

RESEARCH

A Two-Part Strategy for Using Genomic Selection to Develop Inbred Lines

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ABSTRACT

We propose a strategy for implementing genomic selection in plant breeding programs for developing inbred lines that reorganizes traditional breeding programs into two distinct components. These components are: (i) a population improvement component to develop improved germplasm through rapid recurrent selection and (ii) a product development component to identify new inbred varieties or parents for hybrids using traditional breeding program designs. Stochastic simulations of entire breeding programs over 40 yr were used to evaluate the effectiveness of this strategy relative to a conventional program without genomic selection and programs using three standard strategies of implementing genomic selection. Cost effectiveness was measured by constraining all programs to approximately equal annual operating costs and directly comparing each program's overall performance. Programs using the two-part strategy generated between 2.36 and 2.47 times more genetic gain than the conventional program and between 1.31 and 1.46 times more genetic gain than the best performing standard genomic selection strategy. These results indicate that the two-part strategy is a cost-effective strategy for implementing genomic selection in plant breeding programs.

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Abbreviations: 2Part, two-part program with PYT genomic selection; 2Part+H, two-part program with headrow genomic selection; AYT, advanced yield trial; CI, confidence interval; Conv, conventional breeding program; Conv GS, conventional program with genomic selection; EYT, elite yield trial; $G \times Y$, genotype-by-year interaction; Head GS, headrow genomic selection program; PYT, preliminary yield trial; PYT GS, preliminary yield trial genomic selection program; QTN, quantitative trait nucleotides; SNP, single nucleotide polymorphism.

PLANT BREEDING PROGRAMS producing inbred lines have two concurrent goals: (i) identifying new inbreds (either for varieties or parents of hybrids) and (ii) identifying parents for subsequent breeding cycles. We believe the most effective strategy for using genomic selection in these plant breeding programs would address each goal separately. This two-part strategy would reorganize traditional breeding programs into two distinct components: a product development component, to develop and screen for inbred lines, and a population improvement component, to increase the frequency of favorable alleles through rapid recurrent genomic selection.

Genomic selection uses estimates of genetic value from a genomewide set of molecular markers to make selections (Meuwissen et al., 2001; Bernardo and Yu, 2007). The process involves training a statistical model for associations between molecular

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markers and traits of interest using a set of genotyped and phenotyped individuals that are ideally closely related to the selection candidates (Clark et al., 2012; Hickey et al., 2014). The trained model is then used to estimate the genetic value of selection candidates using only their genotypic information. The selection candidates are then selected on the basis of their estimated genetic values.

Estimated genetic values can be used in place of phenotyping to drastically restructure existing breeding programs. Most breeding programs producing inbred lines use crossing to develop new germplasm and then perform selfing to derive new inbred lines (Bernardo, 2014). Alternatively, doubled haploid technology can be used to rapidly derive inbred lines (Forster et al., 2007). These newly derived inbred lines are then phenotyped for one or more cycles before final selection to fulfill one or both of the previously stated goals of product development and germplasm improvement. Within this framework, genomic selection can be used to identify promising lines sooner, thereby reducing cycle time and increasing genetic gain per year (Heffner et al., 2009). The use of inbred lines as parents is arguably only a by-product of the need to phenotypically identify new parents. With the advent of genomic selection, the use of inbred lines as parents could be eliminated entirely (Heffner et al., 2009). Strategies incorporating this idea have been described for both crops that are easy to cross (Bernardo and Yu, 2007; Bernardo, 2009) and those that are difficult to cross because of self-pollination (Bernardo, 2010). The two-part strategy that we propose is an extension of these strategies and aims to maximize the potential of genomic selection in an entire breeding program.

The population improvement component of the two-part strategy uses genomic selection to perform rapid recurrent selection. The goal is to minimize breeding cycle time to maximize genetic gain per year. Each population improvement cycle begins with a large number of genetically distinct plants. These plants are genotyped, genomic selection is applied, and the best individuals are intercrossed to produce a new generation. The cycle is then repeated. Thus, population improvement in the two-part strategy is simply a recurrent genomic selection scheme. A portion of the seed produced in some or all cycles is passed to the product development component to ensure a constant supply of improved germplasm.

