Past family selection in *Populus nigra* leads to an amplification of rust damages when neighbors are related

Authors: Mathieu Tiret[1,3*], Leopoldo Sánchez[2], Martin Lascoux[1].

Affiliations:

- 1: Program in Plant Ecology and Evolution, Department of Ecology and Genetics, EBC and SciLife Lab, Uppsala University, Uppsala, Sweden
- 2: BioForA, INRAE, ONF, Orléans, France.
- 3: IGEPP, INRAE, Institut Agro, Université de Rennes, Domaine de la Motte, Le Rheu, France.

* corresponding author: mathieu.tiret@inrae.fr

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Abstract:

Selfish individuals might prevail in the wild, but they often cause waste of nutritional resources and decrease the population yield in breeding schemes. Re-orienting past evolution towards less competitive paths (e.g., group selection) can limit negative interactions between elite material and leads to a more efficient transformation of resources into economically interesting traits. In particular, there are reasons to believe that, in the presence of kin, individuals either will cooperate (kin selection) or over-compete (niche partitioning). In either case, local relatedness might reveal interaction that can enhance the predictive abilities of genomic models when accounted for. However, recurrent family selection during domestication can have blurred the effect of relatedness on neighbors' phenotypes. To overcome this difficulty, we analyzed data from the French breeding program of *Populus nigra* L., where 1,452 genotypes were replicated six to eight times, each time encountering a different neighborhood. We assessed local relatedness and investigated genomic estimated breeding values on tree height and rust vulnerability with a single-step GBLUP incorporating local relatedness as a covariate. We found that, when surrounded by related individuals, rust damage is significantly more important, whereas no interaction was visible on tree height.

Introduction

Breeding is redirecting evolutionary trade-offs towards new equilibria that often decrease fitness in favor of other economically interesting traits, or towards paths that were insufficiently explored in the wild, such as group selection (Weiner, 2019). When implemented artificially, group selection would favor candidates with a high performance in a group (e.g., robust productivity, tolerance to biotic or abiotic stress, or long-term response to selection). It is then not surprising that overall performance can be higher when performing group selection than when selecting candidates on their own individual performances (Wade et al, 2010). This is especially true when intraspecific competition is strong among candidates (Weiner et al., 2017). Indeed, selfish strategies, although increasing individual fitness, are often costly for the population - and breeding circumventing such strategies ended up with very successful results (e.g., Donald, 1981; Donald and Hamblin, 1983; Weiner et al., 2010). Despite the early success of some ideotypes optimizing group performance, current breeding programs do not necessarily focus on integrating this dimension (e.g., Denison et al., 2003; Murphy et al., 2017; Montazeaud et al., 2020). Consequently, the pool of elite genotypes used nowadays might be mostly composed of selfish genotypes, since early mass selection, bypassing group dynamic, favored them for their vigorous phenotypes (Murphy et al., 2017). It is therefore increasingly important for a sustainable breeding program to counteract the effect of natural selection, and, perhaps, a bit of past artificial selection.

In the specific case of plant breeding, resource competition between candidates occurs with the nearest plants (Casper et al., 1997; Milbau et al., 2007; File et al., 2011). The intensity of competition among neighbors may depend on the relatedness among them, or local relatedness, either because of kin selection, which predicts that relatives may cooperate

(Hamilton, 1964), or niche partitioning, which predicts, on the contrary, that similar individuals may compete for similar resources (Silvertown, 2004). There are therefore reasons to believe that local relatedness impacts phenotypes in one way or the other (Cahill et al., 2011), so that understanding plasticity to the presence of related neighbors might provide valuable information for the implementation of group selection in plant breeding. Although kin recognition was reported to be not particularly relevant in crop species because of their already high relatedness (e.g., Murphy et al., 2017), we might be able to detect it nonetheless in breeding schemes that have been less intensive, such as forest tree breeding. Group selection is in fact expected to give better genetic progress for perennial species, as (i) inbreeding coefficient is much lower than in crop species, and (ii) positive or negative interactions between neighbors lasts a lifetime.

Local relatedness creates micro-environment to which genotypes can respond to, so that, even within a plot, strong genotype x environment interaction (GxE) can be expected. Breeding on GxE is, however, known to be challenging (Diouf et al., 2020), especially for non-model species where small SNP chips can lead to ascertainment bias or low heritability. To meet this challenge, we chose to conduct our analyses on *Populus nigra* L.. Black poplar is a Eurasian riparian forest tree that contributes, as a parent along with *Populus deltoides* Bartr. ex Marsh., to one of the most widely used hybrid tree in forest breeding (*Populus x canadensis*). The dataset of the French breeding program with 1,452 genotypes replicated six to eight times created different neighborhoods for each replicate of a genotype (Pégard et al., 2020). To detect any signal of group selection, we focused on two quantitative traits relevant for breeding, tree height and rust vulnerability. Growth of Black poplar is particularly susceptible to foliar rust, caused by a fungus (*Melampsora larici-populina* Kleb.) that leads to a reduction in photosynthesis efficiency, and might lead to the death of susceptible clones (Legionnet et al., 1999). Rust development is strongly influenced by vulnerability among neighbors, whereas tree height is a poor proxy of competition among neighbors and a good one of the fertility of the trial. In order to assess the contribution of local relatedness in

