1   **Title: Correlational selection and genetic architecture promote the leaf economics spectrum**

2   **in a perennial grass**

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Abstract

15

16   The leaf economics spectrum (LES) is hypothesized to result from a trade-off between

17   resource acquisition and conservation. Yet few studies have examined the evolutionary

18   mechanisms behind the LES, perhaps because most species exhibit relatively specialized leaf

19   economics strategies. In a genetic mapping population of the phenotypically diverse grass

20   *Panicum virgatum*, we evaluate two interacting mechanisms that may drive LES evolution: 1)

21   genetic architecture, where multiple traits are coded by the same gene (pleiotropy) or by genes in

22   close physical proximity (linkage), and 2) correlational selection, where selection acts non-

23   additively on combinations of multiple traits. We found evidence suggesting that shared genetic

24   architecture (pleiotropy) controls covariation between two pairs of leaf economics traits.

25   Additionally, at five common gardens spanning 17 degrees of latitude, correlational selection

26   favored particular combinations of leaf economics traits. Together, these results demonstrate

27   how the LES can evolve within species.

28

Introduction

30   Globally, plants exhibit a correlated suite of functional traits known as the worldwide leaf

31   economics spectrum (Wright et al. 2004, Shipley et al. 2006, Reich 2014). This spectrum is

32   hypothesized to result from a trade-off between resource acquisition in high resource

33   environments and resource conservation in low resource environments (Donovan et al. 2011).

34   The acquisitive end of this spectrum is characterized by a quick-return on investment strategy of

35   short-lived leaves with high nutrient content and metabolic requirements. The conservative end

36   is characterized by a slow-return on investment strategy of long-lived leaves with low nutrient

37   content and metabolic requirements. While evidence for the worldwide leaf economics spectrum

38   (LES) is highly consistent at the global scale (Wright et al. 2004, Díaz et al. 2016), results have

39   been mixed at smaller spatial and taxonomic scales (e.g., Heberling and Fridley 2012, Edwards

40   et al. 2014, Messier et al. 2017, Anderegg et al. 2018). These results have prompted several

41   studies to examine how the LES originates, using either statistical (e.g., Shipley et al. 2006,

42   Mason et al. 2016) or anatomical (e.g., Blonder et al. 2011, John et al. 2017, Onoda et al. 2017)

43   perspectives. An important step toward resolving discrepancies among studies is to shift from

44   describing the LES at different scales to examining the evolutionary forces that form the LES

45   (Donovan et al. 2011). Two evolutionary mechanisms that can interact to promote or constraint

46   LES evolution are correlational selection and genetic architecture.

47   Selection for favored trait combinations, or against maladapative trait combinations (i.e.,

48   correlational selection), is likely an important driver shaping functional trait correlations

49   (Donovan et al. 2011). But the evolutionary responses to selection are also shaped by underlying

50   genetic variation and covariance among traits (i.e., genetic architecture). Patterns of genetic

51   covariance determine the possible trajectories of evolution and can constrain or facilitate the

52   evolution of trait combinations (Walsh and Blows 2009). Thus, studying how adaptive

53   syndromes like the LES evolve, requires accounting for both genetic architecture and

54   correlational selection. Genetic architecture can arise from pleiotropy, where the same gene

55   codes for multiple traits; from linkage, where genes coding for different traits are in close

56   physical proximity and thus, are usually inherited together; or through linkage disequilibrium

57   (LD), where alleles at different loci, and impacting different traits, are in statistical association

58   (Lynch and Walsh 1998, Mackay 2001). Correlational selection occurs where selection acts on

59   the covariance between two or more traits and those trait combinations reduce or enhance fitness

60   (Lande and Arnold 1983). By favoring traits in combination, rather than isolation, correlational

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61   selection can promote phenotypic integration (Svensson et al. 2021), potentially explaining the

62   presence of correlated leaf economics strategies. Moreover, the two mechanisms, correlational

63   selection and genetic architecture (pleiotropy, linkage, LD), can interact to promote or constraint

64   LES evolution (Sinervo and Svensson 2002). When selection favors certain trait combinations,

65   but disadvantageous combinations are genetically linked, evolution of the LES will be

66   constrained (Donovan et al. 2011). Over time though, selection can break down linkage and LD

67   between these disadvantageous combinations (Guilherme Pereira and Des Marais 2020).

68   Correlational selection can also promote the evolution of genetic linkage between traits

69   (Svensson et al. 2021), generating and maintaining the genetic foundation for the leaf economics

70   spectrum. When selection and genetic architecture simultaneously favor the same trait

71   combinations, LES evolution can proceed more rapidly (Donovan et al. 2011).

72   For the LES to evolve, leaf economics traits must exhibit genetic variation. But leaf

73   economics traits and correlations between traits can also be driven by environmental factors, like

74   fertilization and drought (Sherrard and Maherali 2006, Fajardo and Siefert 2018, Ji et al. 2020).

75   Conventional approaches to studying the LES cannot separate these genetic and environmental

76   components (Swenson et al. 2020, Ahrens et al. 2021). However, the potential for LES evolution

77   can be assessed using quantitative genetics techniques (Donovan et al. 2011). Metrics like

78   heritability and genetic correlations, which quantify the genetic component of individual traits

79   and the shared genetic contribution to correlations between traits, can show whether leaf

80   economics traits possess sufficient genetic variation to evolve. Going further, genetic constraints

81   on the LES can be identified through genetic mapping and selection on the LES can be tested

82   using a selection gradient analysis (Donovan et al. 2011). A well-established genetic mapping

83   technique—quantitative trait locus (QTL) mapping—involves crossing individuals from opposite

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84   ends of a phenotypic gradient, then testing for associations between the genome and phenotype

85   (Mauricio 2001, Anderson and Mitchell-Olds 2011). Selection gradients can be estimated as the

86   effect of a trait or trait combination on plant fitness and can be used to detect selection (Brodie

87   et al. 1995, Conner and Hartl 2004, Caruso et al. 2020)

88   Recent efforts to quantify intraspecific variation in leaf economics traits has improved

89   our understanding of the LES (Siefert et al. 2015, Messier et al. 2017, Anderegg et al. 2018).

