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Supplementary Material for:

Pest defenses under weak selection exert a limited influence on the evolution of height growth and drought avoidance in marginal pine populations

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Methods S1 Phenotypic trait measurements

*** Selection of lodgepole pine trees for phenotyping

This study used a total of **1,490 trees selected** from four progeny trial test sites. These test sites are part of the lodgepole pine Region C breeding program, initiated in the early 1980s, and currently managed by Blue Ridge Lumber, a Division of West Fraser Ltd. The four test sites were planted in 1982 with open-pollinated (OP) seedlings and represented a variety of site conditions present within the breeding program region. The four sites were planted with 224 OP families and seed sources for these families were collected from phenotypically superior selected parents from five provenances (populations) within the region. Candidate parent trees were phenotypically selected based on superior growth, stem straightness, health, branching, and crown traits [1]. For this study, a subset of 53-59 families growing at each test site was selected based on their 30-year-old height breeding values representing high, average, and low productivity levels to cover the extent of the genetic variability within the total number of families originally collected. The following describes the measurement details of the phenotypic traits studied on **each of the selected trees**.

(1) Carbon isotope discrimination ($\delta^{13}C$) measurement

A 5mm increment core was collected from each of the trees from bark-to-bark at approximately breast height (1.3m) in a north-south direction in June 2017 (age 35). The cores were transported in a cooler with ice packs until further processing in the lab. Each complete core was divided into a north half and

south half at the center, with a complete set of rings from growth at 1.3m to the collection time. Two 'slabs', each contained rings from pith to bark, were removed from the outside of each core. The slabs were dried at 65°C for three days and then ground using a Qiagen Tissuelyser II (Qiagen Inc.-Canada, Toronto, Ontario, Canada) in a 25ml stainless steel jar with a 20mm stainless steel grinding ball at a frequency of 30Hz for one minute. The ground samples were subsequently sent to the Stable Isotope Lab of InnoTech Alberta (Victoria, BC, Canada) for $\delta^{13}C$ analysis. Samples were analyzed using an established method on a MAT253 mass spectrometer with Conflo IV interface (Thermo Fisher Scientific, Waltham, MA, USA) and a Fisons NA1500 EA (Fisons Instruments, Milano, Italy). Briefly, approximately one mg of pulverized sample was weighed into tin capsules and then placed in a combustion reactor that produces CO_2, which was then analyzed by mass spectrometry for isotopic estimates. The results were normalized and reported against Vienna Pee Dee Belemnite (the established standard for carbon-13 estimates; ref. [2]).

(2) WGR data collection

A complete observational assessment of western gall rust (WGR) severity was conducted in the spring of 2018 at age 36. A 0-6 coding score (seven tiers including control) shown below was applied to the assessment of WGR infection for all trees sampled. This protocol provides a much more comprehensive assessment compared to the presence/absence assessment used previously for the WGR age 30 values. As there were very few trees in categories 2, 4, and 6 across test sites, these categories were merged with the original categories 1, 3, and 5, respectively, resulting in a four-tiers resistance rating classification. The four-tiers WGR ratings were further transformed into a continuous normal score following Gianola and Norton (1981) [3] and used as our study trait.

Six-category classification	Four-category classification	Symptom
0	0	No Galls
1	1	Minor branch galls
2		Minor stem galls
3	2	>2 stem or branch galls
4		Multiple stem galls
5	3	Massive infection - large number of stem and/or branch galls
6		Death most likely due to western gall rust attack

(3) MPB suitability bioassay

Ullah et al. [4] developed a mountain pine beetle (MPB) suitability rating by quantifying defense chemistry (mainly monoterpenes) and conducting insect feeding assays. We applied the rating system to all lodgepole pine samples in this study. Briefly, we collected samples in July 2017 at age 35. On the north side of the main stem of each tree, bark tissue containing phloem was sampled from ~1.3 m in height using a leather punch (1.9 cm in diameter). Tissues were immediately wrapped in aluminum foil, placed in liquid nitrogen, transported back to the laboratory in dry ice, and stored at -40 °C until further

