

High Intra-Accession Variation may Limit the Effectiveness of Association Analyses in Out-Crossing Crop Diversity Panels

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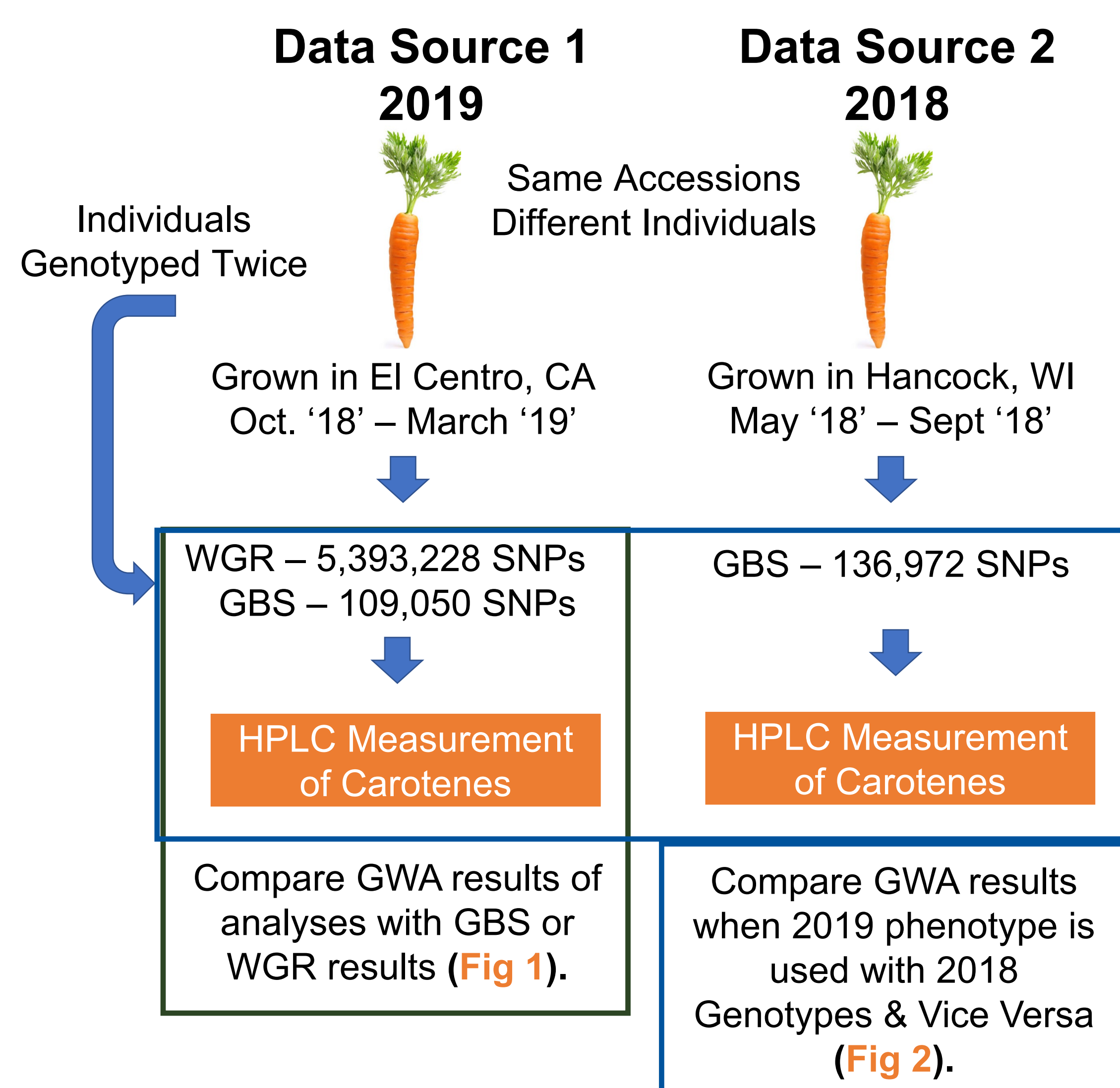
Introduction

