

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**

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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/ cm<sup>2</sup> 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<sub>10</sub>-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 R<sup>2</sup> for mixed models (Nakagawa and Schielzeth, 2013; Nakagawa et al., 2017) to  
234 determine the conditional R<sup>2</sup> (the variance explained by both the fixed and random effects) and  
235 marginal R<sup>2</sup> (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 ( $r^2_{\text{nakagawa}}$  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” ( $r^2_{\text{prt}}$  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  $r^2_{\text{nakagawa}}$ , 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. × bohemica* 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. × bohemica* 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. × bohemica* 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.

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#### 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>

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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)
<b>Total for transplant 1</b>						<b>18</b>	<b>315</b>	<b>110 (35%)</b>
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)
<b>Total for transplant 2</b>						<b>23</b>	<b>240</b>	<b>35 (15%)</b>
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)
<b>Total for transplant 3</b>						<b>22</b>	<b>375</b>	<b>80 (21%)</b>
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)
<b>Total for transplant 4</b>						<b>24</b>	<b>357</b>	<b>165 (46%)</b>

**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.

	<b>r2_nakagawa</b>		<b>rptR</b>			<b>commonalityCoefficients</b>			
	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%
<b>Average</b>		<b>89%</b>
Marshes		
1	CBH	29%
2	CMM	94%
3	WH	65%
4	RHBH	57%
<b>Average</b>		<b>61%</b>
Roadsides		
1	ST	72%
2	CMR	63%
3	HL	74%
4	RHC	34%
<b>Average</b>		<b>61%</b>

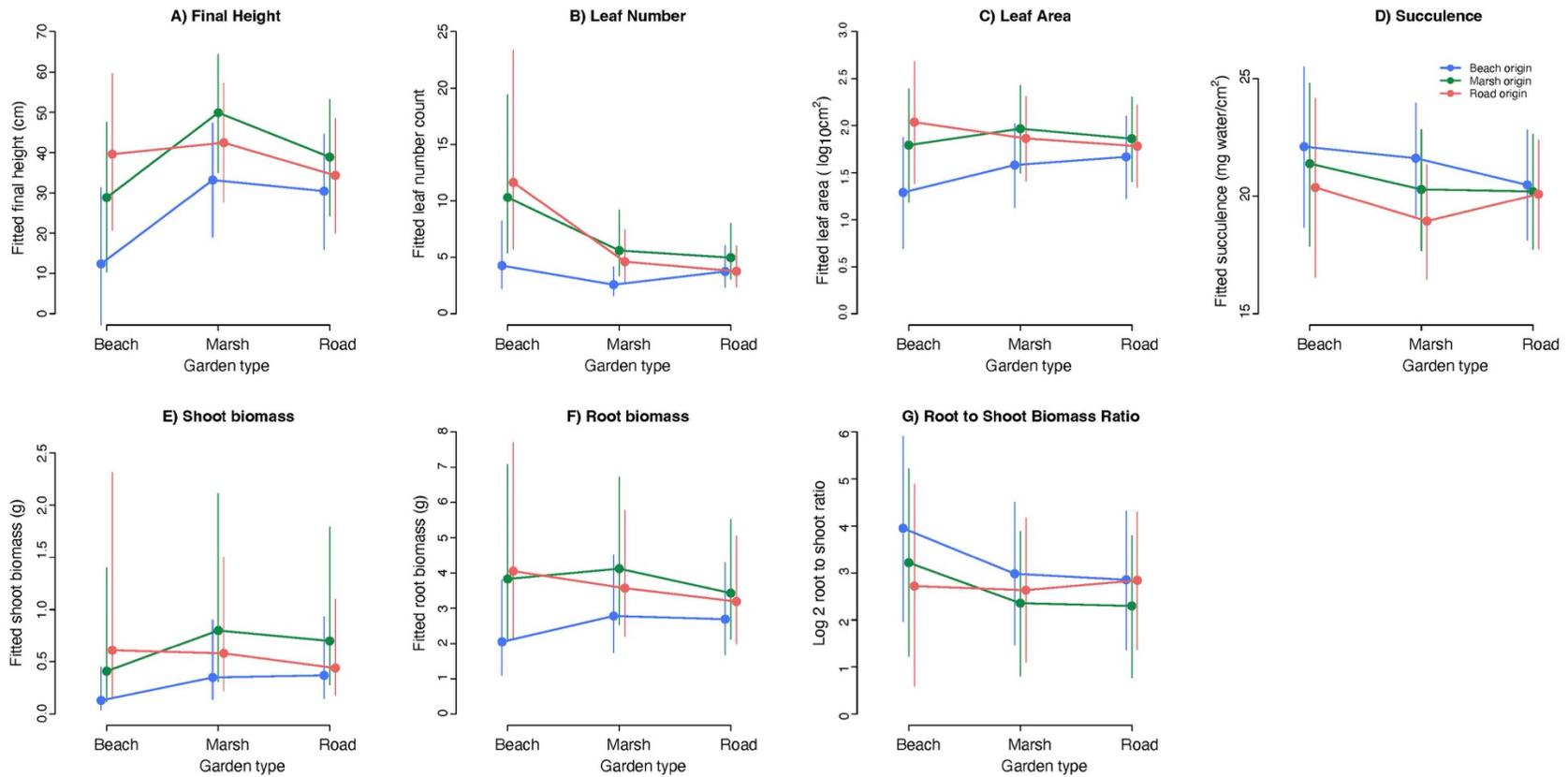
Table S1 Final models for the seven traits.

