

1 Running head: Phenotypic differentiation in Japanese Knotweed

2 **Rapid phenotypic differentiation and local adaptation in**
3 **Japanese knotweed s.l. (*Reynoutria japonica* and *R.* ×**
4 ***bohemica*, Polygonaceae) invading novel habitats**

5 Wei Yuan¹, Massimo Pigliucci² & Christina L. Richards^{3,4,5}

- 6 1. Max Planck Institute for Developmental Biology, Max-Planck-Ring 5 72076 Tübingen,
7 Germany
8 2. City College of New York, 160 Convent Avenue, NY, NY 10468 USA
9 3. Department of Integrative Biology, University of South Florida, 4202 E. Folwer Avenue,
10 Tampa, FL, 33620 USA E-mail: clr@usf.edu, Tel: +1-813-974-5188, Fax: 813-974-3263
11 4. Plant Evolutionary Ecology group, University of Tübingen, D-72076 Tübingen, Germany
12 5. Author for correspondence
13

14 Manuscript received ; revision accepted _____.

15 Running head: Phenotypic differentiation in invasive Japanese knotweed

16 **ABSTRACT (limited to 250 words)**

17 PREMISE: Many plant invaders like the Japanese knotweeds are thought to colonize new
18 habitats with low genetic diversity. Such species provide an opportunity to study rapid adaptation
19 to complex environmental conditions.

20 METHODS: Using replicate reciprocal transplants of clones across three habitats, we described
21 patterns of phenotypic response and assessed degree of local adaptation.

22 KEY RESULTS: We found plants from beach habitats had decreased height, number of leaves,
23 leaf area, and biomass allocation to roots and shoots compared to plants from marsh and roadside
24 habitats when grown in their home habitat. In the marsh habitat, marsh plants were generally
25 larger than beach plants, but not different from roadside plants. There were no differences among
26 plants from different habitats grown in the roadside habitat. Despite this evidence of
27 differentiation in beach and marsh habitats, we found mixed evidence for local adaptation. In
28 their “home site” plants from the marsh habitat had greater biomass than plants from the beaches
29 but not compared to plants from roadsides. Biomass comparisons in other habitats were either
30 maladaptive or not significant. However, plants from the roadside had greater survival in their
31 “home site” compared to foreign plants. There were no differences in survival in the other
32 habitats.

33 CONCLUSIONS: We found phenotypic differentiation associated with habitats despite the low
34 reported genetic diversity for these populations. Our results partially support the hypothesis of
35 local adaptation in marsh and roadside habitats. Identifying whether these patterns of
36 differentiation result from genetic or heritable non-genetic mechanisms will require further work.

37

38 Key words: adaptive evolution; plant invasion; Japanese knotweed (*Reynoutria japonica*,
39 *Reynoutria* × *bohemica*, Polygonaceae); hybridization; natural selection; phenotypic
40 differentiation

41 INTRODUCTION

42 The expansion of invasive species challenges our understanding of the process of
43 adaptation given the likelihood of reduced genetic variation following a population bottleneck
44 (Bock et al., 2015; Colautti and Lau, 2015). The classic population genetic assumption is that
45 dramatically reduced genetic variation will severely constrain the evolutionary potential of a
46 given population or species (Sakai et al., 2001; Allendorf and Lundquist, 2003). The chances of a
47 few individuals or only one genotype landing in a novel location and surviving are expected to
48 be minimal (Dlugosch and Parker, 2007; Bossdorf et al., 2008). Some invasive species benefit
49 from alternative sources of increased genetic variation through multiple introductions (Durka et
50 al., 2005; Lavergne and Molofsky, 2007; Rosenthal et al., 2008; Gammon and Kesseli, 2010;
51 Qiao et al., 2019; Liu et al., 2020) or hybridization (Daehler and Strong, 1997; Bímová et al.,
52 2001; Pysek et al., 2003; Mandák et al., 2004; Salmon et al., 2005; Bailey et al., 2009; Parepa et
53 al., 2014; Qiao et al., 2019). However, many invasive species appear to do well even with low
54 levels of sequence based variation (Hollingsworth and Bailey, 2000; Geng et al., 2007; Dlugosch
55 and Parker, 2008; Loomis and Fishman, 2009). This could be perhaps through chance
56 establishment of “general purpose genotypes” (Baker, 1965; Richards et al., 2006; Oplaat and
57 Verhoeven, 2015), high performance genotypes, or rapid evolution of niche breadth (Matesanz
58 and Sultan, 2013; Matesanz et al., 2015). Vankleunen *et al.* (van Kleunen et al., 2018) argue that
59 adaptive evolutionary processes are at least as common in invasive species as native species,
60 which is surprising given the short amount of time they have to evolve. In fact a meta-analysis of
61 134 plant species in 52 plant families showed that invasive plant species demonstrated patterns
62 of local adaptation just as frequently, and at least as strongly as native plant species (Oduor et al.,
63 2016).

64 Considering the limited genetic diversity of many invasive populations, phenotypic
65 plasticity has often been suggested as a potentially important mechanism in the invasion process
66 (Baker, 1965; Sexton et al., 2002; Sultan, 2004; Bossdorf et al., 2005). Several studies have
67 found support for the importance of plasticity (Cheplick, 2006; Pan et al., 2006; Geng et al.,
68 2007; Muth and Pigliucci, 2007; Bossdorf et al., 2008; Funk, 2008; Richards et al., 2008; Loomis
69 and Fishman, 2009; Walls, 2010; Matesanz and Sultan, 2013; Sultan et al., 2013; VanWallendael
70 et al., 2018), but see (Davidson et al., 2011). Further, epigenetic mechanisms may contribute to
71 the persistence of plastic responses across generations (Herman and Sultan, 2016; Shi et al.,
72 2018; Puy, Carmona, et al., 2021; Puy, de Bello, et al., 2021). Epigenetic effects have been
73 shown to be especially important in response to hybridization and exposure to stressful or novel
74 environments, which are circumstances often experienced by invasive plants (Mounger et al.,
75 2021). The chance sampling of genotypes involved in the invasion process, combined with non-
76 genetic sources of phenotypic variation, can lead to divergence in phenotypes of these
77 populations even in the absence of abundant genetic variation (Keller and Taylor, 2008; Prentis
78 et al., 2008; Neinavaie et al., 2021). Considering the plurality of potential mechanisms of rapid
79 evolution in novel conditions, studies that examine phenotypic response of clonal replicates in
80 natural settings will enhance our understanding of the processes of adaptation.

81 Invasive populations of the *Reynoutria* species complex (referred to as Japanese
82 knotweed *sensu lato* or *s.l.*) are known to occupy a wide range of habitats in Europe (Pyšek et al.,
83 2001; Mandák et al., 2004; Bailey et al., 2009; Zhang et al., 2016), and the United States
84 (Barney, 2006; Grimsby et al., 2007; Richards et al., 2008, 2012; Walls, 2010; Gaskin et al.,
85 2014). On Long Island, NY, in addition to roadside habitats and along railways, they are often
86 growing on beaches and next to or on the terrestrial border of marshes that are inundated with

87 *Phragmites australis* (Richards et al., 2008, 2012; Walls, 2010). These populations provide a
88 unique opportunity to explore rapid adaptation to very different habitat types. The physiological
89 challenges of living in beach and salt marsh habitats might require a dramatic phenotypic shift.
90 For example, plants that are able to tolerate highly saline environments are characterized by traits
91 that specifically ameliorate the toxic and osmotic effects of substrate salinity to allow for growth
92 under these conditions (Flowers et al., 1977; Donovan et al., 1996; Rosenthal et al., 2002; Lexer
93 et al., 2003; Karrenberg et al., 2006; Richards et al., 2010). We used cytology and AFLP markers
94 to show that these populations have extremely low genetic diversity: some are made up of a
95 single *R. japonica* genotype reported across the US and Europe (Hollingsworth and Bailey,
96 2000; Mandák et al., 2005; Grimsby and Kesseli, 2010; Krebs et al., 2010; Gaskin et al., 2014;
97 Groeneveld et al., 2014; Zhang et al., 2016), while the majority consist of a few *R. × bohemica*
98 hybrids (Richards et al., 2008, 2012; Walls, 2010). In the greenhouse, plants from both roadside
99 and marsh habitats were highly plastic in response to treatment with salt, but within and among
100 sites there were significant differences in most traits and trait plasticities (Richards et al., 2008).

101 While controlled experiments allow us to examine response to specific environmental
102 factors, reciprocal transplantation in natural field settings is the most robust approach to identify
103 local adaptation to complex real environments (Kawecki and Ebert, 2004). Plants are considered
104 to be locally adapted if they perform better in their “local site” or “home site” than do plants
105 from other “foreign” sites. A robust quantitative genetics design in a transplant study also allows
106 for investigating which traits are under selection (Lande and Arnold, 1983; Dudley, 1996a; b;
107 Mauricio and Rausher, 1997; Donovan et al., 2007), and characterizing the patterns of
108 divergence of traits across different habitats (Roff and Mousseau, 2005; Doroszuk et al., 2008;
109 Franks and Weis, 2008).

110 In this study, we used reciprocal transplants of Japanese knotweed to investigate 1) how
111 traits vary in response to the different habitat types, and 2) if plants demonstrate patterns of
112 adaptation to the different habitat types. If invasive Japanese knotweed plants have heritable
113 variation for traits that increase fitness in these habitats, selection should act on these traits and
114 lead to locally adapted populations. If these populations are adapted, we expect that they will
115 outperform plants from other habitats, through greater biomass or greater survival, when grown
116 in their local habitat (Kawecki and Ebert, 2004). To test this hypothesis, we established
117 reciprocal transplants among beach, marsh and roadside habitats, planting replicates of each
118 individual back into its home site and into the other two habitat types. We then asked two
119 questions: Are there persistent differences in ecologically important traits in plants from different
120 habitats? Do these populations display evidence of local adaptation?

121 **MATERIALS AND METHODS**

122 *Reynoutria species complex* –

123 Historically, the taxonomy of Japanese knotweeds has been complicated (for a review see
124 Bailey and Conolly, 2000; Bailey and Wisskirchen, 2004; Schuster, Reveal, et al., 2011;
125 Schuster, Wilson, et al., 2011; specifically for the introduction to New York State see Townsend,
126 1997; Del Tredici, 2017). Briefly, the two species *Reynoutria japonica* and *R. sachalinensis* were
127 originally from Japan and were introduced to Europe in the mid 1800's and the United States by
128 the end of the 19th century (Del Tredici, 2017). Although *R. sachalinensis* (2n=44, 66, 88 and
129 132; Tiébré et al., 2007; Park et al., 2018) is distinct morphologically from *R. japonica* (2n=44 or
130 88), the two species are not differentiated in chloroplast DNA (cpDNA, Inamura et al., 2000).
131 Hybridization between them appears to be rare in Japan, where they are not usually sympatric
132 (but see (Park et al., 2018) for evidence of introgression). However, the hybrid *R. × bohemica* is

133 common in the invasive range in Europe (Pysek et al., 2003; Bailey and Wisskirchen, 2004;
134 Mandák et al., 2004; Bailey, 2013; Groeneveld et al., 2014; Parepa et al., 2014) and the U.S.
135 (Forman and Kesseli, 2003; Gammon et al., 2007; Richards et al., 2008, 2012). In the U.S.,
136 studies in New England suggested that spread of all three taxa takes place through both
137 vegetative and sexual reproduction (Forman and Kesseli, 2003; Gammon et al., 2007; Grimsby et
138 al., 2007; Gammon and Kesseli, 2010). However, other studies in the U.S. on *R. japonica* report
139 only the same single female genotype that has also been found throughout Europe (Richards et
140 al., 2012; Gaskin et al., 2014; Groeneveld et al., 2014; but see VanWallerdael et al., 2021).

141 ***Collection sites and experimental gardens*** –

142 In mid-May 2005, we collected Japanese knotweed *s.l.* rhizomes for reciprocal transplant
143 studies between beach, marsh and roadside sites across Suffolk County, Long Island, New York
144 (Table 1; four sites of each habitat type for 12 total sites were used as sources for plant material
145 and as locations of transplants). We created four groups of plants for reciprocal transplant in
146 order to maximize the ability to replicate each genet in one site of each of the habitat types while
147 still testing for superior performance in the original “local” site.