The product development component of the two-part strategy focuses solely on developing inbred lines for release as inbred varieties or hybrid parents. The structure of this component resembles existing breeding programs and can thus be flexibly designed according to existing or new breeding program designs. This flexibility in designing the product development component also allows for different implementations of genomic selection. The key difference between the product development component of the two-part strategy and existing breeding programs is

that lines are not chosen for subsequent cycles of breeding, as this is handled by the population improvement component. Additionally, the product development component must include genotyping of some phenotyped plants. This is necessary for updating the genomic selection training population used in the population improvement component and also allows the application of genomic selection in the product development component. By enabling the construction and updating of the training set, the product development component of the two-part strategy guides the population improvement component.

Determining the cost effectiveness of the two-part strategy requires measuring breeding program performance over a long time period. Such a comparison would ideally use actual breeding programs, but the large investment needed for this is inhibitory. Instead, we use stochastic simulation to provide an initial demonstration of the potential of the two-part strategy.

MATERIALS AND METHODS

Stochastic simulations of entire breeding programs were used to compare cost effectiveness of a conventional breeding program not using genomic selection with two breeding programs implementing the two-part strategy and three breeding programs using alternative genomic selection strategies. The alternative genomic selection strategies are herein referred to as standard genomic selection strategies, because they maintain the traditional structure of a conventional breeding program. The structure of all simulated breeding programs were based on winter wheat (*Triticum aestivum* L.) breeding programs producing doubled haploid lines. The program that used a conventional breeding strategy without genomic selection (Conv) served as a control and is shown in Fig. 1. The standard genomic selection strategies were (. 1): a strategy that uses genomic selection in the preliminary yield trials (PYT) to improve selection accuracy (Conv GS), a strategy that uses genomic selection in the PYT to both improve selection accuracy and decrease cycle time (PYT GS), and a strategy that uses genomic selection in headrows to improve selection accuracy and further decrease cycle time (Head GS). The two-part strategy uses genomic selection to perform recurrent selection on F_1 plants in the population improvement component and to perform selection in either the PYT (2Part) or headrow (2Part+H) stage of the product development component (Fig. 2). Each program was constrained to have approximately equal operating costs, so that direct comparisons between the different breeding programs would represent their relative effectiveness. These comparisons were made using the average of 10 replicates for four levels of genotype-by-year interaction ($G \times Y$) variance. Each replicate consisted of (i) a burn-in phase shared by all strategies so that each strategy had an identical, realistic starting point, and (ii) an evaluation phase that simulated future breeding with each of the different breeding strategies (Fig. 3). Specifically, the burn-in phase was subdivided into three stages: the first simulated the species' genome sequence, the second simulated founder genotypes for the initial parents; and the third simulated 20 yr of breeding using the conventional breeding strategy without genomic selection.