chemical analysis. We removed the outer bark from each sample and only the phloem was ground into a fine powder using liquid nitrogen in mortar and pestle. Hexanes-extractable compounds (mainly monoterpenes) from finely ground inner bark (phloem) of trees were analyzed using a Gas Chromatography/Flame Ionization Detector (GC/FID, Agilent 7890B, Agilent Tech., Santa Clara, CA, USA) based on the method modified from ref. [5]. A total of 15 compounds was identified, including borneol, α-terpinene, γ-terpinene, α-terpineol, 3-carene, terpinolene, α-pinene, β-pinene, limonene, myrcene, camphene, *p*-cymene, 4-allylanisole, bornyl acetate, and β-phellandrene. To select family-level average monoterpene profiles to test against MPB performance (female beetle egg gallery lengths and weight change), a cluster analysis was performed on standardized family estimated breeding values (partitioning around medoids using the *pamk* function from the R package fpc) from 40 families in the Judy Creek test site. Four clusters of family profiles were identified and the family average of individual monoterpene concentrations from three families in each cluster were amended in plant-based MPB media (except β-phellanderene). We allowed the amended media to solidify and then introduced one female beetle in each assay unit. Two experiments were conducted to quantify the impact of defense chemicals on the performance of MPB: profile of family average monoterpenes and dose experiment with β-phellandrene. The weight change of adult female MPB was measured after beetles fed on the media amended with family profile specific monoterpenes. A decision tree analysis was conducted using weight change of MPB (rpart package) that identified that 23.5% limonene (without β-phellandrene) and 16.93 μg/mg fresh weight γ-terpinene as thresholds that separated MPB performance (n = 72, R^2 = 0.48). Furthermore, an ANOVA was performed on the weight change of MPB in the β-phellandrene dose dependence experiment that identified that beetles that fed on 4.079 μg/mg fresh weight gained less weight than lower concentrations ($P < 0.05$). Results from these analyses were combined to develop the MPB suitability rating.

Methods S2 Detrending phenotypic traits using spatial mixed model

To detrend phenotypic traits caused by environmental variation within test site, we analyzed each trait in each test site using a spatial model with a first-order autoregressive residual (co)variance structure (AR1×AR1). The analysis was achieved by the following pedigree-based mixed model with a spatial autocorrelation to predict each trait:

$$y = \boldsymbol{X}\beta + \boldsymbol{Z_r}u_r + \boldsymbol{Z_s}u_s + \boldsymbol{Z_a}u_a + \varepsilon \qquad \text{Eqn 1}$$

where y is the vector of individual-tree trait observations, β is the vector of fixed effects for populations; u_r is the vector of random replicate effects distributed as $u_r \sim \mathcal{N}(0, \boldsymbol{I}\sigma_r^2)$, where $\boldsymbol{I}$ is the identity matrix and σ_r^2 is the replicate variance; u_s is the vector of random set effects distributed as $u_s \sim \mathcal{N}(0, \boldsymbol{I}\sigma_s^2)$, where σ_s^2 is the set variance; u_a is the vector of random effects that represents the genetic effects

distributed as $u_a \sim \mathcal{N}(0, \boldsymbol{A}\sigma_a^2)$, where $\boldsymbol{A}$ is the average numerator relationship matrix derived from the pedigree information [6] and σ_a^2 is the additive genetic variance; and ε is the vector of random residuals, partitioned into spatially dependent (ξ) and independent (η) components. The residual (co)variance matrix is expressed as $\sigma_\xi^2[AR1(\rho_{col}) \otimes AR1(\rho_{row})] + \sigma_\eta^2 \boldsymbol{I}$, where σ_ξ^2 is the spatially dependent variance, σ_η^2 is the spatially independent variance, $AR1(\rho)$ is the first-order autoregressive correlation process, ρ_{col} and ρ_{row} are autocorrelation parameters for tree column and row positions in a test site, respectively, and ⊗ denotes the Kronecker product. The $\boldsymbol{X}$, $\boldsymbol{Z}_\mathbf{r}$, $\boldsymbol{Z}_\mathbf{s}$, and $\boldsymbol{Z}_\mathbf{a}$ terms denote the incidence matrices for respective model-effects. A log-transformation was applied to WGR and MPB data before the adjustment. The detrended phenotypic traits were obtained for each tree at each site by subtracting the estimated replicate, set, and autoregressive residual effects (i.e., ξ term in the model, Eqn 1). We used the *remlf90* function in the R package breedR v.0.12.5 [7] to fit the aforementioned models with the Expectation-Maximization REML algorithm.

