2 Methods**

### 131 **2.1 Study Species 2013-2020 Field Collection and Morphology**

132 Soapberry bugs were collected in 1 to 8 locations in Florida at least once a year from April 2013 to Febru-  
133 ary 2020 (see Appendix A Section 2.5). Each seasonal bug collection contained a mix of soapberry bugs  
134 selected from their native host plant, *Cardiospermum corindum*, and nonnative host plant, *Koeleria elegans*  
135 (Figure 1A). After shipment, collected bugs were either preserved immediately in ethanol in 50 mL fal-  
136 con conical centrifuge tubes grouped by population or preserved individually, with a unique identification  
137 number, in ethanol in 2.0 mL microcentrifuge tubes following laboratory experiments. We took morpho-  
138 logical measurements for each soapberry bug collected, using Mitutoyo digital calipers and a Zeiss Stemi  
139 1000 Microscope 7x – 35x. Measurements of interest included the distance from the rostrum to the tip of the  
140 wing (body length) and forewing length (see Appendix A Section 2.4 for sample microscope images).

### 141 **2.2 Study Species 2019-2020 Field Collection and Flight Trials**

#### 142 **2.2.1 Field Collection**

143 For flight trials, soapberry bugs were collected during the Fall 2019 and Winter 2020 season in eight lo-  
144 cations in Florida and shipped to Chicago, IL. In the three archipelago sites, bugs were collected from *C.*  
145 *corindum*; in the five mainland sites, bugs were collected from *K. elegans* (Figure 1B). To see exact collection  
146 site names and counts for each season, refer to the tables in Appendix A Section 2.5.

#### 147 **2.2.2 Flight Trial Initial Preparation and Care**

148 Of the soapberry bugs collected during Fall 2019 and Winter 2020, 207 and 476 bugs survived shipment,  
149 respectively. All except 147 short-winged bugs collected in Winter 2020 were placed into assembled bug  
150 homes with an assigned unique identification number (see Appendix A section 2.4 comparing a short-  
151 winged adult to a long-winged adult). Bug homes were assembled using a plastic soufflé cup (4 oz) that  
152 was sealed with a mesh lid and lined with filter paper at the base. Each cup contained a 2.0 mL microcen-  
153 trifuge tube filled with deionized (DI) water and stoppered with cotton. Each bug was provided initially  
154 with two different seeds, one from *K. paniculata* (Sheffield’s Seed Company) and one from *C. halicacabum*  
155 (Outsidepride.com, Inc), two congeners of the field host plants that are commercially available. The upper  
156 rim of each plastic soufflé cup was lined with PTFE Fluoropolymer (“Fluon”), creating a slippery surface to  
157 keep bugs primarily in the bottom of the cup (Figure 2B). Identified soapberry bugs in bug homes were then  
158 randomly assorted into boxes and placed into an incubator. The incubator was illuminated with Philips 17  
159 W, 24 in florescent lighting and was set at 28 C/27.5 C (day/night), 70% relative humidity, and a 24 hour  
160 light/0 hour dark cycle.

#### 161 **2.2.3 Repeated Bug Preparation and Care**

162 Daily, dead soapberry bugs were pulled from their assorted homes and preserved in ethanol; then, mor-  
163 phological measurements were taken ( $n = 683$ ) that followed the aforementioned methods in Section 3.1.  
164 Weekly, adult soapberry bugs received general care: their filter paper was replaced, microcentrifuge tubes  
165 were refilled with DI water and capped with a new piece of cotton, and two fresh seeds (one from each host  
166 plant) were added to each bug home. In addition, once a week, eggs laid by female bugs were collected  
167 and discarded. Eggs were only counted during Winter 2020 trials.

## 168 2.2.4 Flight Mill Trials

169 Fall flight trials were conducted over a 27 day period in October and November of 2019. After confidently  
170 confirming that no short-wing bugs fly following different flight trial lengths, we removed all short-wing  
171 bugs from the testing pool and tested the remaining long-winged bugs for an unlimited trial length from  
172 November 5 to November 8. An unlimited trial length is defined as a trial where bugs remained on the flight  
173 mill until they stopped flying of their own volition. The unlimited trial model most successfully captured  
174 the full scope of soapberry bug flight potential, distance, and speed. The unlimited trial experimental setup  
175 was repeated for Winter flight trials.

176 Winter flight trials were conducted over a 22 day period in February and March of 2020. We ran two sets  
177 of trials, the first from February 17 to February 28 on 332 bugs, the second from March 3 to March 10 on the  
178 surviving 282 bugs. Refer to Table 1 for a summary of each season's experimental setup. Only unlimited  
179 trials are described below.

### 180 1. Flight Trial Preparation

181 For both seasons, we randomly ordered bugs by their identification number for each set of flight trials.  
182 The day before their trial, soapberry bugs received sugar water in a 2.0 mL microcentrifuge tube made  
183 from 7 volumes of Fruit Punch Gatorade and 3 volumes of DI water and capped with cotton. We also  
184 painted soapberry bugs' pronotums with Sophisticated Finishes<sup>TM</sup> Iron Metallic Surfacers paint and  
185 set the painted bugs to dry in their bug homes overnight.

### 186 2. Running Flight Trials

187 The day of their flight trial, we assigned a flight mill chamber to each soapberry bug based on their  
188 randomized trial order. The flight mill supported eight individual plexiglass chambers (Figure 2A).  
189 Within each chamber, we magnetically tethered a painted bug to the small N42 neodymium magnets  
190 of a magnetically suspended pivot arm. When the insect flew, the flight mill recorded its flight revolu-  
191 tions using a DATAQ Model DI-1100 data logger and the WinDAQ Acquisition Waveform Recording  
192 and Playback Software. After trials, we processed the recording files into flight distance and instantane-  
193 ous speeds (see Bernat 2021 for details on flight mill construction, functionality, and programming).  
194 The flight mill was located in an incubator set to the same conditions as the rearing incubator except  
195 it ran a 14 hour light/10 hour dark cycle (sunrise at 8 AM and sunset at 10 PM).

196 Before flight trials, Fall bugs ( $n = 207$ , number of mass measurements = 337) and Winter bugs were  
197 massed ( $n = 332$ , number of mass measurements = 611) with an analytical balance ( $0.1 \pm \text{mg}$ ). For each  
198 trial, flight behavior for bugs was recorded every hundredth of a second (sample rate) by the WinDAQ  
199 Software for up to 24 hours. We manually recorded flight response, which was defined as whether a  
200 bug flew (demonstrated rapid wing movement and self-propelled forward motion) or not. If the bug  
201 flew, we recorded the type of flight it displayed. We split flight type into two categories: 'bursting'  
202 and 'continuous' flight behavior. An individual was 'bursting' when it flew but did not maintain  
203 flight behavior for at least 10 minutes while longer flight was labelled as 'continuous'. The majority  
204 of bursting flight was for very short intervals ( $< 30$  seconds). During the trial period, individuals  
205 could display both bursting and continuous flight. We recorded on paper and via Event Markers in  
206 the WinDAQ Software the start and stop time for all bugs.

207 The procedure for unlimited recording was such:

- 208 (a) We began flight trials between 8am and 9am each day. To initiate flight trials, we magnetically  
209 tethered massed bugs onto each of the 8 flight mill arms. For each channel, we entered Event  
210 Markers with the bug's ID into the flight recording. We would then blow on each individual to  
211 motivate flight.
- 212 (b) We made two additional motivational attempts at 10 minutes and 20 minutes into each individ-  
213 ual's flight trial. If the bugs were not exhibiting continuous flight by the 30 minute mark, we  
214 pulled those bugs off the mill and returned them to their individual bug homes.



- 215 (c) Bugs who exhibited continuous flight by the 30 minute mark were left on the flight mill and  
216 checked on every 30 minutes. If a bug stopped flying, its stop time was recorded and it was  
217 pulled from the flight mill and returned to its bug home.
- 218 (d) Vacant chamber(s) were filled by the next bug(s) that followed in their randomized trial order.  
219 We continued adding new bugs and entering their accompanying Event Marker until 5 PM each  
220 flight day. Bugs that were still flying after 5 PM were left on the flight mill until the following  
221 morning.