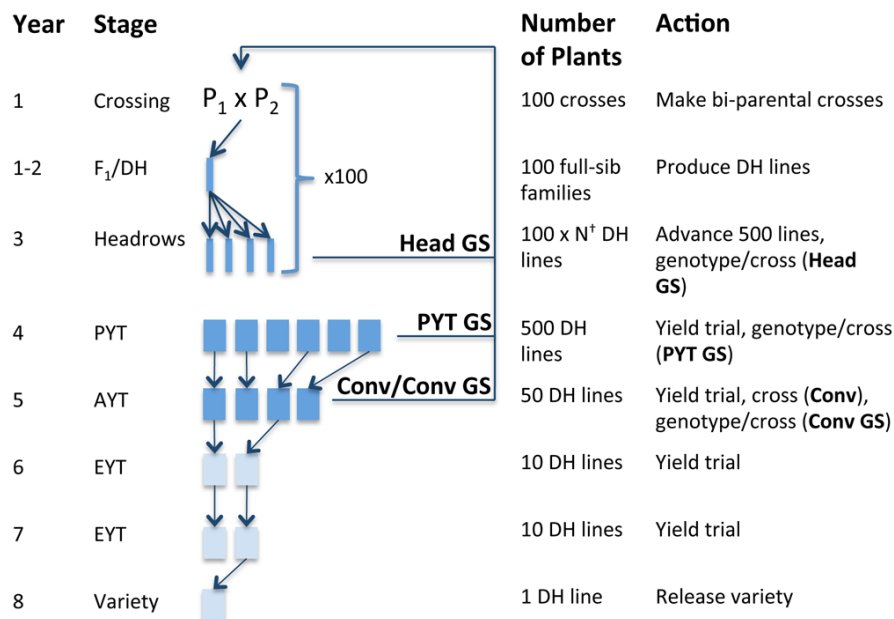
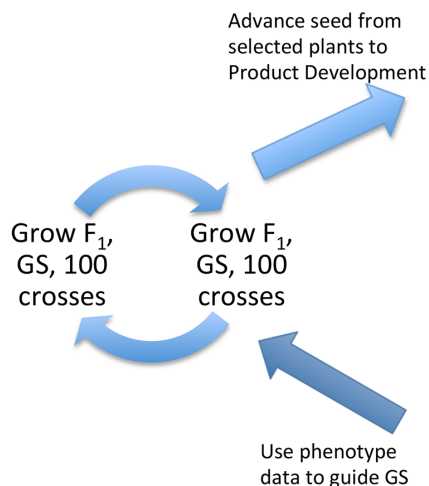


Fig. 1. Overview of breeding schemes for the conventional program (Conv; used in burn-in and as a control) and the programs using standard genomic selection strategies. DH, doubled haploid; PYT, preliminary yield trial; AYT, advanced yield trial; EYT, elite yield trial; Head GS, headrow genomic selection program; PYT GS, preliminary yield trial genomic selection program; Conv GS, conventional program with genomic selection. †The number of DH lines per cross (N) differs for each breeding program to maintain equal operating costs. See Table 1.

Population Improvement



Product Development

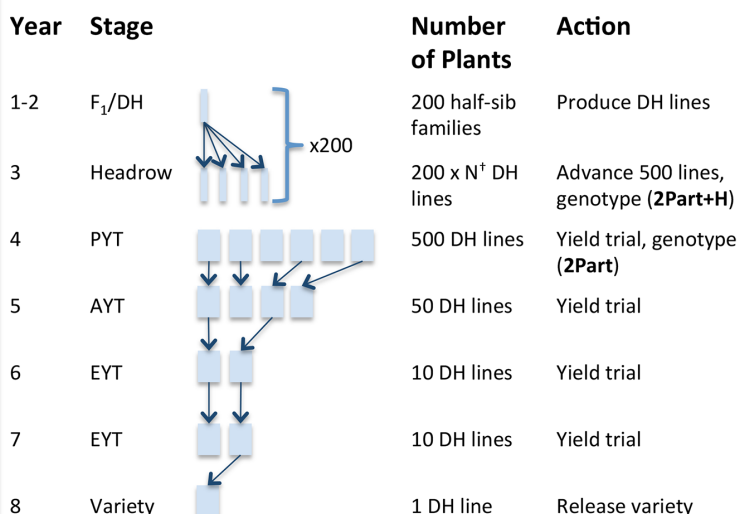


Fig. 2. Overview of the two-part program with PYT genomic selection (2Part) and two-part program with headrow genomic selection (2Part+H). DH, doubled haploid; GS, genomic selection; PYT, preliminary yield trial; AYT, advanced yield trial; EYT, elite yield trial. †The number of DH lines per cross (N) differs for each breeding program to maintain equal operating costs. See Table 1.