Methods S3 Phenotype-based genetic relationship estimation

We utilized pedigree and phenotypic data of all test sites to fit models under a Bayesian framework as implemented in the R package MCMCglmm ver. 2.29 [8]. We fitted multivariate 'animal' models and extracted the posterior distribution of variance-covariance matrices. This yielded a total of four genetic relationship (*G*) matrices (one each site). Each *G*-matrix was estimated to fit the model:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Z_1 u_1} + \mathbf{Z_2 u_2} + \boldsymbol{\varepsilon} \qquad \text{Eqn 2}$$

where **X**, $\mathbf{Z_1}$ and $\mathbf{Z_2}$ are the incidence matrices relating the vectors of traits and population means (**b**), the vector of additive genetic effects ($\mathbf{u_1}$), and the vectors of replicate and set effects ($\mathbf{u_2}$), respectively, to observations in **y**; the vector **ε** contains the residuals. The response vector (**y**) consists of four traits (i.e., height, δ^{13}C, WGR, and MPB) as a multivariate response. To foster model convergence, we scaled height and δ^{13}C across test sites using original non-detrended data. We implemented each model with 5,100,000 MCMC sampling iterations with a burn-in period of 100,000 and a thinning interval of 500. We used a Cauchy prior distribution for random effects [9] with a location parameter of zero and a scale parameter equal to the variance of the raw data (ν = 2.328). To examine the sensitivity of the prior, we adjusted ν to excessively large and small values, but the choice of ν had little influence to the parameter estimates, suggesting that our prior did not strongly affect the parameter values. We visually inspected the model convergence using posterior trace plots and Gelman and Rubin diagnostics [10]. We also checked the effective sample size and autocorrelation of each parameter using the R package coda ver. 0.19-3 [11]. In total, we extracted 10,000 MCMC iterations from each model, which provided the posterior distribution of the four focal traits in each test site. We displayed a summary of these posterior distributions using the posterior mean and the quantiles for each parameter (Fig. S6).

Table S1 Information about the geographical locations of study populations and progeny trial test sites

(1) Population sites

Location	Latitude (°N)	Longitude (°W)	Elevation (m)
Judy Creek	54.38-54.95	-115.28 to -115.95	945 to 1,280
Virginia Hills	54.42-54.95	-115.53 to -115.95	945 to 1,280
Swan Hills	54.42-54.95	-115.25 to -115.95	945 to 1,280
Inverness River	54.95	-115.55	1,000
Deer Mtn	54.92	-115.15	1,080

Note: seed planning zones of Alberta were used to define populations:
https://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/formain15749/$FILE/seed-zones-alberta.pdf

(2) Progeny trial test (common-garden) sites

Location	Abbreviation	Latitude (°N)	Longitude (°W)	Elevation (m)	Soil texture	pH[§]
Timeau	TIME	54.73	-115.30	1,097	Loamy sand	5.4
Swan Hills	SWAN	54.71	-115.50	1,136	Silty loam	5.5
Virginia Hills	VIRG	54.47	-115.85	1,118	Silty loam	5.5
Judy Creek	JUDY	54.44	-115.57	1,110	Clay loam	3.9

[§] measured within 20-90 cm depth

For a better differentiation between population and test site names, we used four capital letters for test sites (grey-shaded), while full names for population sites throughout the study.

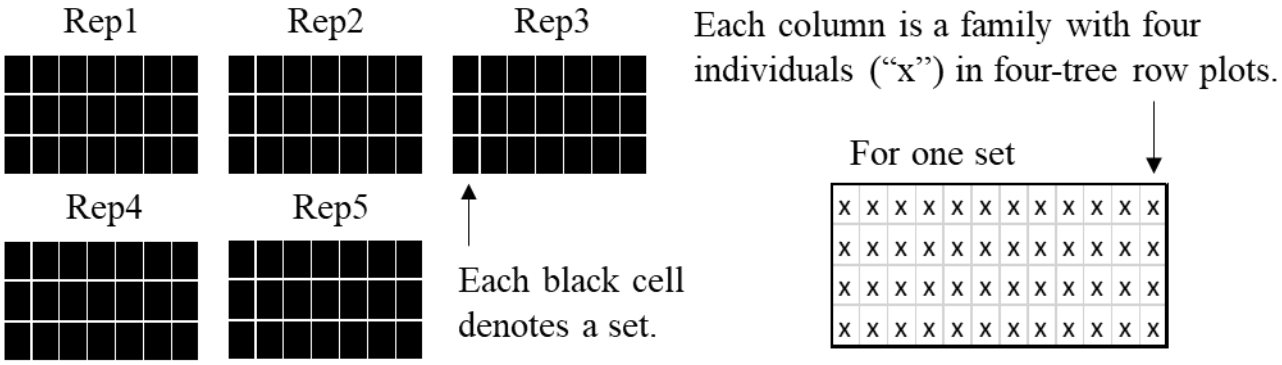


Fig S1 Illustration of the experimental design for a common-garden progeny trial test site