genomic selection (Meuwissen et al., 2001), we performed genomic evaluations with a multitrait single-step Genomic Best Linear Unbiased Predictor (ssGBLUP; Legarra et al., 2009; Christensen et al., 2010). Estimating breeding values with ssGBLUP is a solid baseline in forest trees as recently shown (e.g., Cappa et al., 2019; Ratcliffe et al., 2017), especially its multitrait version that is known to give higher accuracy (Calus et al., 2011). We evaluated the ability of ssGBLUP models to explain phenotypic variance when including local relatedness as a way of measuring the extent to which the phenotype of an individual was impacted by the relatedness with its neighbors. We assessed local relatedness with different subsets of SNPs, each maximizing or minimizing one of the following features: Minor Allele Frequency, Ancestry Informativeness Coefficient, significance in a Genome Wide Association Study, or heritability. We show that local relatedness significantly impacts the phenotype of an individual and, when surrounded by related individuals, rust damage is significantly more important, whereas no interaction was visible on tree height.

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Material and Methods

Plant material. The study was based on previously published data (Pégard et al., 2019, 2020). Seventeen male and 17 female *Populus nigra* were sampled in France, from 21 natural populations and 13 from breeding programs. These individuals were used as parents in a factorial crossing plan. Clones of both parents and progeny, and a hundred additional individuals from breeding programs, were grown in four experimental trials at the same location (Guéméné-Penfao, France, 47°37’59”N, 1°49’59”W), each with a random block design. In total, the experiment comprised 10,301 trees (including 7,169 trees with genotype information), 42 full-sib families, 5 half-sib families, 1,452 genotypes (each replicated on average 7.09 times), and an Unknown Parent Group (UPG) of 105 genotypes (Table S1). Family size ranged from 1 to 119, with an average of 30.2 genotypes per family. Twenty genotypes and 17 families were shared across the four trials.

Phenotype measurements. We focused on two phenotypes of one-year-old trees: height and rust vulnerability (both measured on field). Height was assessed with a graduation rod (in cm), and rust vulnerability was assessed on a 1 to 9 scale (Legionnet et al., 1999). Across the trials, at least 95.29%, 96.05%, and 95.13% of the trees were phenotyped for height, rust vulnerability, or both traits, respectively. Given the low missing rate, we assumed that missing values only marginally unbalanced the block design, and so we used default parameters of the software packages for handling missing values when analyzing the data.

In each trial, every phenotype was first corrected for micro-environmental heterogeneity with a multitrait mixed model, including as explanatory variables a random block effect and a B-spline spatial autocorrelation correction. We used the function *remlf90* (R package *breedR*; Munoz and Sanchez, 2014) with the parameters ‘model’ set to ‘splines’ and ‘method’ set to

'em'. All further analyses were performed on the residuals of this model, i.e., on spatially adjusted phenotypes.

Genomic data. Out of the 1,452 genotypes, 1,034 were genotyped (25 parents and 1,009 offspring), using a *Populus nigra* 12K custom Infinium Bead-Chip (Illumina, San Diego, CA) (Faivre-Rampant et al., 2016). Details of the DNA extraction protocol, bioinformatic pipelines, and SNP mapping on the 19 chromosomes are given in Faivre-Rampant et al. (2016) and Pégard et al. (2019, 2020). It is worth noting that the SNP chip array included SNP associated with, among other traits, rust resistance, but not with vertical growth. We further filtered for a Minor Allele Frequency (MAF) of 5% − corresponding to a threshold of 50 individuals for a genotyping error; in total, 7,129 SNPs were retained for further analyses (out of 7,513 initial SNPs).

Genomic relationship matrix. In order to build the relationship matrix necessary for inferring breeding values, we combined pedigree and genomic information (Legarra et al., 2009; Christensen et al., 2010). The pedigree relationship matrix A^{Γ} was built using the tabular rule method (Emik and Terrill, 1949), modified to account for a single metafounder (assuming a single base population for the Unkown Parent Group or UPG; Legarra et al., 2015), with a self-relationship of $\gamma = 8\sigma_p^2$, with σ_p^2 the variance of allele frequencies across markers (Garcia-Baccino et al., 2017). The combined relationship matrix H was summarized in Aguilar et al. (2019), and is:

$$H^{-1} = A^{\Gamma-1} + (0,0;0;\ G^{-1} - A_{22}^{\Gamma-1}) \qquad (1),$$

where A_{22}^{Γ} was the pedigree relationship matrix for genotyped individuals, $G = (1-\alpha)\ (\omega_a + \omega_b \times G_V) + \alpha\ A_{22}^{\Gamma}$, with G_V the genomic relationship matrix estimated from the SNPs and scaled following the first scaling method of Van Raden (2008), α a scaling parameter (here equal to 0.05), and ω_a and ω_b chosen to equate the average inbreeding and the average

relationships in G_V and A_{22}^{Γ} as in Christensen et al. (2012). As the sample size was relatively small, the matrix H^{-1} was obtained by simply inverting H with the function *solve* (R package *base*).