148 Due to the natural topography of Long Island, the beach sites are all located on the
149 northern shore of Long Island, while the salt marsh sites and roadside sites are more evenly
150 distributed around Suffolk County. The beach sites are separated by 1-65 km, the marsh sites are
151 separated by 14–40 km, and the roadside sites are separated by 20–32 km. At each site, we
152 collected approximately one meter of rhizome from seven (Beach 1) or eight genets (all of the
153 other 11 sites) that were approximately 10 m apart, to maximize the chances of sampling
154 different genotypes and to represent plant distribution at each site. We refer to each of these
155 rhizomes as a separate “genet” because replicates cut from the same rhizome should have the

156 same genotype. However, our previous studies show that most “genets” within a site also have
157 the same AFLP haplotype and most likely belong to the same individual. Rhizomes were brought
158 to the Stony Brook University greenhouse and cut into pieces of 4-8 grams fresh-weight (12 sites
159 x 7 or 8 rhizomes per site for a total of 95 genets x 18-25 replicates = 2225 rhizome pieces). We
160 planted the rhizome pieces in individual wells in 24-well flats with Pro-mix potting medium
161 (Pro-mix Bx, Quakertown, Pennsylvania, USA), and approximately one teaspoon of slow release
162 fertilizer (15-9-12 Osmocote Plus 8-9 month, Marysville, Ohio). The flats were placed in a
163 temperature-controlled greenhouse under conditions approximating mid-summer in Suffolk
164 County, Long Island and watered as needed to keep the soil moist. Day temperature was
165 maintained at 30°C and night temperature at 25°C. We grew the plants in the greenhouse for
166 approximately six weeks to allow for shoots to emerge from the rhizomes and grow to a height
167 of approximately 10-15 cm.

168 The 12 sites were organized into four reciprocal transplant groups, each with one beach,
169 one marsh and one roadside location. Based on the number of plants that emerged, we assigned
170 an equal number of replicate pieces of each rhizome collected at each source site to be
171 transplanted into its home site and into one site of each of the other two habitat types. Therefore,
172 three to eight replicates of each rhizome were assigned randomly across five blocks for each of
173 the three transplant habitats (4 replicate studies x 3 transplant habitats x 3 source habitats x 5-8
174 genets x 2-8 replicates = 1287 plants; Table 1). We prepared the transplant gardens by removing
175 only above ground vegetation (typically other knotweed plants) to a height of less than 2 cm with
176 a machete. The cleared area included a border of 15-30 cm outside of the transplant blocks.

177 Between June 16-21, we transplanted the five blocks for each transplant garden into the
178 field (between 80-125 ramets per transplant garden). Plants were left to grow in the field from

179 the summer of 2005 through the fall of 2006. We measured final height and final number of
180 leaves on all plants and harvested above ground and below ground for all plants between 11-18
181 September 2006. Roots were harvested by carefully digging to unearth the entire root system.
182 Roots were shaken to remove loose dirt in the field and thoroughly washed.

183 *Traits measured* –

184 We measured traits related to salt tolerance and overall performance for each plant:
185 height, total number of leaves, total leaf area (Li-Cor Model LI-3100 Leaf area meter: Li-Cor,
186 Inc., Lincoln, Nebraska, USA), succulence (g water in all leaves/ cm² total leaf area), shoot dry
187 biomass, root dry biomass, root:shoot ratio based on dry biomass, and total biomass at final
188 harvest. For each plant, all live leaf tissue at final harvest was used for calculating total leaf area
189 and succulence. Plants were dried in a forced air oven at 60° C for at least 72 h to determine
190 shoot, root and total dry biomass. We evaluated survival and biomass (as proxies for fitness) to
191 assess the degree of adaptation. These taxa have extensive clonal growth and many individuals
192 may not flower at all in the field, but persist and spread from year to year so biomass is an
193 important indicator of fitness (de Kroon and Groenendael, 1997).

194 *Data Analysis* –

195 We performed all statistical analyses in the R statistical programming environment
196 version 4.0.0 (R Core Team, 2020), using the Linear-Mixed-Model (LMM) or Generalized
197 Linear-Mixed-Model (GLMM) framework (lme4 package, Bates et al., 2015) and the Bayesian
198 simulation package arm (Gelman and Su, 2020). We checked the residuals to assess the
199 appropriateness of the model and performed data transformations on traits as appropriate: we did
200 not transform succulence and final height, but we performed log 10-transformation on leaf area,

201 and log 2 transformation on shoot, root and total biomass. For these traits we fitted LMM with
202 the model:
203 $\text{trait} \sim \text{lmer}(\text{trait} \sim \text{SOURCE.type} + \text{GARDEN.type} + \text{SOURCE.type}:\text{GARDEN.type} + (1|\text{Origin.site}) +$
204 $(1|\text{Transplant.site}) + (1|\text{genetfactor}), \text{data} = \text{data}, \text{REML} = \text{F}).$

205 We used GLMM to model the number of leaves, with the negative binomial distribution with the
206 model:

207 $\text{modlfnum} \sim \text{glmer.nb}(\text{lf.number} \sim \text{SOURCE.type} + \text{GARDEN.type} + \text{SOURCE.type}:\text{GARDEN.type}$
208 $+ (1|\text{Origin.site}) + (1|\text{Transplant.site}) + (1|\text{genetfactor}), \text{data} = \text{data}, \text{REML} = \text{F}).$

209 We did not model root to shoot ratio directly, but instead we used the ratio of the estimates of
210 mean and variance for root and shoot to assess significance within the Bayesian framework
211 (Korner-Nievergelt et al., 2015).

212 In the LMM and GLMM models, “SOURCE.type” is the origin habitat type (beach,
213 marsh , road), “GARDEN.type” is the transplant habitat type (beach, marsh, road). These effects
214 as well as their interaction terms were modeled as fixed effects. The origin site, the transplant
215 site, and the individual genets (“genetfactor”) were initially included as random terms. To avoid
216 overfitting, we removed random effect terms that effectively explained no variance. This was
217 true for the genet term for all traits and for the “Origin.site” term, which was removed for
218 number of leaves, succulence and shoot biomass (see Table S1 for final models).

219 We examined the correlation matrix for each model to evaluate auto correlation between
220 terms. To test the significance of the fixed effects of “SOURCE.type” and “GARDEN.type”, we
221 used 95% credible intervals (CrI), a Bayesian analogue of confidence intervals (Bolker et al.,
222 2009). For each response variable, we obtained the model estimates from the back-transformed
223 effect sizes. We calculated the associated 95% Credible intervals (CrI) for the modeled effects by

224 performing 10,000 iterations of Bayesian simulation of the mean and variance of each estimate,
225 using the sim function in the R package arm with non-informative priors (Korner-Nievergelt et
226 al., 2015). For each model, we examined distributions of simulated fitted values predicted by the
227 fixed effect terms. We used the values corresponding to the 2.5 and 97.5 quantiles of the
228 distribution to designate the lower- and upper-boundary of the 95% CrI. If the CrI of a group did
229 not overlap with the mean of the other group within transplant gardens, we considered the
230 difference between these groups to be significant (sensu Bucharova et al., 2016, 2017).

231 In order to understand how much of the variance was explained by the random and fixed
232 effects in our model, we used several approaches. First, we used the package r2_nakagawa:
233 Nakagawa's R² for mixed models (Nakagawa and Schielzeth, 2013; Nakagawa et al., 2017) to
234 determine the conditional R² (the variance explained by both the fixed and random effects) and
235 marginal R² (the variance explained by the combined fixed effects). The random effect variances
236 calculated in this package are the mean random effect variances, and appropriate for mixed
237 models with nested random effects (Johnson, 2014). We also used the package rptR (Stoffel et
238 al., 2017) to further evaluate the components of variance for each of the random effects
239 separately (i.e., “Origin site” and “Transplant site”). We used the package
240 commonalityCoefficients (Nimon et al., 2008) to examine the amount of variance explained by
241 the separate fixed effects of the habitats of the source and transplant gardens (i.e.,
242 “SOURCE.type” and “GARDEN.type”). This approach does not include information about the
243 random effects of origin site and transplant site which are nested within the source type and
244 garden type. However, this approach is valuable for evaluating the relative contribution of each
245 separate fixed effect.

246 Our design is constrained by the fact that origin site and transplant sites are nested within
247 levels of “Transplant group” (see discussion here Long, 2021). To examine the importance of
248 this design constraint, we also reran the LMER and GLMER models for each trait with the fixed
249 term “Transplant group”. To properly test for the effects in this nesting design, we should ideally
250 fit random intercepts for the sites nested within groups, but we did not have enough replication
251 within groups to do so. We assume that fitting the fixed effect of the “Transplant group” also
252 controls for the non-independence of the origin site and transplant site within groups (Long,
253 2021). By comparing the modeling with and without the fixed term of Transplant group, we
254 evaluated how these random terms impact the main effects of interest which are the fixed effects
255 of the habitats of the source and transplant gardens (i.e., “SOURCE.type” and “GARDEN.type”).

256 We tested for local adaptation with two fitness proxies: total biomass and survival. We
257 ran a “local vs. foreign” test using the Bayesian fitted values for biomass obtained from the same
258 LMM model:

```
259 totalbiomass<-lmer(trait~SOURCE.type+GARDEN.type+SOURCE.type:GARDEN.type+  
260 (1|Origin.site)+(1|Transplant.site)+(1|genetfactor),data=data,REML=F).
```

261 For each garden type we performed random pairwise contrasts as the differences between
262 Bayesian fitted values of the local plants with those from foreign habitats. For survival, we
263 performed random pairwise contrast by calculating the log of odds ratio between local and
264 foreign plants. We reported the mean, 95% CrI, and percentage of contrasts showing superior
265 performance of plants grown in their home site compared to plants from each of the other
266 habitats as magnitude of local adaptation.

267 **RESULTS**

268 *Phenotypic response to reciprocal transplants –*

269 We found that analyses of all traits resulted in large credible intervals (CrI) around the
270 estimates of the means within source-by-garden combinations (Figure 1). This could be due to
271 the large variance among transplant sites (Table 2, Table S2). Despite this large variance, we
272 found differences in responses depended on the source habitat and the garden habitat. For every
273 trait, at least one comparison met our significance criteria based on nonoverlapping of CrI of a
274 group with the mean of another group.

275 In the beach gardens, plants originally from this habitat had only one-third the height, less
276 than half the number of leaves, 30% less leaf area, one-fourth the shoot biomass and half as
277 much root biomass as plants from the roadside habitats (Figure 1; Table S3). Plants from the
278 beach habitat also had half as many leaves and nearly half the root biomass of plants from the
279 marsh habitat when grown in the beach gardens. In the marsh gardens, plants from marsh
280 habitats had two and half times the height and twice the number of leaves as plants from beach
281 habitats. In addition, plants from beach habitats had greater succulence than plants from the
282 roadside habitats (but not greater than plants from marsh habitats) when grown in the marsh
283 gardens. In roadside garden, we found no differences among the groups for any of these traits.
284 We also discovered that the responses of plants from marsh and roadside habitats were largely
285 indistinguishable in any garden (Figures 1 and 2).

286 These findings were supported by examining simulated values of the differences between
287 the groups of plants for each trait (Figure 2). In the beach habitat, beach plants were almost
288 always shorter, had fewer leaves, had less leaf area, less root and shoot biomass than plants from
289 marsh or roadside habitats (beach < marsh plants in 99 or 100% of the simulations). In the marsh

290 garden, marsh plants were taller, had more leaves, greater leaf area, shoot and root biomass than
291 beach plants in 100% of the simulations. Compared to roadside plants transplanted in the marsh
292 garden, marsh plants were also usually taller (97% of the simulations), had more leaves (90%),
293 greater leaf area (79%), shoot (91%) and root (87%) biomass but the differences in response
294 were not as strong as comparisons to beach plants and did not meet our threshold for significance
295 (Figure 1). In the roadside garden, again simulations did not support significant differences in
296 pairwise comparisons of plants from different habitats.

297 *Explanatory power of the models of phenotypic variance –*

298 Our linear mixed models explained 18 to 45% of the variation in the traits we measured
299 (Table 2). However, we found that the majority of the variance was explained by the random
300 effects (r^2_{nakagawa} in Table 2) and in particular the “Transplant.site” which alone explained
301 14-43% of the variance in these traits. Only 2 to 8% of the variance was explained by the fixed
302 effects of “Source type” or “Garden type” (r^2_{prt} in Table 2) which were our main interest to test
303 the general effects of habitats. Together the fixed effects were best able to explain variance in
304 height and number of leaves (8%) and least predictive of succulence (2%). We used the
305 commonalityCoefficients program to further examine the amount explained by each of the fixed
306 effects. The source type explained twice as much of the variance as transplant garden type for
307 height, succulence and total biomass, and three times the variance in shoot biomass, but less of
308 the variance than transplant garden for the number of leaves. Source type explained a similar
309 amount of the variance as transplant garden for leaf area.

