Title: Dissecting the genetic architecture of shoot growth in carrot (*Daucus carota* L.) using a diallel mating design

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Article Summary

Breeding for improved competitive ability is a priority in carrot, which suffers yield losses under weed pressure. However, improvement and in-depth genetic studies for these traits relies on knowledge of the underlying genetic architecture. This study estimated heritable and non-heritable components of carrot shoot growth from a diallel mating design using a Bayesian mixed model. Results directly contribute to improvement efforts by providing estimates of combining ability, identifying a useful tester line, and characterizing the genetic and non-genetic influences on traits for improved competitive ability in carrot.

ABSTRACT

Crop establishment in carrot (*Daucus carota* L.) is limited by slow seedling growth and delayed canopy closure, resulting in high management costs for weed control. Varieties with improved growth habit (i.e. larger canopy and increased shoot biomass) may help mitigate weed control, but the underlying genetics of these traits in carrot is unknown. This project used a diallel mating design coupled with recent Bayesian analytical methods to determine the genetic basis of carrot shoot growth. Six diverse carrot inbred lines with variable shoot size were crossed in WI in 2014. F1 hybrids, reciprocal crosses, and parental selfs were grown in a randomized complete block design (RCBD) with two blocks in CA (2014, 2015) and in WI (2015). Measurements included canopy height, canopy width, shoot biomass, and root biomass. General and specific combining abilities were estimated using Griffing's Model I. In parallel, additive, inbreeding, epistatic, and maternal effects were estimated from a Bayesian linear mixed model, which is more robust to dealing with missing data, outliers, and theoretical constraints than traditional biometric methods. Both additive and non-additive effects significantly influenced shoot traits, with non-additive effects playing a larger role early in the growing season, when weed control is most critical. Results suggest that early season canopy growth and root size express hybrid vigor and can be improved through reciprocal recurrent selection.

53 **Author Contribution Statement**

54 SDT and PWS conceived and designed this study. SDT performed the crosses, collected
55 phenotypic data, ran analyses, and wrote the manuscript. PLM and WV contributed to
56 descriptions of BayesDiallel and assisted with corresponding analyses and interpretations. BSY
57 helped perform imputation and contributed to the presentation and interpretation of results. PLM,
58 WV, BSY, and PWS provided comments on the manuscript prior to submission.

59

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66 **INTRODUCTION**

67 Carrots are the 7th most consumed fresh vegetable in the United States, with an annual
68 per capita consumption of 3.9 kg (USDA-ERS 2016) and a production value of $762 million
69 USD in 2015 (USDA-NASS 2016). In the US, the high alpha- and beta-carotene content in
70 carrots is the leading source of dietary provitamin A (Block 1994; Simon et al. 2009), which is
71 essential for healthy immune function, vision, reproduction, and cellular communication
72 (Institute of Medicine, Food and Nutrition Board 2001; Johnson and Russel 2004; Solomons
73 2012). Despite the economic and dietary importance of carrots, crop establishment and
74 productivity remains limited by erratic germination, slow seedling growth, and delayed canopy
75 closure (Rubatzky et al. 1999). This growth habit, coupled with thin, highly segmented leaf
76 laminae, competes ineffectively with weeds for nutrients, water, and light, resulting in yield
77 losses caused by reductions in root size and marketability (Bellinder et al. 1997; Bell et al. 2000).
78 Furthermore, in a survey of weed competitiveness in 25 crops, carrot had the largest reduction in
79 yield under weed pressure (van Heemst 1985).

80 To limit yield loss, carrots have an extended critical weed-free period ranging from three
81 to six weeks, during which chemical and hand weeding are necessary (Swanton et al. 2010).
82 Hand weeding, while effective, is disruptive to plant growth and requires intensive labor, with
83 estimated costs exceeding $4000 USD/ha (Bell et al. 2000). For organic systems, which
84 constitute 14.4% of carrot acreage in the US (USDA-ERS 2016), hand weeding is typically the
85 primary method of weed management. Even in conventional systems, few herbicides are labeled
86 for carrots and can only be applied when plants reach a threshold height (e.g. linuron) or have
87 five to six true leaves (e.g. metribuzin), by which point weeds have exceeded control stages
88 (Bellinder et al. 1997).

89 Cultivars with increased weed competitiveness offer a low cost, nonchemical, and
90 sustainable addition to an integrated weed management program (Pérez de Vida et al. 2006;
91 McDonald and Gill 2009). Improved competitive ability has been linked to traits that increase
92 resource allocation, such as height and biomass accumulation, in other densely planted crops
93 such as maize (Mohammadi 2007; Zystro et al. 2012), rice (Ni et al. 2000; Fischer et al. 2001;
94 Pérez de Vida et al. 2006), wheat (Lemerle et al. 1996; Murphy et al. 2008), and soybean
95 (Jannink et al. 2000; da Silva et al. 2013). While improvement of these traits offers a potential
96 solution for weed management, it is unknown how these traits are inherited in carrot or how they
97 influence marketability (e.g. root biomass accumulation).

98 The diallel mating design, which consists of pairwise combinations among a group of
99 inbred parents, is a classical breeding approach to identify informative testers, select desirable
100 hybrid combinations, and to determine the primary genetic control for complex traits (Hayman
101 1954a; Hayman 1954b; Gardner and Eberhart 1966). First introduced to plant breeding by
102 Sprague and Tatum in 1942, the relationships generated in a diallel crossing scheme allow
103 estimation of general (GCA) and specific combining abilities (SCA), which correspond to the
104 proportion of additive and non-additive genetic variation, respectively (Sprague and Tatum 1942;
105 Hayman 1954a; Hayman 1954b; Griffing 1956a; Griffing 1956b). However, the diallel mating
106 design is underutilized in genetic studies due to resource constraints, the complexity of the
107 analysis, and untenable assumptions (e.g. no epistasis; independent distribution of genes among
108 parents) (Baker 1978). These challenges often lead to misinterpretation of the estimates obtained
109 from diallel analysis and have been the subject of much debate in the literature (Baker 1978;
110 Hallauer and Miranda 1981; Christie and Shattuck 1992).

111 Several methods of diallel analysis have been described, of which the methodology
112 proposed by Griffing (1956a) is one of the most common. This method estimates the significance
113 of GCA, SCA, and reciprocal cross effects using a general linear model approach and can be
114 modified to test interactions of main effects across environments (Lin et al. 1977; Zhang and
115 Kang 1997). However, these traditional methods are not robust in addressing common issues
116 encountered in field experiments, such as missing data, imbalance, and outliers. Missing data for
117 a single cross is often addressed by list- or pair-wise deletion, thereby substantially reducing the
118 number of observations and power of the analysis.

119 The challenges of missing data and theoretical ambiguity have been addressed by the
120 development of Bayesian methods for diallel analysis, which improve biological interpretability
121 and expand the types of questions that can be addressed (Greenberg et al. 2010; Lenarcic et al.
122 2012). In this study, we used the methodologies developed by Griffing (1956a) and by Lenarcic
123 et al. (2012) to elucidate the relative importance of genetic parameters for shoot growth in
124 carrots. The primary goals of this work were (1) to estimate the inheritance of shoot growth in
125 carrots as a resource to inform selection strategies, identify useful testers, and assess hybrid
126 performance and (2) to present an applied framework for the analysis of multiple environment
127 data using advanced multiple imputation and Bayesian methods.

128

129 **MATERIALS AND METHODS**

130 **Plant material and measurements**

131 Six inbred lines, with canopy heights ranging from short (31.4 cm) to tall (52.8 cm), were
132 selected from the USDA-VCRU carrot breeding program and included L6038, L7550, P0159,
133 Nbh2189, P6139, and B7262 (**Table 1, Figure 1, Figures S1-S2**). Inbred parents were combined

134 in all pairwise combinations for a total of 36 combinations. The resulting F1 progenies,
135 reciprocals, and parental selfs were grown in a randomized complete block design (RCBD) with
136 two blocks. Field sites included the Hancock Agricultural Research Station (Hancock, WI, USA;
137 2015) and the University of California Imperial County Cooperative Extension Station
138 (Holtville, CA, USA; 2015 & 2016). Carrots were grown on 1.5 meter (m) plots with 1 m
139 between-row spacing.

