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21 developed the gene-to-phenotype network, developed the *in-silico* selection experiments,
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Abstract

Predictive breeding is now widely practiced in crop improvement programs and has increased selection response (i.e., the amount of genetic gain between generations) for complex traits. However, world food production needs to increase further to meet the demands of the growing human population. Even with leading current methods, the prediction of complex traits can be inconsistent across different genetic, environmental, and agronomic management contexts because the relationship between genomic and phenotypic variation is not well accounted for. Therefore, developing gene-to-phenotype (G2P) network models that integrate the knowledge of molecular networks from systems biology with population genomics has been proposed as a strategy to close this gap in predictive modelling. Here, we use shoot branching, a critical developmental pathway underpinning harvestable yield for many crop species (e.g., grain yield for cereals), as a case study to explore the value of G2P networks to enhance understanding of selection responses for complex traits. We observed that genetic canalization is an emergent property of the complex interactions among shoot branching G2P network components, leading to the accumulation of cryptic genetic variation, reduced selection responses, and large variation in selection trajectories across populations. As genetic canalization is expected to be pervasive in traits, such as grain yield, that result from interactions among multiple genes, traits, environments, and agronomic management practices, the need to model traits in crop improvement programs as outcomes of networks is highlighted as an emerging opportunity to advance our understanding of selection response and the efficiency of developing crops for future climates.

Introduction

Due to an increasingly harsh and unpredictable climate, improving the consistency and scope of predictions is crucial for global agriculture to meet the challenge of feeding a global population of 10+ billion people (1). Current breeding prediction methods assume a simplified

relationship between genotype and phenotypes (2, 3), thus limiting the realized selection response of crop improvement programs. Although this simplified genotype-to-phenotype relationship (G2P map) is sufficient to successfully model the average selection trajectory of large populations (2), this approach captures only a subset of all performance outcomes, potentially leading to misalignments between predicted performance and realized performance in the field. Such simplified G2P relationships can hinder accurate predictions for crop performance in specific management and environment combinations (4).

Developing G2P network models that integrate knowledge of molecular networks from systems biology with population genomics may improve modelling of the genotype-to-phenotype relationship (5). For crop improvement programs, the detected interactions in molecular network models can unmask existing genetic variation or identify intermediate traits to increase the selection accuracy and efficiency of developing novel varieties. The contributions of the interactions (epistasis) to total genetic variation must be converted into additive genetic variation to deliver a selection response. A strong theoretical understanding exists of the importance of epistasis for selection response when based on G2P models without molecular network models (6, 7). However, relevant knowledge of selection response for G2P models based on networks is lagging due to the lack of adequately characterized empirically based examples.

Here, we use shoot branching as a case study of an agronomically important, well-characterized and empirically described network (8). The structure of this network is largely conserved across herbaceous model plants and divergent crops, including garden pea and rice, and involves interactions among plant hormones (auxin, cytokinins, and strigolactones) and sucrose (8–10). To enhance understanding of how variation from interactions in network-based G2P models is translated into selection responses for complex traits, we created a shoot branching G2P network (Fig. 1) underpinned by genomic variation. *In-silico* selection experiments were performed on a large, segregating plant population to quantify direct (time to bud outgrowth) and

indirect (intermediate traits; hormones and sucrose) selection responses. The results are discussed in terms of practical implications for established and future crop improvement programs.

Results

The *in-silico* selection experiments with the shoot branching G2P network revealed the presence of cryptic genetic variation for the intermediate traits, hormones, and sugars that the selection for time to bud outgrowth struggled to access. Cryptic genetic variation led to many repeated selection cycles with reduced selection response and large variation in selection trajectories across different populations (Fig. 2A, C). Despite the large magnitudes of cryptic genetic variation for the intermediate traits under indirect selection, we only observed small differences in the selection trajectories of time to bud outgrowth under direct selection.