310 When we evaluated the “Transplant group” as a fixed effect, the overall R^2 changed very
311 little (Table S2). On average the models changed by only 0.2%. The largest change in R^2 was in
312 the model for succulence which decreased from 29% in the original model without the effect of

313 transplant group (Table 2) to 26% with the effect (Table S2). On average the Transplant group
314 effect increased the amount of variance explained by fixed effects by 17%. Using
315 commonalityCoefficients, we found that the unique contribution of transplant group was 29-65%
316 of the variance explained by the combined fixed effects. For several traits (e.g., height,
317 succulence, shoot root and total biomass) most of the variance explained by fixed effects was due
318 uniquely to the transplant group effect (Table S2). Adding this effect also changed the amount of
319 variance explained by “Source type” or “Garden type”. When the transplant group was included,
320 the unique contribution of source type still explained twice as much of the variance as that of
321 transplant garden type for shoot biomass, but a similar amount of variance as garden type for
322 height, leaf area, succulence and total biomass. Using this model, source type explained half as
323 much of the variance as transplant garden for the number of leaves.

324 *The effect of transplant habitats on fitness proxies –*

325 We investigated the fitness proxies of total biomass (g) and survival. In the beach
326 gardens, plants from beach habitats accumulated less biomass than plants from either marsh or
327 roadside habitats in 100% of the simulations, contrary to predictions of local adaptation. On the
328 other hand, in the marsh gardens, plants from the marsh habitat accumulated more biomass than
329 plants from beaches (100% of the simulations) and tended to grow bigger than plants from
330 roadsides (in 90% of the simulations but the effect size was smaller; Figure 3a). We found little
331 support for differences in biomass among groups when grown in the roadside gardens.

332 Our model explained approximately 38% of the variance in total biomass. This variance
333 was largely determined by the random term “transplant garden site”: 43% of the variance was
334 attributed to “transplant site” when “transplant group” was not included (Table 2), 36% when
335 “transplant group” was included as a fixed effect (Table S2). The fixed effect of origin habitat

336 type explained almost twice as much as that of transplant garden habitat type, but combined they
337 explained only 6% of the variance in biomass (Table 2). When transplant group is included as a
338 fixed effect, the R^2 jumps to 24% explained by combined fixed effects (according to results of
339 r^2_{nakagawa} , Table S2) and the effect of origin habitat type still explains more than that of
340 transplant garden habitat type (19% compared to 13% of the variance due to fixed effects which
341 translates to approximately 4% and 3% of the overall variance in this model).

342 Mortality was high across the experiment, but particularly in the beach habitat garden
343 sites (average 89% mortality; Table 3), where one site was completely washed away in a storm
344 (PJB) and two other sites suffered 95-97% mortality. Plants from marsh and roadside habitats
345 showed an average of 5.2 – to 14.2 – fold decrease of survival odds when transplanted into beach
346 habitats (probability 0.98-1; Figure 4.). Plants from the marsh habitats suffered less in survival
347 odds than roadside plants (Log Odds Ratio 2.24 vs 3.8). In contrast, the beach plants had an
348 average 7-fold increase in survival odds when transplanted to either of the other habitats (Figure
349 4). The survival odds in reciprocal transplants between road and marsh habitats were similar. In
350 sum, the survival data further suggested that the beach habitat was the most challenging
351 environment out of the three tested, leading to reduced odds of survival for plants from all three
352 habitats. Meanwhile, marsh and roadside habitats were similar.

353 We found no support for local adaptation in plants from beach habitats or marsh habitats
354 when comparing survival in their home habitat to foreign plants in that garden (Figure 3b).
355 However, plants from roadside habitats showed better survival when compared to foreign plants
356 from the beach or the marsh (Figure 3b).

357 **DISCUSSION**

358 In this study, we investigated how one of the world’s most invasive plants may be
359 adapting to three different habitats on Long Island, NY. Many studies have demonstrated that
360 significant differences in habitat characteristics can result in adaptive differentiation within
361 species, even under high levels of gene flow between habitats (Antonovics and Bradshaw, 1970;
362 Linhart and Grant, 1996; Sambatti and Rice, 2006; Papadopulos et al., 2021; Zerebecki et al.,
363 2021 but see Leimu and Fischer, 2008). Introduced species in particular have been highlighted
364 because they can evolve rapidly in response to novel conditions (Lee, 2002; Leger and Rice,
365 2007; Dlugosch and Parker, 2008). We took advantage of replicate populations to test the
366 generality of adaptation to different habitats. Despite the wide variation among sites within
367 habitat types, we revealed differentiation between plants from beach, marsh and roadside
368 populations for most of these purportedly adaptive traits, as well as for fitness. In addition, we
369 found some support for local adaptation.

370

371 *Phenotypic plasticity in Japanese knotweed –*

372 Overall, the variation in phenotypes in our study was most often best explained by the
373 local conditions of the transplant garden site. These findings indicate the importance of
374 plasticity, which has often been highlighted in invasion ecology (Bossdorf et al., 2005, 2008;
375 Freeman and Byers, 2006; Richards et al., 2006; Geng et al., 2007; Muth and Pigliucci, 2007;
376 Funk, 2008; Loomis and Fishman, 2009; Walls, 2010). In our previous greenhouse study, plants
377 from both roadside and marsh habitats were also highly plastic in response to treatment with salt,
378 and within sites there were significant differences in most traits and trait plasticity.

379 ***Habitat differences*** –

380 In order for adaptive differentiation to occur, habitats must select for different trait
381 means, plasticities or relationships among traits. The beach habitat was typically open with
382 plenty of sun. In contrast, the marsh habitats were typically under a canopy of tall (2.5-3 m), well
383 established knotweed or *Phragmites* plants, and the roadside sites were typically under a canopy
384 of trees. Our pairwise comparisons of plants from different habitats across transplant gardens
385 indicated that plants were significantly different for every trait except R:S. Most of the
386 differences were manifest in the beach common gardens where beach plants were significantly
387 shorter, with fewer leaves, and less biomass than plants from marsh and roadside sites. This
388 finding of more differences in the beach habitat is similar to our previous study where we found
389 differences in succulence and root to shoot biomass ratio only under salt addition, but not under
390 pure water conditions (Richards et al., 2008; see similar results in *Borrchia frutescens* Richards
391 et al., 2010). Similarly, a recent reciprocal transplant study in the salt marsh cordgrass *Spartina*
392 *alterniflora* identified more extreme differences in the “Tall *Spartina* zone” habitat for survival,
393 maximum height, root to shoot biomass ratio and total biomass (Zerebecki et al., 2021). In these
394 examples, the conditions that were more challenging for the plants also elicited more differences
395 between plants.

396 We did find a few differences elicited in the marsh transplant gardens where marsh plants
397 tended to be larger than the others. We expected that succulence could be important for invasion
398 of the saline marsh habitat because the ability to become succulent, and dilute the toxic effect of
399 concentrated salt ions, is essential for many species in salt environments (Flowers et al., 1977).
400 For example, *Salsola kali* originating from different habitats was found to have dramatic
401 intraspecific variation in succulence and the salt tolerant subspecies *S. kali traga* was able to

402 increase succulence more than the non-salt tolerant *S. kali ruthenica* (Reimann and Breckle,
403 1995). However, we did not find consistent response in succulence in our previous work with
404 these knotweed taxa. Instead, we found a lot of variation among knotweed genets for succulence
405 in response to salt treatments, including several genets that seemed to display no change in
406 succulence (Richards et al., 2008). In this study, the only difference in succulence we found was
407 that the beach plants were more succulent than the roadside plants in the marsh transplant
408 garden. Succulence could aid in the adaptation to saline habitats in *Reynoutria*, but could be
409 specific to certain genets or conditions that we did not explore with our current design. The
410 plants with by far the highest amount of succulence in this study were those from the Marsh 3
411 site grown in their home marsh environment and plants from the Marsh 1 site and Beach 1 site
412 grown at the beach. This increased level of succulence may have been an important trait
413 contributing to the slight advantage in biomass exhibited by Marsh 3 plants at the marsh and the
414 increased survival of Marsh 1 and Beach 1 plants at the beach where plants from the roadside did
415 not survive at all.

416 The differences in phenotype elicited by these habitats could result in adaptive
417 differentiation if there is heritable variation for these traits within the populations. We did not
418 detect any genet-level variation within populations in this study. However, our power to detect
419 this level of variation was limited by the high mortality. The random effect of source site did not
420 explain much of the variation, but the source habitat type was a better predictor of variation than
421 transplant garden habitat type for most traits.

422 ***Signature of local adaptation*** –

423 We compared performance of local plants with that of the foreign plants for fitness
424 proxies (total biomass and survival) in each of the common gardens to assess local adaptation.

425 Previously, a similar reciprocal transplant study in knotweed found little support for local
426 adaptation along a latitudinal gradient in three populations from similar temperate deciduous
427 forest habitats (VanWallendael et al., 2018). Comparing responses to different habitat types on a
428 more local scale, we found support for local adaptation in plants from the marsh: they out-
429 performed plants from both beach and roadside habitats in biomass. This is surprising given the
430 previous work in the greenhouse which did not support adaptive response to salt treatments. In
431 the current study, the salt marsh plants were the only plants that accumulated more biomass in
432 their home site. This growth advantage, however, did not translate into advantage in survival of
433 marsh plants over foreign plants, in the marsh garden. Instead, there were no differences in
434 survival due to habitat of origin in the marsh transplant garden.

435 Plants from beach and roadside habitats failed to accumulate more total biomass in their
436 home environment compared to foreign plants. Beach plants tended to allocate more resources to
437 roots, but such allocation did not increase their odds of survival compared to foreign plants. In
438 fact, beach plants seemed to be mal-adapted, at least during the time frame of our study. It is
439 unclear whether preferential allocation to roots could eventually lead to an advantage, or if this
440 response is constrained by other factors, or could have been detected in a longer-term study.
441 Plants from the beach habitat have a greater probability to grow larger in either of the away
442 habitats. Growing on the beach on average reduced the biomass accumulation by 1.03-1.1 grams,
443 indicating that beach habitats are suboptimal for plant growth.

444 We also found support for local adaptation among roadside plants, which were better able
445 to survive in their home sites. Roadside plants had the best survival odds when compared to
446 foreign plants. This is somewhat surprising considering that plants from roadside habitats are
447 largely indistinguishable from plants from marsh habitats for most traits that we measured.

448 ***Sources of phenotypic differentiation –***

449 In our previous work, we used cytology and AFLP markers to show that most of these
450 populations consist of a few *R. × bohémica* hybrids (Richards et al., 2008, 2012). Plants from
451 both roadside and marsh habitats were highly plastic in response to treatment with salt in the
452 greenhouse, and even though clonal diversity was low in these populations, within sites there
453 were significant differences in most traits and trait plasticities. Several studies have demonstrated
454 that hybridization can result in significant changes in trait expression (e.g., transgressive traits)
455 with important ecological consequences (Gaskin and Schaal, 2002; Rosenthal et al., 2002, 2008;
456 Lexer et al., 2003; Johnston et al., 2004; Karrenberg et al., 2006; Parepa et al., 2014). In fact,
457 novel traits resulting from hybridization are considered an important feature that allows
458 expansion into novel habitats in several systems (Ellstrand and Schierenbeck, 2000; Lexer et al.,
459 2003; Johnston et al., 2004; Karrenberg et al., 2006). For example, hybrids between the invasive
460 *Carpobrotus edulis* and the native *C. chilensis* have higher biomass in response to low salinity
461 treatments under low nutrient conditions (Weber and D’Antonio, 1999), indicating that they may
462 have an advantage in nutrient poor soils. These and other studies suggest that recombination of
463 different traits may allow for rapid adaptation to new environments (Anderson and Stebbins,
464 1954; Ellstrand and Schierenbeck, 2000; Facon et al., 2005; Gross and Rieseberg, 2005).