140 Measurements of each trait were taken for three biological replicates per plot and are
141 summarized in **Table 1**. Canopy height (cm) and width (cm) were measured at midseason, 80
142 days after planting (DAP), and at harvest, 130 DAP. At harvest, fresh and dry biomass (g) were
143 recorded separately for both shoot and root tissue. For dry biomass, samples were dried at 60°C
144 in a forced-draft oven until reaching a constant weight. A natural log transformation, ln(x), was
145 applied to biomass measurements to make the data distribution symmetric and stabilize the
146 variance.

147

148 **Statistical Analyses**

149 Diallel data for each phenotype was analyzed using two complementary approaches: a
150 traditional analysis after Griffing (1956a), which, owing to its requirement that data is complete
151 and balanced, was combined here with a multiple imputation procedure; and the recent Bayesian
152 mixed model decomposition of Lenarcic et al. (2012), performed on the raw (unimputed) data.
153 These are described in detail below. All analyses were performed in R. 3.2.2 (R Core Team
154 2016). Data and source code are available at https://github.com/mishaploid/carrot-diallel.

155

156 **Multiple imputation of missing data for Griffing's analysis:** To compensate for imbalance,
157 missing data (**Figure S3**) was imputed using the Multivariate Imputation by Chained Equations
158 package (R package mice; R/mice) (van Buuren and Groothuis-Oudshoorn 2011), and
159 specifically using that package's predictive mean matching method (PMM), which is a general
160 purpose, stochastic regression technique that is suitable for numeric data (Little 1988). The
161 predictors used for PMM were chosen based on recommendations in the R/mice documentation,
162 and included male parent, female parent, cross, year, replication, planting density, and numeric
163 measurements with complete data. The values imputed by the PMM were generated by running
164 its associated Markov chain Monte Carlo (MCMC) sampler until it reached a stationary
165 distribution (usually at around 40 iterations; **Figure S4**), and then recording sampled values from
166 a later iteration (e.g. iteration 70). This was repeated m = 50 times to generate m imputed data
167 sets.
168
169 **Griffing's analysis:** Each of the m imputed data sets was analyzed using Griffing's Method I,
170 Model I (1956a), which treats genotypes and blocks as fixed effects and has the base model:
171
172 $$Y_{ijk} = \mu + g_j + g_k + s_{jk} + r_{jk} + \varepsilon_{ijk}$$
173
174 where μ is the population mean, g_j and g_k are the GCA effects for the jth and kth parents,
175 respectively, s_{jk} is the SCA effect for the cross of the jth and kth parents ($s_{jk} = s_{kj}$), r_{jk} is the
176 reciprocal effect for the cross of the jth and kth parents ($r_{jk} = -r_{kj}$), and ε_{ijk} is the
177 environmental effect for the ijkth observation. Analysis was run using the diallele1 function in the
178 R package plantbreeding (Rosyara 2014), which we modified to include environmental effects

179 and genotype x environment interactions (GxE). The proportion of additive to non-additive
180 genetic variation was estimated from the fixed model by comparing the ratio of mean squares for
181 GCA ($\frac{MS_{GCA}-MS_{Error}}{2\times n}$) and SCA ($MS_{SCA} - MS_{Error}$), where n is the number of parental lines
182 (Baker 1978). Values close to unity suggest higher predictability based solely on GCA. Mean
183 squares and approximate F-tests were pooled following the method proposed by Raghunathan
184 and Dong (2011, unpublished manuscript) (**Table S1**). Estimates for GCA, SCA, and reciprocal
185 effects were combined according to Rubin's rules (Rubin 1987) and as implemented in R/mice
186 (van Buuren and Groothuis-Oudshoorn 2011).
187
188 **Bayesian mixed model for diallel analysis:** Raw data for each phenotype (y_i), measured for
189 individuals $i \in \{1, \dots, n\}$ with female parent j and male parent k, were decomposed into additive
190 (a), inbreeding (b), maternal (m), symmetric cross-specific (v), and asymmetric cross-specific
191 effects (w), as described by Lenarcic et al. (2012) and Crowley et al. (2014) and implemented as
192 a Gibbs sampler in the R package BayesDiallel. Specifically, we used BayesDiallel's full
193 unsexed model ('fullu'):
194
195 $$y_i = \mu + \underbrace{x_i^T\beta}_{\text{user fixed}} + \underbrace{\sum_{r=1}^{R} u_i^{(r)}}_{\text{user random}} + \underbrace{a_{j[i]} + a_{k[i]}}_{\text{additive}} + \underbrace{I_{\{j[i]=k[i]\}}(\beta_{\text{inbred}} + b_{j[i]})}_{\text{inbreeding}} + \underbrace{m_{j[i]} - m_{k[i]}}_{\text{maternal}}$$
196 $$+ \underbrace{I_{\{j[i]\neq k[i]\}} v_{(jk)[i]}}_{\text{symmetric}} + \underbrace{I_{\{j[i]<k[i]\}} w_{(jk)[i]}}_{\text{asymmetric}} + \varepsilon_i \,,$$
197
198 where $j[i]$, $k[i]$ and $(jk)[i]$ respectively denote the female, male and female-male combination
199 relevant to individual i, each group of effects parameters is modeled from its own random effects

200 distribution, e.g. $a_j \sim N(0, \tau_a^2)$, fixed covariates x_i, and R additional random-effect components
201 are included as $u_i^{(r)} \sim N(0, \tau_r^2), \forall\, r \in \{1, \ldots, R\}$. Planting density (1-3 scale, 1=low, 3=high) was
202 included as a fixed covariate to capture linear trends and as a random effect to estimate
203 deviations from linearity. Year (CA2015, WI2015, CA2016) was also included as a random
204 effect. This model was fitted in BayesDiallel using a MCMC Gibbs sampler with 5 chains,
205 10000 iterations, and a burn-in of 1000.

206 Although convention is to model heterosis as a dominance effect, this method instead
207 models inbred-specific deviations from heterozygote-based predictions; that is, homozygotes (i.e.
208 parental selfs), which are a minority in the diallel, are treated as a special class. Thus, additive
209 effects are a dosage effect of parent *j* in combination with another sampled parent. Epistasis,
210 modeled as deviations from additivity, is represented by symmetric and asymmetric effects.
211 Symmetric effects model differences specific to a given cross, regardless of parental inheritance
212 and independent of reciprocal effects (i.e. crosses *jk* and *kj* have the same effect). Asymmetric
213 effects model deviations from symmetric effects due to differences between reciprocal crosses
214 (i.e. *jk* and *kj* have different effects).