Each Rep (replicate) consists of 21 sets. Each set contains 12 families arrayed in four-tree row plots. A total of 53-59 families was selected from each test site. As we selected only 1 individual (2 for several families) from a plot if any, we did not consider the plot effect in detrending phenotypes (Methods S2).

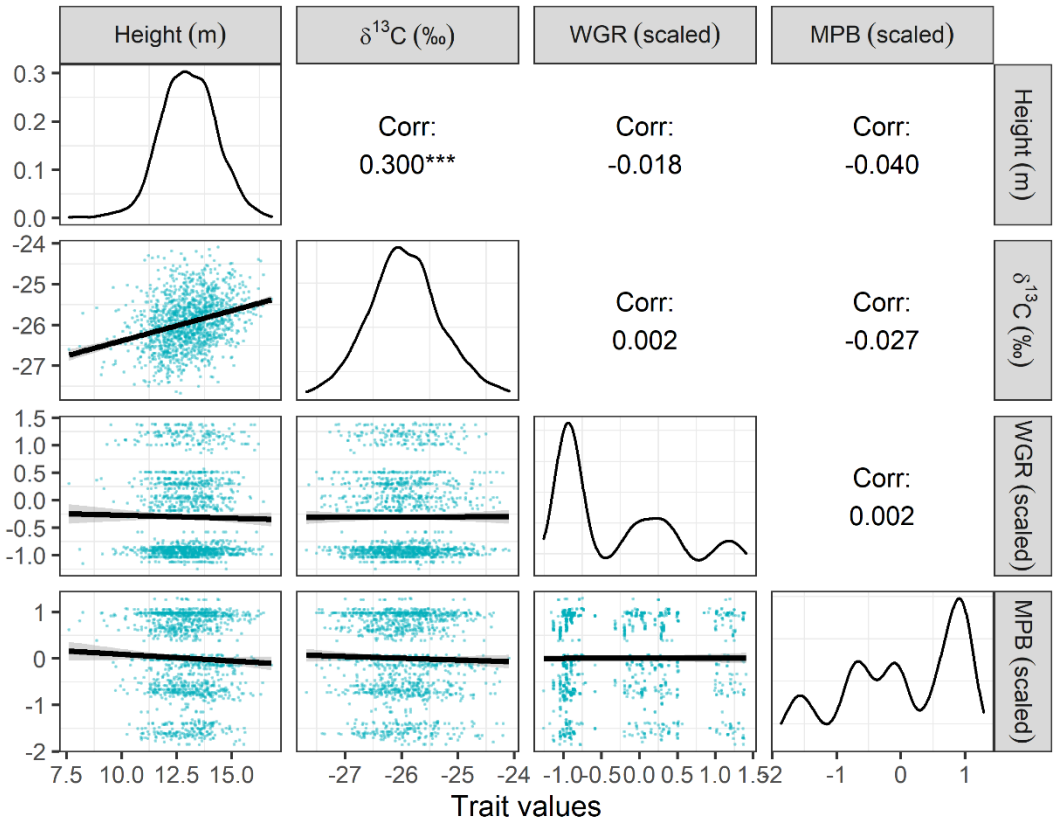


Fig. S2 Distribution of four focal traits and correlations thereof

Each panel of the lower triangle shows trait values for a pair of traits. Density plots (smoothed histograms; N = 1,490) are shown on the diagonal. Upper triangle cells indicate the correlation coefficient for each pair of traits. Significance: '***' $P < 0.0001$; no asterisk for not significant. WGR: western gall rust; MPB: mountain pine beetle.