## 222 **2.3 Statistics**

223 All statistical analyses were conducted in R (R Core Team, 2020). Analyses included multiple R scripts  
224 that conducted regressions for bug morphology from 2013-2020 and for flight response across and between  
225 flight trials. Model selection was based on Akaike information criterion (AIC) and an analysis of variation  
226 (ANOVA) test where  $p > 0.05$  favored the simpler model. Our models included four predictor variables at  
227 most that were based on a priori hypotheses; therefore, we assumed low family-wise error. See appendices  
228 and publicly available scripts (<https://github.com/mlcenzar/SBB-dispersal/tree/master/avbernat>).

### 229 **2.3.1 Morphological Analyses From 2013-2020 Collections**

230 Our morphological traits of interest were primarily concerned with the wing of the soapberry bug. Because  
231 we expected all morphology measurements to be correlated with the overall size of the soapberry bug, we  
232 calculated the ratio of wing length to body length (wing-to-body).

233 Because wing-to-body ratios and long-wing morph frequencies were recorded by month and year, we  
234 first tested for stationarity using the augmented Dickey-Fuller (ADF) test. After finding no significant  
235 temporal dependencies, we concluded that the values from each collection date were independent of each  
236 other (i.e. AR(0) process) for both wing-to-body ratio and long-wing morph frequency. We then performed  
237 multiple regression analyses to identify which predictors (sex, host plant, month, year, and all possible  
238 pairwise interactions) best predicted these two morphological traits, following the aforementioned model  
239 selection process. Finally, for visual purposes, we fit a local polynomial regression to each data set, using the  
240 lowess and geom\_smooth functions in R. We applied smoothers with increasing weights until the residuals  
241 appeared to have constant variance. In turn, all local polynomial regressions were fit with a span of 0.4  
242 ( $\alpha$ , the smoothing parameter), a degree of zero ( $\lambda$ ), and 95% confidence intervals. The locally-weighted  
243 scatterplot smoothing (LOESS) helped depict the non-linear fluctuations in wing-to-body ratio and long-  
244 wing morph frequency across time. See Appendix A for a tidy version of the analyses.

### 245 **2.3.2 Across-Trial and Between-Trial Flight Response From Fall 2019 and Winter 2020 Collections**

246 To prepare the data for analyses, the recording files were manually converted and processed via three  
247 main python scripts (see Bernat 2021). We then ensured that all flight recordings matched our handwritten  
248 records of whether a bug flew or not during its trial.

249 Across flight trials, we performed multiple regression analyses to test whether mass, host plant, wing-to-  
250 body ratio, or latitudinal distance from the sympatric zone was associated with either sex's flight probability  
251 (Appendix B section 2.5). Because soapberry bugs exhibit sexual dimorphism, we hypothesized that the  
252 sexes may exhibit differing effects on flight potential, so we analyzed the sexes separately. We also applied  
253 single-variate regressions to test for any experimental effects, such as days elapsed since the start of trials  
254 (Appendix B section 2.4).

255 Between flight trials, we conducted multinomial logistic regression models to predict the probabilities  
256 of different possible flight outcomes as a function of sex, wing-to-body-ratio, and mass percent change  
257 (Appendix B section 3.5). There were four flight outcomes possible between trials, which we termed as  
258 'flight cases'. Each flight case describes a soapberry bug's change in flight response between trial 1 (T1) and  
259 trial 2 (T2): a bug either flew in both trials, only in T2, in neither trial, or only in T1 (Appendix B section

260 3.4). We also ran multinomial logit models for a subset of the data with females only in order to assess  
261 the impact of egg production as a predictor (Appendix B section 3.6). For females, we predicted various  
262 flight cases using mass percent change, wing-to-body ratio, and 'egg case'. Egg case denotes the change  
263 in egg laying activity between trials: either a female laid eggs in both trials, only in T2, in neither trial, or  
264 only in T1 (Appendix B section 3.6.1). For all flight trial datasets, we used maximum likelihood to estimate  
265 multinomial logit models through the `nnet` library in R. Model selection followed the same aforementioned  
266 process. Each best fit model allowed us to then calculate the predicted probability of a bug's flight case as  
267 well as the odds (OR) ratio between flight cases. The OR signifies the ratio of the probability that a flight  
268 case will happen compared to the probability that another flight case (i.e. the 'baseline') will happen. Refer  
269 to Appendix B section 3.5 to see how the OR is calculated from the prediction equations of a multinomial  
270 logistic model. Finally, we used each best fit model to predict flight case probabilities for bugs flight tested  
271 in Fall 2019 in order to assess the accuracy and overall performance of each best fit model. See Appendix B  
272 for a tidy version of the analyses.

## 273 3 Results

### 274 3.1 Wing Morphology of Field Bugs Over Time

#### 275 3.1.1 Long Wing Morph Frequency

276 The best fit model for wing morph included sex, host plant, month, and year as single factors and interac-  
277 tions ( $n = 3532$ ; Appendix A section 3.1.1). Male soapberry bugs were 30% more likely to be long-winged  
278 than females ( $\beta = -0.26$ ,  $SE = 0.09$ ,  $P = 0.004$ ). Soapberry bugs collected from *K. elegans* were 208% more  
279 likely to be long-winged than those collected from *C. corindum* ( $\beta = 1.13$ ,  $SE = 0.11$ ,  $P \ll 0.05$ ). Within  
280 a given year, long wing morphs were approximately 11% more likely to appear for each month passed ( $\beta$   
281  $= 0.10$ ,  $SE = 0.03$ ,  $P \ll 0.05$ ; Figure 3A, B). Long wing morph frequency also increased by 1% for each  
282 year passed, but this was a relatively weak effect ( $\beta = 0.01$ ,  $SE = 0.003$ ,  $P \ll 0.05$ ; Figure 3C). Yearly and  
283 monthly interactions were also relatively weak but nuanced. Long wing morphs were increasingly more  
284 likely to appear in later months than earlier months before 2019 but less likely to appear in later months  
285 than earlier months after 2019 ( $\beta = -0.002$ ,  $SE = 0.0005$ ,  $P = 0.001$ ; Appendix A section 3.1.1)

286 The best fit model included three additional interaction terms, as follows: sex and host plant, sex and  
287 year, host plant and month. Female soapberry bugs from *K. elegans* or males from *C. corindum* were 4.2%  
288 more likely to be long-winged compared to their host plant counterparts within their sex group ( $\beta = 0.10$ ,  
289  $SE = 0.05$ ,  $P = 0.05$ ; Appendix A section 3.1.1). Female soapberry bugs also were 0.4% more likely to be long-  
290 winged each proceeding year since the first collection unlike their male counterparts which are becoming  
291 less likely to be long-winged. However, the effect of this interaction is relatively small ( $\beta = 0.004$ ,  $SE =$   
292  $0.002$ ,  $P = 0.02$ ; Figure 3C). Finally, soapberry bugs collected from *C. corindum* were 3.7% more likely to be  
293 long-winged for each month passed, whereas individuals collected from *K. elegans* decreased by 3.9% each  
294 month (Appendix A section 3.1.1 & 4.1.3).

#### 295 3.1.2 Wing-to-Body Ratio

296 Bugs that were short-winged or had torn wings were then filtered from the dataset. From this subset of  
297 the data, the best fit model for wing-to-body ratio similarly included sex, host plant, month, and year as  
298 single factors and interactions ( $n = 1903$ ; Appendix A section 3.3.1). Males had larger wing-to-body ratios  
299 ( $\bar{x} = 0.731$ ,  $SE = 6e-04$ ) than females ( $\bar{x} = 0.729$ ,  $SE = 7e-04$ ) by 0.002 units, on average ( $\beta = -0.002$ ,  $SE =$   
300  $0.0005$ ,  $P \ll 0.05$ ; Figure 3E). Soapberry bugs collected from *K. elegans* had larger wing-to-body ratios  
301 by approximately 0.004 units, on average, than those collected from *C. corindum* ( $\beta = 0.004$ ,  $SE = 0.0005$ ,  
302  $P \ll 0.05$ ; Figure 3). However, there was a significant interaction between sex and host plant, with female  
303 soapberry bugs collected from *K. elegans* having the larger wing-to-body ratios, on average, than their male  
304 or *C. corindum* counterparts ( $\beta = 0.002$ ,  $SE = 0.0005$ ,  $P \ll 0.05$ ; Appendix A section 3.3.1).