90   But, although intraspecific variation is required for evolution, these intraspecific studies rarely

91   address how the LES evolves. To examine LES evolution, we leverage the strong ecotypic

92   divergence in leaf economics strategies in *Panicum virgatum* L, a widespread C_4 grass

93   (Aspinwall et al. 2013). First, we examined the genetics of three leaf economics traits—leaf mass

94   per area (LMA), leaf nitrogen content (N_{MASS}), and photosynthetic rate (A_{MASS})—in a genetic

95   mapping population of *P. virgatum* in a single common garden in central Texas. Then, to test for

96   correlational selection on leaf economics traits, we examined the fitness of clones of these plants

97   at five sites spanning 17 degrees of latitude in the central United States. Together, this allowed us

98   to answer four questions: 1) are leaf economics traits under detectable genetic control? 2) do leaf

99   economics traits covary? 3) is covariation amongst traits genetically driven? 4) do particular

100   combinations of leaf economics traits increase fitness in the field?

101

102   **Methods**

103   *Study system*

104   *Panicum virgatum* is an ecologically and economically important species possessing

105   large phenotypic variation in leaf economics traits. *Panicum virgatum* is common throughout

106   central North American grasslands, occupying habitats that vary considerably in season length

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107   and mean annual temperature and precipitation (Lowry et al. 2014). To occupy these diverse

108   habitats, *P. virgatum* has diverged into three ecotypes—upland, coastal, and lowland—with the

109   coastal ecotype exhibiting characteristics intermediate between the other two (Casler 2012,

110   Lovell et al. 2021). The upland ecotype typically occurs in northern regions with short growing

111   seasons, and possesses an acquisitive strategy. The lowland ecotype, occurs in southern regions

112   with long growing seasons, and possesses a conservative strategy (Aspinwall et al. 2013,

113   Heckman et al. 2020).

114   *Experimental setup*

115   This experimental design is described in detail by Milano et al. (2016) and (Lowry et al.

116   2019). In summary, a genetic mapping population was developed by crossing the southern

117   lowland genotype AP13 × the northern upland genotype DAC and the southern lowland

118   genotype WBC × the northern upland genotype VS16. A single F_1 offspring from each of these

119   two crosses was then crossed to produce a four-way outbred mapping population, which

120   contained 400 full sibling F_2 offspring. By recombining upland and lowland alleles, this cross

121   generated F_2 individuals that carry either two lowland alleles, two upland alleles, or one lowland

122   and one upland allele at each locus. The F_2 offspring were then clonally propagated in 3.8L pots

123   at Brackenridge Field Laboratory, Austin, TX.

124   In February 2014, individuals of each genotype were transplanted into a common garden

125   at Brackenridge Field Lab, where the soil is Yazoo sandy loam (Milano et al. 2016). The field

126   was first covered with weed cloth and each individual was planted into a hole in the weed cloth.

127   Plants were randomly assigned to locations in the field in a honeycomb design, with each plant

128   located 1.25 m from its four nearest neighbors. To prevent edge effects, the field was surrounded

129   with a border row of plants of the lowland genotype AP13.

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130 *Leaf economics measurements*

131   On 1-7 July 2014, we measured three leaf economics traits: mass-based photosynthetic

132 capacity (A_{MASS}), mass-based leaf nitrogen content (N_{MASS}), and leaf mass per area (LMA). To

133 calculate LMA, we measured the area of one penultimate fully expanded sun leaf per plant using

134 a LI-3000C leaf area meter (LI-COR Biosciences, Lincoln, NE, USA), then dried leaves at 65°C

135 to constant mass and weighed them. LMA is the ratio of leaf dry mass to leaf area. To calculate

136 N_{MASS}, these dried leaves were ground to a fine powder, then combusted in an elemental analyzer

137 (Flash 2000 Organic Elemental NC Analyzer). To measure photosynthetic capacity, we enclosed

138 two penultimate fully expanded sun leaves in the 2×3 cm cuvette of a LI-6400XT (LI-COR

139 Biosciences, Lincoln, NE, USA) between 10:30 and 14:00. PAR was maintained within the

140 cuvette at 1750 µmol m^{-2} s^{-1} using an actinic light source; chamber CO_2 supply was set to 400

141 ppm, and leaf temperature and water vapor were allowed to track ambient conditions. We

142 converted from an area- (A_{AREA}) to mass-basis (A_{MASS}) by dividing A_{AREA} by LMA.

143 *Fitness measurements*

144   Clonally propagated individuals of each F_2 line were planted in spring 2015 at five

145 locations throughout central North America (Kingsville, TX – Brookings, SD) that spanned 17

146 degrees of latitude (details in Lowry et al 2019). Plantings at these five sites were identical in

147 layout to the Austin, TX planting described above (see Lowry et al. 2019 for details). In 2017

148 and 2018, plants were harvested each fall ~ 15 cm above ground level and aboveground biomass

149 was dried and weighed. Biomasses measured in 2017 also appear in Lowry et al. (2019).

150 Previous work has found that aboveground biomass is highly correlated with seed production in

151 *P. virgatum* (Palik et al. 2016), making it a useful proxy for fitness (Lowry et al. 2019).

152 *Quantitative genetic and other statistical analyses*

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153   To assess whether leaf traits in *P. virgatum* represent a leaf economics spectrum, we

154   performed standardized major axis (SMA) regression on each pair of leaf traits using the `sma`

155   function in smatr (Warton et al. 2012). SMA regression is commonly used in functional ecology

156   because, unlike least squares regression, it assumes that both variables are measured with error

157   (Warton et al. 2006).

158   To quantify the degree of genetic control of leaf economics traits, we calculated broad-

159   sense heritability (H^2) of leaf economics traits using the `mmer` function in the sommer package

160   (Covarrubias-Pazaran 2016). Heritability was the ratio of variance among lines (genetic variance,

161   V_G) to total variance (V_G + environmental variance, V_E) in each trait ($V_G / V_G + V_e$), calculated

162   from a model that accounted for the relatedness among individuals by incorporating an additive

163   genetic relatedness matrix. Throughout, we refer to V_G and H^2 rather than additive genetic

164   variance (V_A) and narrow-sense heritability (h^2) because additive and dominance genetic

165   variance are confounded in this full-sib cross (Hill 2013).