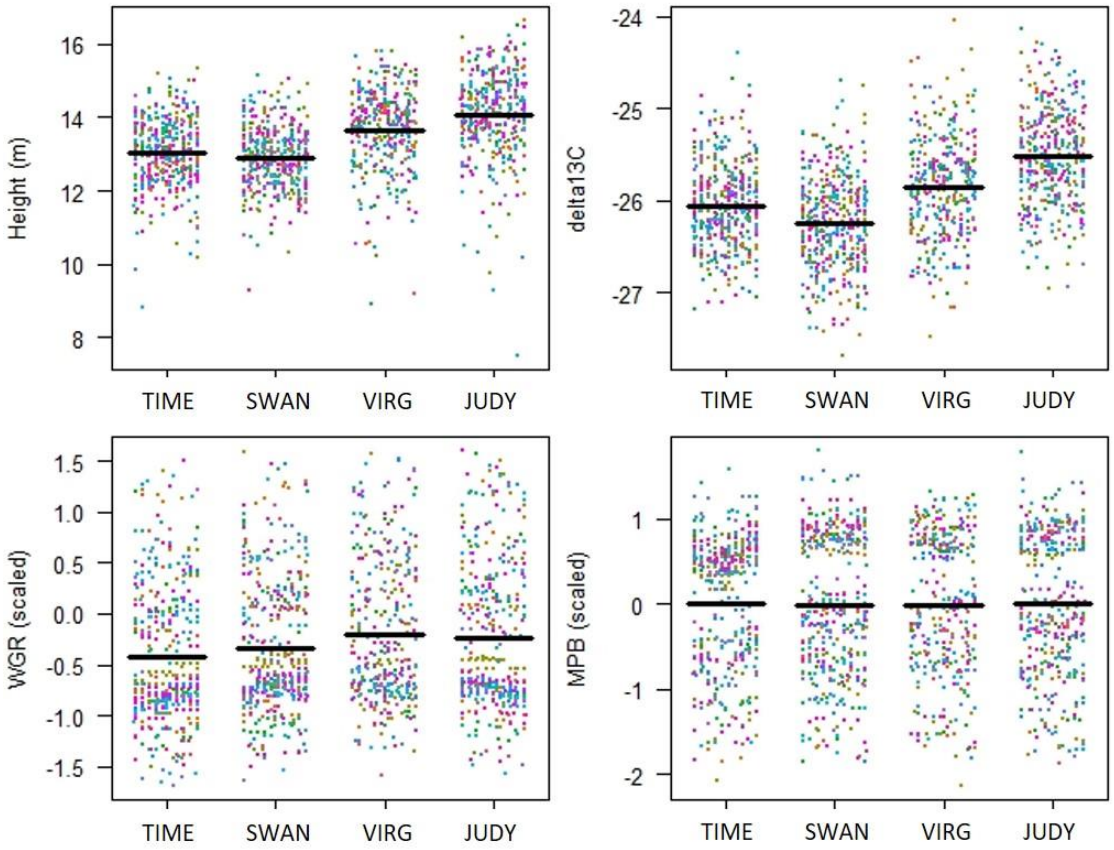


Fig. S3 Detrended trait values in each of the four progeny trial test sites colored by family
Black lines mark the mean trait values in each experimental garden. Note that each site has similar family components from each population [see Table S1-(2)].

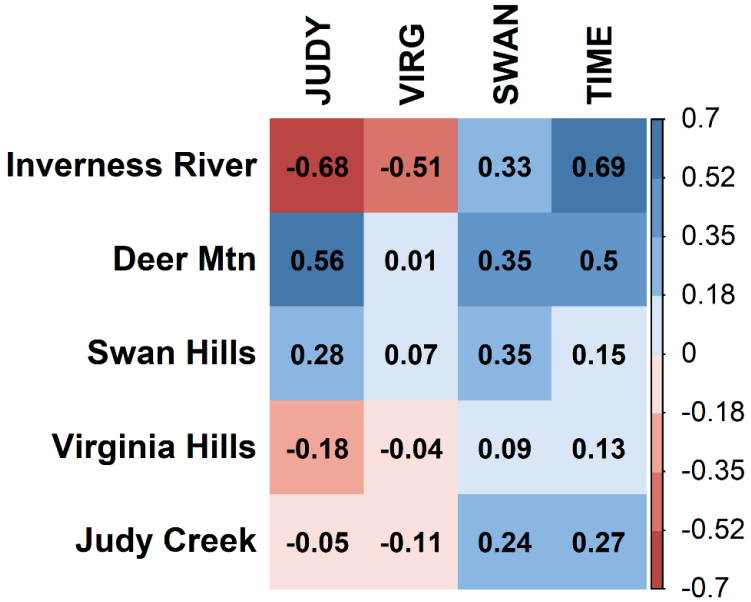


Fig. S4 Pearson product-moment correlation between height and $\delta^{13}C$ for each population and test site
Values in grid cell give correlation coefficients, in addition to via color key.