215

216 **Diallel variance projection as a heritability-like measure:** In order to report the relative
217 contribution of each diallel inheritance class to a given phenotype, Crowley et al. (2014)
218 proposed the diallel variance projection (VarP). This approach uses the posterior predictive
219 distribution of effects from BayesDiallel to simulate future, complete, perfectly balanced diallels
220 of the same parental lines. In each simulated dataset, the contribution of each inheritance class
221 (additive, inbreeding, etc.) is then calculated as its sum of squares (SS) divided by the total
222 phenotype SS. The resulting proportion, the VarP, is similar to the traditional heritability of

223 Mather and Jinks (1982) and Lynch and Walsh (1998) but with two important differences: 1) it is
224 explicitly prospective, in that it seeks to describe how much additive effects, say, would impact a
225 future experiment; and 2) its estimation is more precise, since it is calculated as a function
226 primarily of the effects parameters (e.g. $a_1, a_2, \ldots, a_6$), which are well informed by the data,
227 rather than of the variance components (τ_a^2, τ_m^2, etc.), which are typically not. VarPs are
228 calculated from multiple posterior draws leading to a complete posterior distribution of the VarP
229 for each inheritance class, summarized here as highest posterior credibility intervals. Credibility
230 intervals that include zero are interpreted as not contributing positive, nonzero information to the
231 prediction of y_i, whereas credibility intervals excluding zero provide strong evidence that an
232 effect is important to the model.
233

234 **RESULTS**

235 **Imputation of missing data**

236 There was a high incidence of missing data due to variation in seed production and
237 disease pressure (**Table 1, Figure S3**). A large proportion of missing data occurred in the
238 WI2015 environment, which was subject to severe infestation by Alternaria leaf blight, a fungal
239 pathogen that causes leaf necrosis and plant death in carrots (Pryor and Strandberg 2001).
240 Distributions of imputed data matched those expected from observed data when accounting for
241 environmental variation (**Figures S5-6**).
242

243 **Additive and non-additive gene action contributed to observed phenotypes**

244 Most phenotypes were positively correlated and significant at α=0.001, with the
245 exception of the ratio for shoot:root biomass with both canopy height and width at 80 DAP

246 (**Figure 2**). Griffing's analysis revealed significant genotypic differences for all phenotypes
247 (**Table 2**), which are reflected in posterior predicted means from BayesDiallel (**Figure 3 a, b;**
248 **Figures S7-12**). For all traits, both GCA and SCA contributed significantly to the observed
249 genotypic variation, suggesting that both additive and non-additive effects are important. The
250 ratio of GCA to SCA varied for each trait but was less than one for all phenotypes except
251 shoot:root ratio, indicating a more prominent role of non-additive gene action for most traits
252 (**Table 2**). This is reaffirmed by the results from BayesDiallel, in which the highest posterior
253 density intervals for inbreeding effects were greater and more dispersed than for additive effects
254 (**Figure 3 c, d; Figures S13-19**).
255
256 **Inbreeding effects differed across genetic backgrounds**
257 Results from Griffing's analysis indicated that the observed phenotypes, with the
258 exception of shoot:root ratio, were largely under non-additive genetic control (**Table 2**), which is
259 also reflected in the posterior predicted means and highest posterior density intervals from
260 BayesDiallel (**Figures S7-19**). These effects are illustrated by canopy height at 130 DAP, for
261 which inbred lines were an average of 8.4 cm shorter than their hybrid counterparts (overall
262 inbreeding, β_{inbred}, in **Figure 3**). Additionally, the intensity of inbreeding also varied across
263 genetic background (**Figure 3**, **Figures S13-19**). Relative to heterozygotes, line L6038 had a
264 minimal net reduction in canopy height of 3.0 cm ($\beta_{inbred} + b_{j[1]}$), while line B7262 had a net
265 22 cm reduction in canopy height ($\beta_{inbred} + b_{j[5]}$) (**Figure 3**).

266 **Identification of superior parents, hybrids, and testers for applied breeding**

267 GCA estimates were compared to determine the relative performance of each parent
268 (**Table 3**). Parent L6038 had negative and significant GCA for all traits except canopy height and
269 width at 80 DAP. Low and significant GCA was also observed in parent L7550 for height (130
270 DAP), shoot biomass, and the ratio of shoot:root biomass. For canopy height, parents with
271 positive and significant GCA included Nbh2189 (130 DAP), P6139 (80 and 130 DAP), and
272 B7262 (130 DAP). Parent Nbh2189 was the only inbred with significant and positive GCA for
273 canopy width (130 DAP). Parents P0159 and B7262 had high and significant GCA for both
274 shoot biomass and the ratio of shoot:root biomass. Positive and significant GCA for root biomass
275 was only observed for parent P0159.

276 GCA estimates largely agree with the results from BayesDiallel, which provided similar
277 rankings based on posterior predicted means (**Figure 3 a, b; Figures S7-12**) and HPD intervals
278 (**Fig. 3 c, d; Figures S13-19**). For canopy height (130 DAP), hybrids with parents L6038 and
279 L7750 were, on average, about 6.5 cm shorter, while hybrids with parents P6139 and B7262
280 were an average of 5.0 cm taller. (**Figure 3**). The observed and posterior predicted means for
281 canopy height (130 DAP) also demonstrate relatively higher values for hybrids with parents
282 P6139 and B7262, as well as lower values for hybrids containing parents L6038 and L7550
283 (**Figure 3 a, b**).

284 SCA effects were identified as crosses that performed better or worse than expected
285 based on the GCA values of the contributing parents (**Table 4**). Hybrid Nbh2189 x P6139 had
286 high SCA for both height and width (80 and 130 DAP). For shoot biomass, the largest SCA was
287 observed in hybrid Nbh2189 x B7262. Hybrids with high SCA for root biomass included L7550
288 x B7262, P0159 x Nbh2189, and Nbh2189 x B7262. No significant positive effects were

289 observed for shoot:root ratio. Line Nbh2189 was the most discriminating parent in hybrid
290 combination for all phenotypes except root biomass, suggesting it can serve as a valuable tester
291 for shoot growth in applied breeding.
292
293 **Non-genetic factors and interactions influenced observed variation**
294 Highly significant reciprocal effects were detected for all traits in Griffing's analysis
295 (**Table 2**), suggesting parent-of-origin influences phenotypic expression. For increasing height
296 and width (80 and 130 DAP), lines L6038 and P0159 tended to perform best as female parents
297 and lines L7550 and B7262 tended to perform best as male parents. Significant increases were
298 also observed for shoot biomass, root biomass, and shoot:root ratio when line L7550 was used as
299 a female parent and when lines P0159 and Nbh2189 were used as male parents.
300 Genotype by environment interaction (GxE) was significant for canopy height (80 and
301 130 DAP), shoot biomass (fresh and dry), and shoot:root ratio (**Table 2**). For corresponding
302 traits with significant GCAxE, SCAxE, and ReciprocalxE interactions, estimates for each year
303 are provided in **Tables S2-4**. Significant GCAxE interactions were observed for canopy height
304 (80 and 130 DAP), shoot biomass, and shoot:root ratio. For canopy height (130 DAP), GCA
305 ranked consistently negative across environments for parents L6038 and L7550 (**Figure 4**).
306 Parent Nbh2189 had positive GCA in all environments, but effects were only significant for the
307 CA2015 and CA2016 locations (**Table S2**). In general, relative rankings for parents remained
308 consistent across environments, but the ability to distinguish significant effects was diminished
309 in the WI2015 environment. The performance of parents P0159 and B7262 was notably
310 inconsistent and fluctuated between negative and positive values of GCA (**Figure 4**). SCAxE
311 interactions were significant for height (80 DAP), shoot biomass, and shoot:root ratio, but it was

312 still possible to identify consistently high performing hybrids across environments (**Table S3**).
313 Similarly, significant ReciprocalxE interactions were observed for canopy height (130 DAP) and
314 fresh shoot biomass (**Table S4**). Differences across replications within a year were not
315 significant.

316 Additional effects estimated in BayesDiallel included planting density (fixed and
317 random) and year (random) (**Figure S20**). On average, planting density increased plant height in
318 a mostly linear fashion, with a greater effect at 80 DAP (5.39 cm) compared to 130 DAP (3.51
319 cm) (**Figure 5**). Similarly, year had a greater influence at 80 DAP than at 130 DAP, with the
320 highest mean in the WI2015 season and the lowest mean in the CA2016 season (**Figure 5**).

321

322 **Genetic architecture varied across traits and over developmental time**

323 Although most correlations among phenotypes ranged from moderate (r>0.3) to high
324 (r>0.5) (**Figure 2**), genetic architecture varied substantially by trait and across developmental
325 time (**Figure 6**). For canopy height and width, the GCA/SCA ratio increased approximately
326 three-fold from 80 to 130 DAP, demonstrating a higher proportion of non-additive variation
327 early in the growing season (**Table 2**). Results from BayesDiallel exhibit a similar relationship,
328 with overall inbreeding (β_{inbred}) explaining more variation than additive effects for height and
329 width at 80 DAP (**Table 6, Figure 6**).