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)

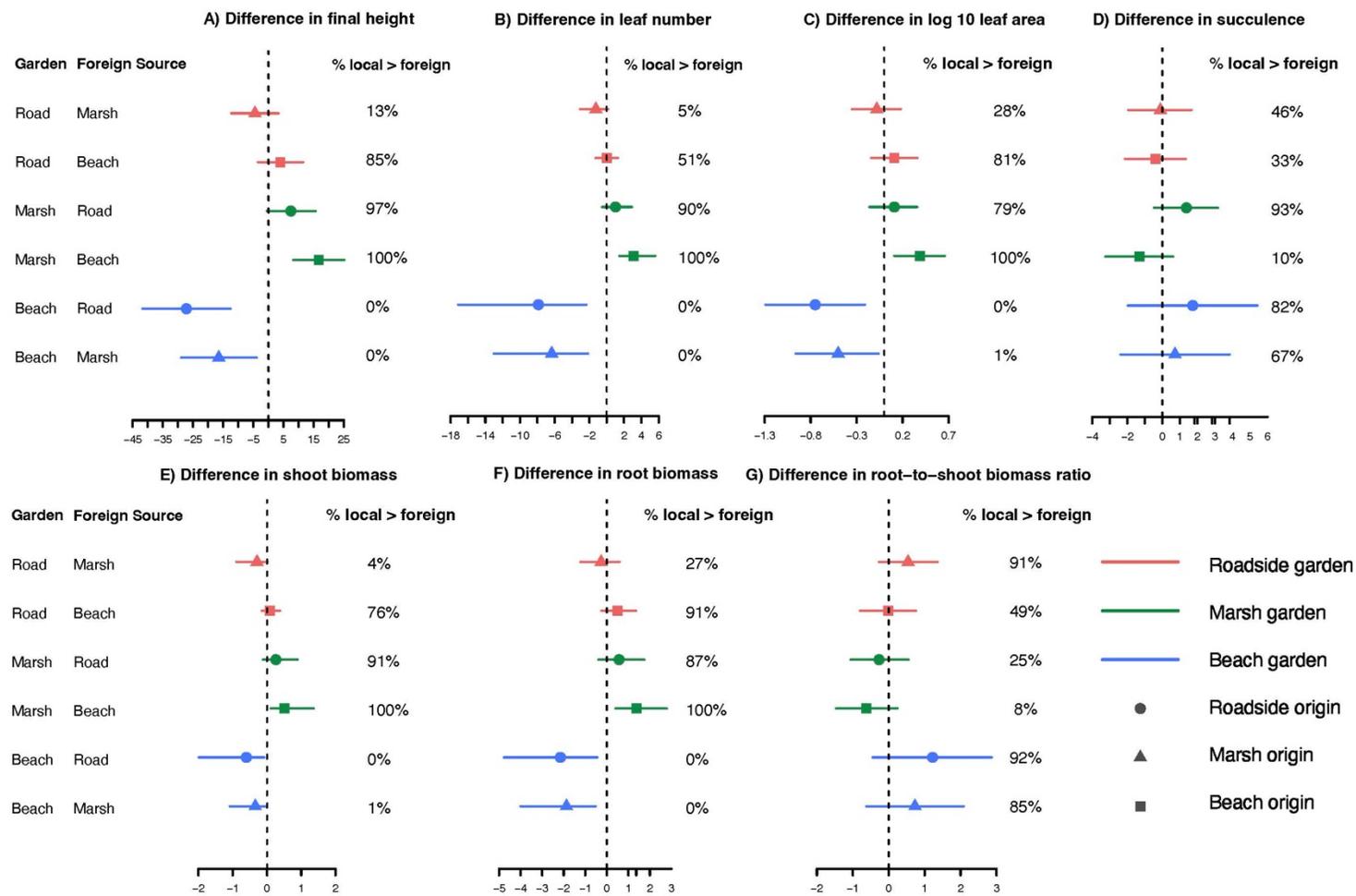
**Table S2.** As with table 2 in the main text, we provide tests for components of variance for each trait with random effects of origin site and transplant site, and fixed effects of source habitat type and transplant garden habitat type as well as the fixed effect of transplant group. The three test 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]