Cryptic genetic variation for the intermediate traits resulted in temporary and permanent plateaus in selection response (Fig. 2A, C). The emergence of these plateaus began after only three selection cycles. In scenarios with a simulated broad-sense heritability of 1 (no error during selection), the average genetic mean for sucrose began to increase again around selection cycle nine when the genetic mean for strigolactones reached approximately 0.1. However, even with this perfect selection accuracy, a few populations reached permanent plateaus at local maxima for two intermediate traits, sucrose and strigolactones. In scenarios with error variance included in the direct selection of time to bud outgrowth, selection response was decreased for all components of the shoot branching G2P network, with the largest reductions observed for the genetic mean of sucrose. For example, with a broad-sense heritability of 0.3, the average genetic mean for sucrose across the 100 population replicates reached a permanent plateau at less than 90% of the maximum theoretical value after 30 selection cycles (Fig. 2C), with several individual populations achieving less than 60%.

Different magnitudes of cryptic genetic variation across the intermediate traits of hormones and sucrose resulted in different allele frequency changes for causal loci with similar genetic effect sizes (Fig. 2B, D). This trend was most apparent for causal loci occupying the bottom 60% of

genetic effect sizes. In this G2P network simulation, alleles of causal loci with moderate genetic effect sizes (0.2–0.6) for cytokinins and auxin reached fixation (allele frequency of 0 or 1). However, causal loci for sucrose and strigolactones, also with moderate genetic effect sizes, were still segregating after 30 selection cycles (Fig. 2D). In the most extreme cases, causal loci with small genetic effect sizes (<0.2) underwent genetic drift, with allele frequency changes in the opposite direction from that expected. For example, we observed increases in allele frequencies of causal loci for strigolactones until at least selection cycle 10 (Fig. 2B, D) even though these would intuitively be expected to be selected against under the direct selection for faster bud outgrowth (Fig. 1). This property of intermediate traits was independent of the heritability of the trait under direct selection.

Discussion & Conclusion

In this study, cryptic genetic variation accumulated over selection cycles for the intermediate traits (hormones and sucrose) of the shoot branching G2P network, which resulted in reduced selection responses. The cryptic genetic variation for sucrose can be explained by the complex interaction between sucrose and strigolactone signalling (Fig. 1), which resulted in genotypes with completely different combinations of strigolactone and sucrose levels producing similar values for time to bud outgrowth (Fig. 3).

The accumulation of cryptic genetic variation is not specific to the shoot branching G2P network. It can occur whenever non-linear relationships exist among traits or casual genetic loci due to genetic canalization (11). Therefore, we expect genetic canalization to be pervasive in complex target traits under selection in crop improvement programs, such as grain yield, that result from interactions among multiple interacting genes, traits, environments, and agronomic management practices.

Reductions in detectable genetic variation for complex traits under selection due to genetic canalization have important implications for the selection responses achieved by crop improvement

programs. Reductions in detectable genetic variation for complex target traits reduce the accuracy of identifying the best performing individuals, and decanalization of genetic variation for intermediate traits could contribute to the unexpected, continued selection responses seen in long-term selection experiments (12). Additionally, the combination of reduced selection accuracy and reduced estimates of genetic variation could cause crop improvement programs to mistake temporary selection plateaus at local maxima for true, permanent selection limits.

Therefore, crop improvement programs require strategies to improve the prediction of selection response for complex traits in the presence of genetic canalization. In this study, decanalization and the subsequent increases in selection response occurred via the fixation of causal loci by chance (genetic drift). A more targeted strategy would be to restructure improvement programs to promote and control the conversion of epistatic genetic variance into additive genetic variance. In maize (*Zea mays*), Technow et al. (13) demonstrated that a decentralized structure of multiple, smaller crop improvement programs interconnected by a few key parents is required to facilitate selection response under high levels of genetic complexity. Another complementary strategy involves direct selection on traits at intermediate layers of the G2P network to circumvent the complex interactions that generate canalization of traits at higher levels of the network hierarchy. In the case of shoot branching, decanalization was achieved by direct selection on sucrose levels (data not shown). Such strategies could be designed via *in-silico* simulations, akin to our approach, and then implemented within empirical, longitudinal, "select and sequence" studies (14, 15) to improve understanding of G2P networks and the influence of genetic canalization on selection response for complex traits in nature and agriculture.