166   To estimate the proportion of variance shared among leaf traits due to genetic causes

167   (genetic correlations, r_g), we performed a multivariate analysis using `mmer`. The model

168   included all three leaf economics traits as responses with an additive genetic relatedness matrix

169   as a random effect. To estimate the significance of these genetic correlations, we compared a

170   model in which the genetic covariance between a pair of traits was estimated freely to a model in

171   which the genetic covariance between those traits was constrained to 0 (i.e., no genetic

172   covariance between traits was allowed) using a likelihood ratio test. A significant likelihood ratio

173   test indicates that genetic covariance differs from 0.

174   *QTL mapping*

175   We conducted QTL mapping on leaf economics traits using the qtl2 package (Broman et

176   al. 2019). Methods for constructing the linkage map used in this analysis are described by Lovell

177   et al. (2020). First, we assessed the likelihood that each genetic marker is associated with a

178   phenotype of interest by using the `scan1` function with a leave-one-chromosome-out kinship

179   matrix. This provided log-odds (LOD) profiles for every marker in the genome. From these LOD

180   profiles, we identified putative QTL using the `find_peak` function with drop = 1.5 and peakdrop

181   = 2.5 at a significance threshold of α = 0.15. We determined the LOD score corresponding to

182   this significance threshold via permutation test with 1000 iterations using the `scan1perm`

183   function. We chose a relaxed significance threshold because a stricter threshold could hinder a

184   primary goal of this study—to detect and evaluate QTL overlap among leaf traits. To maintain

185   statistical rigor when evaluating putative QTL, we further assessed the significance and

186   explanatory power of all QTL by fitting a multiple-QTL model for each phenotype using the

187   `makeqtl` and `fitqtl` functions in the qtl package (Broman et al. 2003).

188   When QTL confidence intervals for two traits overlapped, we tested whether this overlap

189   was due to pleiotropy (one QTL) or separate QTL using a pipeline from the qtl2pleio package

190   (Boehm et al. 2019). To do this, we first used the `scan_pvl` function to identify the region of

191   QTL overlap, then used the `find_pleio_peak_tib` function to identify the marker corresponding

192   to the peak of the pleiotropy trace, then used the `boot_pvl` function with 1000 iterations to

193   perform a bootstrapped multivariate QTL scan that evaluates the evidence for separate QTL. In

194   this analysis, the null hypothesis is one QTL (i.e., pleiotropy). Thus, $P > 0.05$ indicates a lack of

195   evidence for separate QTL and is consistent with pleiotropy.

196   *Selection gradient analysis*

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197   To assess linear and non-linear selection on leaf economics traits in the field, we

198   performed a selection gradient analysis. Standard selection gradient analysis pairs a fitness

199   component (or fitness proxy) with traits measured on the same individuals (Lande and Arnold

200   1983). Here, however, we take a somewhat different approach: we measured leaf economics

201   traits on plants at a single site, then pair these predictors with a fitness proxy measured on

202   clonally propagated individuals at multiple sites. Because individuals grown at each site are

203   genetic clones of the individuals measured for leaf economics traits, this is essentially a repeated-

204   measures design. Importantly, we assume that plasticity and genotype-by-environment effects on

205   LES traits are low. For selection gradients, we first mean-standardized genotype-level

206   aboveground biomass separately for each of our five sites and two years by dividing each

207   genotype's biomass by the site-level mean biomass that year (Franklin and Morrissey 2017).

208   This results in a relativized proxy for fitness, such that average fitness at each site is equal to 1;

209   above average fitness > 1; and below average fitness < 1. Plants that died during the study were

210   retained for analysis with biomass = 0. At each of the three northern sites (Brookings, KBS,

211   Columbia), only 0-4 plants were recorded as dead in any year. This corresponds with a mortality

212   rate of 0-1.3%. At the two southern sites, mortality was higher: 5.7% and 16.2% in Kingsville in

213   2017 and 2018, respectively, and 0.3% and 8.2% in Austin in 2017 and 2018, respectively. We

214   then examined how three mean-centered and variance-standardized (mean = 0, sd = 1) leaf

215   economics traits impacted relative biomass in a single linear mixed model using the `lme`

216   function in nlme (Pinheiro et al. 2016). This model included main effects (i.e., linear directional

217   selection), quadratic effects (i.e., non-linear stabilizing or disruptive selection), and all two-way

218   interactions between these three traits (i.e., non-linear correlational selection) and interactions

219   between all leaf economics predictors and site identity. Year of biomass measurement did not

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220   significantly interact with any other model parameters and was removed from the final model.

221   To account for heteroscedasticity, this model included an identity variance structure (varIdent),

222   which allows residual variance to differ by site.

223   To complement phenotypic selection gradients and circumvent the issue of measuring

224   LES traits and biomass on different clonal propagates, we also performed genetic selection

225   gradient analysis (Rausher 1992). To do this, we first calculated genomic best linear unbiased

226   predictors (GBLUPs) for LES traits and biomass using the `mmer` model described above for

227   estimating heritability. GBLUPs represent the additive genetic contribution to a given phenotype.

228   We then performed selection gradient analysis with GBLUPs instead of phenotypic values using

229   the `gls` function. This approach assesses only the genetic relationship between traits, which

230   more accurately describes the evolutionary pressure on LES traits.

231

232   **Results**

233   *Are leaf economics traits under detectable genetic control?*

234   All three traits exhibited moderate broad-sense heritability (H^2; Table 1). Of the three,

235   A_{MASS} had the lowest heritability ($H^2 = 0.26$), while LMA and N_{MASS} had similar, and higher,

236   heritability ($H^2 = 0.51$ and 0.46, respectively). For each trait, we detected 4-5 significant QTL

237   (Table 2), which combined to explain 19-33% of variation in leaf economics traits (Table 1).

238   Together, these results confirm that the leaf economics traits evaluated here have a substantial

239   genetic basis.

240   *Do leaf economics traits covary phenotypically?*

241   In F_2 individuals, there were significant bivariate relationships amongst all leaf

242   economics traits. LMA covaried negatively with N_{MASS} ($R^2 = 0.06$, $P < 0.001$; Fig. 1a) and with

243   A_{MASS} ($R^2 = 0.29$, $P < 0.001$; Fig. 1b), which is consistent with global LES relationships.

244   Similarly, N_{MASS} and A_{MASS} covaried positively ($R^2 = 0.06$, $P < 0.001$; Fig. 1c). Thus, in this

245   system, leaf economics traits significantly, although sometimes weakly, covary phenotypically.