Population structure. In order to control for population structure and avoid spurious associations, we computed a distance matrix 1 − H*, where H* is the correlation matrix obtained from H using the function *cov2cor* (R package *stats*), and the minus sign is to convert similarities to distances. We then used Multidimensional Scaling (MDS) on the distance matrix with the function *cmdscale_lanczos* (R package *refund*; Goldsmith et al., 2022; Miller, 2022) with the parameter 'k' set to 5, and 'eig' set to 'TRUE'. Negative eigenvalues were set to null, and the variance explained by an axis was computed as the ratio between its squared eigenvalue and the sum of the squared eigenvalues. Additionally, to assess how well the family structure was captured, we measured the variable V_F defined as the average within family variance on the first five axes (the lower the V_F, the better the family structure is captured). To make MDSs comparable, we normalized V_F by the sum of squares of the first five eigenvalues.

Breeding value inferences. The spatially adjusted phenotypes were then used to infer the breeding values with a multitrait single-step Genomic Best Linear Unbiased Predictor (ssGBLUP), as follows:

$$Y_{ijk} = \mu + mds1_j + mds2_j + TRIAL_i + ANIMAL_j + e_{ijk} \quad , \qquad (2)$$

where 'Y_{ijk}' is the phenotype of the k-th replicate of the j-th genotype in the i-th trial, μ is the grand mean, '$mds1_j$' and '$mds2_j$' the fixed effects on the j-th genotype of the first and second axes of the MDS respectively, '$TRIAL_i$' the random effect of the i-th trial, '$ANIMAL_j$' the polygenic effect of the j-th genotype, and 'e_{ijk}' the residuals. Both the trial effect and the residuals follow a Gaussian distribution with diagonal covariance matrices, and the polygenic

effect follows a Gaussian distribution with a covariance matrix equal to k.H x Σ, where k is a scaling parameter equal to 1 - γ/2 (Legarra et al., 2015), H the combined relationship matrix, x the Kronecker product, and Σ the 2x2 covariance matrix between height and rust vulnerability. Estimates of coefficients and variance components of model (2) were obtained with the function *remlf90* (R package *breedR*) through one run with the 'method' parameter set to 'em' until convergence, then another run with the parameter 'method' set to 'ai' and the parameter 'progsf90.option' set to 'maxrounds 1'. The output estimate of '$ANIMAL_j$' is the Estimated Breeding Value (EBV) of the j-th genotype. The heritability of the traits was estimated as the diagonal of Σ divided by the sum of the diagonals of Σ and the estimated variance of the residuals. The predictive ability (PA) of the model was defined as Pearson's correlation coefficient between the breeding values re-estimated from the effect sizes (see below) and the adjusted phenotypes corrected by the trial effect (i.e., $Y_{ijk} - TRIAL_i$).

Genome-Wide Association Study (GWAS). Following the equations of Aguilar et al. (2019), the effect size of the SNPs was backsolved from the EBVs, along with their standard error and their p-values. This process was performed trait by trait (i.e., without accounting for the multitrait dimension) without loss of information, as the Pearson's correlation between the ssGBLUP's EBV and the breeding values re-estimated by the effect sizes was greater than 0.999.

With the estimated effect sizes and the standard errors, we also computed an alternative to the p-value accounting for multiple testing: the local false sign rate (lfsr) estimated with an empirical Bayes approach for adaptive shrinkage using the function *ash* (R package *ashr*; Stephens, 2017). In order to detect GWAS peaks (regions in the chromosomes with a high density of significant SNPs), we used a custom peak detection script on the profile of lfsr (Tiret & Milesi, 2021) with default parameters.

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Neighborhood. In order to account for the effect of the neighborhood, we estimated the breeding values with an additional fixed effect, the local relatedness, defined as the average relationship (from the matrix H) between a focal individual and its neighbors (eight or less if on boundaries; as the king's moves on chess). Local relatedness was orthogonal to any micro-environmental effect, because of the random block design. The re-estimated breeding values were denoted nEBV.

SNP subsets. We investigated the effect of sampling SNPs on the ssGBLUP (i.e., weighted ssGBLUP). We selected subsets based on population and quantitative genetics features computed after a first run of ssGBLUP: MAF, Ancestry Informativeness Coefficient (AIM; Rozenberg et al., 2003), and for each trait, significance in the GWAS, and heritability. For each feature, we kept one-tenth of the whole set (713 SNPs, a sample size close to previous studies on weighted GBLUP, e.g., Li et al., 2018) with either the smallest or the largest values, ending up with 12 subsets. AIM was estimated per family with the R script provided by Cappa et al. (2022), and SNP heritability was estimated as $2p(1-p)a^2$, where p is the MAF, and a the effect size (assuming no dominance nor interaction deviation of the genetic variance; p.129, Falconer, 1989). We re-estimated the combined relationship matrix for each SNP subset by re-estimating in the equation (1) the genomic relationship matrix G_V with the SNP subset. With the re-estimated matrices H, we re-performed the MDSs, and re-estimated the PAs of the EBVs and nEBVs (where local relatedness were measured with the re-estimated H).

Statistical tests. For each SNP subset, we computed the confidence interval of the Predictive Ability (PA) with a 1000 iteration bootstrap (re-sampling individuals), without re-estimating the covariance matrix Σ. Here, the bootstrap was only used to assess the variance, so that we centered the bootstrapped PA around the true PA. To assess the effect of sampling, these subsets were compared to 1000 random samples of 713 SNPs (the size of the SNP subsets). Comparisons were performed with a Student's one-sample *t*-test

(denoted t_1 with a degree of freedom or df of 999 corresponding to the number of bootstrap iterations), or a Welch two-sample *t*-test (denoted t_2 with a varying df), both using the function *t.test* (R package *stats*). The sign of the *t* statistic is arbitrarily reported as positive when greater than a baseline (e.g., the true PA), and negative when not. Models were compared with a likelihood ratio test with a df of 1 using the function *lrtest* (R package *lmtest*; Zeileis & Hothorn, 2002). All scripts were written in R v4.1.3 (R Core Team, 2022).