465 Hybridization between *R. japonica* and *R. sachalinensis* to form *R. × bohémica* has also
466 been considered an important mechanism in the Japanese knotweed *s.l* invasion (Pysek et al.,
467 2003; Bímová et al., 2004; Mandák et al., 2005; Bailey et al., 2009). We considered that
468 transgressive trait segregation might therefore be an important contributor to success in this
469 diversity of habitats and that we would find particularly aggressive *R. × bohémica* genotypes.
470 We found that a few of our populations were characterized as *R. japonica* (Marsh 2, Roadside 2

471 and Beach 4). According to ANOVA they did not respond differently to these habitats than *R.* ×
472 *bohemica*. However, the ANOVA was performed only on surviving individuals and a logistic
473 regression of taxon on survival suggests that taxon alone explains a small, but significant amount
474 of the variation in survival. For the long-term success of *R. japonica*, it is meaningful that plants
475 from these populations did not *survive* as well as the *R.* × *bohemica* hybrids in our field
476 transplants. Plants from Marsh 2 and Roadside 2, for instance, did not survive at the beach and
477 marsh transplants had low survival on the roadside. This suggests that the hybrids have an
478 increased ability to establish in the most diverse and stressful habitats. In combination, these
479 studies show that complex ecologically relevant environments elicit differences in phenotype
480 that are not detectable by manipulation of salinity alone under controlled conditions, which
481 further underscores the importance of conducting studies in the field (Endler, 1986; Kingsolver
482 et al., 2001; Kawecki and Ebert, 2004; Sambatti and Rice, 2006; Leimu and Fischer, 2008).

483 Even considering that the few genotypes that have invaded these habitats may have
484 benefited from transgressive traits, the dramatically varied response to the transplant habitats
485 from what should only be a few genotypes is surprising. For example, the plants from Marsh 4
486 and Roadside 4 were identical across AFLP markers (Richards et al., 2012), and they have
487 almost identical survival at the roadside site. However, at the marsh transplant the marsh plants
488 had a significantly higher survival rate. Under different circumstances, this could reflect the
489 importance of maternal effects or provisioning. However, in our study we took care to start
490 plants with similar initial rhizome weights.

491 A potentially important possibility is that persistent epigenetic effects may have resulted
492 from the hybridization process or may have been induced by exposure to these dramatically
493 different environments. We have reported a surprising level of epigenetic variation in these

494 populations compared to levels of sequence-based variation found with AFLP (Richards et al.,
495 2012). Epigenetic effects have been suggested as a source of phenotypic variation in ecologically
496 relevant traits, but they have not yet been explored extensively in studies of invasive species
497 (Mounger et al., 2021; Hawes et al. 2018). In some cases, environmentally-induced epigenetic
498 changes may be inherited by future generations (Jablonka and Raz, 2009; Verhoeven et al., 2010;
499 Richards et al., 2017; Bonduriansky and Day, 2018; Richards and Pigliucci, 2020) and therefore
500 could contribute to explaining short-term adaptation to novel environments. Moreover,
501 epigenetic processes are an important component of hybridization events (Rapp and Wendel,
502 2005; Salmon et al., 2005; Flowers and Burton, 2006).

503 **CONCLUSIONS**

504 Although we found only limited support for local adaptation, this is not so unusual
505 (VanWallendael et al., 2018). Leimu & Fischer (2008) reported a meta-analysis of local
506 adaptation studies where they found that plants from “home” populations outperformed the
507 “foreign” plants in both habitat types in only 51% of the studies surveyed. These findings were
508 independent of plant longevity, mating system, clonality or habitat type and the authors
509 concluded that local adaptation may not be as common as it is assumed. Considering the
510 potentially random sampling of genotypes during the invasion process, genetic drift may play a
511 large role in shaping the evolutionary trajectory of these populations (Keller and Taylor, 2008;
512 Prentis et al., 2008). Identifying adaptive changes in a small founding population is difficult
513 because it requires identifying the source of the invasion and comparing responses of the
514 invaders to those of the source material (Bossdorf et al., 2008; Keller and Taylor, 2008; Prentis
515 et al., 2008; Colautti and Lau, 2015); e.g. (Bock et al., 2018; Exposito-Alonso et al., 2018).

516 Despite our mixed evidence, adaptive processes could still be important for most of the
517 populations, since transplants maintained biomass across at least two if not all three habitats. The
518 current study confirms our findings from the greenhouse that there is phenotypic differentiation
519 among these populations of Japanese knotweed, some of which is attributed to their source
520 habitat. Some of the plasticity in these traits and in fitness are likely to be passive responses to
521 resource limitation and stress, but “active” or adaptive plasticity in underlying morphological
522 and physiological traits may help to minimize the fitness loss in these environments (van
523 Kleunen and Fischer, 2005; Van Kleunen and Fischer, 2007; Murren et al., 2015; Bock et al.,
524 2018). Whatever the mechanisms of divergence, which could include drift, selection,
525 transgressive segregation, nongenetic effects, and genetic accommodation, this study
526 demonstrates that there is persistent phenotypic variation present in the populations of interest.
527 This variation in ecologically important traits provides the potential for future adaptation that
528 could increase the already high rate of spread of this species complex in North America, and in
529 salt marsh and beach habitats in particular. Understanding the components that contribute to the
530 success of this extensively clonal plant with little or no genetic variation could require
531 reevaluating how we measure adaptation.

532 **Acknowledgments**

533 The authors thank Ramona Walls for valuable assistance in finding field sites and making
534 connections with local government agencies and organizations. We also thank Larry
535 Gottschamer, Kristi Adams, Oliver Bossdorf, Tara George, Norris Muth and Radha
536 Parameswaran for assistance in the field and with harvesting as well as Stony Brook University
537 greenhouse staff Mike Axelrod and John Clumpp. We thank Fränzi Korner-Nievergelt, Ramona
538 Irimia, Madalin Parepa, Robert Rauschkolb and Oliver Bossdorf for critical advice on data

539 analysis. This work was supported by the Research Foundation of the State University of New
540 York (to MP), New York SEA Grant (to MP and CLR), and the Federal Ministry of Education
541 and Research (BMBF; MOPGA Project ID 306055 to CLR).

542 **Author Contributions**

543 C.L.R. and M.P. designed the study. C.L.R. implemented and maintained the experiment and
544 collected all data. W.Y. and C.L.R. completed statistical analyses. C.L.R. wrote the manuscript
545 and all authors contributed to the writing.

546 **Data Availability Statement**

547 Data and code for data analysis have been submitted for review on the Dryad Digital Repository:
548 <https://doi.org/10.5061/dryad.wdbrv15qz>

549 **Literature Cited**

550 Allendorf, F. W., and L. L. Lundquist. 2003. Introduction: Population biology, evolution, and control of
551 invasive species. *Conservation biology: the journal of the Society for Conservation Biology* 17: 24–
552 30.

553 Anderson, E., and G. L. Stebbins. 1954. Hybridization as an Evolutionary Stimulus. *Evolution;*
554 *international journal of organic evolution* 8: 378–388.

555 Antonovics, J., and A. D. Bradshaw. 1970. Evolution in closely adjacent plant populations VIII. Clinal
556 patterns at a mine boundary. *Heredity* 25: 349–362.

557 Bailey, J. P. 2013. The Japanese knotweed invasion viewed as a vast unintentional hybridisation
558 experiment. *Heredity* 110: 105–110.

559 Bailey, J. P., K. Bímová, and B. Mandák. 2009. Asexual spread versus sexual reproduction and evolution
560 in Japanese Knotweed s.l. sets the stage for the ‘Battle of the Clones’. *Biological invasions* 11:
561 1189–1203.

562 Bailey, J. P., and A. P. Conolly. 2000. Prize-winners to pariahs - a history of Japanese knotweed s.l.
563 (Polygonaceae) in the British Isles. *Watsonia* 23: 93–110.

564 Bailey, J. P., and R. Wisskirchen. 2004. The distribution and origins of *Faúopia × bohémica*
565 (Polygonaceae) in Europe. *Nordic Journal of Botany* 24: 173–199.

566 Baker, H. G. 1965. Characteristics and modes of origin of weeds. In H. G. Baker, and G. L. Stebbins
567 [eds.], *The genetics of colonizing species*, 147–172. Academic Press Inc., NY.

568 Barney, J. N. 2006. North American History of Two Invasive Plant Species: Phytogeographic

- 569 Distribution, Dispersal Vectors, and Multiple Introductions. *Biological invasions* 8: 703–717.
- 570 Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models Using lme4.
571 *Journal of statistical software* 67.
- 572 Bímová, K., B. Mandák, and I. Kašparová. 2004. How does Reynoutria invasion fit the various theories
573 of invasibility? *Journal of vegetation science: official organ of the International Association for*
574 *Vegetation Science* 15: 495–504.
- 575 Bímová, K., B. Mandák, and P. Pyšek. 2001. Experimental control of Reynoutria congeners: a
576 comparative study of a hybrid and its parents. *Plant invasions: species ecology and ecosystem*
577 *management*, 283–290. Backhuys Publishers.
- 578 Bock, D. G., C. Caseys, R. D. Cousens, M. A. Hahn, S. M. Heredia, S. Hübner, K. G. Turner, et al. 2015.
579 What we still don't know about invasion genetics. *Molecular ecology* 24: 2277–2297.
- 580 Bock, D. G., M. B. Kantar, C. Caseys, R. Matthey-Doret, and L. H. Rieseberg. 2018. Evolution of
581 invasiveness by genetic accommodation. *Nature ecology & evolution* 2: 991–999.
- 582 Bolker, B. M., M. E. Brooks, C. J. Clark, S. W. Geange, J. R. Poulsen, M. H. H. Stevens, and J.-S. S.
583 White. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. *Trends*
584 *in ecology & evolution* 24: 127–135.
- 585 Bonduriansky, R., and T. Day. 2018. *Extended heredity*. Princeton University Press, Princeton, NJ.
- 586 Bossdorf, O., H. Auge, L. Lafuma, W. E. Rogers, E. Siemann, and D. Prati. 2005. Phenotypic and genetic
587 differentiation between native and introduced plant populations. *Oecologia* 144: 1–11.
- 588 Bossdorf, O., A. Lipowsky, and D. Prati. 2008. Selection of preadapted populations allowed *Senecio*
589 *inaequidens* to invade Central Europe. *Diversity & distributions* 14: 676–685.
- 590 Bucharova, A., W. Durka, N. Hölzel, J. Kollmann, S. Michalski, and O. Bossdorf. 2017. Are local plants
591 the best for ecosystem restoration? It depends on how you analyze the data. *Ecology and evolution* 7:
592 10683–10689.
- 593 Bucharova, A., M. Frenzel, K. Mody, M. Parepa, W. Durka, and O. Bossdorf. 2016. Plant ecotype affects
594 interacting organisms across multiple trophic levels. *Basic and applied ecology* 17: 688–695.
- 595 Cheplick, G. P. 2006. A modular approach to biomass allocation in an invasive annual (*Microstegium*
596 *vimineum*; Poaceae). *American journal of botany* 93: 539–545.
- 597 Colautti, R. I., and J. A. Lau. 2015. Contemporary evolution during invasion: evidence for differentiation,
598 natural selection, and local adaptation. *Molecular ecology* 24: 1999–2017.
- 599 Daehler, C., and D. Strong. 1997. Hybridization between introduced smooth cordgrass (*Spartina*
600 *alterniflora*; Poaceae) and native California cordgrass (*S. foliosa*) in San Francisco Bay, California,
601 USA. *American journal of botany* 84: 607.
- 602 Davidson, A. M., M. Jennions, and A. B. Nicotra. 2011. Do invasive species show higher phenotypic
603 plasticity than native species and, if so, is it adaptive? A meta-analysis: Invasive species have higher
604 phenotypic plasticity. *Ecology letters* 14: 419–431.
- 605 Del Tredici, P. 2017. The introduction of Japanese knotweed, *Reynoutria japonica*, into North America.