Materials and Methods

We created a G2P network for bud outgrowth by connecting a published, empirical shoot branching network (8) to underlying allelic variation in the genome (Appendix A). The shoot branching network models the trait "time to bud outgrowth" as the outcome of the intermediate traits, auxin, cytokinins,

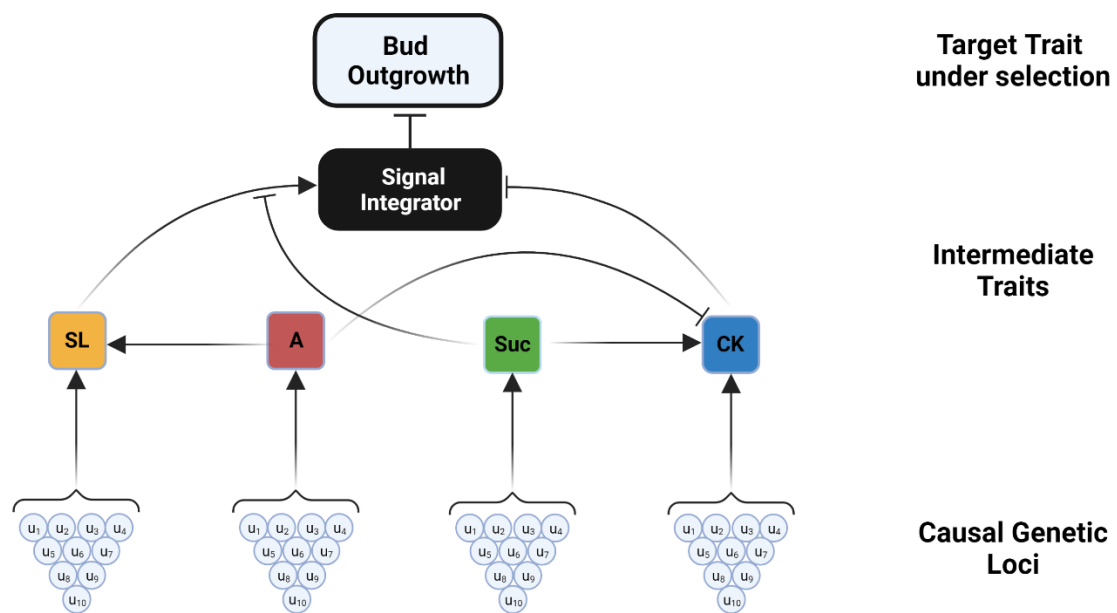
strigolactones, and sucrose, and their interactions (Fig.1). We simulated 10 non-pleiotropic, causal genetic loci for each intermediate trait and calculated additive genetic values for individual genotypes. These additive genetic values replaced the synthesis term of the hormones and sucrose in Bertheloot et al. (8) (Appendix A). Therefore, the trait under selection, time to bud outgrowth (y_{BO}), can be viewed as a function F of the levels of genotype-dependent intermediate traits (g) and a random error term (e). To quantify response to selection, we performed *in-silico* divergent selection experiments over 100 selection cycles (Appendix A). The results presented are generated from 100 replicates of the selection experiment. Code for the shoot branching network, genetic simulations and figures can be accessed in the following repository: <https://github.com/powellow/GeneticCanalizationOfG2PNetworks>.

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Selection For Faster Bud Outgrowth