330 As described by Crowley et al. (2014), the variance projection of the additive diallel
331 inheritance class, VarP[a], can be likened to narrow sense heritability (h^2). Traits with significant
332 additive effects included canopy height at 80 DAP (VarP[a] = 0.22), canopy height at 130 DAP
333 (0.43), canopy width at 130 DAP (0.23), shoot biomass (0.31), root biomass (0.12), and
334 shoot:root ratio (0.29) (**Table 6, Figure 6**).

335 The influence of non-additive variation was largely due to overall inbreeding, which
336 contributed significantly to canopy height at 80 DAP (VarP[B] = 0.20), canopy width at 80 DAP
337 (0.21) and at 130 DAP (0.14), shoot biomass (0.09), and root biomass (0.27) (**Table 6, Figure**
338 **6**). However, parent-specific inbreeding effects, symmetric epistasis, and asymmetric epistasis
339 did not contribute significantly to the predicted phenotypes (**Table 6, Figure 6**). While parent-
340 of-origin is not a genetic effect, it did explain variation for canopy height at 80 DAP (Var[m] =
341 0.07) and 130 DAP (0.04), canopy width at 80 DAP (0.07) and 130 DAP (0.02), and shoot
342 biomass (0.05) (**Table 6, Figure 6**).
343

344 **DISCUSSION**

345 **Primary gene action**

346 In this study, we estimated genetic, parent-of-origin, and environmental effects on carrot
347 growth traits for six carrot inbred lines and their combinations in a 6x6 diallel framework.
348 Significant genetic variation contributed to all carrot growth attributes, suggesting that there is
349 potential to improve these traits in carrot.

350 Apart from canopy width at 80 DAP, all phenotypes had some level of predictability
351 based on inbred performance, as evidenced by the presence of non-zero additive effects. Traits
352 with high additivity included canopy height (130 DAP) and shoot biomass, both of which are
353 well documented as highly heritable polygenic traits that play a fundamental role in plant fitness
354 and adaptation (Khush 2001; Meyer et al. 2004; Peiffer et al. 2014).

355 For the parental lines in this study, we observed varying sensitivity to inbreeding, which
356 could be due to genetic divergence and/or differing levels of prior inbreeding (East 1936;
357 Birchler et al. 2003). This matches expectations based on the biological constraints of

358 outcrossing in carrot, which has putative susceptibility to inbreeding depression (Simon 2000).
359 Consequently, hybrid vigor was evident for canopy height (80 DAP), canopy width (80 DAP),
360 and root biomass, which had high proportions of non-additive genetic variation and significant
361 estimates of inbreeding, suggesting that causative loci may have heterozygote advantage
362 (Charlesworth and Willis 2009). This result coincides with widespread evidence of heterosis in
363 plants, whereby hybrids demonstrate increased developmental speed and greater biomass relative
364 to their inbred parents (East 1936; Birchler et al. 2003; Meyer et al. 2004), and agrees with
365 previous observations of hybrid vigor for root weight in carrot (Simon et al. 1982).
366
367 **Breeding strategies**
368 With discovery of cytoplasmic male sterility (CMS) in carrot (Welch and Grimball
369 1947), breeding strategies transitioned from selection in open-pollinated populations to an
370 inbred-hybrid system, thereby improving crop uniformity and vigor (Peterson and Simon 1986).
371 We expect that traits with a significant contribution of inbreeding, such as canopy height (80
372 DAP), canopy width (80 DAP), and root biomass, will be responsive to commonly used hybrid
373 breeding strategies in carrot, such as reciprocal recurrent selection. Alternatively, selection for
374 traits with high additivity, such as canopy height (130 DAP), canopy width (130 DAP), and
375 shoot biomass, may allow more rapid genetic gain while indirectly selecting for positively
376 correlated traits under non-additive control. For all phenotypes in this study, we identified
377 promising parental lines and hybrids for use in applied breeding. Additionally, line Nbh2189 was
378 notable as a favorable tester for future breeding efforts on shoot growth.
379 Accounting for genotype by environment interaction (GxE) is especially important in
380 biennial crops like carrot, as breeding programs rely on winter nurseries to achieve an annual

381 breeding cycle (Simon and Goldman 2007). We conducted trials in CA and WI, which are two of
382 the leading carrot production regions and representative of common, but contrasting, breeding
383 environments. In general, significant GxE interactions did not affect the ability to identify high
384 and low rankings among parents and hybrids. Thus, we anticipate that environmental differences
385 are important, but should have a minimal impact on selection efforts.
386
387 **Source-sink relationships**
388 Biomass partitioning between the shoot and root is a major consideration in carrot
389 breeding and has been extensively studied, both in a breeding and an ecological context (Barnes
390 1979; Currah and Thomas 1979; Hole et al. 1983; Thomas et al. 1983; Hole and Dearman 1991).
391 The ideotype for carrot shoot growth is rapid initial growth that plateaus following canopy
392 closure, simultaneously reducing the critical weed free period and promoting taproot
393 development. Equally important is avoiding growth habits with large, dense canopies, which
394 foster a microclimate that is conducive to the development of foliar diseases (Simon et al. 2008).
395 Consistent with findings by Hole et al (1983), we found a strong linear relationship
396 between the log transforms of shoot and root biomass (r = 0.69, $P<0.001$). However, the ratio of
397 shoot:root biomass had a wide range across parents (0.19-0.72), providing evidence that high
398 shoot biomass is not necessary to produce roots with high biomass, and vice versa.
399 Previous work has demonstrated rapid and early acquisition of dry matter in carrot
400 storage roots, with the taproot constituting 42% of the plant dry weight at 67 days after sowing
401 (Benjamin and Wren 1978). Interestingly, our results demonstrate that canopy height and width
402 at 80 DAP were negatively correlated with the ratio of shoot:root biomass (r = -0.26 and -0.29,
403 respectively, $P< 0.001$) and positively correlated with root biomass at harvest (r = 0.58 and 0.55,

404 respectively, $P<0.001$). Conversely, canopy height at harvest (130 DAP) was positively
405 correlated with shoot:root ratio (r = 0.39, $P<0.001$) and not significantly correlated with root
406 biomass (r = 0.10, $P=0.18$). This suggests that early shoot growth is important for root biomass
407 accumulation and agrees with previous conclusions that these traits are subject to hybrid vigor.
408

409 **Method of analysis**

410 When applied appropriately, traditional diallel analysis provides valuable information on
411 combining abilities for parental lines and the underlying gene action for complex traits (Griffing
412 1956a). However, the benefits of diallel mating designs are often overshadowed by practical and
413 theoretical constraints of the analysis. The requirement for complete, balanced data is especially
414 restrictive, particularly in crops with poor seed set and limited availability of inbred lines. In this
415 study, we performed classical evaluation using Griffing's Method I and a modern analysis using
416 a general Bayesian approach (BayesDiallel). Both methods provided similar conclusions
417 regarding primary gene action, parental rankings, and hybrid performance.

418 Although the underlying model is more complex, BayesDiallel provided numerous
419 practical advantages over Griffing's analysis, which are thoroughly discussed by Lenarcic et al
420 (2012). Of relevance in this experiment was the robustness of BayesDiallel to missing data,
421 which was a substantial challenge when applying Griffing's model and is pervasive in field
422 experiments. For the latter, we chose to address missing data using multiple imputation, which
423 produces a set of plausible values to replace missing information (Rubin 1987). However, there
424 are several caveats and compromises regarding multiple imputation in that there are inadequate
425 or vague diagnostics and, although simple in principle, methods to pool multi-factor ANOVA
426 results are often vague or are not widely accessible (van Ginkel and Kroonenberg 2014; Grund et

427 al. 2016). Furthermore, the ability of BayesDiallel to analyze data sets in the presence of missing
428 data allows breeders to leverage data from partial diallels, which are often generated by proxy in
429 a breeding program.