- 606 *The Journal of the Torrey Botanical Society* 144: 406–416.
- 607 Dlugosch, K. M., and I. M. Parker. 2008. Invading populations of an ornamental shrub show rapid life
608 history evolution despite genetic bottlenecks. *Ecology letters* 11: 701–709.
- 609 Dlugosch, K. M., and I. M. Parker. 2007. Molecular and quantitative trait variation across the native range
610 of the invasive species *Hypericum canariense*: evidence for ancient patterns of colonization via pre-
611 adaptation? *Molecular ecology* 16: 4269–4283.
- 612 Donovan, L. A., S. A. Dudley, D. M. Rosenthal, and F. Ludwig. 2007. Phenotypic selection on leaf water
613 use efficiency and related ecophysiological traits for natural populations of desert sunflowers.
614 *Oecologia* 152: 13–25.
- 615 Donovan, L. A., J. H. Richards, and M. W. Muller. 1996. Water relations and leaf chemistry
616 of *Chrysothamnus nauseosus* ssp. *consimilis* (Asteraceae) and *Sarcobatus*
617 *vermiculatus* (Chenopodiaceae). *American journal of botany* 83: 1637–1646.
- 618 Doroszuk, A., M. W. Wojewodzic, G. Gort, and J. E. Kammenga. 2008. Rapid divergence of genetic
619 variance-covariance matrix within a natural population. *The American naturalist* 171: 291–304.
- 620 Dudley, S. A. 1996a. Differing selection on plant physiological traits in response to environmental water
621 availability: a test of adaptive hypotheses. *Evolution; international journal of organic evolution* 50:
622 92–102.
- 623 Dudley, S. A. 1996b. The response to differing selection on plant physiological traits: evidence for local
624 adaptation. *Evolution; international journal of organic evolution* 50: 103–110.
- 625 Durka, W., O. Bossdorf, D. Prati, and H. Auge. 2005. Molecular evidence for multiple introductions of
626 garlic mustard (*Alliaria petiolata*, Brassicaceae) to North America. *Molecular ecology* 14: 1697–
627 1706.
- 628 Ellstrand, N. C., and K. A. Schierenbeck. 2000. Hybridization as a stimulus for the evolution of
629 invasiveness in plants? *Proceedings of the National Academy of Sciences of the United States of*
630 *America* 97: 7043–7050.
- 631 Endler, J. A. 1986. *Natural Selection in the Wild*. Princeton University Press.
- 632 Exposito-Alonso, M., C. Becker, V. J. Schuenemann, E. Reiter, C. Setzer, R. Slovak, B. Brachi, et al.
633 2018. The rate and potential relevance of new mutations in a colonizing plant lineage. *PLoS genetics*
634 14: e1007155.
- 635 Facon, B., P. Jarne, J. P. Pointier, and P. David. 2005. Hybridization and invasiveness in the freshwater
636 snail *Melanoides tuberculata*: hybrid vigour is more important than increase in genetic variance.
637 *Journal of evolutionary biology* 18: 524–535.
- 638 Flowers, J. M., and R. S. Burton. 2006. Ribosomal RNA Gene Silencing in Interpopulation Hybrids of
639 *Tigriopus californicus*: Nucleolar Dominance in the Absence of Intergenic Spacer Subrepeats.
640 *Genetics* 173: 1479–1486.
- 641 Flowers, T. J., P. F. Troke, and A. R. Yeo. 1977. The mechanism of salt tolerance in halophytes. *Annual*
642 *review of plant physiology* 28: 89–121.
- 643 Forman, J., and R. V. Kesseli. 2003. Sexual reproduction in the invasive species *Fallopia japonica*

- 644 (Polygonaceae). *American journal of botany* 90: 586–592.
- 645 Franks, S. J., and A. E. Weis. 2008. A change in climate causes rapid evolution of multiple life-history
646 traits and their interactions in an annual plant. *Journal of evolutionary biology*.
- 647 Freeman, A. S., and J. E. Byers. 2006. Divergent induced responses to an invasive predator in marine
648 mussel populations. *Science* 313: 831–833.
- 649 Funk, J. L. 2008. Differences in plasticity between invasive and native plants from a low resource
650 environment. *The Journal of ecology* 96: 1162–1173.
- 651 Gammon, M. A., J. L. Grimsby, D. Tsirelson, and R. Kesseli. 2007. Molecular and morphological
652 evidence reveals introgression in swarms of the invasive taxa *Fallopia japonica*, *F. sachalinensis*, and
653 *F. xbohemica* (Polygonaceae) in the United States. *American journal of botany* 94: 948–956.
- 654 Gammon, M. A., and R. Kesseli. 2010. Haplotypes of *Fallopia* introduced into the US. *Biological*
655 *invasions* 12: 421–427.
- 656 Gaskin, J. F., and B. A. Schaal. 2002. Hybrid *Tamarix* widespread in U.S. invasion and undetected in
657 native Asian range. *Proceedings of the National Academy of Sciences of the United States of*
658 *America* 99: 11256–11259.
- 659 Gaskin, J. F., M. Schwarzländer, F. S. Grevstad, M. A. Haverhals, R. S. Bouchier, and T. W. Miller.
660 2014. Extreme differences in population structure and genetic diversity for three invasive congeners:
661 knotweeds in western North America. *Biological invasions* 16: 2127–2136.
- 662 Gelman, A., and Y.-S. Su. 2020. arm: Data Analysis Using Regression and Multilevel/Hierarchical
663 Models. R package version 1.11-2.
- 664 Geng, Y.-P., X.-Y. Pan, C.-Y. Xu, W.-J. Zhang, B. Li, J.-K. Chen, B.-R. Lu, and Z.-P. Song. 2007.
665 Phenotypic plasticity rather than locally adapted ecotypes allows the invasive alligator weed to
666 colonize a wide range of habitats. *Biological invasions* 9: 245–256.
- 667 Grimsby, J. L., and R. Kesseli. 2010. Genetic composition of invasive Japanese knotweed s.l. in the
668 United States. *Biological invasions* 12: 1943–1946.
- 669 Grimsby, J. L., D. Tsirelson, M. A. Gammon, and R. Kesseli. 2007. Genetic diversity and clonal vs.
670 sexual reproduction in *Fallopia* spp. (Polygonaceae). *American journal of botany* 94: 957–964.
- 671 Groeneveld, E., F. Belzile, and C. Lavoie. 2014. Sexual reproduction of Japanese knotweed (*Fallopia*
672 *japonica* s.l.) at its northern distribution limit: new evidence of the effect of climate warming on an
673 invasive species. *American journal of botany* 101: 459–466.
- 674 Gross, B. L., and L. H. Rieseberg. 2005. The ecological genetics of homoploid hybrid speciation. *The*
675 *Journal of heredity* 96: 241–252.
- 676 Hawes, N. A., A. E. Fidler, L. A. Tremblay, X. Pochon, B. J. Dunphy, and K. F. Smith. 2018.
677 Understanding the role of DNA methylation in successful biological invasions: a review.
678 *Biological invasions* 20: 2285–2300.
- 679 Herman, J. J., and S. E. Sultan. 2016. DNA methylation mediates genetic variation for adaptive
680 transgenerational plasticity. *Proceedings of the Royal Society B: Biological Sciences* 283.

- 681 Hollingsworth, M. L., and J. P. Bailey. 2000. Evidence for massive clonal growth in the invasive weed
682 *Fallopia japonica* (Japanese Knotweed). *Botanical journal of the Linnean Society. Linnean Society of*
683 *London* 133: 463–472.
- 684 Inamura, A., Y. Ohashi, E. Sato, Y. Yoda, T. Masuzawa, M. Ito, and K. Yoshinaga. 2000. Intraspecific
685 Sequence Variation of Chloroplast DNA Reflecting Variety and Geographical Distribution of
686 *Polygonum cuspidatum* (Polygonaceae) in Japan. *Journal of plant research* 113: 419–426.
- 687 Jablonka, E., and G. Raz. 2009. Transgenerational epigenetic inheritance: prevalence, mechanisms, and
688 implications for the study of heredity and evolution. *The Quarterly review of biology* 84: 131–176.
- 689 Johnson, P. C. 2014. Extension of Nakagawa & Schielzeth’s R2GLMM to random slopes models.
690 *Methods in ecology and evolution / British Ecological Society* 5: 944–946.
- 691 Johnston, J. A., L. A. Donovan, and M. L. Arnold. 2004. Novel phenotypes among early generation
692 hybrids of two Louisiana iris species: flooding experiments. *The Journal of ecology* 92: 967–976.
- 693 Karrenberg, S., C. Edelist, C. Lexer, and L. Rieseberg. 2006. Response to salinity in the homoploid
694 hybrid species *Helianthus paradoxus* and its progenitors *H. annuus* and *H. petiolaris*. *The New*
695 *phytologist* 170: 615–629.
- 696 Kawecki, T. J., and D. Ebert. 2004. Conceptual issues in local adaptation. *Ecology letters* 7: 1225–1241.
- 697 Keller, S. R., and D. R. Taylor. 2008. History, chance and adaptation during biological invasion:
698 separating stochastic phenotypic evolution from response to selection. *Ecology letters* 11: 852–866.
- 699 Kingsolver, J. G., H. E. Hoekstra, D. Berrigan, S. N. Vignieri, C. E. Hill, A. Hoang, P. Gibert, and P.
700 Beerli. 2001. The Strength of Phenotypic Selection in Natural Populations. *The American Naturalist*
701 157: 245–261.
- 702 van Kleunen, M., O. Bossdorf, and W. Dawson. 2018. The Ecology and Evolution of Alien Plants.
703 *Annual review of ecology, evolution, and systematics* 49: 25–47.
- 704 van Kleunen, M., and M. Fischer. 2005. Constraints on the evolution of adaptive phenotypic plasticity in
705 plants. *The New phytologist* 166: 49–60.
- 706 Korner-Nievergelt, F., T. Roth, S. von Felten, J. Guélat, B. Almasi, and P. Korner-Nievergelt. 2015.
707 Bayesian Data Analysis in Ecology Using Linear Models with R, BUGS, and Stan. Academic Press.
- 708 Krebs, C., G. Mahy, D. Matthies, U. Schaffner, M.-S. Tiébré, and J.-P. Bizoux. 2010. Taxa distribution
709 and RAPD markers indicate different origin and regional differentiation of hybrids in the invasive
710 *Fallopia complex* in central-western Europe. *Plant biology* 12: 215–223.
- 711 de Kroon, H., and J.-M. Groenendael. 1997. The Ecology and Evolution of Clonal Plants. Backhuys
712 Publishers.
- 713 Lande, R., and S. J. Arnold. 1983. The measurement of selection on correlated characters. *Evolution;*
714 *international journal of organic evolution* 37: 1210–1226.
- 715 Lavergne, S., and J. Molofsky. 2007. Increased genetic variation and evolutionary potential drive the
716 success of an invasive grass. *Proceedings of the National Academy of Sciences of the United States*
717 *of America* 104: 3883–3888.

- 718 Lee, C. E. 2002. Evolutionary genetics of invasive species. *Trends in ecology & evolution* 17: 386–391.
- 719 Leger, E. A., and K. J. Rice. 2007. Assessing the speed and predictability of local adaptation in invasive
720 California poppies (*Eschscholzia californica*). *Journal of evolutionary biology* 20: 1090–1103.
- 721 Leimu, R., and M. Fischer. 2008. A meta-analysis of local adaptation in plants. *PLoS one* 3: e4010.
- 722 Lexer, C., M. E. Welch, O. Raymond, and L. H. Rieseberg. 2003. The origin of ecological divergence in
723 *Helianthus paradoxus* (Asteraceae): selection on transgressive characters in a novel hybrid habitat.
724 *Evolution; international journal of organic evolution* 57: 1989–2000.
- 725 Linhart, Y. B., and M. C. Grant. 1996. Evolutionary significance of local genetic differentiation in plants.
726 *Annual review of ecology and systematics* 27: 237–277.
- 727 Liu, W., Y. Zhang, X. Chen, K. Maung-Douglass, D. R. Strong, and S. C. Pennings. 2020. Contrasting
728 plant adaptation strategies to latitude in the native and invasive range of *Spartina alterniflora*. *The*
729 *New phytologist* 226: 623–634.
- 730 Long, R. 2021. Crossed random effects: how do we model multiple reciprocal transplants in lme4?
731 *stackexchange.com*. Website <https://stats.stackexchange.com/users/7486/robert-long>, [Crossed](https://stats.stackexchange.com/questions/530923/crossed-random-effects-how-do-we-model-multiple-reciprocal-transplants-in-lme4)
732 [random effects: how do we model multiple reciprocal transplants in lme4?](https://stats.stackexchange.com/questions/530923/crossed-random-effects-how-do-we-model-multiple-reciprocal-transplants-in-lme4), URL (version: 2021-06-
733 [16](https://stats.stackexchange.com/q/530923)): <https://stats.stackexchange.com/q/530923> [accessed 17 June 2021].
- 734 Loomis, E. S., and L. Fishman. 2009. A Continent-Wide Clone: Population Genetic Variation of the
735 Invasive Plant *Hieracium aurantiacum* (Orange Hawkweed; Asteraceae) in North America.
736 *International journal of plant sciences* 170: 759–765.
- 737 Mandák, B., K. Bímová, P. Pyšek, J. Štěpánek, and I. Plačková. 2005. Isoenzyme diversity in Reynoutria
738 (Polygonaceae) taxa: escape from sterility by hybridization. *Plant systematics and evolution =*
739 *Entwicklungsgeschichte und Systematik der Pflanzen* 253: 219–230.
- 740 Mandák, B., P. Pyšek, K. Bímová, and Others. 2004. History of the invasion and distribution of
741 Reynoutria taxa in the Czech Republic: a hybrid spreading faster than its parents. *Preslia* 76: 15–64.
- 742 Matesanz, S., T. Horgan-Kobelski, and S. E. Sultan. 2015. Evidence for rapid ecological range expansion
743 in a newly invasive plant. *AoB plants* 7.
- 744 Matesanz, S., and S. E. Sultan. 2013. High-performance genotypes in an introduced plant: insights to
745 future invasiveness. *Ecology* 94: 2464–2474.
- 746 Mauricio, R., and M. D. Rausher. 1997. Experimental manipulation of putative selective agents provides
747 evidence for the role of natural enemies in the evolution of plant defense. *Evolution; international*
748 *journal of organic evolution* 51: 1435–1444.
- 749 Mounger, J., M. L. Ainouche, O. Bossdorf, A. Cavé-Radet, B. Li, M. Parepa, A. Salmon, et al. 2021.
750 Epigenetics and the success of invasive plants. *Philosophical transactions of the Royal Society of*
751 *London. Series B, Biological sciences* 376: 20200117.
- 752 Murren, C. J., J. R. Auld, H. Callahan, C. K. Ghalambor, C. A. Handelsman, M. A. Heskell, J. G.
753 Kingsolver, et al. 2015. Constraints on the evolution of phenotypic plasticity: limits and costs of
754 phenotype and plasticity. *Heredity* 115: 293–301.
- 755 Muth, N. Z., and M. Pigliucci. 2007. Implementation of a novel framework for assessing species