246   *Is covariation amongst traits genetically driven?*

247   We detected 13 QTL for leaf economics traits, all of which were highly significant

248   predictors of LES traits in multiple-QTL models ($P < 0.05$; Table 2). These 13 QTL included

249   three pairs of colocalized QTL (i.e., confidence intervals for the QTL overlapped; Fig. 2a):

250   LMA-A_{MASS} on Chr9K and A_{MASS}-N_{MASS} at two locations on Chr2K. For all three pairs of

251   colocalized QTL, our results are consistent with pleiotropy (i.e., one QTL for both traits) because

252   we did not find evidence that these colocalized QTL were produced by distinct loci ($P = 0.14$, P

253    $= 0.254$, and $P = 0.429$, respectively; Fig. 2b). Further supporting pleiotropy, marker regression

254   showed that genotype effects at colocalized QTL were in the same direction for both traits (i.e.,

255   both conservative or both acquisitive). For instance, individuals possessing two upland alleles on

256   Chr2K@12 had lower A_{MASS} and N_{MASS} than other genotypes. Similarly, at the QTL on Chr9K,

257   individuals with two upland alleles had significantly higher A_{MASS} and significantly lower LMA

258   than other genotypes. In total, these results show that leaf economics traits exhibit some degree

259   of genetic coordination.

260   Despite seeing colocalized QTL for LMA-A_{MASS} and N_{MASS}-A_{MASS}, a significant genetic

261   correlation existed only between LMA and A_{MASS} ($r_g = -0.72$, $\chi^2 = 15.87$, $P < 0.001$; Table 3).

262   Neither LMA-N_{MASS} ($r_g = -0.12$, $\chi^2 = 0.79$, $P = 0.37$) nor A_{MASS}-N_{MASS} ($r_g = 0.26$, $\chi^2 = 1.11$, $P =$

263   0.29) had significant genetic correlations. These contrasting effects may be related to the

264   directionality of QTL for the three LES traits. All five significant QTL for LMA were in the

265   same direction and consistent with a conservative leaf economics strategy among the lowland

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266   ecotype and an acquisitive strategy among the upland ecotype (i.e., individuals with both

267   lowland alleles had high LMA and individuals with both upland alleles had low LMA; Figs. 2d,

268   S2a-d). Conversely, three of the four N_{MASS} QTL and two of the four A_{MASS} QTL behaved

269   opposite to LES predictions: individuals with both upland alleles had low N_{MASS} and low A_{MASS},

270   while individuals with both lowland alleles had high N_{MASS} and high A_{MASS} (Figs 2b-c, S2g).

271   When QTL differ in directionality, as LMA and N_{MASS} did, it may weaken the aggregate genetic

272   correlations (Gromko 1995). Similarly, despite detecting two colocalized QTL for A_{MASS} and

273   N_{MASS}, these pairs of colocalized QTL were all in the opposite direction of typical leaf

274   economics strategies, which could also weaken genetic correlations.

275   *Do particular combinations of leaf economics traits increase fitness in the field?*

276   There was a significant interactive effect of LMA and N_{MASS} on relative biomass across

277   all sites (γ_{ij} = -0.05; P = 0.015; Table S2, S3; Fig. 3a). The relative biomass of individuals

278   possessing conservative values of both traits (i.e., high LMA, low N_{MASS}) was higher than that of

279   individuals with acquisitive values of both traits (i.e., low LMA, high N_{MASS}). Both conservative

280   and acquisitive individuals had greater relative biomass than individuals with mismatched trait

281   combinations (high LMA, high N_{MASS} or low LMA, low N_{MASS}). This correlational selection for

282   particular combinations of LMA and N_{MASS} could promote LES evolution. Additionally, when

283   controlling for other trait values, LMA had a marginally significant direct positive effect on

284   relative biomass across all sites (LMA: β = 0.03; P = 0.081; Table S2, S3).

285   Selection gradients differed among sites, but not systematically by latitude (Table S3, S4;

286   Fig. 3b). Directional selection favored high LMA plants at four of five sites (Site × LMA: P =

287   0.42; KBSM: β = 0.06, P = 0.014; CLMB: β = 0.04, P = 0.095; PKLE: β = 0.09, P = 0.046;

288   KING: β = 0.12, P = 0.063; Table S3, S4), and high A_{MASS} plants at two of five sites (Site ×

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289   A_{MASS}: P = 0.008; KBSM: β = 0.06, P = 0.015; KING: β = 0.13, P = 0.053; Table S3, S4).

290   Selection favoring higher values of both LMA and A_{MASS} combined with a negative genetic

291   correlation between the two traits would generate a genetic constraint on the evolution of the

292   LES in *P. virgatum*. Finally, correlational selection favored matched phenotypic combinations of

293   LMA and N_{MASS} at two sites (Site $\times$ LMA $\times$ N_{MASS}: P = 0.27; BRKG: P = 0.059; PKLE: P =

294   0.045; Table S4; Fig. S2) and matched combinations of A_{MASS} and N_{MASS} at two other sites (Site

295    $\times$ N_{MASS} $\times$ A_{MASS}: P = 0.009; KBSM: P = 0.007; CLMB: P = 0.018; Table S4; Fig. S3).

296   Genetic selection gradients largely supported these results: LMA $\times$ N_{MASS} and N_{MASS} $\times$

297   A_{MASS} interacted to impact biomass among GBLUPs (P = 0.080 and P = 0.032, respectively;

298   Table S5; Fig 4). Together, these results demonstrate that correlational selection on particular

299   leaf economics trait combinations can occur in the field and is genetically based.

300

301   **Discussion**

302   We found evidence that the LES exists in *P. virgatum*, and may be driven by a

303   combination of genetic architecture and correlational selection. These results provide the

304   strongest evidence to date for the evolution of the LES within a species. Overall, we found that

305   correlational selection and genetic architecture generally promote the hypothesized leaf

306   economics strategies, but that some genetic constraints may limit LES evolution in this system.