- Carrots are a highly nutritious vegetable crop and are one of the best sources of vitamin A precursors due to their high accumulation of α - and β -carotene¹.
- There are many breeding targets for improvement of carrot production including biofortification and reducing losses due to biotic² and abiotic stressors³.
- Diverse carrot germplasm (e.g., USDA-NPGS) have been assayed to identify accessions that contain novel and beneficial traits⁴.
- Subsequent genomic analyses, including genome wide association studies (GWAS), have identified regions of the genome that contain beneficial alleles.
- GWAS in USDA-NPGS germplasm has resulted in the detection of loci related to traits such as root shape⁵, stand vigor⁶ and carotenoid accumulation⁷.
- GWAS may have limitations because carrots from the USDA germplasm collection have markedly low linkage disequilibrium with an $r^2 < 0.2$ within 500bp, observed heterozygosity ~ 0.18 and frequent recombination⁷.
- Given the large amount of intra-accession phenotypic variation, genotyping 15-20 individuals may be needed to capture the genetic variation in carrot accessions from the USDA collection⁸.

Objectives

- Test if reduce representation sequencing, such as genotyping-by-sequencing (GBS), results in enough marker density to have comparable results to whole-genome resequencing (WGR).
- Evaluate the value of genotyping a random single plant to represent the genotype of an accession from the USDA germplasm collection in a GWAS.

Methods



GWA Analyses DNA Isolation, Sequencing & HPLC Genotype Filtering

References

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Results & Conclusions

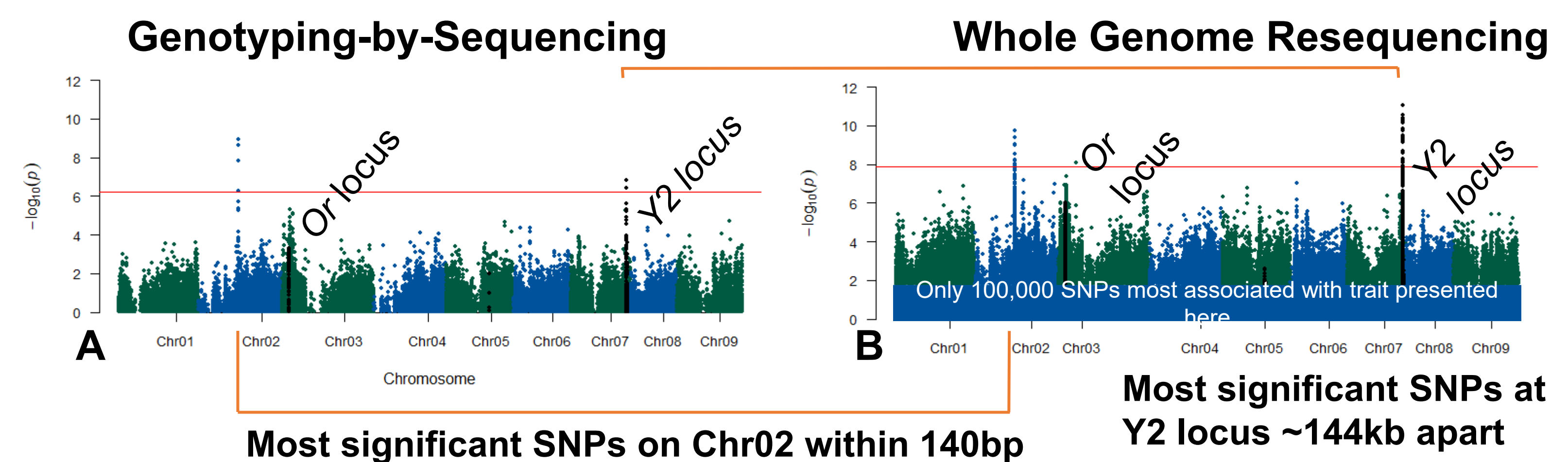


Figure 1. GWAS using the same phenotypic data (Date Source 1) from 354 different individuals comparing GBS and WGR. GWAS methods were identical except 109,050 markers were included in analyses with GBS (A) and 5,393,228 SNPs with WGR (B). Previously identified loci are indicated via black colored dots.