305 There was no effect of year ( $P = 0.43$ ), but there was a significant year and host plant interaction where  
306 soapberry bugs collected from *K. elegans* have larger wing-to-body ratios each year since 2013 by  $6e-05$  units,  
307 on average ( $\beta = 6e-05$ ,  $SE = 2e-05$ ,  $P = 0.007$ ; Figure 3F; Appendix A section 3.3.1). Larger wing-to-body  
308 ratios were also increasingly likely later in the year ( $\beta = 0.0007$ ,  $SE = 0.0001$ ,  $P \ll 0.05$ ) where each month  
309 wing-to-body ratios increased by  $0.0007$  units, on average (Figure 3D, E).

## 310 **3.2 Winter 2020 Across-Trial and Between-Trial Flight Response**

### 311 **3.2.1 Across Trials**

312 One factor from our experimental design affected flight response across trials: days since the start of trials.  
313 On average, soapberry bugs tested on day 20 compared to day 1 of trials had a 15% drop in flight prob-  
314 ability ( $\beta = -0.008$ ,  $SE = 0.002$ ,  $P = 0.017$ ; Appendix B section 2.4), suggesting a possible age affect [xxxx].  
315 Individuals were randomized across date and start times; however, to control for the impact of days since  
316 the start of trials on flight response, we calculated and used each individual's mean trial date in our models  
317 (Appendix B section 2.5.1).

318 The best fit model for flight response across trials included sex as a significant single factor as well as  
319 significant interactions of mass with host plant and mass with latitudinal distance from the sympatric zone  
320 ( $n = 333$ ; Appendix B section 2.5.3). Male soapberry bugs were 59% more likely to fly than females ( $\beta = -0.46$ ,  
321  $SE = 0.17$ ,  $P = 0.006$ ). Soapberry bugs from *K. elegans*, which resides in the mainland of Florida, were more  
322 likely to fly if they were heavier; individuals  $0.05$  g heavier than average were 50% more likely to fly than  
323 their lighter counterparts ( $\beta = 1.86$ ,  $SE = 0.59$ ,  $P = 0.002$ ; Figure 4A). The opposite was true for soapberry  
324 bugs from *C. corindum*, which dominates the islands of Florida; here, bugs that were  $0.05$  g heavier than  
325 average were 31% less likely to fly than their lighter counterparts (Figure 4B). Additionally, the effect of  
326 mass depended on latitudinal distance from the sympatric zone: for bugs two latitudinal degrees further,  
327 individuals were 25% less likely to fly than bugs one degree closer ( $\beta = -1.41$ ,  $SE = 0.69$ ,  $P = 0.04$ ; Figure 4  
328 and Appendix B section 2.5.3).

329 When models were separated by sex, we found wing-to-body ratio primarily drove male flight and  
330 mass drove female flight. The best fit multiple regression model for females included average mass, host  
331 plant, wing-to-body ratio, and average days since the start of trials ( $n = 120$ ; Appendix B section 2.5.4). We  
332 found that a female tested a day later on average would be 12% more likely to fly ( $\beta = 0.12$ ,  $SE = 0.05$ ,  $P$   
333  $= 0.015$ ), but later testing dates could be masking changes in female egg laying throughout the trials. We  
334 also found that, for every  $0.05$  unit increase in wing-to-body ratio, females experienced a 31% increase in  
335 flight potential ( $\beta = -5.37$ ,  $SE = 2.66$ ,  $P = 0.044$ ). As with the full dataset, we found a mass by host plant  
336 interaction: a female that was  $0.06$  g heavier than average and from *K. elegans* would be 83% more likely to  
337 fly than those from *C. corindum* ( $\beta = 3.02$ ,  $SE = 1.40$ ,  $P = 0.031$ ).

338 The best fit multiple regression model for males included wing-to-body ratio, host plant, and distance  
339 from the sympatric zone ( $n = 213$ ; Appendix B section 2.5.4), but notably did not include mass. For males,  
340 every  $0.05$  unit increase in wing-to-body ratio corresponded to a 53% increase in flight potential (a higher  
341 rate than females;  $\beta = -15.21$ ,  $SE = 5.22$ ,  $P = 0.004$ ). Additionally, wing-to-body ratio interacted with host  
342 plant: a  $0.05$  unit longer-than-average winged male from *C. corindum* would be 38% more likely to fly than  
343 those from *K. elegans* ( $\beta = -9.46$ ,  $SE = 4.28$ ,  $P = 0.027$ ) and 37% more likely to fly if it was 1 latitudinal degree  
344 farther from the sympatric zone ( $\beta = 6.31$ ,  $SE = 3.03$ ,  $P = 0.037$ ).

### 345 **3.2.2 Between Trials**

346 The best fit multinomial logit model used to explain a soapberry bug's observed flight case included mass  
347 percent change, sex, and wing-to-body ratio. Host plant was not significant. Results of the multinomial  
348 regression analysis are presented in Table 2; also, see Appendix B sections 3.5.4 and 3.5.5 for each prediction  
349 equation and for a heatmap adaptation of the results.

350 We found that if a soapberry bug had a 20% increase in mass, the odds of flying in T1 rather than twice  
351 ( $\beta = 0.02$ ,  $SE = 0.01$ ,  $P = 0.032$ ) as well as the odds of flying twice rather than not flying ( $\beta = 0.04$ ,  $SE = 0.01$ ,

352  $P \ll 0.05$ ) were both 30% lower compared to a bug that flies only in T1 rather than not flying ( $\beta = -0.02$ ,  
353  $SE = 0.01$ ,  $P = 0.005$ ). In other words, between flying twice, flying once in T1, and not flying at all, a bug  
354 had the highest odds of flying in T1 when a bug gained 20% of its original mass.

355 Sex and wing-to-body ratio were also significant for prediction equations where a bug could either fly  
356 once or twice rather than not fly at all. For bugs that had a wing-to-body ratio 0.01 units higher than  
357 average, the odds of flying twice ( $\beta = 0.28$ ,  $SE = 0.10$ ,  $P = 0.004$ ) were 4% higher compared to the odds of  
358 flying in T1 ( $\beta = 0.24$ ,  $SE = 0.12$ ,  $P = 0.049$ ). Males had a 78% higher odds of flying twice ( $\beta = -0.76$ ,  $SE = 0.17$ ,  
359  $P \ll 0.05$ ) and a 68% higher odds of flying in T1 only ( $\beta = -0.57$ ,  $SE = 0.21$ ,  $P = 0.007$ ) compared to females.  
360 Likewise, the odds of flying twice compared to flying in T1 was 17% higher for males but approximately  
361 17% lower for females. In summary, females not only had a lower odds of flying twice compared to males,  
362 but females also had the highest odds of not flying at all whereas males had the highest odds of flying  
363 twice.

364 Predicted probabilities showing the likelihood of a soapberry bug exhibiting one flight case over another  
365 can be seen in Figure 5. Figure 5B depicts males' propensity to fly repeatedly and their narrower mass range  
366 compared to females' larger mass ranges and propensity to fly either only in T1 or not at all. Figures 5A and  
367 5B also display the stochasticity in flight case probability introduced by wing-to-body ratio where, across  
368 the sexes, larger wing-to-body ratios led to greater probabilities that bugs would fly twice or fly in T1 rather  
369 than not fly at all. Additionally, probability thresholds can be extracted from Figure 5. For example, if a  
370 female were to gain more than 41% of her original body mass, then she would be most likely to fly in  
371 T1. Such results reinforce previous analyses that female flight potential is consistently more dependent on  
372 mass, whereas male flight potential is strongly influenced by wing morphology.

373 For females, the timing of egg laying also impacted certain flight case outcomes. In the best fit multinomial  
374 logit model for females only, predictor variables included mass percent change and egg case. Results  
375 of the multinomial regression analysis are presented in Table 3; also, see Appendix B section 3.6.5 and 3.6.6  
376 for each prediction equation and for a heatmap adaptation of the results.