307   Few studies have explored the mechanisms driving the evolution of leaf economics

308   strategies (Donovan et al. 2011). This results, in part, from the fact that most species, and even

309   many genera, occupy a small portion of the LES (Edwards et al. 2014). Because most species

310   possess relatively specialized leaf economics strategies, they typically lack sufficient variation

311   for genetic constraints and correlational selection to promote LES evolution (Agrawal 2020).

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312   But, when species exhibit distinct ecotypes or occupy a broad range of habitats, it is possible to

313   evolve a clear LES at lower taxonomic levels, as we observe in *P. virgatum*. Thus, species like *P.*

314   *virgatum*, that do possess distinct leaf economic strategies, offer a unique opportunity to evaluate

315   microevolution of the LES. By crossing genotypes that exhibit divergent leaf economics

316   strategies, we can break up these leaf economics strategies and examine how selection acts on

317   combinations of traits that rarely occur naturally. This is especially valuable because it is

318   impossible to independently manipulate two or more LES traits in a macroevolutionary context

319   (Shipley et al. 2006).

320   Our approach is not the only valuable way to study the evolution of the LES. One

321   important method for understanding genetic correlations between traits is artificial selection on

322   extreme trait combinations. Donovan et al. (2011) advocate for selection on high N_{MASS} – low

323   A_{MASS} lines (and vice versa) for multiple generations to determine whether a positive correlation

324   between N_{MASS} and A_{MASS} can be broken up. Another way to evaluate selection on leaf

325   economics strategies is to grow plants under different resource conditions, then examine how

326   fitness changes. For instance, to test the hypothesis that acquisitive strategies are favored under

327   high resource conditions and conservative strategies are favored under low resource conditions,

328   one could evaluate the fitness of conservative and acquisitive genotypes along an experimental

329   nutrient gradient. This approach could show not only whether the LES is present within a

330   species, but also, whether it should evolve through the hypothesized acquisition-conservation

331   trade-off.

332   The selective pressures acting upon individual species may differ from those that produce

333   the LES among species, potentially limiting the evolution of the LES within species (Agrawal

334   2020). For instance, when species specialize in particular habitats or evolve distinct ecotypes

335   adapted to habitats differing in water availability, these selective pressures may not align with

336   the LES. Thus, LES evolution within species may be rare (Agrawal 2020). In *P. virgatum*, the

337   evolution of distinct leaf economics strategies may be driven by large differences in growing

338   season lengths across its range. The northern upland ecotype, which exhibits an acquisitive

339   strategy (e.g., smaller, narrower leaves with higher N_{MASS} and A_{MASS} and lower LMA), may have

340   evolved to take advantage of the relatively short growing season in the northern US and Canada.

341   Short growing seasons may promote a quick return-on-investment strategy of low tissue

342   construction costs and high photosynthetic capacity (Baird et al. 2021). In the southern habitat

343   typical of the lowland ecotype, the long growing season may select for longer-lived leaves with

344   higher LMA.

345   Season length as a driver of leaf economics strategies may be particularly important if

346   divergent selection pressures in the northern and southern portions of *P. virgatum*'s range drive

347   selection for different leaf economics strategies. All five putative QTL for LMA exhibited

348   divergence in the same direction—plants with lowland alleles exhibited higher LMA than plants

349   with upland alleles—suggesting that directional selection may have promoted divergence in

350   LMA across the range of *P. virgatum* (Milano et al. 2016). Although all significant QTL for

351   LMA had the same directionality, QTL for A_{MASS} and N_{MASS} did not. This suggests that selection

352   on these two traits may depend on infrequent, severe abiotic or biotic stress, such as cold,

353   drought, or high consumer pressure (e.g., some strategies may be advantageous only under

354   severe stress), or stabilizing selection that maintains moderate values of the traits. This may slow

355   the evolution of leaf economics strategies in *P. virgatum*. Evolution of leaf economics strategies

356   may also be constrained by the LMA-A_{MASS} relationship: high LMA and high A_{MASS} were both

357   favored in selection gradient analysis, but the strong negative genetic correlation between these

358   traits should limit the ability of this combination to evolve. On the other hand, constraints on the

359   evolution of N_{MASS}-LMA should be minimal. These traits exhibited a weak negative genetic

360   correlation and correlational selection in the same direction (i.e., high LMA and low N_{MASS} are

361   favored). Together, these results suggest that the conservative strategy should be favored under

362   most circumstances, but the acquisitive strategy (low LMA, high N_{MASS}) could be favored under

363   more extreme conditions that did not occur over the duration of this study. While it is impractical

364   to follow these individuals over their entire lifetimes, future studies that incorporate climatic

365   extremes, either naturally or experimentally, could better determine the conditions that favor the

366   acquisitive strategy in *P. virgatum*.

367   Our results showed some similarities with the small number of previous studies on this

368   topic. Like Donovan et al. (2011), we found that genetic correlations between leaf economics

369   traits were variable and while directional selection occurred on many leaf economics traits in the

370   studies they surveyed, selection differentials and gradients were not consistent between traits or

371   across studies. However, due to limitations in the data available to Donovan et al. (2011), there

372   were also some notable differences between our studies. For instance, we found evidence for

373   pleiotropic loci controlling both N_{MASS}-A_{MASS} and A_{MASS}-LMA and a potential genetic constraint

374   on the correlated evolution of LMA and A_{MASS}. Our results are also somewhat consistent with

375   recent work on the LES in *Arabidopsis thaliana*, which showed that pleiotropic loci control some

376   leaf economics correlations (Vasseur et al. 2012, Hanemian et al. 2020) and that leaf economics

377   strategies differ across the geographic range of the species, possibly due to climate-driven

378   selection (Sartori et al. 2019).