Burn-In: Genome Sequence

For each replicate, a genome consisting of 21 chromosome pairs was simulated for the hypothetical plant species similar to wheat. These chromosomes were assigned a genetic length of 1.43 Morgans and a physical length of 8×10^8 base pairs. Sequences for each chromosome were generated using the Markovian coalescent simulator (MaCS; Chen et al., 2009) and AlphaSim (Faux et al., 2016). Recombination rate was inferred

from genome size (i.e., 1.43 Morgans/ 8×10^8 base pairs = 1.8×10^{-9} per base pair), and mutation rate was set to 2×10^{-9} per base pair. Effective population size was set to 50, with linear piecewise increases to 1000 at 100 generations ago, 6000 at 1000 generations ago, 12,000 at 10,000 generations ago, and 32,000 at 100,000 generations ago. These values were chosen to roughly follow the evolution of effective population size in wheat (Thuillet et al., 2005; Peng et al., 2011).

Simulation Stage	Key Features
Burn-in	Genome Sequence
	100,000 generations of evolution Wheat historical effective population size 21 chromosome pairs 1.43 Morgans per chromosome 8x10 ⁸ base pairs per chromosome
	Founder Genotypes
	2x10 ⁻⁹ mutation rate 50 inbred founders 21,000 SNP markers 21,000 QTN Normally distributed QTN effects
Evaluation	Recent Breeding
	20 years of modern breeding (-19 to 0) Double haploid lines No genomic selection
	Future Breeding
	20 years of breeding (1 to 20) Testing alternative breeding programs Equal cost programs Ridge regression for genomic selection

Fig. 3. Simulation stages for a single replicate. SNP, single nucleotide polymorphism; QTN, quantitative trait nucleotides.

Burn-In: Founder Genotypes

Simulated genome sequences were used to produce 50 founder lines. These founder lines served as the initial parents in the burn-in phase. This was accomplished by randomly sampling gametes from the simulated genome to assign as sequences for the founders. Sites segregating in the founders' sequences were randomly selected to serve as 1000 single nucleotide polymorphism (SNP) markers per chromosome (21,000 total) and 1000 quantitative trait nucleotides (QTN) per chromosome (21,000 total). The founders were converted to inbred lines by simulating formation of doubled haploids.

Burn-In: Phenotype

A single trait representing grain yield was simulated for all doubled haploid lines. The genetic value of this trait was determined by the summing its QTN allele effects. The allele effects depended on the value of an environmental effect. The environmental effect changed over years to model $G \times Y$. For a given year, the allele effects followed this formula:

$$a_i(w_j) = b_i + m_i w_j,$$

where a_i is the allele effect for QTN i , w_j is the environmental effect for year j , b_i is the QTN intercept and m_i is the QTN slope on the environmental effect. The slope, intercept, and environmental effects were sampled from the following normal distributions:

$$b_i \sim N\left(0, \frac{\sigma_G^2}{n_{QTN}}\right),$$

$$m_i \sim N\left(0, \frac{\sigma_{G \times Y}}{\sqrt{n_{QTN}}}\right),$$

$$w_j \sim N\left(0, \frac{\sigma_{G \times Y}}{\sqrt{n_{QTN}}}\right),$$

where n_{QTN} is 21,000, σ_G^2 is genetic variance due to additive gene action, which was set to 1, and $\sigma_{G \times Y}$ is the square root of the $G \times Y$ variance. Four levels of $G \times Y$ variance were examined: 0, 2, 4, and 10 times σ_G^2 .

The genetic values of each doubled haploid line were used to produce phenotypic values by adding random error. The random error was sampled from a normal distribution with mean 0. The variance of the random error varied according to the stage of evaluation in the breeding program. This was done to account for increasing accuracy in evaluation as the plot size and number of replications per entry increases. The values for these error variances were set so as to achieve target levels of heritability. The levels of heritability represented heritability on an entry-mean basis for the 50 founder genotypes when $G \times Y$ variance equals 0. The used levels of heritability are given in the next subsection. Realized entry-mean heritability changed depending on genetic variance of the evaluated genotypes.

Recent (Burn-In) Breeding and Conventional Program (Conv)

Recent (burn-in) breeding for yield in the species was simulated using 20 yr of breeding in a conventional program without genomic selection. The design of the burn-in program was modeled after existing winter wheat breeding programs. The key features of this breeding program were (i) a crossing block consisting of 50 doubled haploid lines used to develop 100 biparental populations each year, (ii) development of new doubled haploid lines from each biparental cross, (iii) a 4-yr cycle time from crossing to selection of new parents, and (iv) an 8-yr production interval from crossing to release of a new variety.