Results

Once relatedness among neighbors is actually shown to be correlated with focal phenotypes, the difficulty in telling apart kin selection from niche competition lies in finding outwhether relatedness is the cause, as the expression of kin selection or niche competition, or a consequence, as the expression of a confusion between relatedness and phenotypic interaction. In the latter case, the focal phenotype would be modified by that of its neighbors through phenotypic interaction, and if phenotypic interaction increases when phenotypes are similar (i.e., directional selection, e.g., competition for resource uptake), it can be confused with the effect of relatedness, as genetically related individuals tend to have similar phenotypes. Tree height and rust vulnerability have been the choice of our focus; both exhibit high heritabilities (0.469 and 0.666 respectively), high predictive abilities (0.677 and 0.755 respectively), and a strong independence, with an estimated genetic correlation of 0.022. The low correlation is consistent with some previous reports (e.g., Beaulieu et al., 2020; Liu et al., 2022), though not all (e.g., Lenz et al., 2021; Cappa et al., 2022); we will assume in our case that orthogonality is strong enough to show that the traits are non-redundant. Whether investigating highly heritable and orthogonal traits can help detecting kin selection is yet unknown, and will be developed further on, but these preliminary results make a solid case for tree height and rust vulnerability, and the choice of the SNP chip array.

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Kin selection is a local relationship that can be hard to evaluate accurately (e.g., blurred by micro-environmental effects); we posit that this problem can be overcome by investigating the extent to which SNPs structuring families (i.e., kins) control phenotypes of the focal individual, compared to other subsets of SNPs. Genetic variance explained by the markers was low for both tree height (0.051) and rust vulnerability (0.058), displaying an expected missing heritability, because of the size of the SNP chip (Yang et al., 2010); association studies on our dataset was then expected at best to underestimate SNP contributions. On the one hand, the GWAS was not solely artefactual, as it did not overfit phenotypic variation: swapping phenotypes among family members of the same block (i.e., only randomly mismatching Mendelian sampling) significantly reduced the average significance of the SNPs ($t_1 < -2.59 \times 10^2$, $p < 0.001$). On the other hand, only one genetic variant was significantly associated with rust vulnerability, located on the chromosome 1, and none for tree height (Fig. 1A, 1B). The Q-Q plot on rust vulnerability revealed that some SNPs were significantly associated with a level of significance well above the 95% confidence interval (Fig. 1D); most of them being in the identified peak. None of the SNPs were significantly associated with tree height after Bayesian shrinkage, and Q-Q plot revealed a classic population structure overcorrection (Fig. 1C). We ascribe the difference in significance between tree height and rust vulnerability to the SNP chip that was designed over QTLs and expressional candidate genes related to rust resistance among other traits, but not tree height (Faivre-Rampant et al., 2016). Altogether, although the genomic evaluation (i.e., when using all SNPs) seems robust and consistent, SNP-wise approaches such as GWAS were noticeably not robust to the SNP chip design. From here onwards, to account for this design bias, we will only work with the subsets of SNPs described above, not single SNPs.

Common and most heritable alleles structured families

The population under study is structured in a hierarchical way: first into population structure, then in family structure. Population structure, captured by the first eigenvalues (Patterson et al., 2006), substantially shaped genetic diversity, as the first two eigenvalues alone

explained more than 50% of the variance (40.0% and 24.5% respectively). Because the population underwent family selection during breeding in the past, family structure is strong and can even be apprehended visually (Fig. 2). Several statistics significantly supports the strong family structure: (i) randomly shuffling the family ID increased by 9.96 times the within "family" variance ($t_1 = 3.70 \times 10^3$, $p < 0.001$); (ii) when restricting to common alleles, the first two eigenvalues of the PCA still explained a large part of the variance (56.2%), showing that family structure is bound to selection, as expected under family selection.

Population structure is generally assumed to be reflected by polymorphism atneutral genes scattered across the genome, to the point where sufficiently large samples of SNPs can reveal population structure quite accurately. That is not the case for family structure in our dataset, as random samples of SNPs performed quite poorly compared to the whole set of SNPs ($t_1 = 2.12 \times 10^2$, $p < 0.001$), suggesting a non-random distribution of the family structure information across the genome. Actually, the "most" neutral and the "most" selected SNPs had the strongest structuring power on the family level (Fig. 3): SNPs with the largest AIM, performing better than random samples ($t_1 = 3.86 \times 10^2$, $p < 0.001$), and even than the whole set as can be expected under neutrality; and SNPs putatively under selection, i.e., with the largest MAF and the largest heritability, performing better than random samples ($t_1 > 1.09 \times 10^2$, $p < 0.001$). Additionally, and supporting the aforementioned bias of SNP-wise approaches, both significant and non-significant SNPs (in the GWAS) were only slightly different from random samples in regards to family structure though significant ($|t_1| > 5.52$, $p < 0.001$).