430 A notable advantage of BayesDiallel was the option to add covariates for year and
431 planting density as random and fixed effects. Posterior distributions for year matched
432 expectations based on observed data, with higher means observed in WI2015 compared to
433 CA2015 and CA2016. The inclusion of planting density was especially advantageous and
434 matched expectations from previous studies, which demonstrated significant effects of planting
435 density on canopy height and biomass partitioning in carrot (Bleasdale 1967; Benjamin and
436 Sutherland 1992; Traka-Mavrona 1996; Li et al. 1996)

437 Precise estimates of heritability are useful when determining which breeding strategy will
438 result in the most genetic gain. While it is possible to estimate heritability through variance
439 component decomposition using Griffing's model II, this method requires that the parents are
440 selected from a random mating population (at least according to its traditional interpretation; see
441 discussion in Lenarcic et al. 2012), and is not accompanied by a measure of estimation
442 uncertainty. As an alternative, the diallel variance projection (VarP) in BayesDiallel has benefit
443 for applied breeding by (1) describing how several inheritance classes will influence future
444 experiments composed of the same parents, and (2) providing a 95% credibility interval as a
445 measure of uncertainty, which affords more flexibility when designing future experiments and
446 estimating potential for genetic gain.

447

448

449 **Conclusions**

450 The rise of sustainable agriculture has tasked breeders to develop cultivars with improved
451 weed competitive ability. Using traditional and modern approaches, we analyzed diallel data to
452 describe the quantitative inheritance of previously uncharacterized traits in carrot, which have
453 been demonstrated to confer improved competitive ability in other crops. However, future
454 trialing for weed competitive ability in carrot will be essential to validate the utility of these
455 traits, to determine the underlying mechanism of competitive ability (i.e. tolerance or
456 suppression), and to assess relative fitness costs (e.g. trade-offs with yield).

457 Results from this study support applied breeding efforts for carrot shoot growth in
458 numerous ways, most notably through the quantification of inbred performance, the
459 identification of a useful tester line (Nbh2189), and the assessment of potential hybrid
460 combinations. Furthermore, the detailed characterization of the inbred parents in this study
461 provides a foundation for the development of a multi-parental advanced generation intercross
462 (MAGIC) population in carrot, which will facilitate future in-depth genetic studies (Huang et al.
463 2015).

464 Lastly, we demonstrate the utility of the BayesDiallel framework for modeling heritable
465 and non-heritable components of carrot phenotypes. This example will make BayesDiallel more
466 accessible as a resource in the plant breeding community to maximize the potential of diallel
467 experiments, especially in under-resourced crops.

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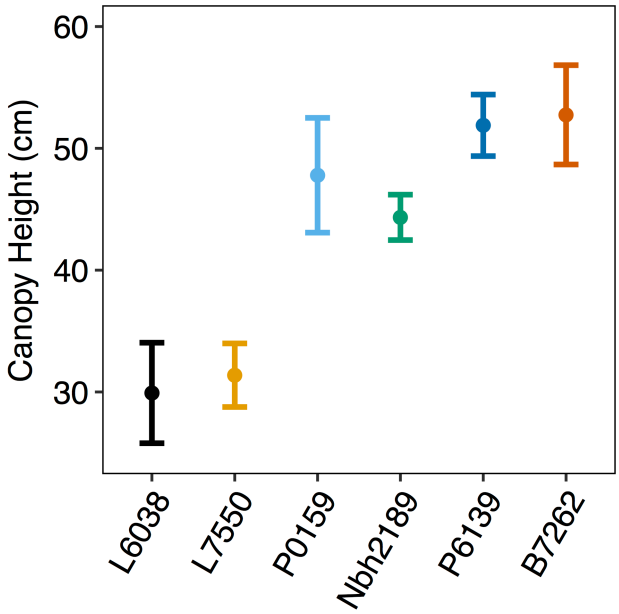


Figure 1. Variation in means and 95% confidence intervals for canopy height (130 DAP) among carrot inbred lines used in this study.

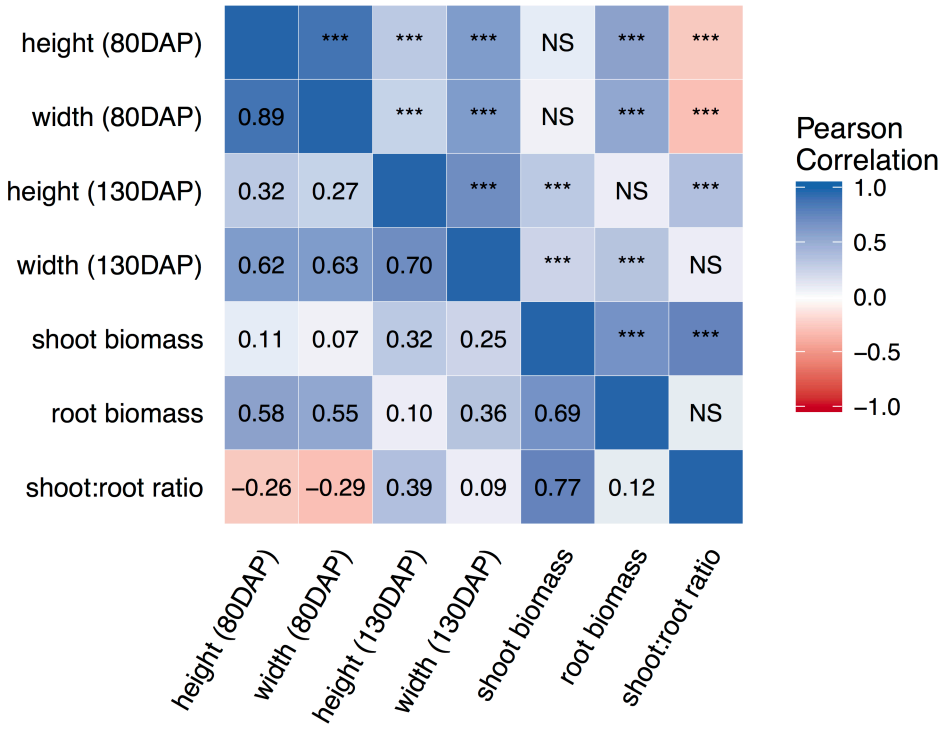


Figure 2. Pearson's correlations (lower diagonal) and significance (upper diagonal) among carrot growth traits measured in this study. Significance codes: *** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$, NS not significant.