377 We found that if a female had a 20% increase in mass and she had the chance of flying either once or  
378 twice rather than not flying at all, she was 33% less likely to fly twice ( $\beta = 0.02$ ,  $SE = 0.01$ ,  $P = 0.04$ ) compared  
379 to the odds of flying only in T1 ( $\beta = 0.04$ ,  $SE = 0.01$ ,  $P = 0.001$ ). Additionally, the odds of flying twice were  
380 67% lower for a female that had laid no eggs, 89% lower for a female that laid eggs only in T2, and 96%  
381 lower for a female that laid eggs twice compared with those that had only laid eggs in T1 ( $\beta = -1.10$ ,  $SE =$   
382  $0.30$ ,  $P \ll 0.05$ ). It appears that for females who have completed oviposition or are not close to oviposition,  
383 they will most likely fly twice, irrespective of changes in mass (Figure 5D and 5F); their flight behavior also  
384 mimics the flight behavior and mass changes of males (Figure 5B). For females who are in the middle of  
385 oviposition or nearing the beginning of oviposition, they appear to be most likely to not fly if not enough  
386 mass is gained or mass is lost (mass range: -30%, ~30%; Figure 5C and 5E). However, if enough mass is  
387 gained (mass range: 40%, 110%), females have higher chances of at least flying once Figure 5C. Refer to  
388 Appendix B section 3.6.7 for an alternative display and reading of Figure 5.

### 389 3.3 Predicting Fall 2019 Flight Potential

390 To test the performance of our models, we calculated flight case accuracy for the Fall 2019 flight tested bugs  
391 (Appendix B Section 4). For the best fit model for all Winter 2020 soapberry bugs, the overall prediction ac-  
392 curacy was 0.60 ( $n = 45$ ), with female prediction and male prediction accuracy at 0.38 and 0.69, respectively.  
393 Because the model favored repeated flight events and not all male bugs always flew twice, this model over-  
394 estimates flight capability. It over predicts when bugs fly twice (73.3%) and misses events when they only  
395 fly once (0%). For the best fit model for Winter 2020 female bugs, the overall prediction accuracy was 0.46  
396 ( $n = 13$ ). Females in the Fall mostly laid eggs twice ( $n = 10$ ), flew twice, and had a narrower mass range  
397 than females tested in the Winter (mass range: -20%, 30%; Appendix B section 3.6.7). The model greatly  
398 overestimates flight capability by overpredicting that female will only fly twice (100%) even though females  
399 in the Fall flew twice approximately half of the time (46.2%). This suggests that the narrower mass changes  
400 could be advantageous for females to fly repeatedly despite being in the middle of oviposition.

## 401 4 Discussion

402 In this study, we examined patterns of dispersal behavior that demonstrated plastic flight responses as well  
403 as recent trends in wing morphology in soapberry bugs from Florida, USA. We found plastic vs. fixed  
404 dispersal behavior to be sex-biased and wing morph-biased. We also found a latitudinal cline and host  
405 plant differences in flight response and demonstrated plastic flight responses largely mediated by mass.  
406 In particular, we identified macropterous, island-native males as the dispersal unit of this soapberry bug  
407 species as well as identified its complementing plastic dispersal unit: macropterous, mainland-native fe-  
408 males. Furthermore, we modeled mobility characteristics that reveal links between a population's evolu-  
409 tionary history and its environmentally-induced phenotypic variation [28,60]. Finally, given how plasticity  
410 impacts the ability of animals not only to adapt but physically maneuver through degraded habitats or in-  
411vasive species [27,61], we provide a step towards empirically predicting mobility for organisms undergoing  
412 HIREC.

### 413 4.1 Sex Differences in Flight Potential

414 Our results unequivocally showed sex differences in dispersal-behavioral plasticity. Males were consistent  
415 flyers and their flight response was positively driven by their wing-to-body ratio. They invested more in  
416 flight morphology and they were more likely to fly and fly again irrespective of changes in mass. In contrast,  
417 females were more variable, mass-dependent flyers because of trade-offs with egg production. Male-biased  
418 dispersal and trade-offs between investments in flight structures and reproduction have been demonstrated  
419 in various wing-polymorphic species [54, 62]. For instance, short-wing morphs of the cricket *Gryllus rubens*  
420 more efficiently assimilate nutrients into ovarian mass and overall biomass, compared to their long-winged  
421 counterparts who invest in flight muscle mass [63]. Bush crickets, as measured via microsatellite DNA  
422 analyses, also largely favored long-winged males as the dispersal unit of their species, which were more  
423 frequently found at their range margins compared to longer-established populations in their range core [54].  
424 Our observations that males are more dispersive and exhibit more fixed, as opposed to plastic, dispersal  
425 behavior suggest males similarly can be held accountable for the range expansion of their species. In turn,  
426 metapopulation connectivity, colonization, and spatial sorting [49] are likely to be driven primarily by  
427 males.

428 Female flight potential, on the other hand, was strongly associated with mass changes, host plant, and  
429 reproductive state. Generally, female soapberry bugs exhibited a negative mass effect that differed by host;  
430 heavy females from the invasive host were far more likely to fly than those from the native host. However,  
431 reproductive state could reverse negative mass relationships, making females more closely resemble their  
432 male counterparts (Figure 5). Females that were at the end of oviposition were most likely to fly repeatedly  
433 and they sustained short mass changes. Conversely, females in the middle or beginning oviposition had  
434 large mass ranges and were either prone to fly once or not fly at all.

435 These findings raise one possible interpretation of female dispersal. Female flight response, evidently  
436 plastic, could be characterized as more strategic and time-sensitive as females coordinate their flight be-  
437 havior based on their reproductive activity. Time-sensitive dispersal behavior has been seen for monarch  
438 butterflies in Texas and northern Mexico that consistently increase their lipid levels before Fall migrations  
439 [64]; however, none to our knowledge have connected insect flight behavior to mass and egg laying changes  
440 observed during a two week span of testing. This observation demonstrates that brief trade-offs between  
441 dispersal and egg-laying would demand optimizing the right time to be relatively mass stable in order  
442 make a strong one-time flight attempt before laying eggs. Likewise, changes in mass – a fluctuating met-  
443 ric throughout an insect's life – could temporarily induce trade-offs with dispersal but also provide the  
444 necessary physiological advantage before takeoff.

445 From these sex-biased plastic dispersal patterns, we can favorably characterize males as the dispersal  
446 unit of this soapberry bug species and females as the plastic dispersal unit. This characterization can relate  
447 to how soapberry bugs traverse and interact with their environment. We found that males and females  
448 raised on on *C. corindum* and males collected from the southern end of the Floridian islands had higher  
449 tendencies to fly. With the Atlantic Ocean acting as a spatial barrier, island-native males or females who

450 have ended or not begun oviposit would favor flying towards the mainland or northern islands, taking  
451 advantage of range edges as a means to drive phenotypic change where “space per se is an evolutionary  
452 agent” [49]. Likewise, females who leave their host to oviposit on the invasive host can facilitate rapid  
453 directional gene flow from *C. corindum* to *K. elegans* [51] or, in theory, they can induce rapid gene flow  
454 reversals [65], making populations between the islands and mainland more insular in response to HIREC.

455 However, it is uncertain whether fast-dispersing soapberry bugs at the edges of their dispersing fronts  
456 will breed and lead to successive generations that evolve to be even faster than the last. It is also un-  
457 clear whether spatial sorting or adaptive radiation leads to ecological opportunities [66], especially under  
458 a rapidly changing environment. This is relevant considering that our Winter 2020 model’s ability to pre-  
459 dict Fall 2019 female response was always below 50% as opposed to 69% for male flight response. Thus,  
460 sex-biased evolutionary-dispersal mechanisms behind soapberry bug gene flow warrant further study ac-  
461 companied by a phylogeographic analysis [67,68] or observations on additional traits (e.g. age, thorax  
462 muscle mass, thorax muscle histolysis, or diapausing status) [47,69].

## 463 4.2 Host Plant Differences and Latitudinal Cline in Flight Potential

464 To then better understand how host plant or latitudinal distance from the sympatric zone affected soap-  
465 berry bug dispersal, we included environmental factors in our models and found another case of dispersal-  
466 behavioral plasticity dependent on mass. Specifically, dispersal was more dependent on mass and latitudi-  
467 nal distance for mainland-native bugs collected from *K. elegans* than for island-native bugs from *C. corindum*.  
468 The deeper a bug from *K. elegans* was into the mainland, then the more likely it will fly if it was heavier but  
469 if it was closer to the islands, then the more likely it will fly if it was lighter. Such a relationship was sub-  
470 stantially variable compared to island-native bugs collected from *C. corindum* where, instead, heavier bugs  
471 would be less likely to fly, regardless of where it was located.