All selection in the burn-in program was performed using phenotypes. These phenotypes represented either direct selection on yield using a yield trial or indirect selection for yield using visual selection on correlated traits. The levels of heritability at a particular selection stage were set in consultation with breeders and based on observed values in the field.

A schematic for the overall design of the burn-in program is given in Fig. 1 and a detailed description follows. The progression of germplasm through the breeding program was simulated using AlphaSim (Faux et al., 2016) and R (R Development Core Team, 2014).

Year 1

Crossing is performed to produce 100 biparental populations. Parental combinations are chosen from all possible combinations for the 50 parental lines in the crossing block (1225 possible combinations) using random sampling without replacement.

Years 1 and 2

One hundred doubled haploid lines are produced from each biparental family.

Year 3

The newly developed doubled haploids are planted in headrows to increase seed and perform visual selection. Visual selection in the headrows is modeled as selection on a yield phenotype with heritability of 0.1 to represent the breeder selecting on correlated traits. The breeder advances 500 lines.

Year 4

The 500 lines are evaluated in the PYT. The PYT represents evaluation in an unreplicated trial that is mechanically harvested to measure yield. Selection in the PYT is modeled as selection on a yield phenotype with heritability of 0.2. The best performing 50 lines are advanced to the next trial. The best performing 20 lines are also advanced to the next year's crossing block, thereby completing a crossing cycle.

Year 5

The 50 lines advanced from the PYT are evaluated in an advanced yield trial (AYT). The AYT represents evaluation in a small, multilocation replicated yield trial. Selection in the AYT is modeled as selection on a yield phenotype with heritability of 0.5. This value of heritability was based on the assumption that an AYT represents four effective replications of the PYT. The best performing 10 lines in the AYT are advanced to the next trial. These 10 lines are also considered as candidates for next year's crossing block. The next year's crossing block of 50 lines is composed of the 20 best PYT lines, the 10 best AYT lines, and the best 20 lines selected from the current crossing block's 30 non-PYT lines.

Year 6

The 10 advanced lines are evaluated in an elite yield trial (EYT). The EYT represents evaluation in a large, multilocation replicated yield trial. Selection in the EYT is modeled as selection on a yield phenotype with heritability of 0.67. This value of heritability was based on the assumption that an EYT represents eight effective replications of the PYT. All 10 lines are kept in the EYT to be reevaluated in the following year. Any of the 10 lines used in the current year's crossing block have their phenotypes updated to reflect their performance in this year's EYT before lines are chosen for next year's crossing block.

Year 7

The 10 lines from the previous year's EYT are reevaluated. Any of those still in the current crossing block have their phenotypes updated to reflect their performance in both years of evaluation in the EYT before lines are chosen for next year's crossing block.

Year 8

The line with the best average performance over the previous 2 yr of EYT evaluation is released as a variety.

Future Breeding

The future breeding phase of the simulation modeled breeding using alternative breeding program designs. Each design was simulated for an additional 20 yr following the recent breeding burn-in phase, so that each design could be evaluated with an equivalent starting point. At the end of these 20 yr,

Table 1. Summary of breeding program sizes and costs.

Program†	Crosses per year	Lines per cross	Head-rows	i‡	Cost \$
Conv	100	100	10,000	1.75	350,000
Conv GS	100	97	9700	1.74	349,500
PYT GS	100	97	9700	1.74	349,500
Head GS	100	63	6300	1.52	346,500
2Part	200	31	6200	1.52	347,000
2Part+H	200	20	4000	1.27	340,000

† Conv, conventional breeding program; Conv GS, conventional program with genomic selection; PYT GS, preliminary yield trial genomic selection; Head GS, headrow genomic selection program; 2Part, two-part program with PYT genomic selection; 2Part+H, two-part program with headrow genomic selection.

‡ Standardized selection intensity for PYT entries selected from headrows (Bernardo, 2014)

the breeding programs continued without making new crosses. This was done to allow germplasm in different stages of the program to progress to the end. For example, crosses made in year 20 were evaluated as headrows in year 22 and progressed through the entire program by year 27.