Local relatedness caused an amplification of rust damage

Although the growth of focal individuals was not affected by their neighbors, damage due to rust was more important the more related the focal individuals were with their neighbors. Indeed, the genomic models incorporating local relatedness as a covariate had significantly higher likelihood than without for rust vulnerability ($\chi^2 = 9.83$, $p = 0.002$), but not for tree

height (χ^2 = 0.09, p = 0.236). As for family structure which is driven by some SNPs, different categories of SNPs had a different impact on focal phenotypes. When accounting for relatedness via the different subsets of SNPs, the Akaike Information Criterion (AIC) generally decreased (Fig. S2). However, for rust vulnerability, only the subset of SNPs with the largest MAF significantly increased the likelihood of the model (χ^2 = 4.02, p = 0.04), that is the one that best captured the family structure. Likelihood was not significantly improved by SNPs with the largest AIM (χ^2 = 0.15, p = 0.703) or the largest heritability (χ^2 = 2.65, p = 0.104, respectively), and was significantly worse with other subsets (χ^2 > 6.66, p < 0.010). For tree height, no subset of SNPs improved the likelihood (χ^2 < 3.48, p > 0.062). In other words, when the probability of focal individuals and neighbors to be in the same family was high, focal phenotypes were more affected by their neighbors; in this case, family neighboring caused significantly more damage by rust.

One of the differences between tree height and rust vulnerability is the genetic architecture: the GWAS shows a monogenic architecture of rust vulnerability as opposed to a putative polygenic architecture of tree height, hence a “simpler” architecture for rust vulnerability. Even if it should be taken cautiously, it is not uncommon to encounter a simpler genetic architecture for response to biotic constraints compared to complex quantitative traits (e.g., gene-for-gene resistance; Flor, 1971). Consequently, many haplotype combinations can lead to tall individuals, but few to rust resistance, because a polygenic architecture is redundant; in other words, when individuals are related, their probability of both being resistant is higher than that of both being tall. Local relatedness thus reveals a shared vulnerability within the neighborhood. Results suggest that shared vulnerability is the cause of rust damage amplification in related neighborhood. Supporting this claim, the covariance between random pairs of neighboring individuals’ rust vulnerability was enriched by local relatedness (from 0.28 for the whole set to 0.43 for the highest quartile), suggesting again that local relatedness was a proxy of phenotypes being correlated due to a correlation of genotypes.

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In spite of genomic models being sensitive to local relatedness, improvement of predictive abilities (PA) was limited - probably due to a lack of power. Indeed, incorporating local relatedness did not significantly change the PA neither with the whole set of SNPs ($|t_2| < 0.594$, $p > 0.553$), nor with any of the subset ($|t_2| < 1.03$, $p > 0.301$), with a slightly better PA when incorporating local relatedness for SNPs with the smallest MAF on rust vulnerability ($t_2 = 1.73$, df = 2.00×10^3, $p = 0.0841$), and SNPs with the smallest tree height significance on rust vulnerability ($t_2 = 1.97$, df = 2.00×10^3, $p = 0.0491$). See the Supplementary materials for further details.

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Discussion

Genomic selection in forest tree breeding has successfully predicted growth and wood quality for many species (Lenz et al., 2020), such as Eucalyptus (e.g. Resende et al., 2012), pines (e.g., Isik et al., 2016), or spruces (e.g., Beaulieu et al., 2014). The predictive ability (PA) of the underlying genomic models relies on the ability of genotypic data to capture the covariance between candidate loci, thereby reflecting how much genetic similarity explains phenotypic similarities (Powell et al., 2010). An additional source of phenotypic covariances is micro-environmental effects, such as local relatedness, and results clearly demonstrates that in some cases, interaction between neighbors cause an individual to grow differently. In the case of the Black poplars under study, for rust vulnerability the interaction was more likely due to phenotypic interaction rather than kin selection/niche competition.

SNP chip designs strongly impact the analysis

In order to understand what drove the phenotypic covariance between individuals, we subset 10% of the SNPs according to their MAF, AIM, significance in a GWAS, or heritability, thus redefining local relatedness with a different set of linkage disequilibrium. Subsetting SNPs is commonplace in genomic selection, mainly to reduce costs when, for instance, commodity value per individual is low (e.g., Luo et al., 2021), or when imputation is powerful enough to make genomic predictions possible with low-density markers (e.g., Kijas et al., 2017; Sanchez et al., 2017; Tsai et al., 2017). So far, different subsetting techniques have been proposed: AM-BLUP whereby selecting relevant regions of chromosomes (Astle & Balding, 2009; but see critiques from Gualdrón Duarte et al., 2020), TA-BLUP equivalent to our set of heritable SNPs (Zhang et al., 2010), from GWAS (Luo et al., 2021), or from machine learning methods (Li et al., 2018). In the present paper, we tried to integrate this large spectrum of methods in a common framework to assess their relative efficiency.