472 Previous studies have suggested that, in locations where dispersal between the divergent environments  
473 is the highest, local adaptation might not be apparent because of high migration and gene flow [30, 70].  
474 In response, plasticity can evolve to allow these populations to display locally adaptive phenotypes in  
475 the absence of genetic differentiation. This was observed in a study tracking brain mass variability and  
476 dispersal potential in African cichlid fish in the oxygen-diverse rivers, lakes, and swamps of East Africa [30].  
477 However, we observe a more complex scenario. Even though heavy soapberry bugs from the mainland  
478 exhibited the highest flight potentials, low-weight bugs from the islands still consistently exhibited high  
479 flight potentials; and yet, only mainland bugs exhibited a plastic latitudinal cline. This discrepancy could be  
480 due to whether soapberry bugs across Florida differentially recognize adaptive vs. maladaptive responses  
481 to rapid shifts in host plant utilization. In this case, maladaptive responses would suggest soapberry bugs  
482 are dampening their abilities to disperse for resources and mates; highly variable flight could then be less of  
483 a phenotypic display and more of an evolutionary trap [12] because mainland bugs don’t readily recognize  
484 the potential for island resources.

485 Additionally, given that the ability to fly is an act that is highly mechanistic, metabolic, and spatial  
486 [55,56,57,58,59], it may be that the energetics and physics of dispersal cannot be readily divorced from the  
487 larger narrative of plastic potential and its costs. As such, a narrower, more fixed range of flight potential  
488 in the islands could be necessary if the costs of failing to traverse to the mainland, which results in death,  
489 outweighs the benefit of being plastic flyers. Meanwhile, mainland-native bugs without such irreversible  
490 costs may show less consistent investments in flight performance. To better evaluate the costs of dispersal-  
491 behavioral plasticity, it then becomes important to measure additional insect flight performance metrics  
492 (e.g. distance or duration) [unpublished data collected by AVB and MLC], especially for insects undergoing  
493 HIREC who may adapt or sink in response to human influences in unexpected ways.

## 494 4.3 Wing Morphology Through Time

495 Our analyses of soapberry bug wing morph and wing-to-body ratio from 2013 to 2020 identified annual  
496 and seasonal trends in soapberry bug wing morphology. Such trends show how dispersal could change



497 in the future, as this insect-host system continues to undergo evolutionary responses to HIREC. First, we  
498 found strong, positive within-year trends in long-wing morph frequency and wing-to-body ratio. Second,  
499 we found that males, which significantly depend on larger wing-to-body ratios for higher flight potentials,  
500 are gradually becoming more short-winged over time. Likewise, soapberry bugs collected from the native  
501 host are gradually exhibiting smaller wing-to-body ratios. Even though males are consistent flyers and  
502 would most likely be the first in a population to traverse between the islands and mainland, these trends  
503 suggest that the traits favoring frequent flight are being selected against. Possibly, this pattern implicates  
504 another case of maladaptive plasticity induced by host plant on the system [50] where flightlessness is im-  
505 peding some dispersal agents from traversing between the mainland and islands for resources and mates.  
506 This could lead to narrower windows of high dispersal for males and for soapberry bugs from *C. corindum*.  
507 Conversely, it would appear that female and mainland dispersal agents are either generally unaffected or  
508 actively resisting evolutionary responses to HIREC in the last decade due to their plastic dispersal behav-  
509 iors; specifically, their plasticity may be dampening natural selection for less flight-capable traits [71]. As  
510 similarly observed by studies on birds, high phenotypic plasticity, behavioral flexibility, and/or greater  
511 within-species variation in behavioral tendencies allow a species to cope well with environmental variation  
512 [72] and extinction [26], and the same could be occurring in Floridian soapberry bugs.

#### 513 4.4 Conclusions

514 Dispersal-behavioral plasticity determines the ability of animals to physically maneuver and cope with  
515 new or unusual challenges such as rapid host invasions or extreme climate events. Dispersal-behavioral  
516 plasticity thus has the potential to reduce species vulnerability and enhance population fitness following  
517 environmental changes. However, in light of the speed and force of human culture evolution, we have  
518 put into question how biota will weather and response to these rapid changes, how does dispersal link  
519 plasticity to HIREC, and how we can better predict who will adapt or maladapt? Additionally, as ethol-  
520 ogy reconciles losing the contexts in which organisms originally evolved in, it may become increasingly  
521 necessary to not speak too broadly about environmental changes but instead become abundantly more  
522 specific about an organisms' evolutionary history, especially if it has been significantly mediated by human  
523 activities. This specialization may seem counterintuitive to a discipline defined by broad patterns in behav-  
524 ioral ecology, and it may be tempting to advise that studies like ours simply be assimilated into ethology;  
525 however, it may be worthwhile to instead consider the evolutionary force of humans as imperatively self-  
526 identifying because of its scope. To consolidate this, empirical studies would need to continue to quantify  
527 and demonstrate the links and patterns formed between plasticity and HIREC as well as invest in tracking  
528 direct phenotypic plastic changes over time.

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## 680 **7 Author Contributions**

681 MLC conceived and designed the study; AVB enhanced the design of the study, performed the data collec-  
682 tion of flight trials, and assisted the data collection of morphology; MLC performed the data collection of  
683 morphology and assisted the data collection of flight trials; AVB and MLC drafted code; AVB analyzed the  
684 data and MLC code reviewed; AVB drew or generated the figures; AVB wrote the paper and appendices.  
685 All authors contributed to the final draft.

## 686 **8 Competing Interests**

687 The authors declare no competing interests. Upon submission of this manuscript, AVB is employed at the  
688 Pacific Northwest National Laboratory (PNNL), but there is no competing research interest or financial  
689 stake from PNNL's perspective.

## 690 **9 Data Availability Statement**

691 See Dryad (<https://doi.org/10.5061/dryad.XXXX>.) for a full description of each experimental setup and its  
692 accompanying data.

## 693 **10 Tables**



Season	Year	Dates	Experimental Setup	N Tested	Wing Morph
Fall	2019	October 15 to October 23	30 min	182	short and long
Fall	2019	October 23 to October 29	60 min	130	short and long
Fall	2019	October 30 to November 4	90 min	126	short and long
Fall	2019	November 5 to November 8	unlimited	66	long
Winter	2020	February 17 to February 28	unlimited	332	long
Winter	2020	March 3 to March 10	unlimited	282	long

**Table 1: Experimental setup of Fall 2019 and Winter 2020 flight trails.**

Variables in the Model	Flew twice rather than flew in T1	Flew twice rather than did not fly	Flew in T1 rather than did not fly
Mass % Change *	<b>0.67 (0.66, 0.68)</b>	<b>1.49 (1.46, 1.52)</b>	<b>2.23 (2.18, 2.27)</b>
Sex (female = 1)	0.83 (0.56, 1.22)	<b>0.47 (0.34, 0.65)</b>	<b>0.57 (0.37, 0.85)</b>
(male = -1)	1.21 (0.82, 1.79)	<b>2.14 (1.53, 2.98)</b>	<b>1.77 (1.17, 2.67)</b>
Wing-to-Body *	1.04 (0.84, 1.29)	<b>1.32 (1.09, 1.6)</b>	<b>1.27 (1, 1.61)</b>

\* The mass percent change estimates and wing-to-body estimates were multiplied by 20 and 0.01, respectively, before calculating the odds. These transformations better represent experimental observations and offer a more realistic odds (e.g. 20% mass increase and a 0.01 unit increase in wing-to-body ratio).

Table 2: **Results of multinomial logistic (ML) regression for predicting flight case for all soapberry bugs.** The odds of a soapberry bug exhibiting a particular flight case rather than another flight case (i.e. the base category) are presented below each column. T1 denotes trial 1 and T2 denotes trial 2. Statistically significant results ( $p < 0.05$ ) are in boldface and 95% confidence intervals are adjacent to the odds. Only ML prediction equations with at least one significant main effect are shown; however, ML equations comparing whether a soapberry bug flew in T2 to another flight case are not shown despite sex being significant. This is because there were no flight cases where females only flew in T2 during Winter 2020 trials.