379   *Limitations*

17

380   This study had several limitations. First, we did not measure leaf economics traits and

381   fitness on the same plants. This would be important if plasticity in leaf economics traits is high,

382   particularly if genotypes differ in plasticity (i.e., genotype-by-environment interactions). In a

383   previous study using different genotypes of *P. virgatum*, we found significant genotype-by-

384   environment effects on N_{MASS}, but not on leaf dry matter content, which is highly correlated with

385   LMA (Heckman et al. 2020). Given the low heritability of A_{MASS}, this trait is likely to exhibit

386   higher plasticity, although it is unclear whether A_{MASS} should exhibit strong GxE. Because

387   genetic selection gradients were largely consistent with phenotypic selection gradients, plasticity

388   or environmental correlations in traits should be a minor concern. However, genetic selection

389   gradients could still be biased by large genotype-by-environment effects. Second, we use

390   biomass as a proxy for fitness (Franklin and Morrissey 2017). While biomass production is

391   highly correlated with seed set (Lowry et al. 2019), we cannot assess fitness over the entire

392   lifespan of this relatively long-lived species. Third, because most ecologically important

393   quantitative traits are polygenic (Barghi et al. 2020), we probably failed to detect many important

394   QTL. This is made clear by the fact that significant QTL explained only ~50% of the genetic

395   variation in these traits. Thus, these QTL results should be considered a hypothesis-generating

396   tool. Future work could examine in new populations whether the same QTL are detected.

397   Moreover, future studies could further explore candidate genes in the overlapping QTL regions

398   that suggest pleiotropy.

399   *Conclusions*

400   The worldwide LES is one of the most striking patterns in plant ecology, yet little work

401   has been done to examine how it evolves. Here, we show that the LES can evolve within a

402   widespread grass species through a combination of genetically linked traits and correlational

403   selection favoring individuals possessing particular LES combinations. While it is unclear

404   whether evolution of the LES in *P. virgatum* is driven by the same resource conservation-

405   acquisition trade-off that is hypothesized to underlie the worldwide LES, this system provides a

406   rare opportunity to address this longstanding hypothesis in a tractable system.

407

408   **Acknowledgments**

417

418   **Author contributions**

419   RWH analyzed the data and led writing with input from TEJ and PAF; TEJ conceived the

420   experiment; JEB and BEC collected data and contributed to writing.

421

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557

Table 1 Genetic basis of three leaf economics traits. H^2 is broad-sense heritability [V_G / (V_G + V_e)] ± standard error; variance explained by QTL is calculated from a multiple-QTL model using all identified putative QTL as predictors of each trait; proportion of heritable variation explained by QTL is the ratio of variance explained by QTL to H^2. LMA is leaf mass per area (g m^{-2}); N_{MASS} is mass-based leaf nitrogen content (%); A_{MASS} is mass-based leaf photosynthetic rate (µmol C g^{-1} s^{-1}).

	H^2	Variance explained by QTL	Proportion of heritable variation explained by QTL
LMA	0.51 ± 0.07	0.33	0.65
N_{MASS}	0.46 ± 0.08	0.24	0.52
A_{MASS}	0.26 ± 0.08	0.19	0.73

Table 2 Significant QTL markers for each leaf economics trait detected at a LOD threshold of α = 0.15. P values are based on F tests calculated by dropping one QTL at a time from a multiple-QTL model that included all putative QTL for each trait.

Trait	Marker	LOD	P
LMA	Chr2N@56.080999	3.972	< 0.001
	Chr5N@4.933063	7.814	< 0.001
	Chr8N@13.652308	3.873	< 0.001
	Chr9K@12.433663	10.359	< 0.001
	Chr9N@79.37425	4.173	< 0.001
A_{MASS}	Chr2K@11.849289	6.079	< 0.001
	Chr2K@59.819311	4.58	0.017
	Chr8K@11.338989	4.771	< 0.001
	Chr9K@13.273228	3.896	0.002
N_{MASS}	Chr2K@10.283923	4.164	< 0.001
	Chr2K@61.75027	5.601	< 0.001
	Chr3N@16.536961	8.592	< 0.001
	Chr5K@21.5219	3.929	< 0.001

Table 3 Genetic correlations between leaf economics traits. LMA is leaf mass per area (g m^{-2}); N_{MASS} is mass-based leaf nitrogen content (%); A_{MASS} is mass-based leaf photosynthetic rate (μmol C g^{-1} s^{-1}).

	LMA	A_{MASS}	N_{MASS}
LMA	---		
A_{MASS}	**-0.76** (0.12)	---	
N_{MASS}	-0.16 (0.17)	0.26 (0.21)	---

558 **Figure legends**

559 **Fig 1** Relationship between **A)** leaf mass-based nitrogen (N_{MASS}; %) and leaf mass per area

560 (LMA; g m^{-2}); **B)** leaf mass-based photosynthetic rate (A_{MASS}; µmol C g^{-1} s^{-1}) and LMA; **C)**

561 A_{MASS} and N_{MASS} calculated using standardized major axis regression. Black dots are *P. virgatum*

562 F_2 individuals; blue triangles are F_0 line means (not included in SMA line fit). Inset plots show

563 the location of *P. virgatum* F_2 individuals (black dots) relative to the worldwide leaf economics

564 spectrum (grey dots; from Wright et al. 2004) on a $\log_{10}$-$\log_{10}$ scale.

565 **Fig 2 A** Location of QTL for three leaf economics traits, LMA, N_{MASS}, and A_{MASS}. Point

566 estimates are the location of highest LOD score and confidence intervals are the region within a

567 1.5 LOD drop. **B** Effects of genotype on leaf economics traits at three colocalized QTL markers

568 (i.e., markers with overlapping confidence intervals) calculated using ordinary least squares

569 regression. Genotypes at each QTL are a consequence of recombination in the experimental

570 cross, resulting in four possible combinations of alleles. In each panel, lowland1 and lowland2

571 denote alleles inherited from genotypes AP13 and WBC, respectively; upland1 and upland2

572 denote alleles inherited from genotypes DAC and VS16, respectively. Error bars represent 95%

573 confidence intervals; shared letters indicate no significant difference between genotypes. For all

574 colocialized QTL pairs, genotype effects are in a consistent direction (i.e., each genotype

575 exhibits conservative or acquisitive values of both traits) and are consistent with pleiotropy. See

576 Fig. 1 legend for units and abbreviations.

577 **Fig 3 A** Effects of standardized LMA and N_{MASS} (transformed such that across all plants, mean =

578 0 and sd = 1) on relative biomass while controlling for A_{MASS}. Following (Stinchcombe et al.