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The use of subsets of SNPs in genomic selection is an ongoing debate, as doing so ended in some studies with better predictive abilities (e.g., Dong et al., 2016; Luo et al., 2020; Cappa et al., 2022), whereas, as our results showed, some studies showed otherwise (e.g., Lu et al., 2020; Thistlethwaite et al., 2020). The second option can be understood when following the infinitesimal model (Fisher, 1918), or its recent development, the omnigenic model (e.g., Boyle et al., 2017). In particular, subsetting reduces the scope of linkage disequilibrium (Thistlethwaite et al., 2020), and deleting common SNPs ends up with a considerable loss of heritability (Yang et al., 2010; Biddanda et al., 2020). Our results suggest that subsetting can be successful, as it was here for rust vulnerability, only because the SNP chip design matched the trait or past selection. Genomic selection should therefore be carefully designed in accordance with the forecasted usage of a low-density marker chips if any, and acknowledging that extending its usage can be fraught with difficulties.

Selection of relatedness or co-selection of relatives?

Interactions between neighbors may depend on local relatedness, and whether this interaction is positive or negative depends on the relative strength of kin selection (Hamilton, 1964) and niche partitioning (Silvertown, 2004). Although the importance of group selection in nature is controversial (Maynard Smith, 1964; Lewontin, 1970; Wilson & Wilson, 2007; Okasha, 2020), local relatedness has been shown to be perceived through kin recognition in plant species (e.g., Dudley et al., 2007; Murphy et al., 2009). Competition can be accounted for in a quantitative genetic model by assigning to an individual one direct genetic effect (DGE) as the effect of its genes on its own phenotype, and one indirect genetic effect (IGE) as the effect of its genes on phenotypes of surrounding individuals (Griffing, 1967; potential applications in Muir, 2005). Although adding complexity to the model, including competition proved to reduce bias in covariance estimation (Costa e Silva et al., 2013). In our case, on top of all our attempts to incorporate interactions, IGE resulted in a lower predictive ability than with the ssGBLUP baseline (results not shown).

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The simpler genetic architecture of rust vulnerability compared to that of tree height might play a role in shaping the phenotypes. Family structure was driving similarity of rust vulnerability, and combination of similar vulnerability resulted in increased rust damage. The amplifying effect is even stronger in perennials as shared vulnerability can be perceived by successive generations of pathogens. In agreement with the higher resilience in regards to diseases confered by varietal association in a field (e.g., Smithson and Lenne, 1996; Burdon and Thrall, 2009), locally pyramiding vulnerability amplified rust damage. Resistance similarity and relatedness were strongly connected, but it is likely that the more complex the genetic architecture, the easier it is to uncorrelate phenotypic similarity and relatedness, and so profitable for breeding schemes when trying to limit local pyramiding while using family selection.

Whether there was kin selection/competition on top of phenotypic interaction or we simply lacked statistical power is hard to decide. Selection on local relatedness due to kin selection or niche partitioning - namely, selection on relatedness - might be confused with the fact that selection leads to phenotypic convergence, that in turn leads to a genotypic convergence - namely, a co-selection of related. The random block design and repetition can partly limit this confusion, but it is evident that interpreting the data is fraught with difficulties, as the tallest individuals showed also a higher relatedness with the rest of the population. The fact that family structure was mainly carried by SNPs likely associated with selection (following the hypothesis of Yang et al., 2010 and Biddanda et al., 2020) suggest that relatives were co-selected, as is often the case in breeding programs implementing family selection. An inevitable consequence is that, as seen in crop selection, interesting genotypes for kin selection/cooperation were lost during domestication (Fréville et al., 2022).

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Author Contributions

MT was responsible for writing the report, conducting the search, extracting and analysing data, interpreting results, updating reference lists. LS was responsible for designing the experiments and screening potentially eligible studies. ML and LS contributed to interpreting results and provided feedback on the report.

Competing Interests

The authors declare that there is no conflict of interest.

Data Archiving

The data that support the findings of this study are openly available on DATA INRAE at https://data.inrae.fr/privateurl.xhtml?token=b79ab1ca-ebb9-47c6-9272-1568c0c33d70.

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Figures

Figure 1. GWAS on tree height and rust vulnerability in Populus nigra. **(A)**. Manhattan plot of the GWAS on tree height. The dots are the log significance of the markers. The dashed horizontal line stands for the 5% significance level, and the solid horizontal line stands for the 5% FDR level. **(B)**. Idem as panel A, for rust vulnerability. **(C)**. Q-Q plot of the GWAS on tree height. The dots were the quantile of the markers; the solid line is the 1-slope segment; the gray cross is the position of the median; and the gray area is the 95% confidence interval. **(D)**. Idem as panel C, for rust vulnerability.

Figure 2. The first two axes of the Multidimensional Scaling (MDS) of Populus nigra. **(A)**. MDS with labels of the fathers (with black large shapes). Colored and smaller shapes are the offspring, the shape corresponding to its father. **(B)**. The same MDS as in panel A., but labeled for the mothers.

Figure 3. Within family variance (V_F) for each subset of SNPs. The black dashed horizontal line is the average V_F of randomly sampled SNPs, the gray dashed horizontal lines delimit the 95% confidence interval, and the black horizontal dotted line is V_F of the entire set of SNPs. Red dots are when selected for tree height, and blue for rust vulnerability. SNP subsets: largest or largest MAF (+ or - MAF), largest or smallest AIM (+ or - AIM), most or least significant (+ or - $\log_{10}(p)$), and most or least heritable for tree height (+ or - h^2).