Variables in the Model	Flew twice rather than did not fly	Flew in T1 rather than did not fly
Mass % Change *	<b>1.49 (1.46, 1.52)</b>	<b>2.23 (2.18, 2.27)</b>
Egg Case (laid eggs twice = 2)	<b>0.11 (0.06, 0.2)</b>	0.35 (0.16, 0.73)
(laid eggs in T2 = 1)	<b>0.33 (0.18, 0.6)</b>	0.59 (0.28, 1.24)
(did not lay eggs = 0)	<b>1 (0.56, 1.8)</b>	1 (0.47, 2.11)
(laid eggs in T1 = -1)	<b>3 (1.67, 5.41)</b>	1.7 (0.81, 3.58)

\* The mass percent change estimates were multiplied by 20 before calculating the odds. This transformation better represents experimental observations and offers a more realistic odds (e.g. 20% mass increase).

Table 3: **Results of multinomial logistic (ML) regression for predicting flight case for female soapberry bugs.** The odds of a soapberry bug exhibiting a particular flight case rather than another flight case (i.e. the base category) are presented below each column. T1 denotes trial 1. Statistically significant results ( $p < 0.05$ ) are in boldface and 95% confidence intervals are adjacent to the odds. Only ML prediction equations with at least one significant main effect are shown.

## 694 **11 Figures**

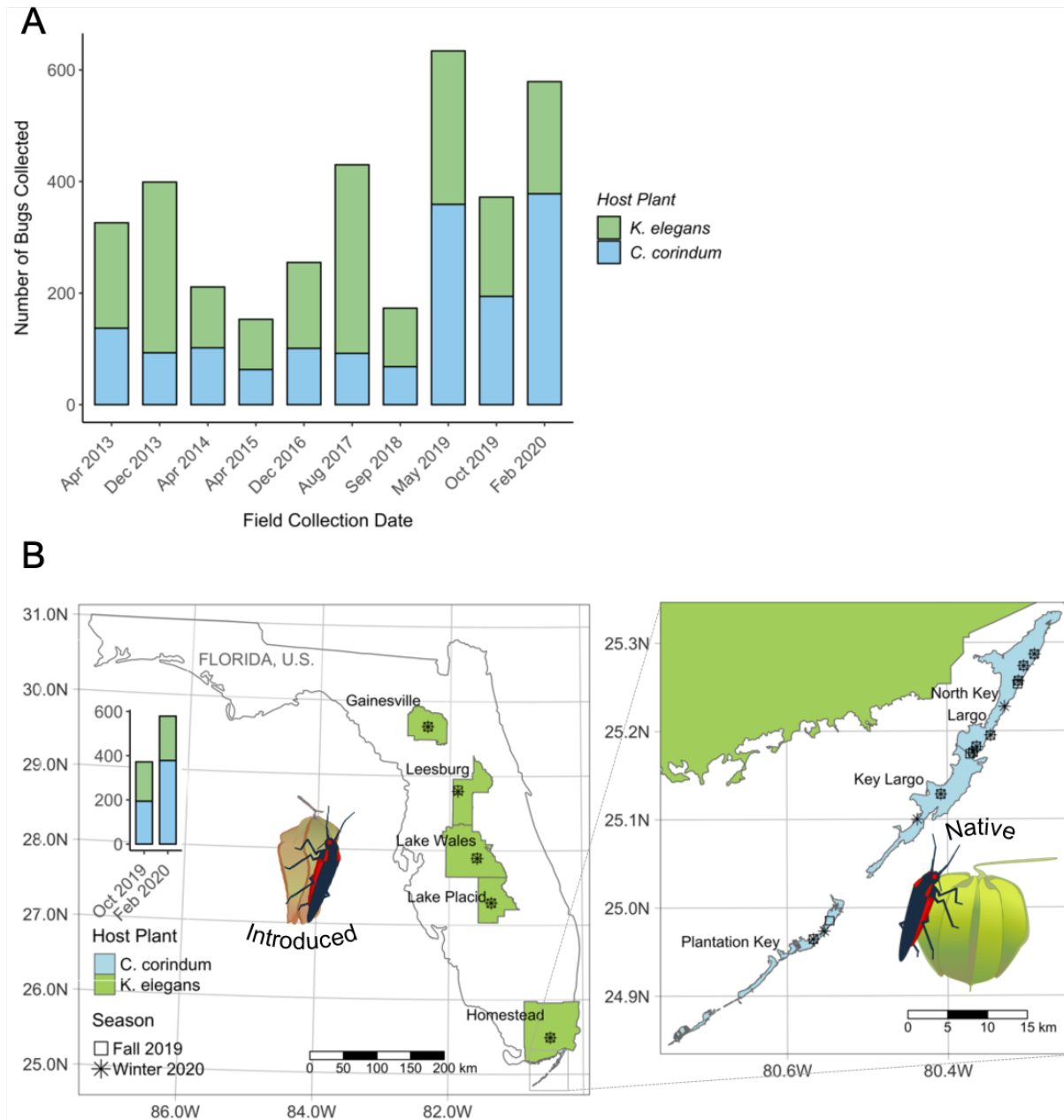
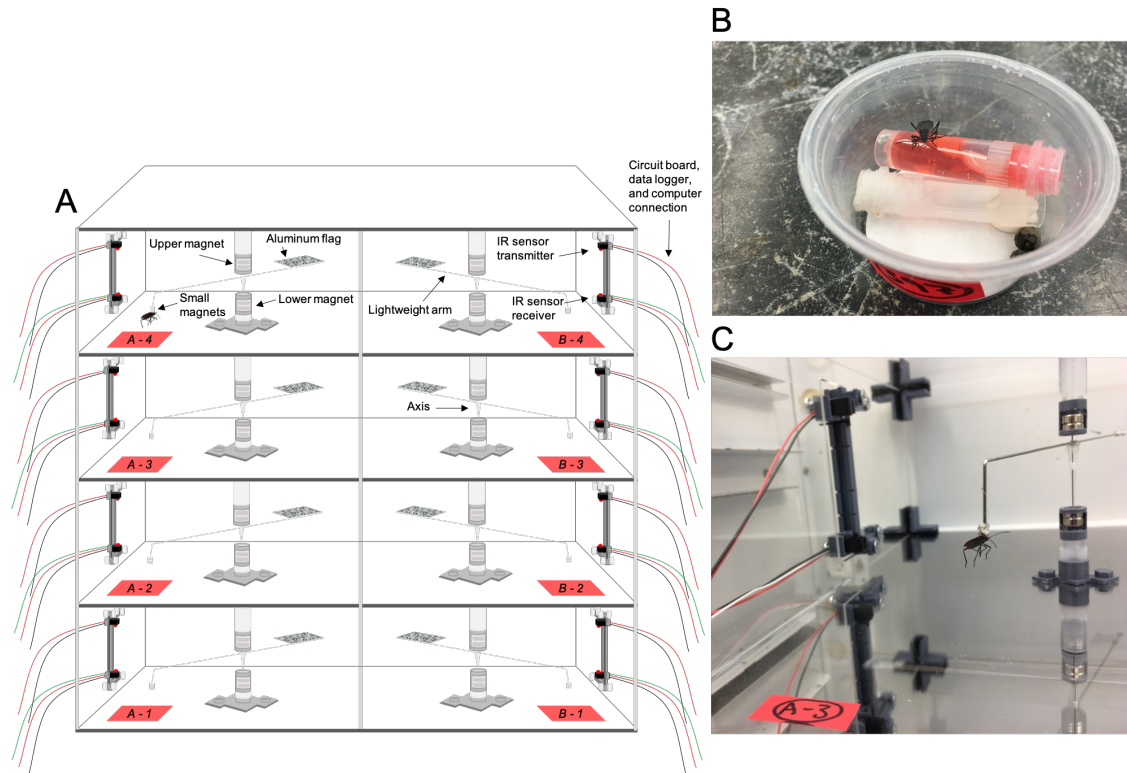


Figure 1: **Soapberry bug collection by host plant and season across Florida U.S. from 2013 to 2020. A.** Stacked barplot of the number of soapberry bugs collected by season and host plant from April 2013 to February 2020. Bars shaded in green represent bugs collected from *K. elegans* and bars shaded in blue represent bugs collected from *C. corindum*. **B.** Map of Fall 2019 and Winter 2020 soapberry bug collections tested for flight trials. Collection sites are marked by season where squares represent Fall 2019 collection sites and stars represent Winter 2020 collection sites. Population names are displayed and named after the nearest city to the collection site(s). Counties shaded in green on the mainland represent bug collections from *K. elegans* and counties shaded in blue in the keys represent bug collections from *C. corindum*. The stacked barplot on the left of Florida graphs the number of soapberry bugs collected and tested for Fall 2019 ( $n = 203$ ) and Winter 2020 ( $n = 332$ ) among each host plant. Images of a soapberry bug feeding on the seeds inside the pods of *K. elegans* (left) and *C. corindum* (right) were illustrated by AVB.