The alternative breeding program designs were based on breeding programs with equivalent operating costs using different genomic selection strategies (Table 1). The design of these programs used the conventional program as a template. Minimal modifications were made to this template to produce initial designs for each strategy. The initial designs were then adjusted for number of doubled haploid lines produced from each biparental cross to ensure that the overall operating cost was approximately equal to the cost used for the conventional breeding program in the recent breeding burn-in stage. Since the two-part strategies used two cycles per year, they resulted in twice as many crosses per year. The remaining components of the breeding programs were kept constant. This meant, for example, that the number of entries in yield trials was kept constant across breeding programs. This was done to avoid changing genomic selection training population size; this factor is outside the scope of the present study.

Equalization of operating costs was performed using estimated costs for genotyping and producing doubled haploid lines. The cost for producing doubled haploid lines was estimated at \$35 on the basis of the lowest publically advertised price from Heartland Plant Innovations (<http://www.heartlandinnovations.com>; accessed 23 June 2017). The cost of increasing seed of these doubled haploid lines in a headrow (progeny row) was considered to be included in this estimate even though this is not part of the advertised service. The cost for genotyping was estimated at \$20. These estimates were intentionally skewed toward a smaller ratio of doubled haploid cost to genotyping cost as a higher ratio requires less of an offset in breeding program design and favors genomic selection breeding programs. Costs for many other stages in the breeding program, such as yield trials, were not estimated since they were kept constant across all programs. The cost for crossing, which in any case would be small, was not considered even though it varied between breeding programs.

Genomic selection in each breeding program used an initial training population containing the last 3 yr of yield trial

data in the recent breeding burn-in phase. This initial training population thus contained 1710 phenotypic records on 1570 genotypes. New data was added to the training population in subsequent years as new yield trials were evaluated. By year 20, the training population expands to contain 12,540 phenotypic records on 11,070 genotypes.

Genomic predictions were made using a ridge regression model (Hoerl and Kennard, 1976; Meuwissen et al., 2001). The model handled the heterogeneous error variance due to different levels of error in each yield trial by weighting for the effective number of replications. The model was implemented in C++ and R using the R library “RcppArmadillo” (Eddelbuettel and Sanderson, 2014). The code was partially adapted from code in the R library “EMMREML” (Akdemir and Godfrey, 2015).

Conventional Program with Genomic Selection

The Conv GS was developed as a control to measure change in selection accuracy due to the use of genomic selection without changes to the conventional breeding program (Fig. 1). As such, the program used the same procedures as the conventional program. The number of doubled haploid lines per cross was reduced from 100 in the conventional scheme to 97 to offset genotyping costs.

Preliminary Yield Trial Genomic Selection Program

The PYT GS program introduced genotyping at the PYT stage (Fig. 1). Genomic selection was used to advance lines in the PYT and AYT stages and to select parental lines for the crossing block. The parental lines were selected by choosing the 50 lines with the highest genomic estimated value from a set of candidates that comprised last year’s crossing block lines as well as all entries from the PYT and later yield trials. This reduced the cycle time for crossing to selection of new parents from 4 yr in the conventional program to 3 yr. The number of doubled haploid lines per cross was reduced from 100 in the conventional scheme to 97 to offset genotyping costs.

Headrow Genomic Selection Program

The Head GS program began genotyping lines for genomic selection in the headrows (Fig. 1). Therefore, genomic selection was used in the headrows, PYT, and AYT. Crossing block lines were chosen by selecting the 50 lines with the highest genomic estimated value from the previous year’s crossing block and all headrow and yield trial entries. This reduced the cycle time from crossing to selection of new parents to 2 yr. The number of doubled haploid lines per cross was reduced from 100 in the conventional scheme to 63 to offset genotyping costs.