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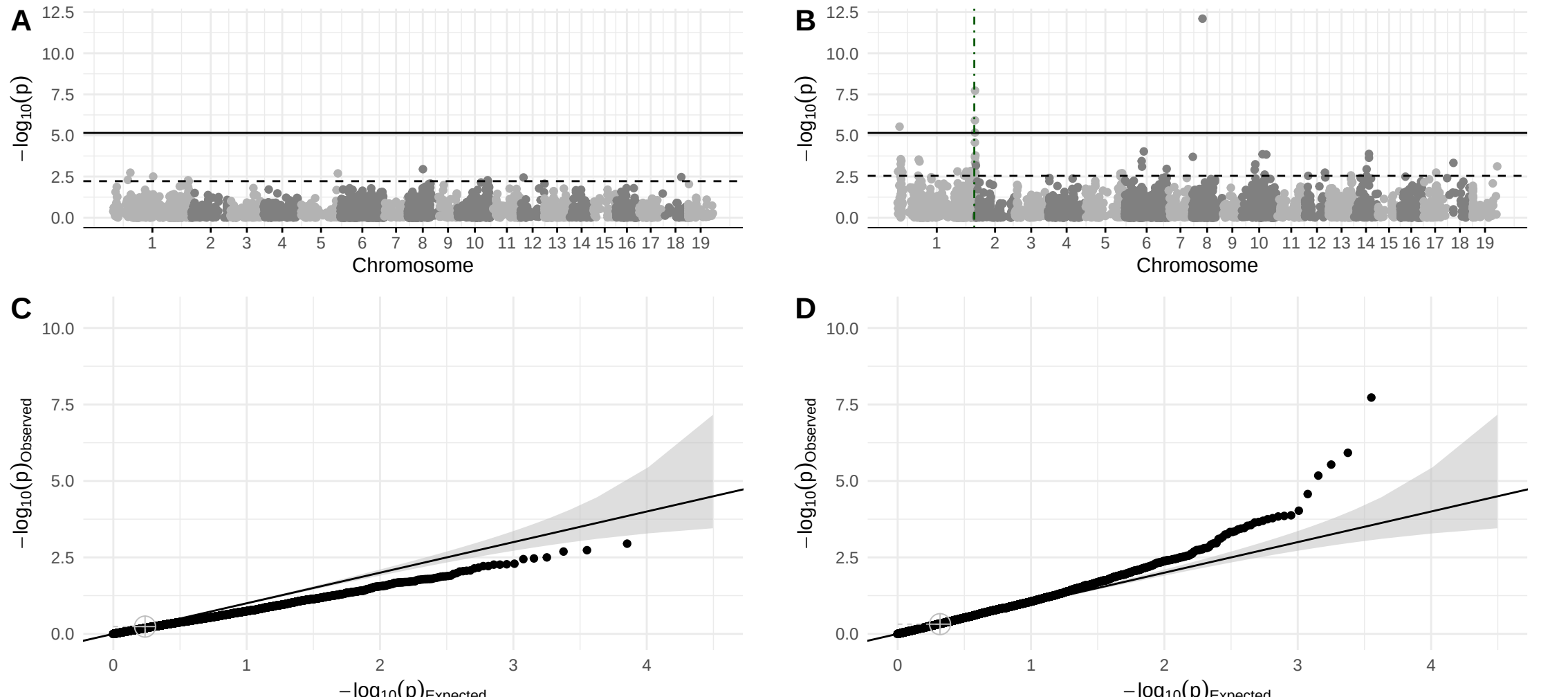
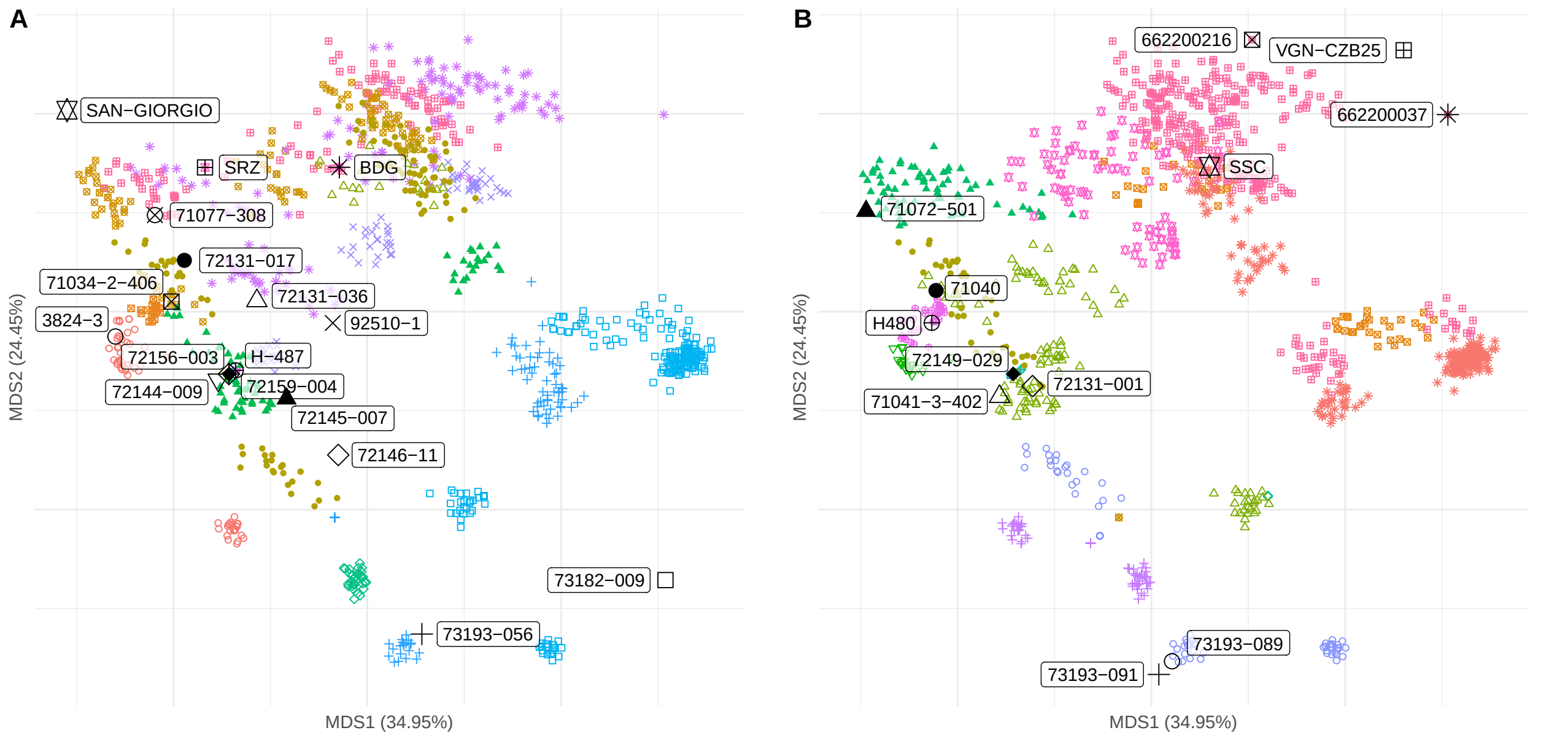
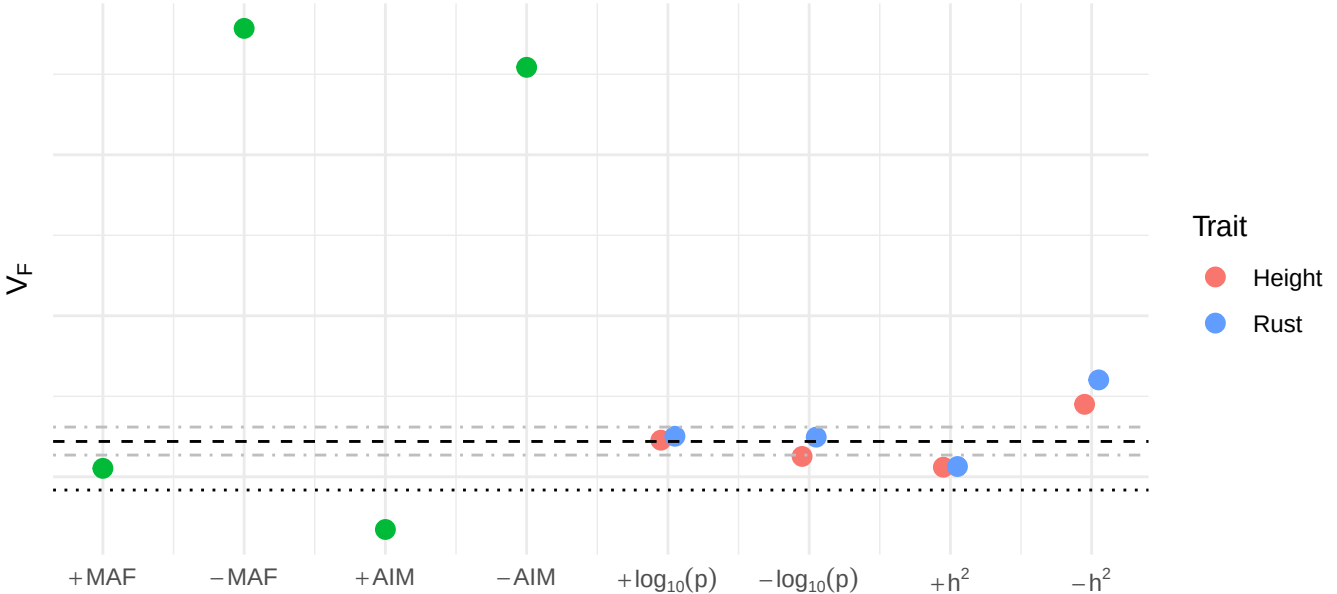

A
B
C
D
$-\log_{10}(p)$
Chromosome
$-\log_{10}(p)_{Observed}$
$-\log_{10}(p)_{Expected}$


A
SAN−GIORGIO
SRZ
BDG
71077−308
72131−017
71034−2−406
72131−036
3824−3
92510−1
72156−003
H−487
72144−009
72159−004
72145−007
72146−11
73182−009
73193−056
MDS2 (24.45%)
MDS1 (34.95%)
B
662200216
VGN−CZB25
662200037
SSC
71072−501
71040
H480
72149−029
72131−001
71041−3−402
73193−089
73193−091
MDS2 (24.45%)
MDS1 (34.95%)


V_F
Trait
Height
Rust
+MAF
−MAF
+AIM
−AIM
$+\log_{10}(p)$
$-\log_{10}(p)$
$+h^2$
$-h^2$