**Figure 2: Flight mill machine and assembled bug home.** **A.** Diagram of the flight mill, **B.** photograph of assembled and prepped bug home, and **C.** photograph of a stationary mounted soapberry bug in chamber A-3. Illustration made by and photos captured by AVB.

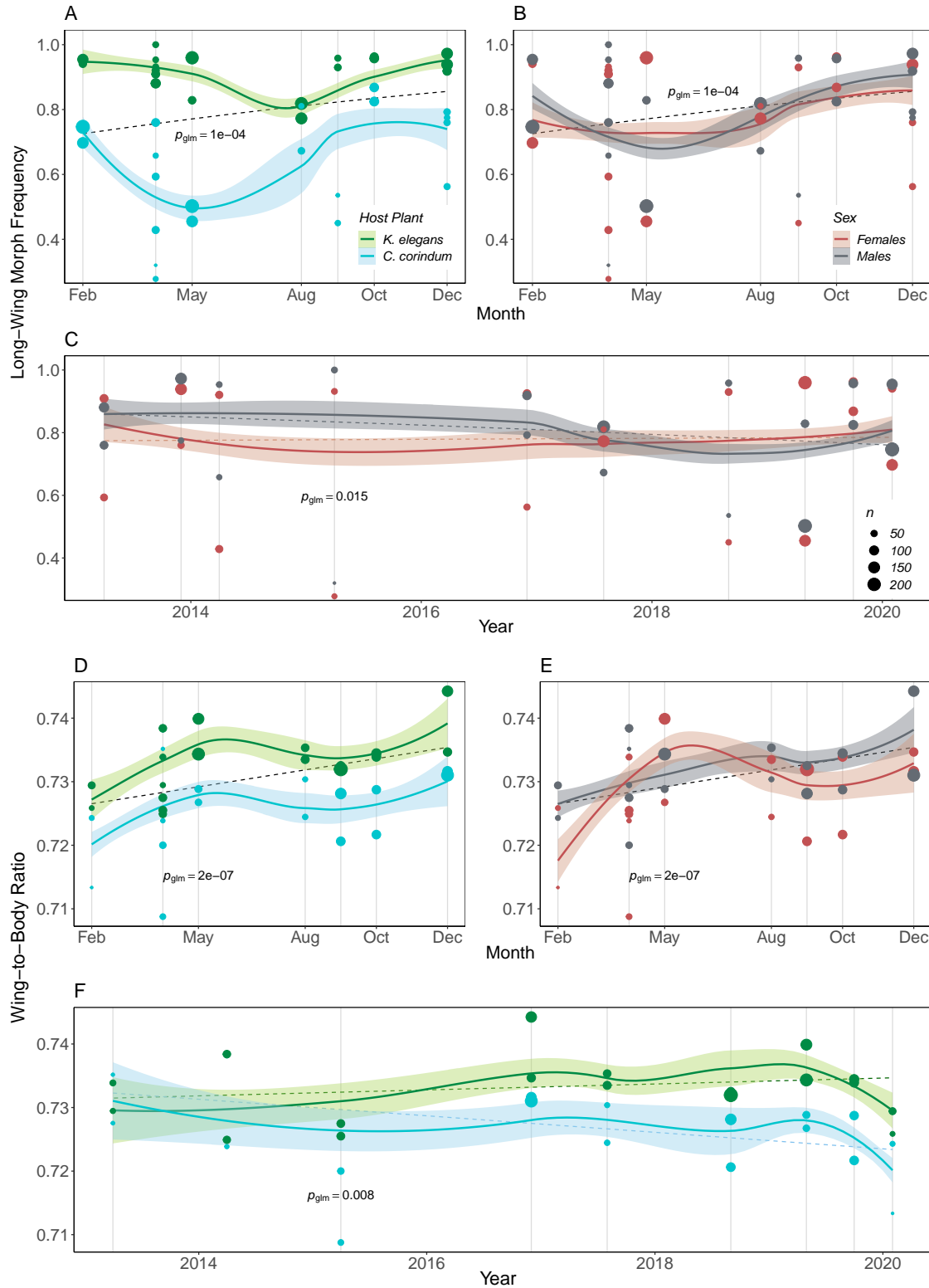


Figure 3: Evaluation of long-wing morph frequency and wing-to-body ratio of soapberry bugs averaged across month and year from April 2013 to February 2020. For each point, the mean long-wing morph

frequency or wing-to-body ratio of each month and year is plotted with LOESS smooth lines (solid lines) and 95% confidence intervals (shading) and linear regression line(s) (dashed line(s)). LOESS plots are only for visual purposes. For LOESS regressions, the smoothing parameter ( $\alpha$ ) was 0.4 and the degree ( $\lambda$ ) was 0. For linear regressions, each P-value was extracted from their corresponding best fit model [**A-C** formula=wing morph  $\sim$  sex \* host + sex \* year + host \* month + month \* year or **D-F** formula=wing2body  $\sim$  sex \* host + host \* year + month]. **A, D, F**. Each point, line, or shading colored green corresponds to soapberry bugs collected from *K. elegans*, while those colored blue correspond to soapberry bugs collected from *C. corindum*. **B, C, E**. Each point, line, or shading colored black corresponds to male soapberry bugs while those colored red correspond to female soapberry bugs.