- 756 plasticity in biological invasions: responses of *Centaurea* and *Crepis* to phosphorus and water
757 availability. *The Journal of ecology* 95: 1001–1013.
- 758 Nakagawa, S., P. C. D. Johnson, and H. Schielzeth. 2017. The coefficient of determination R^2 and intra-
759 class correlation coefficient from generalized linear mixed-effects models revisited and expanded.
760 *Journal of the Royal Society, Interface / the Royal Society* 14.
- 761 Nakagawa, S., and H. Schielzeth. 2013. A general and simple method for obtaining R^2 from generalized
762 linear mixed-effects models. *Methods in ecology and evolution / British Ecological Society* 4: 133–
763 142.
- 764 Neinavaie, F., A. Ibrahim-Hashim, A.M. Kramer, J.S. Brown, and C.L. Richards. 2021. The genomic
765 processes of biological invasions: from invasive species to cancer metastases and back again.
766 *Frontiers in Ecology and Evolution* 9: Article 681100.
- 767 Nimon, K., M. Lewis, R. Kane, and R. M. Haynes. 2008. An R package to compute commonality
768 coefficients in the multiple regression case: an introduction to the package and a practical example.
769 *Behavior research methods* 40: 457–466.
- 770 Oduor, A. M. O., R. Leimu, and M. van Kleunen. 2016. Invasive plant species are locally adapted just as
771 frequently and at least as strongly as native plant species. *The Journal of ecology* 104: 957–968.
- 772 Oplaat, C., and K. J. F. Verhoeven. 2015. Range expansion in asexual dandelions: selection for general-
773 purpose genotypes? *The Journal of ecology* 103: 261–268.
- 774 Pan, X., Y. Geng, W. Zhang, B. Li, and J. Chen. 2006. The influence of abiotic stress and phenotypic
775 plasticity on the distribution of invasive *Alternanthera philoxeroides* along a riparian zone. *Acta*
776 *Oecologica* 30: 333–341.
- 777 Papadopulos, A. S. T., A. J. Helmstetter, O. G. Osborne, A. A. Comeault, D. P. Wood, E. A. Straw, L.
778 Mason, et al. 2021. Rapid Parallel Adaptation to Anthropogenic Heavy Metal Pollution. *Molecular*
779 *biology and evolution*.
- 780 Parepa, M., M. Fischer, C. Krebs, and O. Bossdorf. 2014. Hybridization increases invasive knotweed
781 success. *Evolutionary applications* 7: 413–420.
- 782 Park, C.-W., G. S. Bhandari, H. Won, J. H. Park, and D. S. Park. 2018. Polyploidy and introgression in
783 invasive giant knotweed (*Fallopia sachalinensis*) during the colonization of remote volcanic islands.
784 *Scientific reports* 8: 16021.
- 785 Prentis, P. J., J. R. U. Wilson, E. E. Dormontt, D. M. Richardson, and A. J. Lowe. 2008. Adaptive
786 evolution in invasive species. *Trends in plant science* 13: 288–294.
- 787 Puy, J., F. de Bello, H. Dvořáková, N. G. Medina, V. Latzel, and C. P. Carmona. 2021. Competition-
788 induced transgenerational plasticity influences competitive interactions and leaf decomposition of
789 offspring. *The new phytologist* 229: 3497–3507.
- 790 Puy, J., C. P. Carmona, H. Dvořáková, V. Latzel, and F. de Bello. 2021. Diversity of parental
791 environments increases phenotypic variation in *Arabidopsis* populations more than genetic diversity
792 but similarly affects productivity. *Annals of botany* 127: 425–436.
- 793 Pysek, P., J. H. Brock, K. Bímová, B. Mandák, V. Jarosík, I. Koukolíková, J. Pergl, and J. Stepánek.
794 2003. Vegetative regeneration in invasive *Reynoutria* (Polygonaceae) taxa: the determinant of

- 795 invasibility at the genotype level. *American journal of botany* 90: 1487–1495.
- 796 Pyšek, P., B. Mandák, T. Francírková, and K. Prach. 2001. Persistence of stout clonal herbs as invaders in
797 the landscape: A field test of historical records. In G. Brundu, J. Brock, I. Camarda, L. Child, and M.
798 Wade [eds.], *Plant Invasions: Species Ecology and Ecosystem Management.*, 235–244. Backhuys
799 Publishers.
- 800 Qiao, H., W. Liu, Y. Zhang, Y.-Y. Zhang, and Q. Q. Li. 2019. Genetic admixture accelerates invasion via
801 provisioning rapid adaptive evolution. *Molecular ecology* 28: 4012–4027.
- 802 Rapp, R. A., and J. F. Wendel. 2005. Epigenetics and plant evolution. *The New phytologist* 168: 81–91.
- 803 R Core Team. 2020. R: A language and environment for statistical computing.
- 804 Reimann, C., and S.-W. Breckle. 1995. Salt tolerance and ion relations of *Salsola kali* L.: differences
805 between ssp. *tragus* (L.) Nyman and ssp. *ruthenica* (Iljin) Soó. *The New phytologist* 130: 37–45.
- 806 Richards, C. L., C. Alonso, C. Becker, O. Bossdorf, E. Bucher, M. Colomé-Tatché, W. Durka, et al. 2017.
807 Ecological plant epigenetics: Evidence from model and non-model species, and the way forward.
808 *Ecology letters* 20: 1576–1590.
- 809 Richards, C. L., O. Bossdorf, N. Z. Muth, J. Gurevitch, and M. Pigliucci. 2006. Jack of all trades, master
810 of some? On the role of phenotypic plasticity in plant invasions. *Ecology letters* 9: 981–993.
- 811 Richards, C. L., and M. Pigliucci. 2020. Epigenetic Inheritance. A Decade into the Extended Evolutionary
812 Synthesis. *Paradigmi* 38: 463–494.
- 813 Richards, C. L., A. W. Schrey, and M. Pigliucci. 2012. Invasion of diverse habitats by few Japanese
814 knotweed genotypes is correlated with epigenetic differentiation. *Ecology letters* 15: 1016–1025.
- 815 Richards, C. L., R. L. Walls, and J. P. Bailey. 2008. Plasticity in salt tolerance traits allows for invasion of
816 novel habitat by Japanese knotweed sl (*Fallopia japonica* and *F. ×bohemica*, Polygonaceae).
817 *American Journal of*.
- 818 Richards, C. L., S. N. White, M. A. McGuire, and S. J. Franks. 2010. Plasticity, not adaptation to salt
819 level, explains variation along a salinity gradient in a salt marsh perennial. *Estuaries*.
- 820 Roff, D. A., and T. Mousseau. 2005. The evolution of the phenotypic covariance matrix: evidence for
821 selection and drift in *Melanoplus*. *Journal of evolutionary biology* 18: 1104–1114.
- 822 Rosenthal, D. M., A. P. Ramakrishnan, and M. B. Cruzan. 2008. Evidence for multiple sources of
823 invasion and intraspecific hybridization in *Brachypodium sylvaticum* (Hudson) Beauv. in North
824 America. *Molecular ecology* 17: 4657–4669.
- 825 Rosenthal, D. M., A. E. Schwarzbach, L. A. Donovan, O. Raymond, and L. H. Rieseberg. 2002.
826 Phenotypic Differentiation between Three Ancient Hybrid Taxa and Their Parental Species.
827 *International journal of plant sciences* 163: 387–398.
- 828 Sakai, A. K., F. W. Allendorf, and J. S. Holt. 2001. The population biology of invasive species. *Annual*
829 *review of*.
- 830 Salmon, A., M. L. Ainouche, and J. F. Wendel. 2005. Genetic and epigenetic consequences of recent
831 hybridization and polyploidy in *Spartina* (Poaceae). *Molecular ecology* 14: 1163–1175.

- 832 Sambatti, J. B. M., and K. J. Rice. 2006. Local adaptation, patterns of selection, and gene flow in the
833 Californian serpentine sunflower (*Helianthus exilis*). *Evolution; international journal of organic*
834 *evolution* 60: 696–710.
- 835 Schuster, T. M., J. L. Reveal, and K. A. Kron. 2011. Phylogeny of Polygoneae (Polygonaceae:
836 Polygonoideae). *Taxon* 60: 1653–1666.
- 837 Schuster, T. M., K. L. Wilson, and K. A. Kron. 2011. Phylogenetic Relationships of Muehlenbeckia,
838 Fallopia, and Reynoutria (Polygonaceae) Investigated with Chloroplast and Nuclear Sequence Data.
839 *International journal of plant sciences* 172: 1053–1066.
- 840 Sexton, J. P., J. K. McKay, and A. Sala. 2002. Plasticity and genetic diversity may allow saltcedar to
841 invade cold climates in north America. *Ecological applications: a publication of the Ecological*
842 *Society of America* 12: 1652.
- 843 Shi, W., X. Chen, L. Gao, C.-Y. Xu, X. Ou, O. Bossdorf, J. Yang, and Y. Geng. 2018. Transient Stability
844 of Epigenetic Population Differentiation in a Clonal Invader. *Frontiers in plant science* 9: 1851.
- 845 Stoffel, M. A., S. Nakagawa, and H. Schielzeth. 2017. rptR: repeatability estimation and variance
846 decomposition by generalized linear mixed-effects models. *Methods in ecology and evolution /*
847 *British Ecological Society* 8: 1639–1644.
- 848 Sultan, S. E. 2004. Promising directions in plant phenotypic plasticity. *Perspectives in plant ecology,*
849 *evolution and systematics* 6: 227–233.
- 850 Sultan, S. E., T. Horgan-Kobelski, L. M. Nichols, C. E. Riggs, and R. K. Waples. 2013. A resurrection
851 study reveals rapid adaptive evolution within populations of an invasive plant. *Evolutionary*
852 *applications* 6: 266–278.
- 853 Tiébré, M.-S., J.-P. Bizoux, O. J. Hardy, J. P. Bailey, and G. Mahy. 2007. Hybridization and
854 morphogenetic variation in the invasive alien Fallopia (Polygonaceae) complex in Belgium.
855 *American journal of botany* 94: 1900–1910.
- 856 Townsend, A. 1997. Japanese Knotweed: A Reputation Lost. *Arnoldia Zimbabwe* 57: 13–19.
- 857 Van Kleunen, M., and M. Fischer. 2007. Progress in the detection of costs of phenotypic plasticity in
858 plants. *The New phytologist* 176: 727–730.
- 859 VanWallerdael, A., M. Alvarez, and S. J. Franks. 2021. Patterns of population genomic diversity in the
860 invasive Japanese knotweed species complex. *American journal of botany* 108: 857–868.
- 861 VanWallerdael, A., E. Hamann, and S. J. Franks. 2018. Evidence for plasticity, but not local adaptation,
862 in invasive Japanese knotweed (*Reynoutria japonica*) in North America. *Evolutionary ecology* 32:
863 395–410.
- 864 Verhoeven, K. J. F., J. J. Jansen, P. J. Van Dijk, and A. Biere. 2010. Stress-induced DNA methylation
865 changes and their heritability in asexual dandelions. *The New phytologist* 185: 1108–1118.
- 866 Walls, R. L. 2010. Hybridization and Plasticity Contribute to Divergence Among Coastal and Wetland
867 Populations of Invasive Hybrid Japanese Knotweed s.l. (*Fallopia* spp.). *Estuaries and Coasts* 33:
868 902–918.
- 869 Weber, E., and C. M. D'Antonio. 1999. Germination and growth responses of hybridizing *Carpobrotus*

- 870 species (Aizoaceae) from coastal California to soil salinity. *American journal of botany* 86: 1257–
871 1263.
- 872 Zerebecki, R. A., E. E. Sotka, T. C. Hanley, K. L. Bell, C. Gehring, C. C. Nice, C. L. Richards, and A. R.
873 Hughes. 2021. Repeated Genetic and Adaptive Phenotypic Divergence across Tidal Elevation in a
874 Foundation Plant Species. *The American naturalist*: E000–E000.
- 875 Zhang, Y.-Y., M. Parepa, M. Fischer, and O. Bossdorf. 2016. Epigenetics of colonizing species? A study
876 of Japanese knotweed in Central Europe. *In* S. Barrett, R. I. Colautti, K. M. Dlugosch, and L. H.
877 Rieseberg [eds.], *Invasion Genetics: The Baker and Stebbins Legacy*, 328–340. Wiley-Blackwell
878 UK.