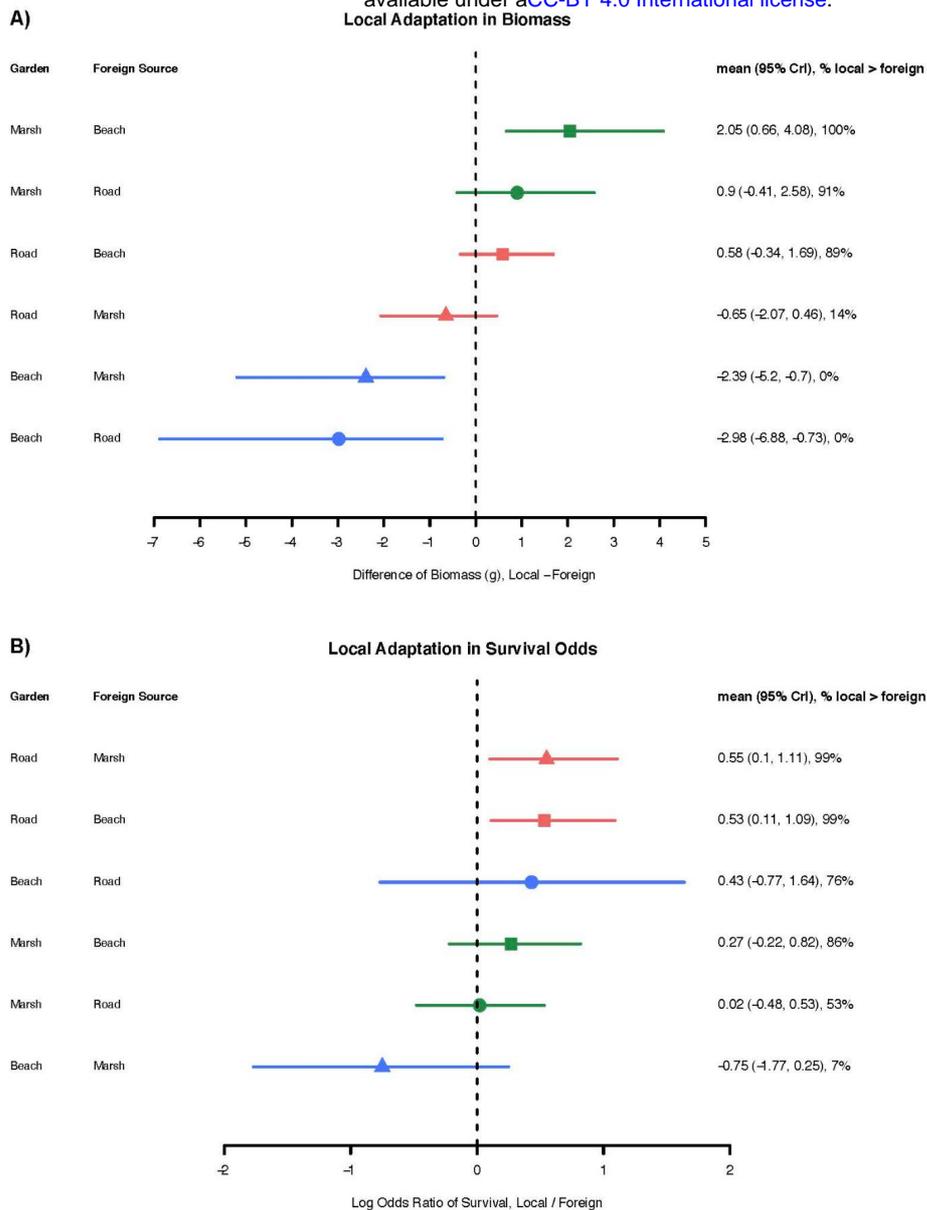
	<b>commonalityCoefficients</b>							Total
	Unique to Transplant group (fixed)	Unique to Source type (fixed)	Unique to Garden type (fixed)	Common to Group & Source type	Common to Group & Garden type	Common to Source type & Garden type	Common to Group, Source & Garden type	
Final height	0.175	0.055	0.062	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