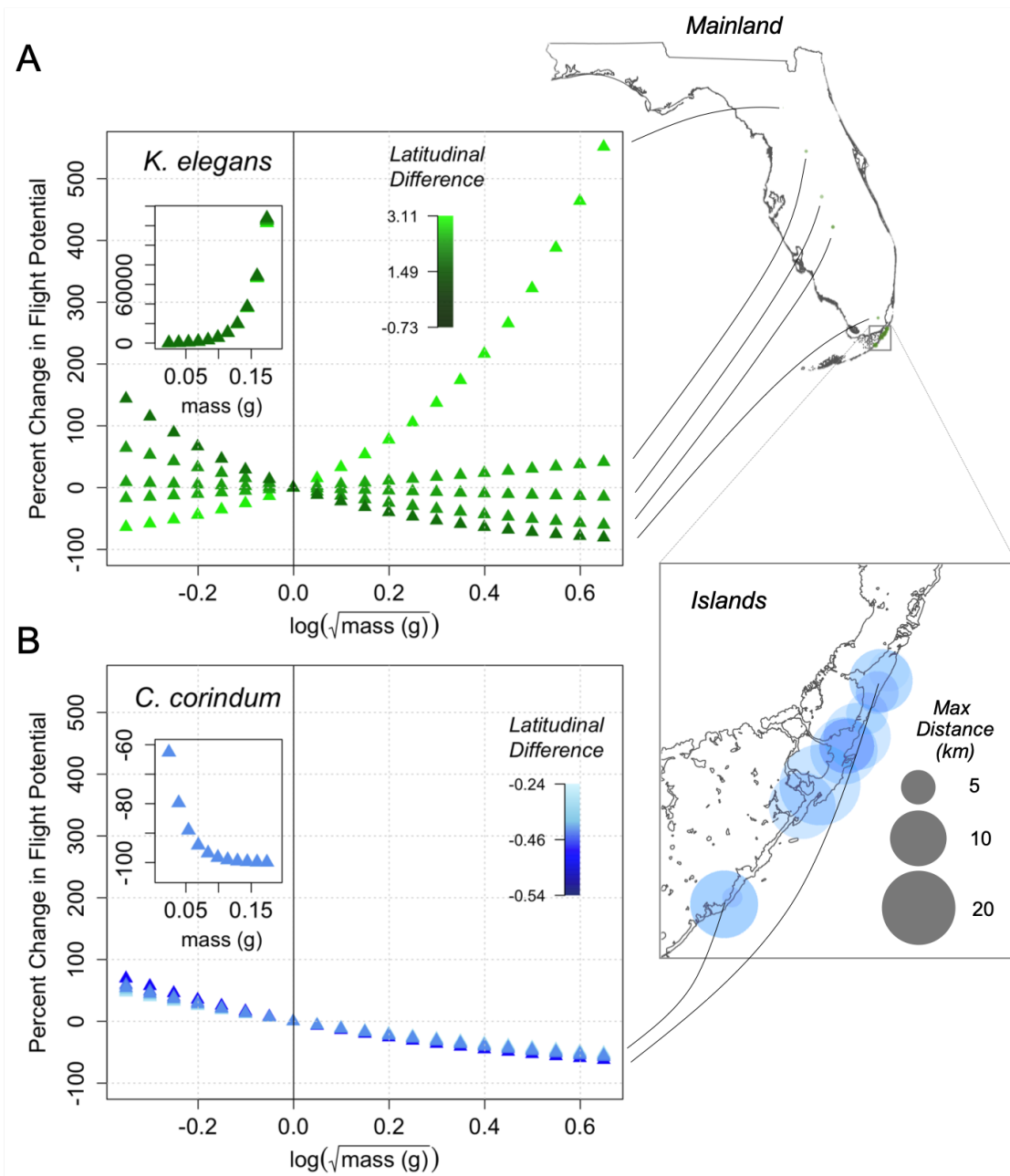
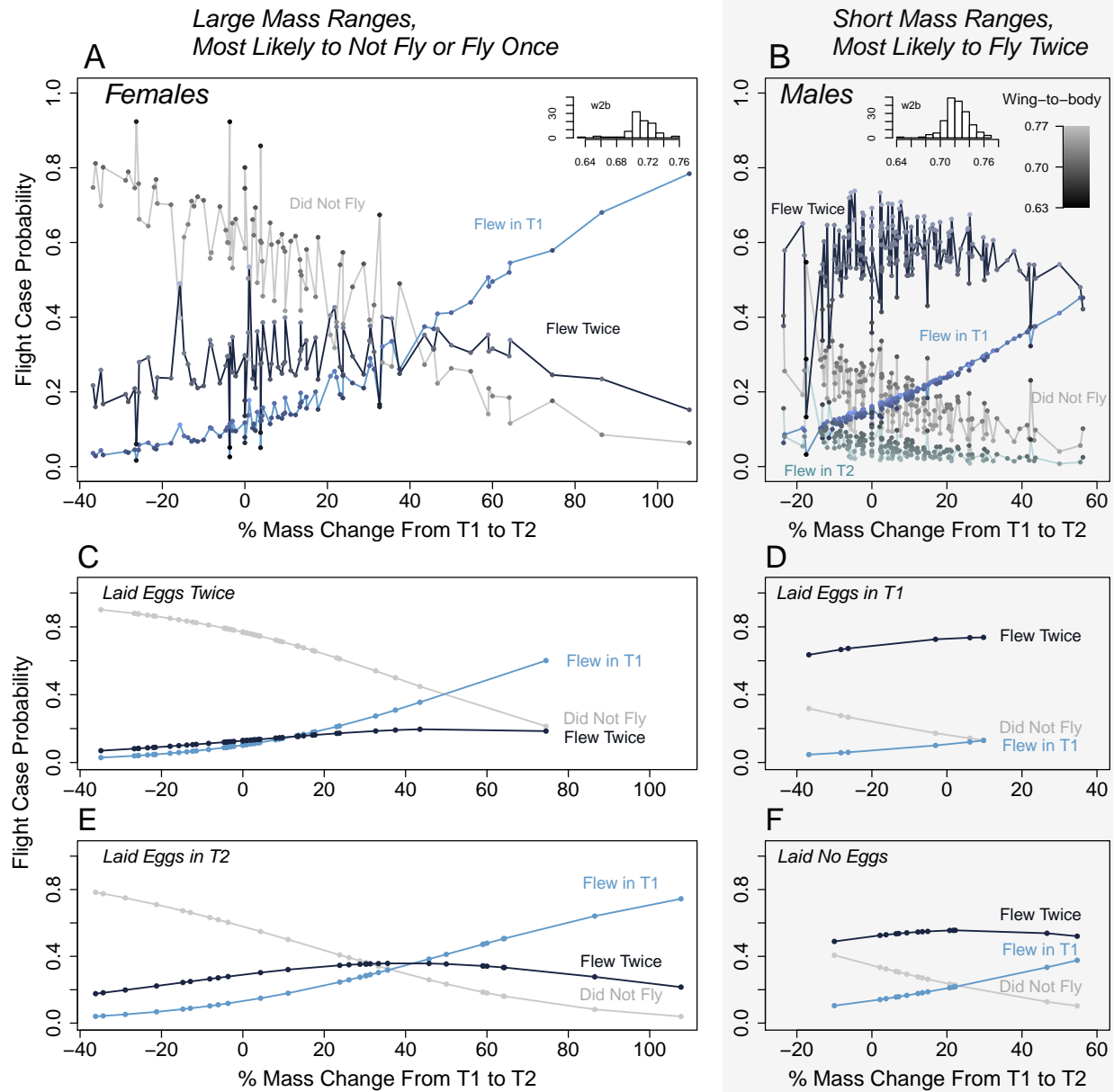


Figure 4: Estimated percent changes of flight potential as a function of soapberry bug mass averaged across trials. Color gradients in the plots represent latitudinal differences from the sympatric zone (Homestead, FL; latitudinal distance = -0.73), where lighter shades indicate a location far from Homestead and darker shades indicate a location close to Homestead. Latitudinal differences are standardized. Percent mass changes in flight response and the average mass for tested soapberry bugs ( $n = 333$ ) were fitted using multiple regression modeling. From the best fit model, selected interaction effects were extracted [ formula=flight potential  $\sim$  latitudinal difference \* mass + host \* mass ].

Both the transformed and back transformed (top-left, smaller graph) coefficients are plotted. The maps of Florida on the right-hand side display the latitudinal locations of most sites and their corresponding fitted line. The map of the islands of Florida emphasizes the maximum flight distance (to scale) reached by a soapberry bug collected from each island site, which helps visualize the bugs' ability to traverse between the island and mainland. **A** Only soapberry bugs collected from *K. elegans* are plotted in green triangles, showing relatively more variation in flight potential between collection sites as well as more variable relationships between flight potential and mass between collection sites. **B** Only soapberry bugs collected from *C. corindum* are plotted in blue triangles, showing little variation in flight potential between collection sites.





**Figure 5: Estimated probabilities of different flight cases as a function of soapberry bug percent mass change between trial 1 (T1) and trial 2 (T2).** The flight case probabilities were fitted using multinomial logit modeling. Solid lines represent estimated probabilities and each line color represents a flight case. Plot shading highlights similarities between short or large mass ranges across sexes or reproductive status. The flight case probabilities of **A.** females ( $n = 93$ ) and **B.** males ( $n = 185$ ) are individually plotted with the histogram of wing-to-body ratio (w2b) in the top-right corner of each plot. Each sex shares the same best fit multinomial logit model [formula=flight case  $\sim$  sex + wing-to-body ratio + mass percent change between trials]. The stochasticity in the plots represent the affect wing-to-body ratio has on flight case probability where lighter shaded circles represent larger wing-to-body ratios and darker shaded circles represent smaller wing-to-body ratios. **C-F.** Estimated probabilities of different flight cases plotted for only female soapberry bugs ( $n = 93$ ) and their corresponding best fit model [formula=flight case  $\sim$  egg case + mass percent change between trials]. Each plot represents a different egg laying case for a female soapberry bug where **C.** ( $n = 45$ ), **D.** ( $n = 6$ ), **E.** ( $n = 28$ ), and **F.** ( $n = 14$ ).