Table 1. Locations of origin sites for the three habitat types within each transplant garden group for the 12 sites (used as sources for plant material and as locations of transplants). The table provides the number of rhizomes from each site (with range of replicates) and total number of replicates per site, as well as the total number of rhizomes and replicates within the four reciprocal transplants. The table also indicates the total number of surviving plants from each origin site (and number of survivors in beach, marsh and roadside transplant gardens).

Transplant garden group	Origin site	Source habitat type	City	Latitude	Longitude	No. of rhizomes (reps/rhizome)	Total no. ramets	Survivors
1	MSH	Beach	Port Jefferson	40 57.7	73 02.6	6 (9-21)	81	35 (3,25,7)
1	CBH	Marsh	Port Jefferson	40 57.2	73 02.7	5 (6-21)	117	32 (2,24,6)
1	ST	Roadside	Smithtown	40 51.5	73 12.6	7 (6-15)	117	43 (0,27,16)
Total for transplant 1						18	315	110 (35%)
2	PJB	Beach	Port Jefferson	40 57.9	73 03.2	7 (9-21)	93	19 (0,5,14)
2	CMM	Marsh	Center Moriches	40 48.0	72 46.4	8 (9-24)	75	8 (0,0,8)
2	CMR	Roadside	Center Moriches	40 48.0	72 46.4	8 (6-21)	72	8 (0,0,8)
Total for transplant 2						23	240	35 (15%)
3	RPB	Beach	Rocky Point	40 58.0	72 57.3	6 (6-21)	147	37 (2,12,23)
3	WH	Marsh	Brookhaven	40 46.2	72 53.9	8 (6-21)	117	8 (0,4,4)
3	HL	Roadside	Rocky Point	40 57.8	72 57.3	8 (6-21)	111	35 (2,17,16)
Total for transplant 3						22	375	80 (21%)
4	HP	Beach	Southold	41 05.2	72 26.7	8 (12-24)	120	39 (11,6,22)
4	RHBH	Marsh	Riverhead	40 54.2	72 37.1	8 (6-19)	132	76 (22,29,32)
4	RHC	Roadside	Riverhead	40 54.5	72 37.5	8 (9-21)	105	50 (9,16,25)
Total for transplant 4						24	357	165 (46%)

Table 2. Tests for components of variance for each trait with random effects of origin site and transplant site, and fixed effects of source habitat type and transplant garden habitat type. The three tests of variance provide information about R^2 of the full model versus just fixed effects ($r2_nakagawa$), R^2 of the two random effects and combined fixed effects ($rptR$) and the contribution of each fixed effect without accounting for random effects ($commonalityCoefficients$). See methods for more details.

	r2_nakagawa		rptR			commonalityCoefficients			
	conditional r2 (random and fixed)	marginal r2 (fixed)	Origin site (random)	Transplant site (random)	Fixed effects	Unique to Source type (fixed)	Unique to Garden type (fixed)	Common to Source & Garden	Total
Final height	0.448	0.12	0.021 [0, 0.073]	0.421 [0.116, 0.612]	0.077 [0.035, 0.35]	0.127	0.063	0	0.186
Total leaf number	0.375	0.16	NA	0.143 [0, 0.212]	0.08 [0.039, 0.236]	0.048	0.072	0.008	0.128
Total leaf area	0.29	0.04	0.006 [0, 0.068]	0.174 [0.011, 0.335]	0.069 [0.03, 0.234]	0.066	0.075	0.006	0.147
Succulence	0.185	0.03	0.019 [0, 0.095]	0.204 [0, 0.379]	0.018 [0.01, 0.166]	0.021	0.008	0.0003	0.166
Shoot biomass	0.341	0.06	NA	0.367 [0.093, 0.561]	0.045 [0.025, 0.258]	0.093	0.023	0.002	0.119
Root biomass	0.366	0.05	0.016 [0, 0.077]	0.423 [0.117, 0.620]	0.027 [0.018, 0.299]	0.088	0.062	0.007	0.157
Total biomass	0.379	0.06	0.013 [0, 0.07]	0.427 [0.098, 0.606]	0.034 [0.022, 0.283]	0.099	0.051	0.007	0.157

Table 3 Breakdown of mortality by group within transplant habitat and average for each habitat type

	Garden	Mortality
Beaches		
1	MSH	95%
2	PJB	100%
3	RPB	97%
4	HP	65%
Average		89%
Marshes		
1	CBH	29%
2	CMM	94%
3	WH	65%
4	RHBH	57%
Average		61%
Roadsides		
1	ST	72%
2	CMR	63%
3	HL	74%
4	RHC	34%
Average		61%

Table S1 Final models for the seven traits.

Trait	Transformation	Type of model	Final model
Succulence	none	LMER	Source type + Garden type + Source type x Garden type + (Transplant.site)
Leaf area	log 10	LMER	Source type + Garden type + Source type x Garden type + (Origin site + Transplant site)
Total leaf number	negative binomial	GLMER	Source type + Garden type + Source type x Garden type + (Transplant site)
Final height	none	LMER	Source type + Garden type + Source type x Garden type + (Origin site + Transplant site)
Root:Shoot	log 2	LMER	Source type + Garden type + Source type x Garden type + (Origin site + Transplant site)?
Shoot biomass	log 2	LMER	Source type + Garden type + Source type x Garden type + (Transplant site)
Root biomass	log 2	LMER	Source type + Garden type + Source type x Garden type + (Origin site + Transplant site)

Table S2. As with table 2 in the main text, we provide tests for components of variance for each trait with random effects of origin site and transplant site, and fixed effects of source habitat type and transplant garden habitat type as well as the fixed effect of transplant group. The three test of variance provide information about R^2 of the full model versus just fixed effects ($r2_nakagawa$), R^2 of the two random effects and combined fixed effects ($rptR$) and the contribution of each fixed effect without accounting for random effects ($commonalityCoefficients$). See methods for more details.

	$r2_nakagawa$		$rptR$		
	conditional	marginal $r2$	Origin site	Transplant site	Fixed effects
	$r2$ (random and fixed)	(fixed)	(random) [CI]	(random) [CI]	[CI]
Final height (396)	0.466	0.334	0.019 [0, 0.072]	0.3 [0.032, 0.497]	0.24 [0.135, 0.563]
Total leaf number (395)	0.374	0.364	NA	0 [0, 0]	0.235 [0.184, 0.321]
Total leaf area (395)	0.264	0.231	0.009 [0, 0.06]	0.165 [0, 0.362]	0.136 [0.077, 0.365]
Succulence (372)	0.185	0.067	0.015 [0, 0.095]	0.253 [0.002, 0.074]	0.045 [0.038, 0.334]
Shoot biomass (395)	0.334	0.275	NA	0.208 [0.005, 0.414]	0.226 [0.116, 0.483]
Root biomass (395)	0.382	0.209	0.015 [0, 0.064]	0.379 [0.045, 0.588]	0.148 [0.071, 0.528]
Total biomass (395)	0.394	0.239	0.013 [0, 0.057]	0.361 [0.027, 0.056]	0.169 [0.086, 0.535]

	commonalityCoefficients							Total
	Unique to Transplant group (fixed)	Unique to Source type (fixed)	Unique to Garden type (fixed)	Common to Group & Source type	Common to Group & Garden type	Common to Source type & Garden type	Common to Group, Source & Garden type	
Final height	0.175	0.055	0.062	0.0713	0.0003	0.003	-0.006	0.359
Total leaf number	0.075	0.039	0.076	0.0091	-0.0046	-0.002	0.0097	0.202
Total leaf area	0.060	0.042	0.056	0.0235	0.0189	-0.002	0.0073	0.147
Succulence	0.053	0.012	0.011	0.009	-0.0038	0.002	-0.002	0.029
Shoot biomass	0.155	0.042	0.017	0.0507	0.0075	0.001	0.0013	0.119
Root biomass	0.103	0.048	0.047	0.0402	0.0156	-0.001	0.0081	0.157
Total biomass	0.121	0.052	0.036	0.0477	0.0148	-0.0004	0.0072	0.157

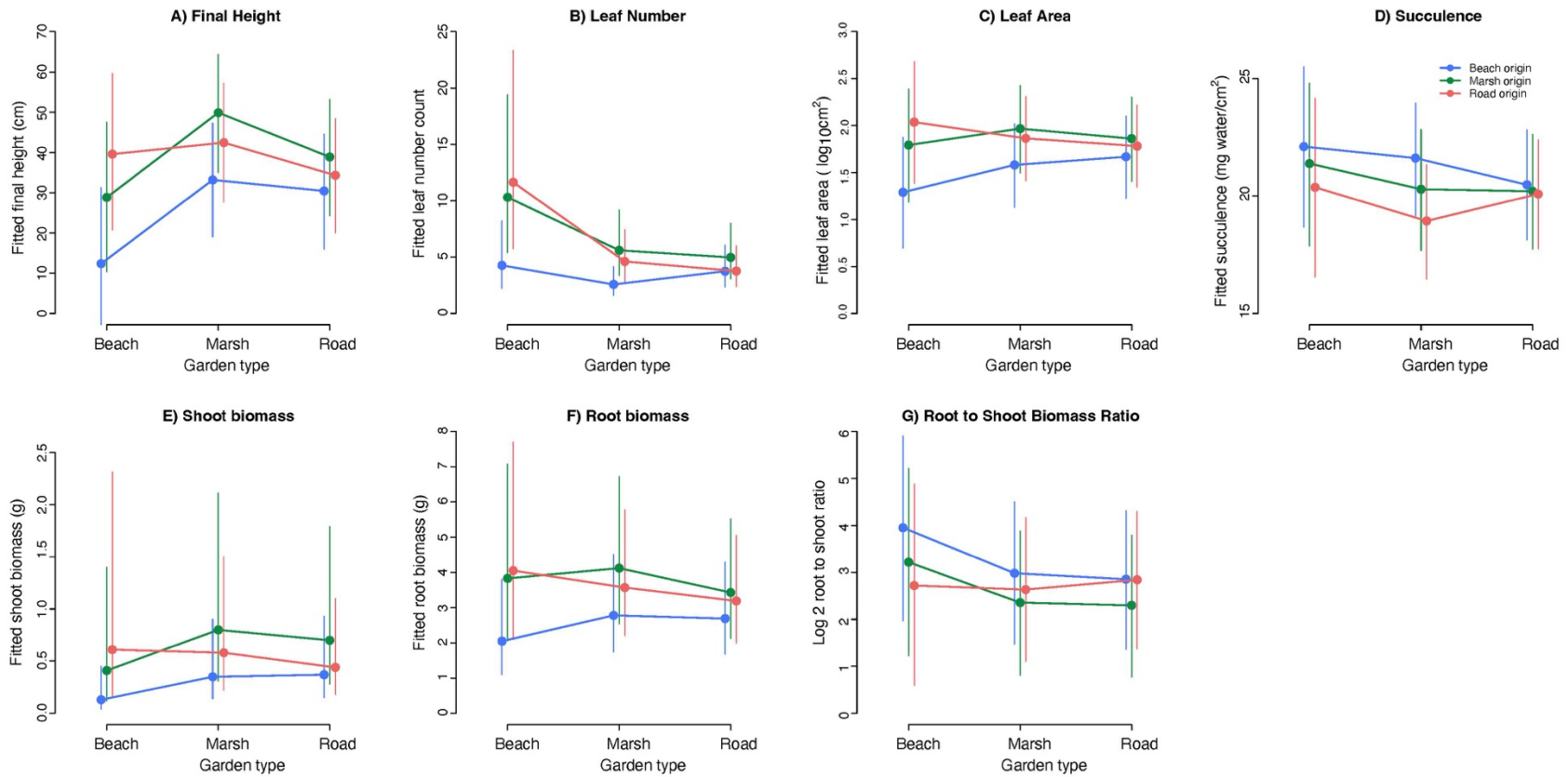


Figure 1. Reaction norms of (means \pm 95% CrI) across three transplant habitat gardens for plants from the three habitat origins: A) final height, B) total number of leaves, C) total leaf area of all leaves at final harvest, D) succulence as measured on all leaves at final harvest, E) dry shoot biomass, F) dry root biomass and G) dry root biomass to dry shoot biomass ratio at final harvest. Beach sites are depicted with blue lines, marsh sites with green and roadside sites with red.