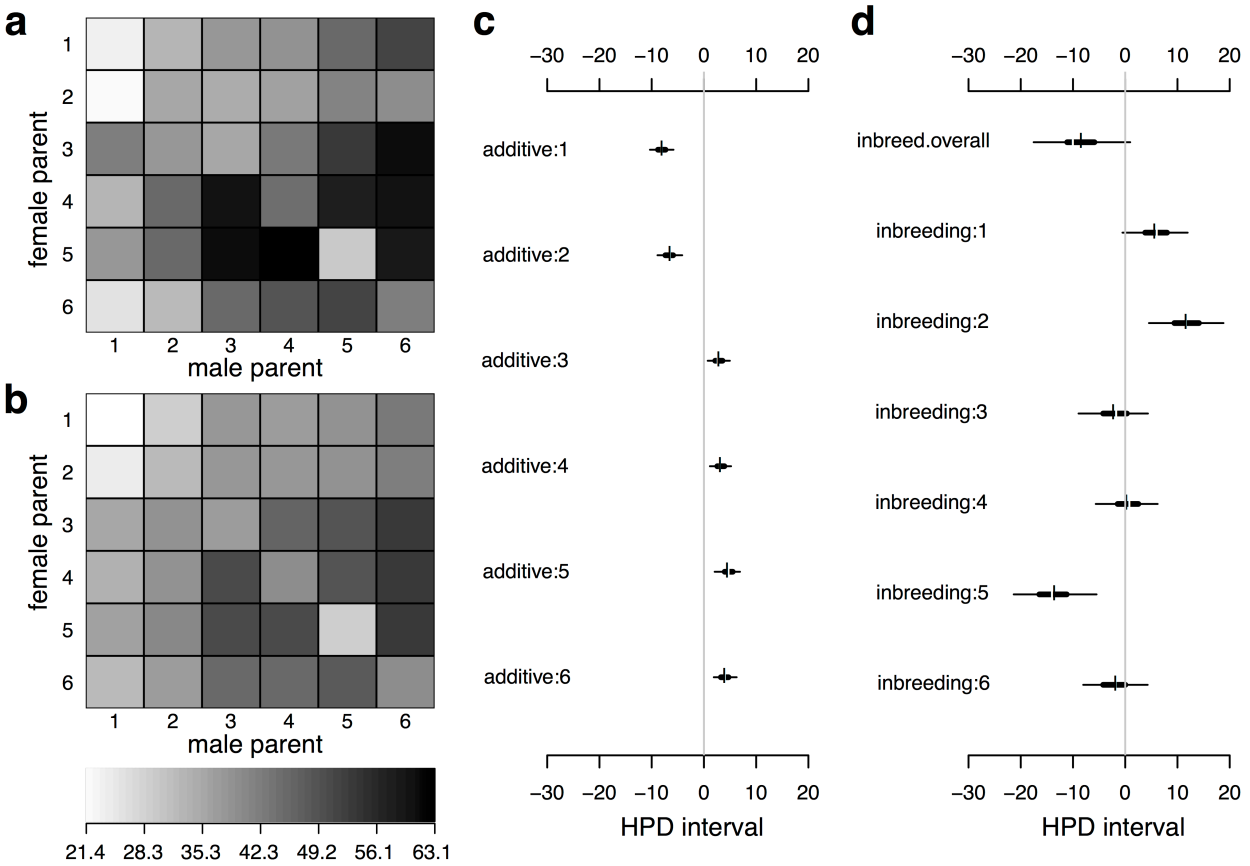
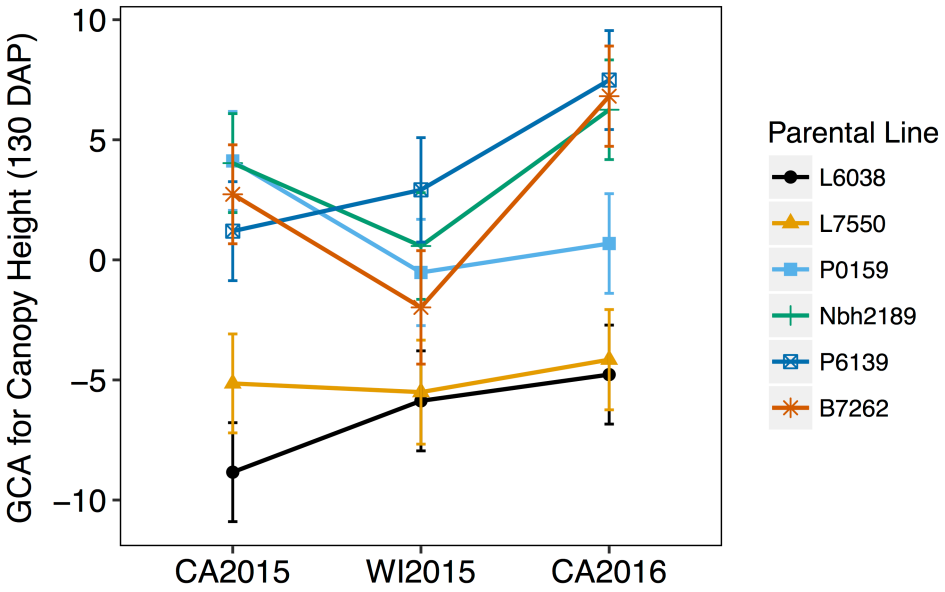


Figure 3. Diallel effects for carrot canopy height (cm) at 130 days after planting. **a** and **b** show observed and predicted means, respectively. Shading indicates height on a scale from 21.4 cm (lighter) to 63.1 cm (darker). Posterior predicted means in **b** are the result of fitting the model in BayesDiallel to the data in **a**. The highest posterior density (HPD) intervals for additive effects are shown in **c** and the HPD intervals for inbreeding effects are shown in **d**. For each effect, thin and thick horizontal lines show the 95% and 50% HPD intervals, respectively, and the vertical break displays the posterior mean. Key: 1 = L6038, 2 = L7550, 3 = P0159, 4 = Nbh2189, 5 = P6139, 6 = B7262.



640

641

642 **Figure 4.** Interaction of general combining ability (GCA) and year (WI2015, CA2015, CA2016)

643 for canopy height at 130 DAP.

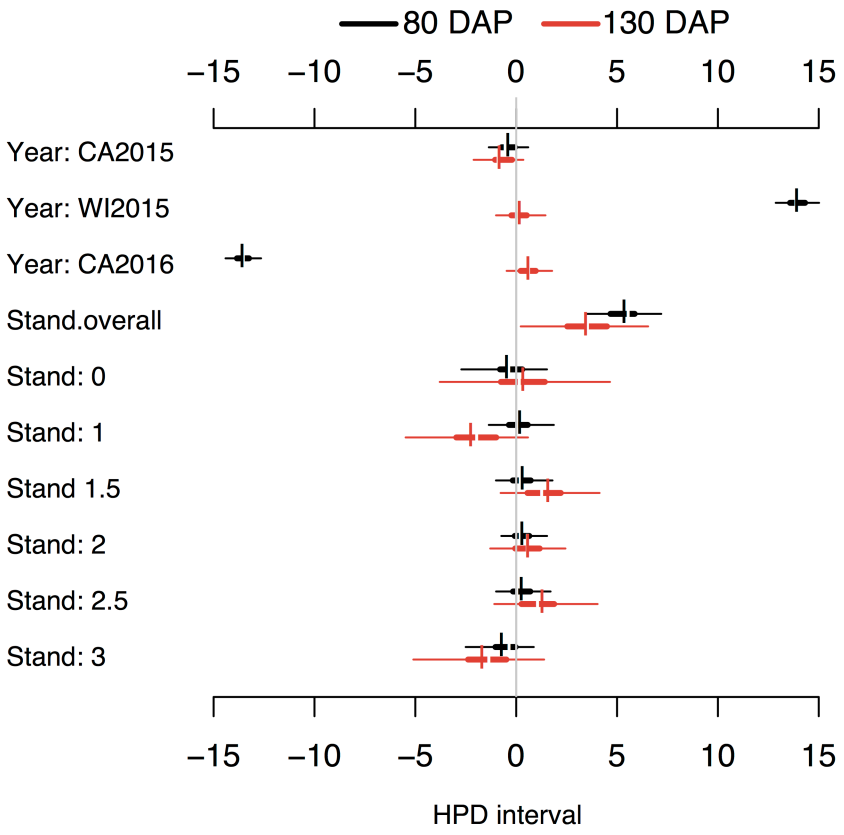


Figure 5. Highest posterior density (HPD) intervals of year and planting density (stand; 1-3 scale, 1 = low, 3 = high) for canopy height at 80 DAP (black) and 130 DAP (red). For each effect, thin and thick horizontal lines show the 95% and 50% HPD intervals, respectively, and the vertical break displays the posterior mean.