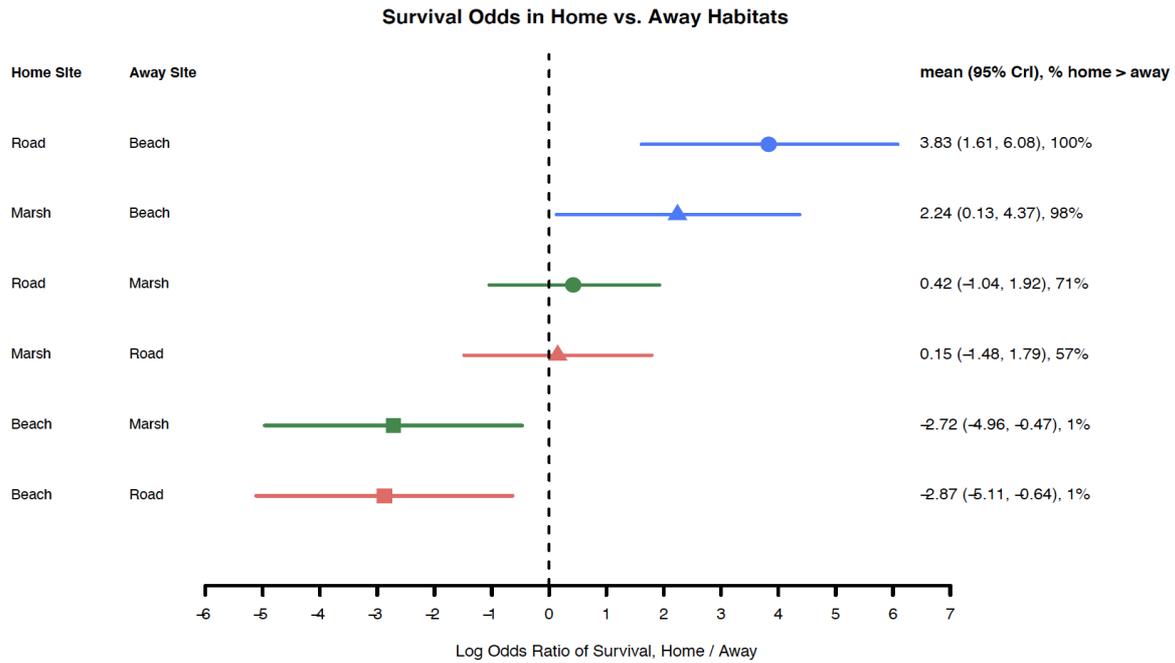
**Figure 1.** Reaction norms of (means  $\pm$  95% CrI) across three transplant habitat gardens for plants from the three habitat origins: A) final height, B) total number of leaves, C) total leaf area of all leaves at final harvest, D) succulence as measured on all leaves at final harvest, E) dry shoot 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.