- Regions on Chr02 and Chr07 (Fig 1A,B) were significantly associated with carotenoid accumulation and are within previously identified loci including the Y2 locus⁹.
- The physical position of significant SNPs was nearly identical on Chr02.
- The significantly associated SNPs at the Y2 locus are separated by nearly 144kb (Fig 1A,B).
- A significant association was identified near the *Or-like* gene in the WGR (Fig 2B).
- GBS results were highly comparable to WGR though WGR provided somewhat higher mapping resolution that could increase the likelihood of identifying loci and positional candidate genes.**

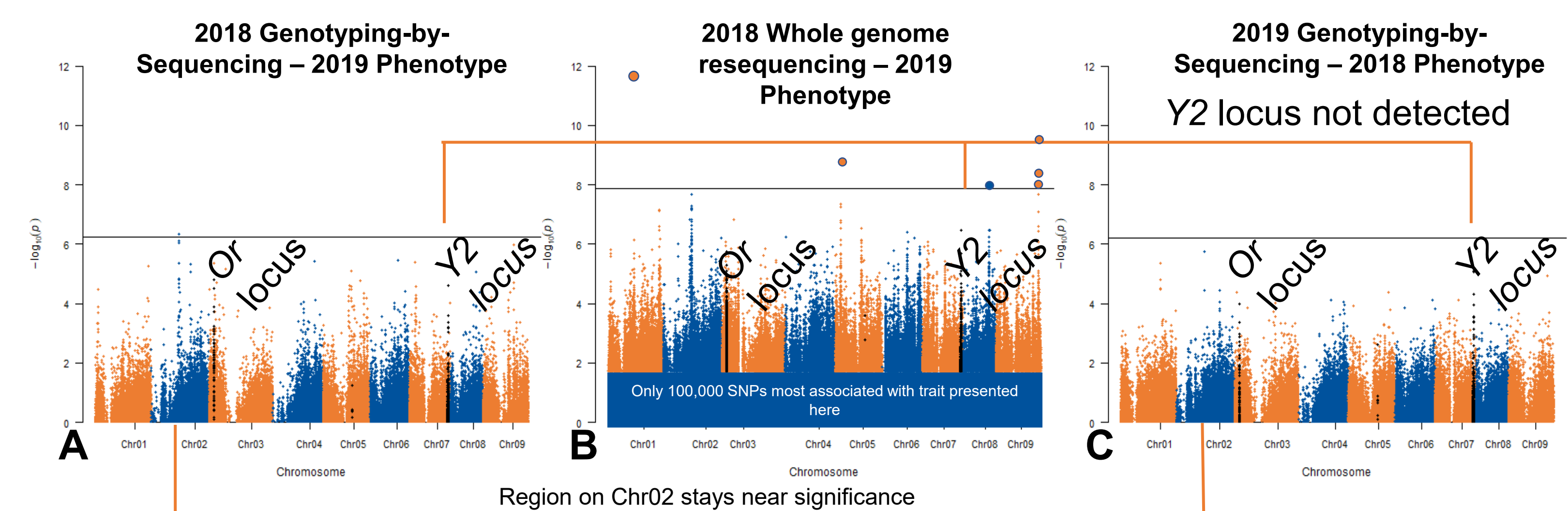


Figure 2. GWAS using genotypic data from plants that were not phenotyped but representing the same accession. (A) Plants genotyped via GBS in 2019 (Date Source 1) with phenotypic data from individuals assayed in 2018 (Date Source 2). (B) Genotypic data from plants genotyped via WGR in 2019 (Date Source 1) and phenotyped in 2018 (Date Source 2). Potential false positives indicated by larger dots. (C) Genotypic data from individuals genotyped via GBS in 2018 (Date Source 2) and different plant phenotyped in 2019 (Date Source 1).

- When genotypes and phenotypes come from different plants the Y2 locus was not identified in GWAS and the locus on Chr02 was only identified in one analysis at lower significance (Fig 2A).
- Several loci were detected in the analyses using WGR data from 2018 individuals and 2019 phenotypes (Fig 2B). These loci have not been previously identified and likely represent false positives.
- No loci were identified in analyses with 2019 GBS data and phenotypes from 2018 (Fig 2C)
- Researchers completing genomic analyses in species with high intra-accession variation should carefully consider how to phenotype and genotype plants for informative results.**
- Genotyping multiple or pooling individuals from an accession may be required to adequately represent an accession especially for traits where multiple individuals need to be phenotyped.**

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