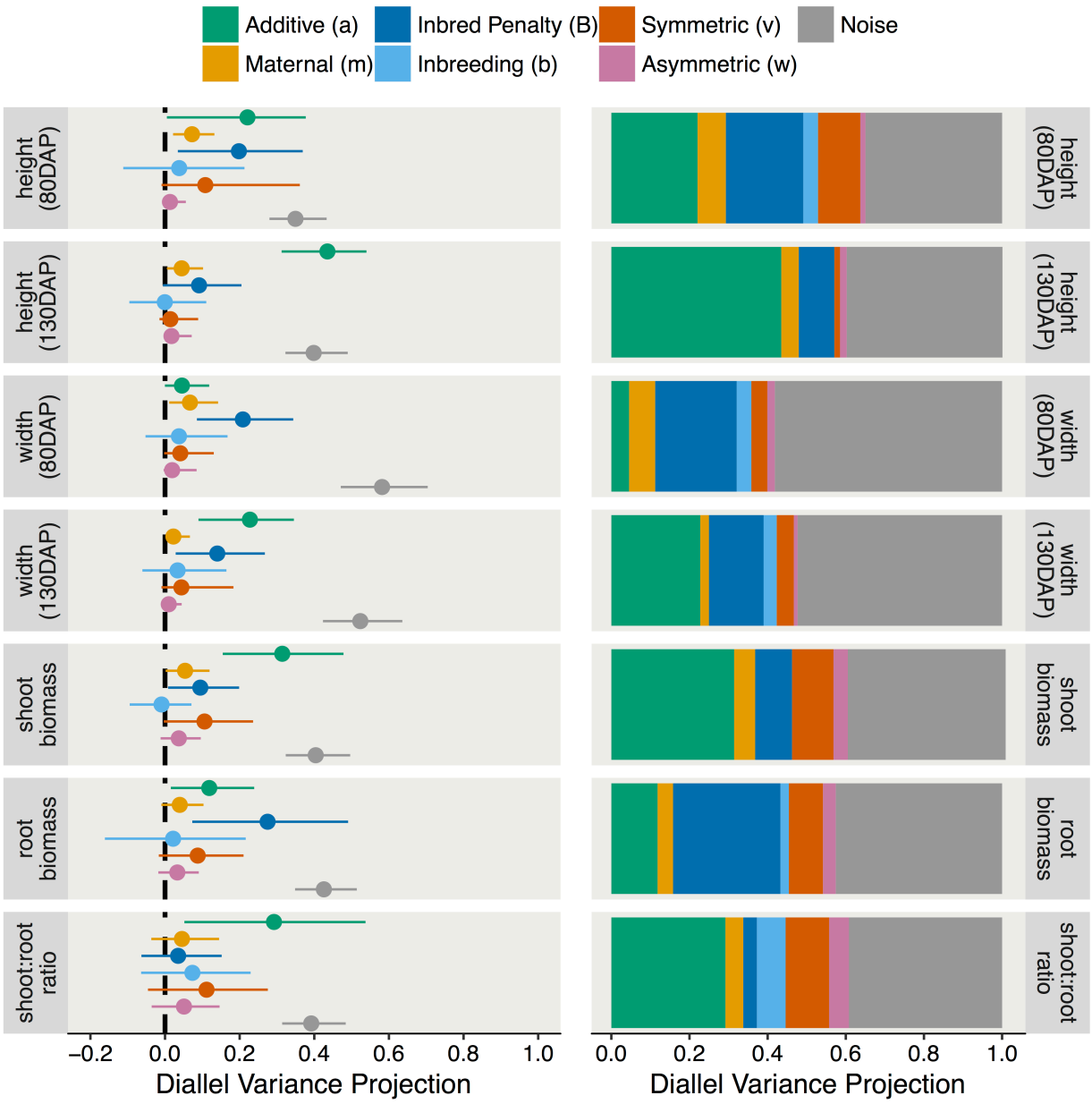


Figure 6. Diallel variance projections characterizing the genetic architecture for each trait, including additive (a), maternal (m), overall inbreeding penalty (B), parent-specific inbreeding (b), symmetric (v), and asymmetric (w) effect classes. **Left:** mean and 95% credibility intervals of effect classes for each trait. **Right:** Mean values showing overall genetic architecture.

654 **Table 1.** Carrot traits evaluated, their range among parents, and number of complete observations for each environment in this study.

655

Phenotype	Measurement	Unit	Parental range	Data Transformation	Number of complete observations[a]		
					CA2015	WI2015	CA2016
Canopy height	Three points within the plot	Centimeters (cm)	29.9 – 52.8	None	72	50	69
Canopy width	Three points within the plot	Centimeters (cm)	41.5 – 61.3	None	72	50	69
Shoot biomass	Fresh and dry	Grams (g)	6.43 – 21.3 (fresh) 1.02 – 3.39 (dry)	ln(x)	72	49	68
Root biomass	Fresh and dry	Grams (g)	29.0 – 64.9 (fresh) 4.22 – 8.64 (dry)	ln(x)	72	49	68
Shoot to root ratio	Shoot biomass/root biomass (dry)	Grams (g)	0.23 – 0.64	ln(x)	72	49	68

[a] 72 observations possible per environment (36 entries x 2 replications)

656 **Table 2.** Griffing's Method I, Model I ANOVA mean square values for carrot growth traits, including: canopy height and width (cm),
657 shoot biomass (g), root biomass (g), and the ratio of shoot:root biomass. The ratio of general combining ability (GCA) to specific
658 combining ability (SCA) variance is also reported.

659

		Height		Width		Shoot Biomass[a]		Root Biomass[a]		Shoot:root ratio[a]
Source	**df**	**80 DAP**	**130 DAP**	**80 DAP**	**130 DAP**	**Fresh**	**Dry**	**Fresh**	**Dry**	
Genotype (G)	35	331.34 ***	489.3 ***	353.57 ***	433.77 ***	1.8 ***	2.13 ***	0.76 ***	0.91 ***	0.24 ***
GCA	5	516.06 ***	1488.95 ***	363.35 ***	1024.37 ***	7.27 ***	8.55 ***	1.13 ***	1.74 ***	1.27 ***
SCA	15	393.69 ***	376.33 ***	362.43 ***	435.66 ***	0.95 ***	1.21 ***	1.11 ***	1.3 ***	0.06 ***
Reciprocal	15	207.42 ***	269.07 ***	341.46 ***	235.02 ***	0.83 ***	0.91 ***	0.28 ***	0.24 **	0.07 ***
Year (E)	2	10794.6 ***	268.7 **	11668.22 ***	944.08 ***	0.11	2.06 ***	17.8 ***	19.71 ***	0.27 ***
G x E	70	25.91 *	68.48 ***	55.6	64.74	0.26 ***	0.28 **	0.12	0.12	0.04 ***
GCA x E	10	39.72 *	134.49 ***	55.63	110.75 *	0.45 ***	0.4 **	0.21 *	0.2	0.08 ***
SCA x E	30	25.26 *	43.07	36.82	50.95	0.26 **	0.31 **	0.14	0.13	0.04 ***
Reciprocal x E	30	21.96	71.88 **	74.37	63.2	0.19 *	0.2	0.09	0.09	0.02
rep(E)	3	24	12.49	56.44	106.03	0.1	0.12	0.14	0.15	0.01
Error	105	15.38	32.14	45.39	53.17	0.12	0.14	0.09	0.1	0.01
GCA/SCA		0.11	0.35	0.08	0.21	0.72	0.65	0.09	0.11	2.35

*** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$

[a] Natural log transformation; measured at harvest (130 DAP)

660 **Table 3.** Pooled estimates of general combining ability (GCA) for carrot growth traits combined across all growing environments.