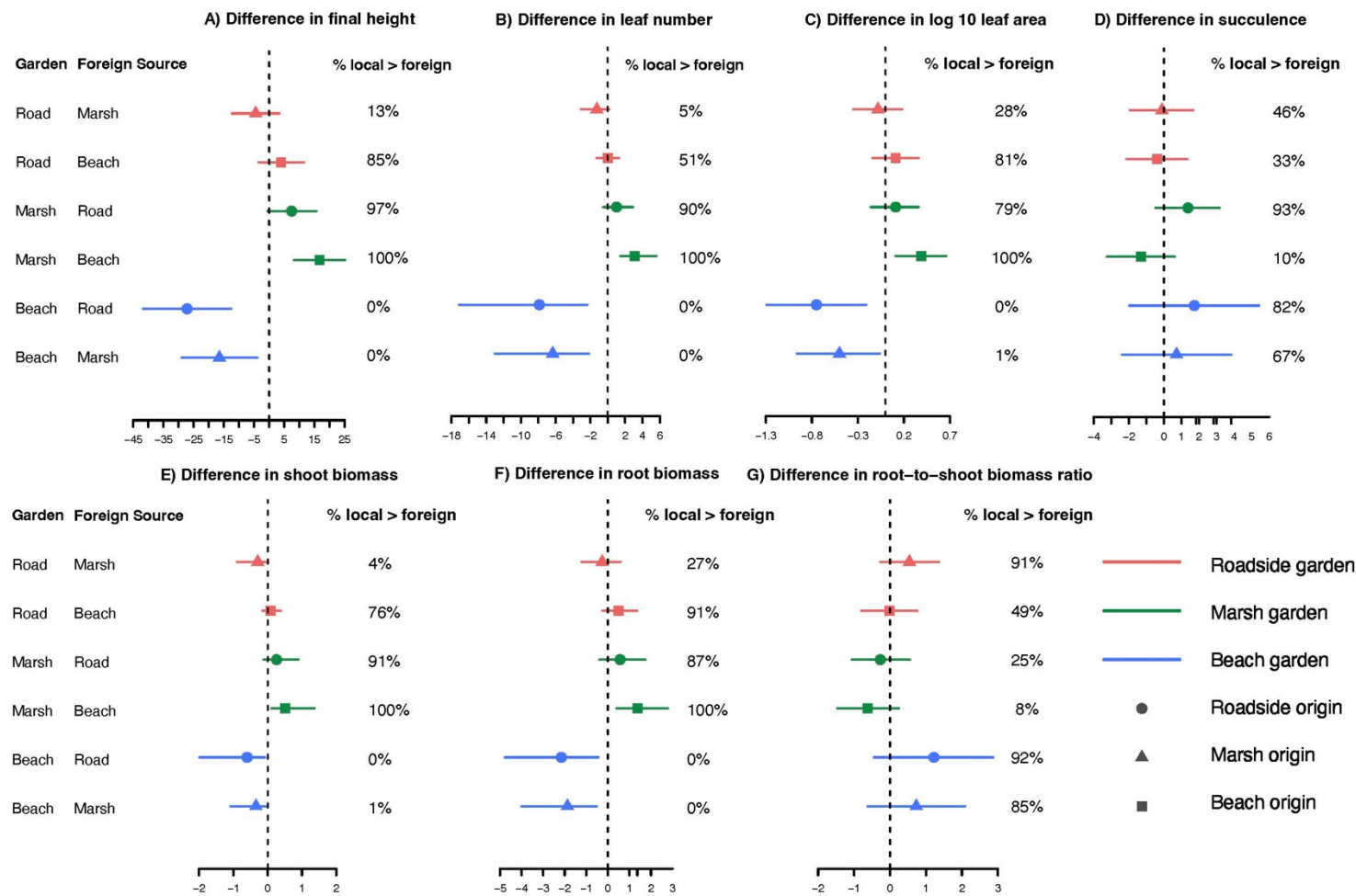


Figure 2. Differences in trait responses across three transplant habitat gardens for plants from the three habitat origins: A) final height, B) total number of leaves, C) total leaf area of all leaves at final harvest, D) succulence as measured on all leaves at final harvest, E) dry shoot biomass, F) dry root biomass and G) dry root biomass to dry shoot biomass ratio at final harvest. Beach sites are depicted with blue lines, marsh sites with green and roadside sites with red.

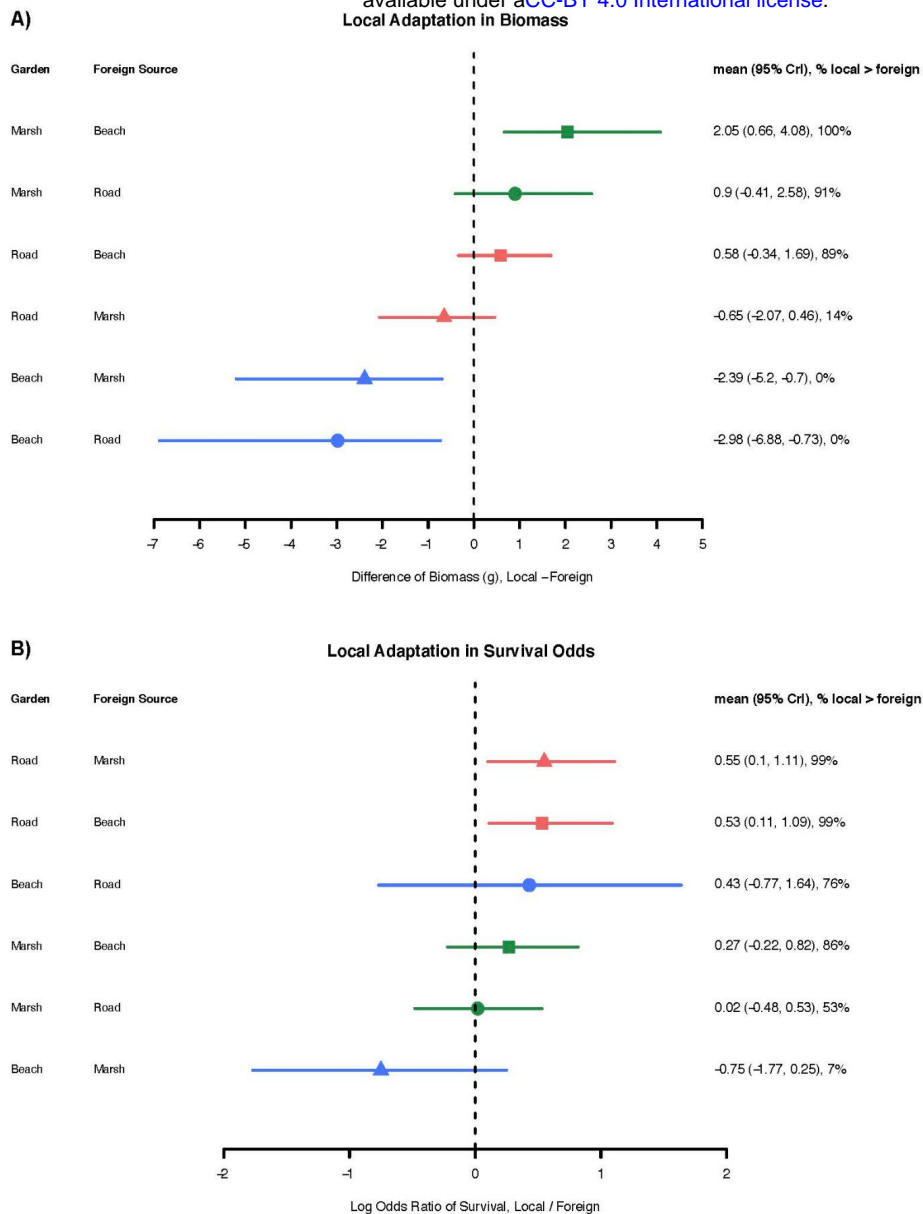


Figure 3. Local adaptation is supported in A) marsh plants compared to beach and roadside plants grown in marsh habitats as measured by total dry biomass (g; top two green lines) and B) survival of roadside plants compared to beach or marsh plants grown in roadside habitats (top two red lines). Symbols and whiskers are differences of fitted estimates and credible intervals estimated from statistical models (see Methods for details).

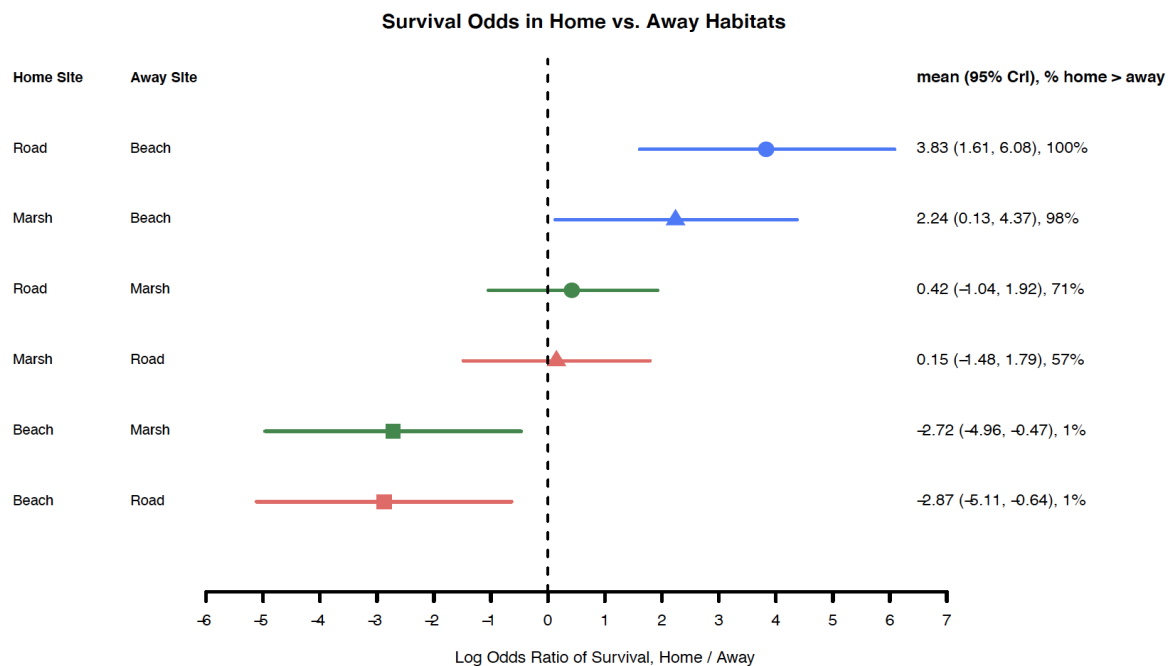


Figure 4. Evidence of differences in survival across habitats. Marsh and roadside plants grow better in their home compared to beach sites while beach plants grow better in marsh and roadside habitats than their home habitat (see Methods for details). Beach sites are depicted with blue lines, marsh sites with green and roadside sites with red.