579   2008), model-derived quadratic parameter estimates are doubled. **B** Selection gradients estimated

580   at each site. Linear selection gradients (β_i) are main effects; non-linear selection gradients (γ_{ii})

581   are quadratic effects (model-derived estimates were doubled); non-linear correlational selection

582   gradients (γ_{ij}) are interactive effects between two leaf economics traits. Red points denote

583   significant effect. See Fig. 1 legend for units and abbreviations.

584   **Fig 4 A** Effects of LMA and N_{MASS} genomic linear unbiased predictors (GBLUPs) on plant

585   biomass GBLUPs **B** Effects of N_{MASS} and A_{MASS} GBLUPs on plant biomass GBLUPs. GBLUPs

586   were calculated separately for each trait from a mixed model that accounted for the relatedness

587   among individuals by incorporating an additive genetic relatedness matrix
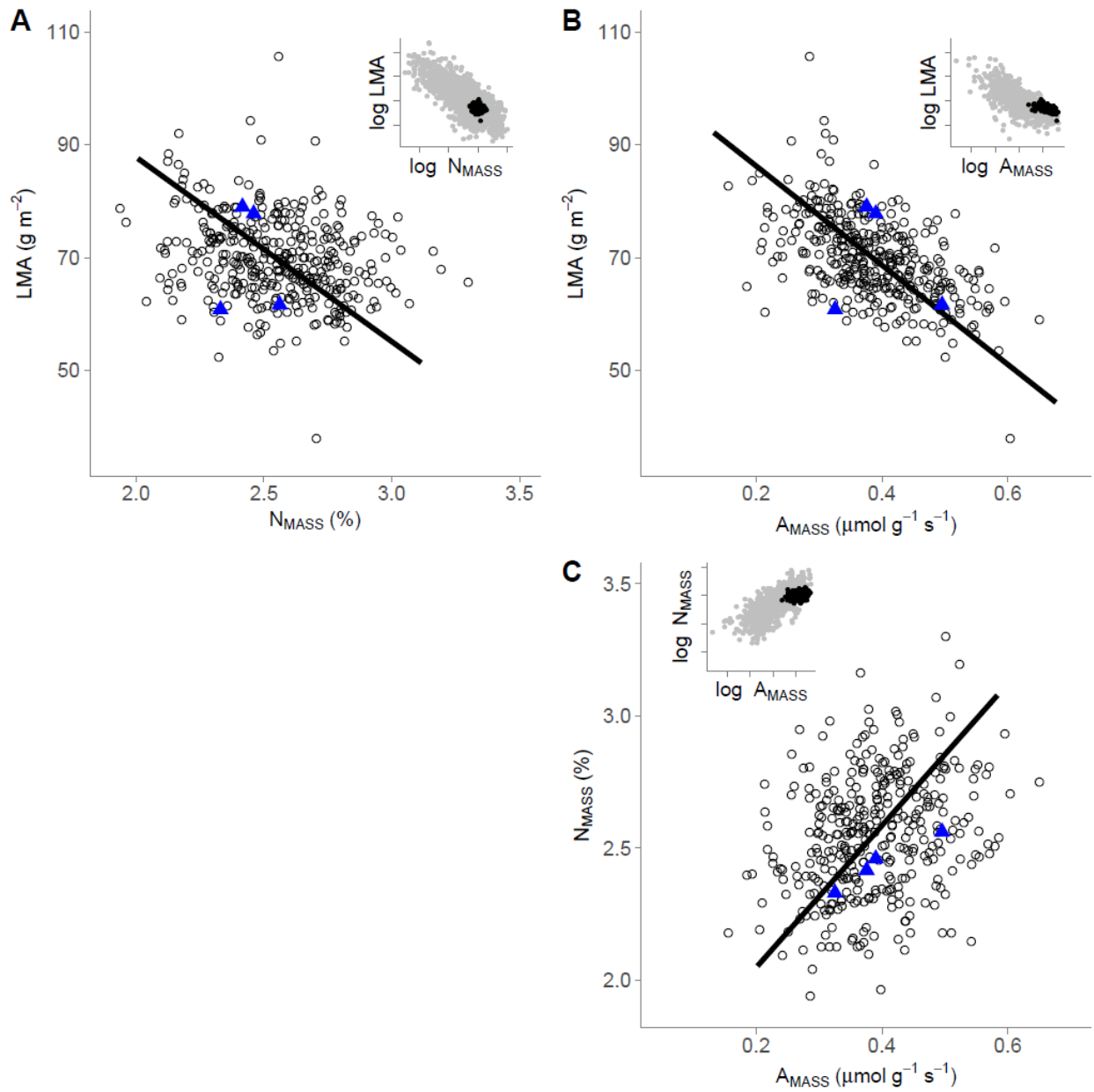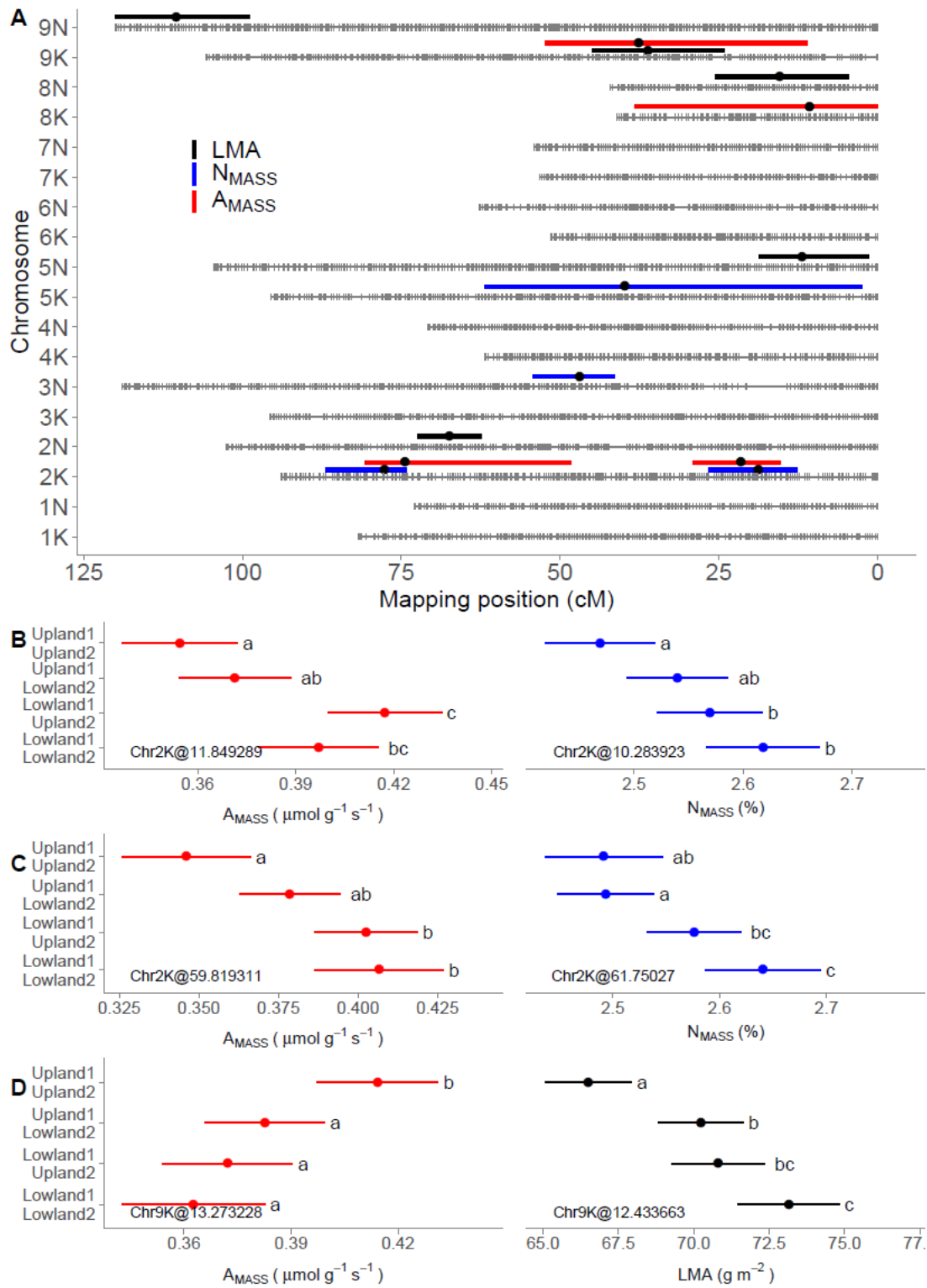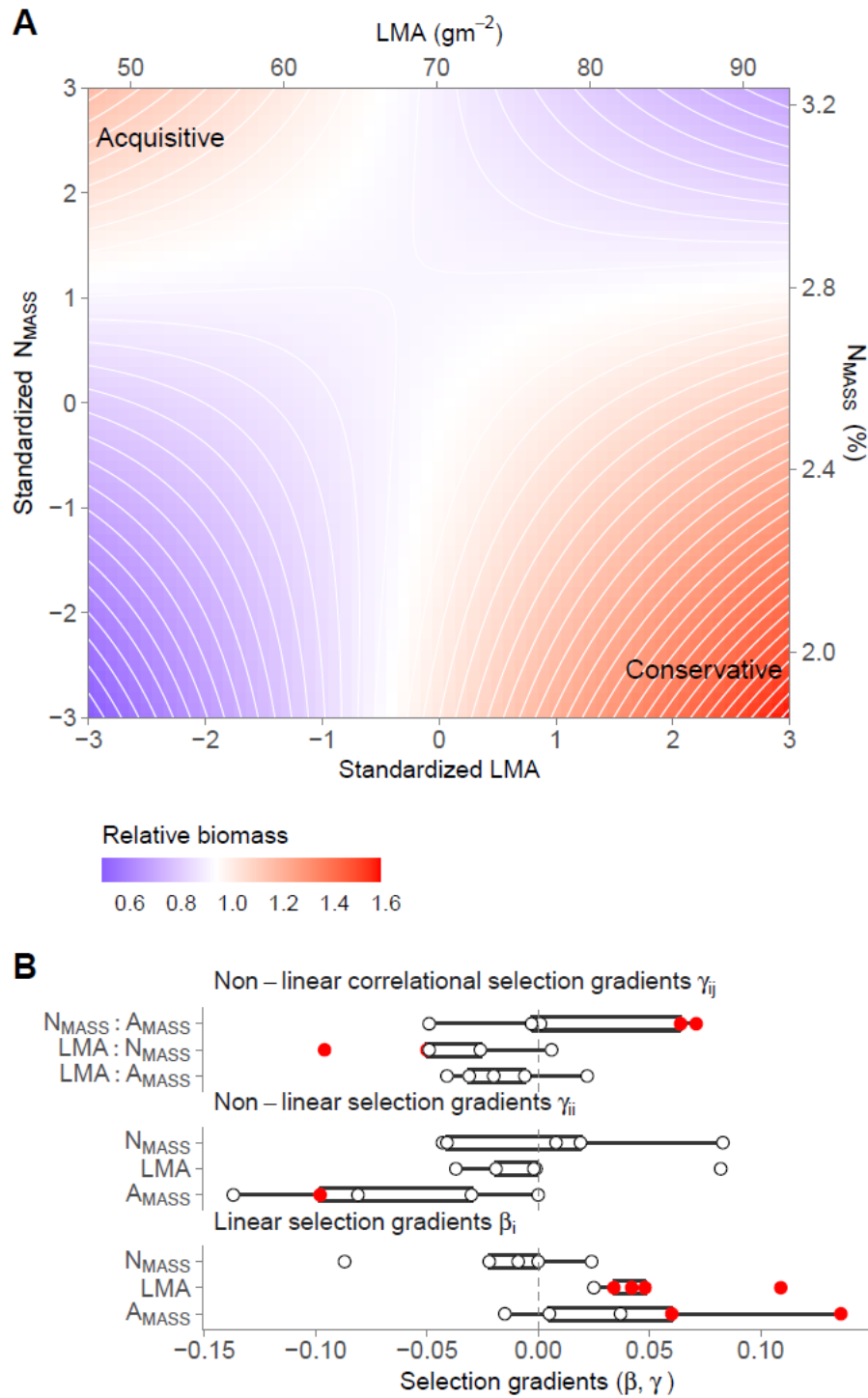
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Fig 1

Fig 2

Fig 3

Fig 4