	Height		Width		Biomass[a]		
Parent	**80 DAP**	**130 DAP**	**80 DAP**	**130 DAP**	**Shoot**	**Root**	**Shoot:root ratio**
L6038	-1.39	-6.5***	0.09	-3.5**	-0.53***	-0.19*	-0.19***
L7550	-2.83	-4.94***	-0.63	-2.23	-0.15	-0.03	-0.08**
P0159	0.85	1.43	-1.56	-1.41	0.39***	0.27***	0.1***
Nbh2189	2.43	3.62**	3.15	7.13***	0.11	0.06	0.03
P6139	3.52*	3.87**	1.86	0.53	-0.15	-0.07	-0.03
B7262	-2.58	2.52*	-2.92	-0.53	0.33***	-0.03	0.18***
Grand Mean	32.27	45.77	43.57	53.74	1.55	2.55	0.6

*** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$

[a] Natural log transformation; dry weight as measured at harvest (130 DAP)

661

662 **Table 4.** Pooled estimates of specific combining ability (SCA) for carrot growth traits combined

663 across all growing environments.

664

	Height		Width		Biomass[a]		
F1 hybrids	**80 DAP**	**130 DAP**	**80 DAP**	**130 DAP**	**Shoot**	**Root**	**Shoot:root ratio**
L6038 x L7550	-0.53	-0.75	-1.4	-2.27	0.1	0	0.06
L6038 x P0159	1.05	1.83	1.82	2.86	0.12	0.17	0.05
L6038 x Nbh2189	1.01	-2.03	0.45	-2.62	-0.24	-0.06	-0.1*
L6038 x P6139	1.66	0.96	-0.8	2.43	0.21	0.15	0.06
L6038 x B7262	2.05	0.95	4.62	2.67	-0.06	0.09	-0.04
L7550 x P0159	-1.08	-1.75	-1.91	0.61	0.1	0.19	-0.01
L7550 x Nbh2189	0.48	-0.63	-1.97	-0.28	0.28	0.04	0.09
L7550 x P6139	0.34	0.68	0.01	-0.76	-0.05	-0.03	0.01
L7550 x B7262	-3.43	-2.92	0.96	-1.45	0.33*	0.39***	0
P0159 x Nbh2189	1.46	1.77	1.38	0.23	0.09	0.28**	-0.05
P0159 x P6139	4.04*	4.99*	3.95	1.24	-0.14	-0.04	-0.05
P0159 x B7262	4.39**	2.93	4.28	4.79*	-0.12	-0.09	-0.08
Nbh2189 x P6139	5.57***	6.26**	7.01**	7.2**	0.17	0.12	0.05
Nbh2189 x B7262	-5.45**	0.6	-1.66	1.7	0.54***	0.3**	0.08
P6139 x B7262	2.32	3.17	2.73	5*	-0.22	-0.04	-0.09*
Parental selfs							
L6038	-5.24*	-1.97	-4.58	-3.73	-0.16	-0.33*	-0.03
L7550	2.35	4.31	1.74	3.14	-0.44*	-0.38**	-0.08
P0159	-13.12***	-12.06***	-10.72**	-11.63**	-0.43*	-0.83***	0.1
Nbh2189	-6.40**	-4.48	-9.11**	-8.38*	-0.47*	-0.52***	0.01
P6139	-18.85***	-19.93***	-16.83***	-19.55***	-0.15	-0.34*	-0.01
B7262	-3.42	-6.22	-8.6*	-10.37**	-0.81***	-0.88***	0.05

*** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$

[a] Natural log transformation; dry weight as measured at harvest (130 DAP)

665

666 **Table 5.** Pooled estimates of reciprocal cross effects for carrot growth traits over all growing

667 environments.

668

	Height		Width		Biomass[a]		
F1 hybrids	**80 DAP**	**130 DAP**	**80 DAP**	**130 DAP**	**Shoot**	**Root**	**Shoot:root ratio**
L6038 x L7550	4***	4.35**	4.51*	5.25**	-0.1	-0.08	-0.02
L6038 x P0159	-0.91	-2.21	-3.37	-2.62	0.19	0.12	0.05
L6038 x Nbh2189	2.97**	2.43	2.22	3.74*	0.12	0.05	0.03
L6038 x P6139	2.96**	2.76	3.01	3.61*	-0.01	0.1	-0.03
L6038 x B7262	7.18***	9.93***	8.75***	7.14***	-0.15	-0.06	-0.06
L7550 x P0159	-2.89**	-1.61	-3.44	0.32	0.58***	0.28***	0.14***
L7550 x Nbh2189	-7.17***	-4.3	-9.45***	-5.42*	0.77***	0.36***	0.22***
L7550 x P6139	-4.58***	-1.5	-7.17***	-4.13*	0.03	0.04	0.01
L7550 x B7262	-0.24	2.46	1.4	1.04	0.11	0.15	-0.02
P0159 x Nbh2189	-1.5	-6.63***	-3.29	-2.81	0.04	0.04	0.01
P0159 x P6139	-4.72***	-2.96	-6.78***	-4.24*	-0.01	-0.01	0.01
P0159 x B7262	3.18**	6.74***	1.31	4.09*	-0.07	-0.03	0.01
Nbh2189 x P6139	0.81	-1.92	-0.97	-0.82	0	0.07	-0.02
Nbh2189 x B7262	6.14***	7.35**	7.15**	8.75***	-0.28*	-0.09	-0.08*
P6139 x B7262	3.96***	2.58	5.39**	1.71	-0.01	-0.06	0

*** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$

[a] Natural log transformation; dry weight as measured at harvest (130 DAP)

669 **Table 6.** Diallel variance projection (VarP) for carrot growth traits. Under each trait (column) is listed the predicted percentage and

670 95% credibility intervals of variance that would be attributable to each class of effect. Predictions are conditional on a future complete

671 diallel composed of the same parental lines.

672

	Height		Width		Biomass[a]		
Diallel inheritance class	**80 DAP**	**130 DAP**	**80 DAP**	**130 DAP**	**Shoot**	**Root**	**Shoot:root ratio**
Additive (a)	22.07 (0.49,37.72)	43.49 (31.24,53.98)	4.56 (-0.04,11.83)	22.75 (8.96,34.53)	31.43 (15.47,47.83)	11.84 (1.55,23.9)	29.21 (5.18,53.73)
Parent of origin (m)	7.24 (2.15,13.23)	4.48 (0.49,10.19)	6.67 (1.12,14.21)	2.22 (0.14,6.66)	5.37 (0.14,11.93)	3.95 (-0.98,10.31)	4.55 (-3.68,14.49)
Overall inbreeding (B)	19.8 (3.43,36.9)	9.1 (-0.57,20.49)	20.87 (8.54,34.36)	13.98 (2.85,26.76)	9.44 (0.82,19.86)	27.48 (7.3,49.06)	3.51 (-6.33,15.18)
Inbreeding (b)	3.79 (-11.2,21.29)	-0.08 (-9.57,11.05)	3.74 (-5.23,16.75)	3.37 (-6.07,16.45)	-0.91 (-9.46,7.08)	2.17 (-16.12,21.62)	7.34 (-6.41,22.94)
Symmetric epistasis (v)	10.82 (-0.93,36.13)	1.44 (-1.49,8.87)	4.09 (-0.24,13.07)	4.36 (-0.95,18.33)	10.6 (-0.34,23.6)	8.73 (-1.67,21.04)	11.12 (-4.57,27.55)
Asymmetric epistasis (w)	1.32 (-0.46,5.57)	1.73 (-0.2,7.15)	1.92 (-0.38,8.46)	1 (-0.1,4.46)	3.68 (-1.19,9.57)	3.27 (-1.8,9.05)	5.08 (-3.58,14.62)
Total variance explained	65.03 (56.69,72.02)	60.15 (51.04,67.74)	41.85 (29.6,52.9)	47.69 (36.41,57.67)	59.62 (50.38,67.66)	57.43 (48.6,65.16)	60.81 (51.58,68.61)
Unexplained variance	34.97 (27.98,43.31)	39.85 (32.26,48.96)	58.15 (47.1,70.4)	52.31 (42.33,63.59)	40.38 (32.34,49.62)	42.57 (34.84,51.4)	39.19 (31.39,48.42)

[a] Natural log transformation; dry weight as measured at harvest (130 DAP)

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684 evaluated.

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697