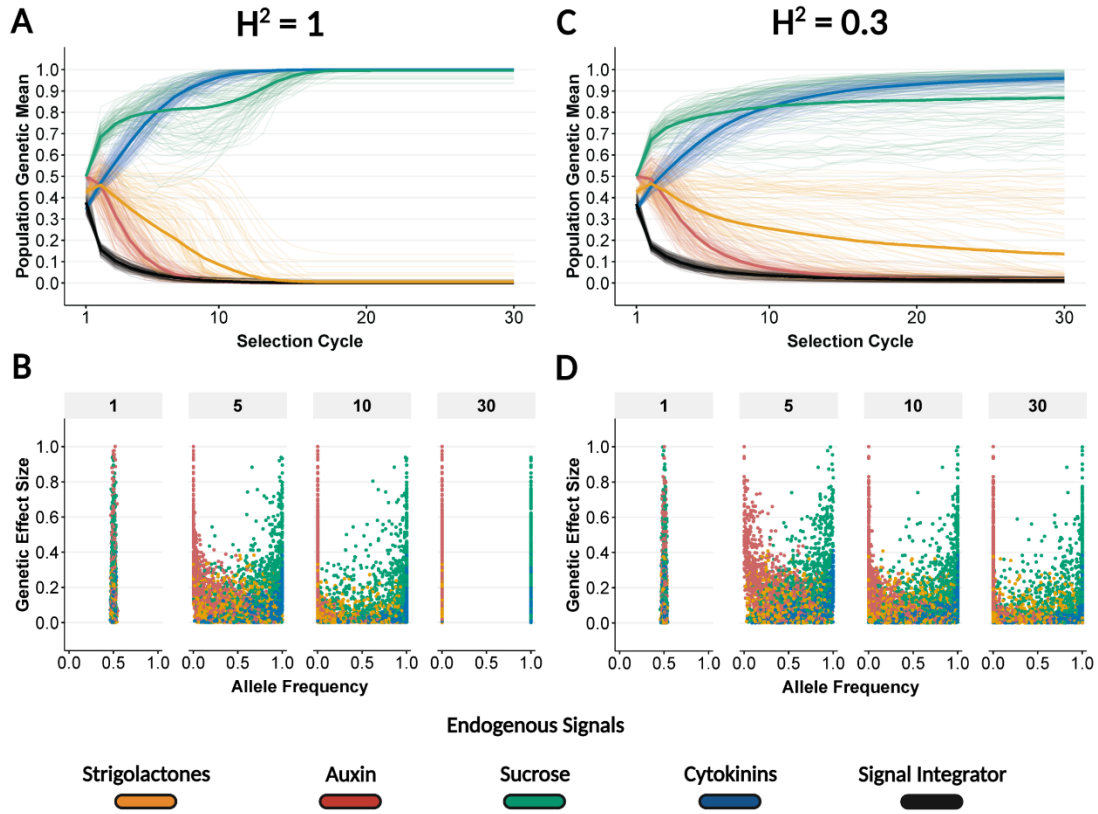
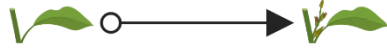


Figure 2. Selection trajectories for the shoot branching G2P network for faster bud outgrowth and the relationships between normalized genetic effect sizes and allele frequency changes at causal genetic loci over selection cycles. (A, B) Results from selection with a broad sense heritability, H^2 , of 1. (C, D) Results from selection with a broad sense heritability, H^2 , of 0.3. (A, C) Normalized total genetic values for the intermediate traits over selection cycles. Thick lines are the normalized genetic means averaged across the population replicates. Thin lines are the normalized genetic means for each population replicate. (B, D) Plot of allele frequency changes at causal genetic loci versus normalized genetic effect sizes. Each point represents a causal genetic locus from the 100 population replicates. Values are presented for selection cycles 1, 5, 10, and 30.

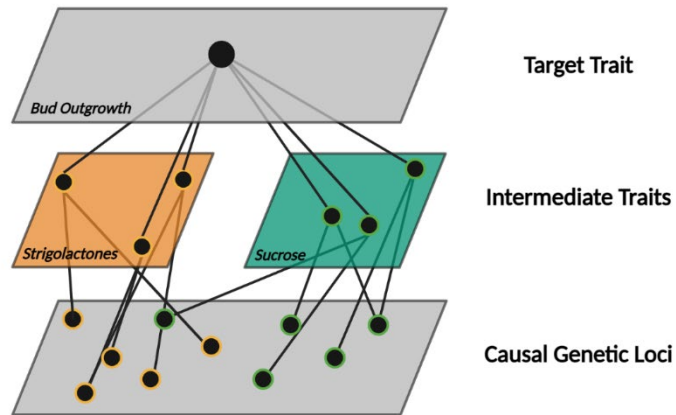


Figure 3. Genetic Canalization of Gene-to-Phenotype Networks. Multiple genetic combinations of intermediate traits produce similar values for the target trait, causing the accumulation of cryptic genetic variation that cannot be accessed by selection.