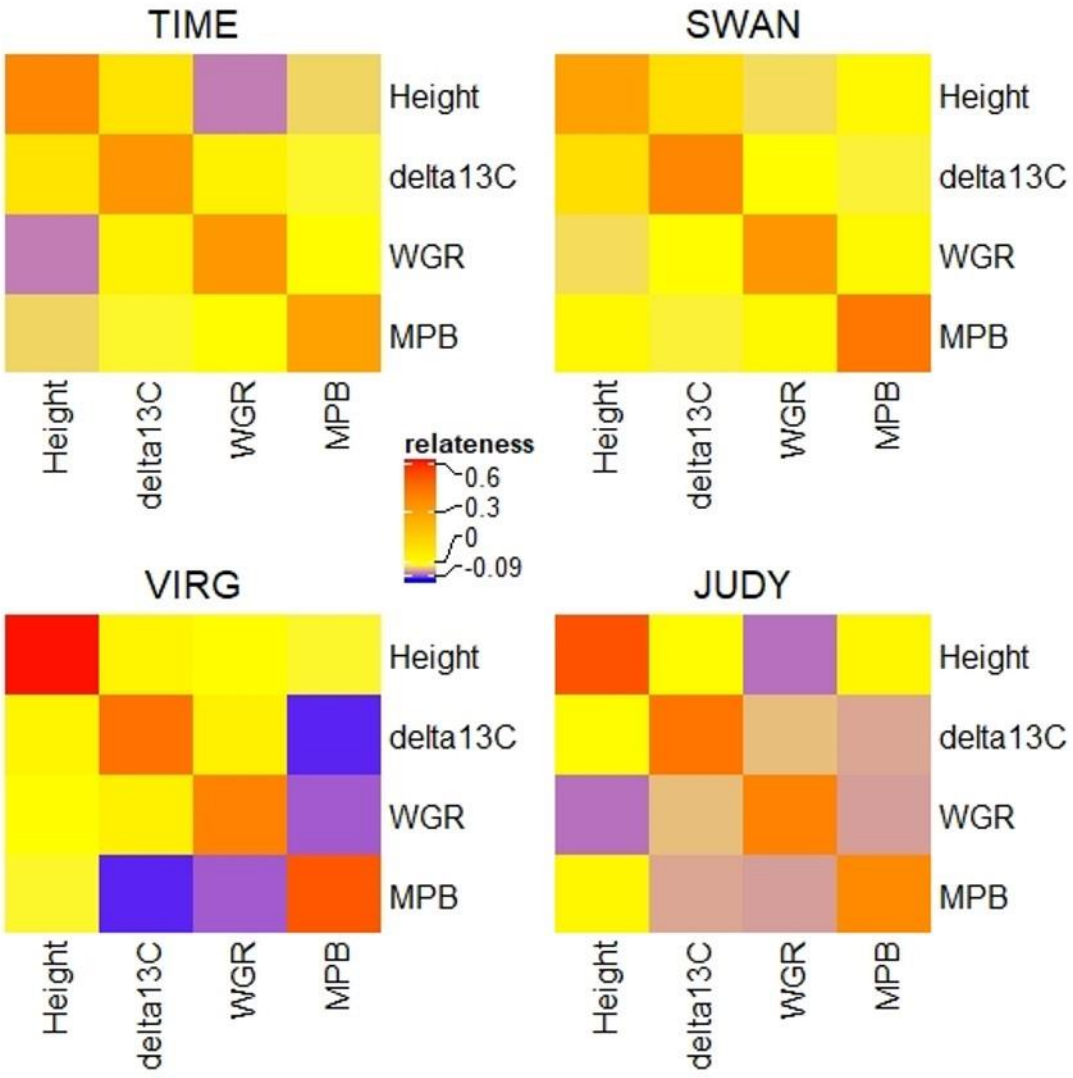
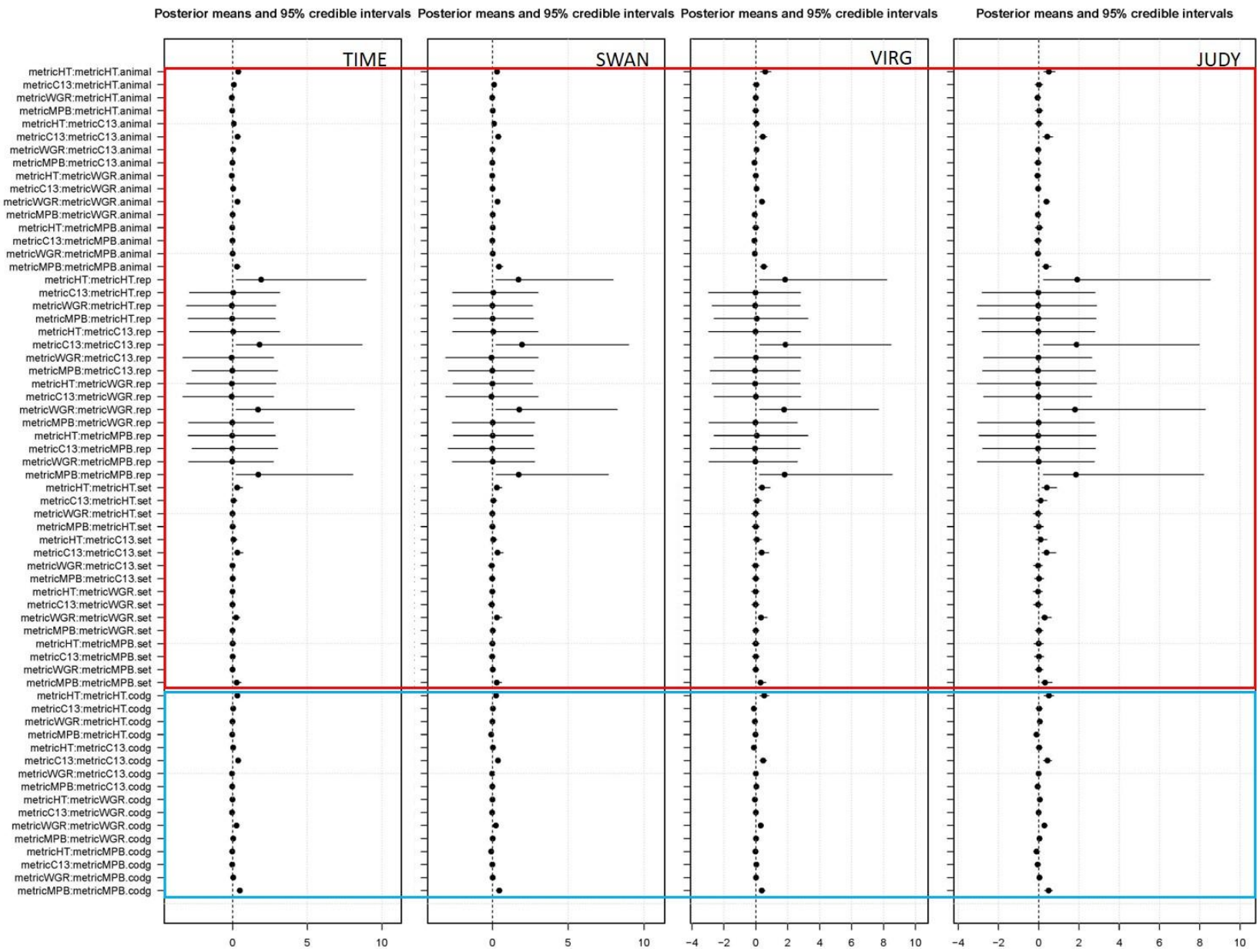


Fig. S5 Heatmap showing *G*-matrices of four focal traits in each of the four progeny trial test sites
G-matrix: the additive genetic variance-covariance matrix of focal phenotypes. Each *G*-matrix displayed is the average across 10,000 MCMC samples.

Fig. S6 Plot of the posterior means along with 95% CIs for each parameter estimated in each test site
The red and blue boxes demarcate random effects and residual covariance structure terms, respectively. The graph panels for test sites show that only the replication (rep) term was highly variable in its posterior probabilities across samples. The posterior distributions of all variance matrices were significant, whereas those of covariance matrices were not significant.


Posterior means and 95% credible intervals
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