Two-Part Program

Additional aspects of the simulated species’ biology were assigned for the purpose of developing the two-part program. First, it was assumed a hybridizing agent could be applied to induce male sterility. Male sterile plants could then be crossed in a greenhouse using open pollination with untreated, fully fertile plants. Each cross produced 40 seeds, and the process from start

to finish was accomplished within half a year. The values were chosen using values believed to be feasible for winter wheat.

Crossing and selection of new parents in the two-part program was handled in the population improvement component (Fig. 2), which consisted of two crossing cycles per year. Each cycle began by randomly dividing progeny from the previous cycle into equal numbers of male and female candidates. Genomic selection was then used on the candidates to choose 100 male and 100 female parents. These parents were then grown in greenhouses. At the appropriate stage, the female plants were treated with a hybridizing agent to induce male sterility and pollinated by multiple randomly chosen males. This modeled open pollination of the females. Seed from each female plant was harvested as half-sib families. Thirty seeds, chosen at random, from each family were used as selection candidates for the next cycle of crossing. The remaining 10 seeds were used to produce new doubled haploid lines. The number of doubled haploid lines per cross was reduced from 100 in the conventional scheme to 31 to offset genotyping costs.

The product development component of the two-part program handled screening of germplasm to identify new varieties (Fig. 2). This process began with the production of the new doubled haploid lines. The doubled haploid lines were screened in the same manner as doubled haploid lines in the PYT genomic selection program with regard to selection and genotyping. Here, none of the lines were selected for the crossing block, but their genomic and phenotypic data were added to the genomic selection training population. This allowed the genomic selection model used in the population improvement component to be updated over time as new material was evaluated.

Two-Part Program with Headrow Genomic Selection (2Part+H)

This breeding program used the same basic design as the two-part program, with the difference of starting with genomic selection in headrows instead of the PYT. Thus, screening of the doubled haploid lines was performed in the same manner as the headrow genomic selection program. The number of doubled haploid lines per cross was reduced to 20 to offset genotyping costs.

Comparison of Breeding Programs

The effectiveness of each breeding strategy was measured by comparing genetic values of newly produced doubled haploid lines (headrow stage) over time in each of the corresponding breeding programs. Genetic values for headrows were examined because it is the earliest stage in which all programs evaluate inbred lines. In addition to genetic gain, we also monitored genetic variance and accuracy of genomic predictions. Genetic gain and genetic variance in the breeding programs was assessed by plotting mean and variance of genetic values for headrow entries over time. Genetic gain for EYT entries was also examined to determine if it differed from genetic gain in headrow entries. To aid in visualization, the mean genetic values were centered at the mean value for headrow entries in Year 0 for each replicate. Year 0 was defined as the last year of the burn-in phase.

Direct comparisons between breeding programs for genetic gain and genetic variance were reported as ratios with 95% confidence intervals (95% CI). These were calculated by

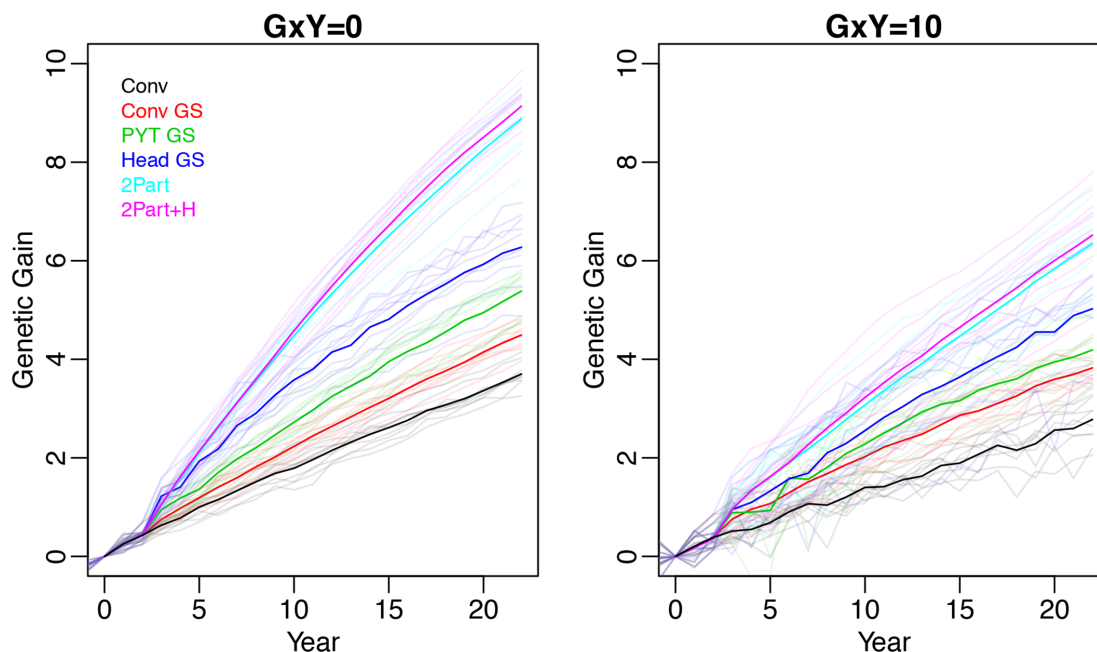


Fig. 4. Genetic gain for all breeding programs when genotype-by-year ($G \times Y$) variance is 0 and 10. Genetic gain is expressed as mean genetic value of headrow entries over time. The mean genetic value for each replicate was centered on 0 in Year 0. Individual replicates are shown with faded lines, and means for all 10 replicates are shown with dark lines. Conv, conventional breeding program; Conv GS, conventional program with genomic selection; PYT GS, preliminary yield trial genomic selection program; Head GS, headrow genomic selection program; 2Part, two-part program with PYT genomic selection; 2Part+H, two-part program with headrow genomic selection.

performing paired Welch's t tests on log-transformed values from the 10 simulation replicates. The log-transformed differences and 95% CI from the t test were then back-transformed to obtain ratios (Ramsey and Schafer, 2002). All calculations were performed using R (R Development Core Team, 2014).

The accuracy of genomic predictions at each time point was assessed in each breeding program. Accuracy was defined as the correlation between the true genetic values of headrow entries and their genomic estimated values. Genomic estimates were produced for all breeding programs, even those that did not use genomic selection at the headrow stage. This was done only to enable comparisons across breeding programs. The genomic estimated values themselves were only used for selection if breeding program design specified doing so. Accuracy of genomic predictions was also assessed in the population improvement components of the two-part programs. This was done to estimate the effectiveness of parent selection using this strategy.

RESULTS

The breeding programs using the two-part strategy generated the most genetic gain and all programs using genomic selection generated more gain than the conventional breeding program. The relative rankings of the different breeding programs for genetic gain remained consistent across both simulation years and different levels of $G \times Y$ variance. The relative rankings of the different breeding programs for genetic variance showed complex interactions between breeding program, level of $G \times Y$ variance, and simulation year. The relative rankings of the different breeding programs for genomic prediction accuracy was also complex.

Genetic Gain

Breeding programs using the two-part strategy showed the most genetic gain regardless of $G \times Y$ variance. This is shown in Fig. 4, which presents two plots for the mean genetic value of headrow entries by year. The first plot shows the trends for individual replicate and the mean of all replicates for each of the breeding programs evaluated in the future breeding component when $G \times Y$ variance is 0. The second plot shows the same trends for $G \times Y$ variance of 10. Both plots show that by Year 4, the breeding programs using the two-part strategy (i.e., 2Part and 2Part+H) have higher mean genetic values than all other programs. The 2Part+H program, which begins genomic selection for product development in the headrows, slightly outperforms the 2Part program, which begins genomic selection for product development in the PYT. The same trends were observed when $G \times Y$ variance equaled 2 and 4 (Fig. S1).

Figure 4 also shows that overall ranking of breeding programs for total genetic gain was consistent across levels of $G \times Y$ variance. This is shown by looking at the average genetic values of headrow entries in the final year (Year 22) of each plot. In both plots the ranking from lowest to highest average genetic value was: Conv, Conv GS, PYT GS, Head GS, 2Part, and 2Part+H. This ranking was also observed when $G \times Y$ variance was 2 and 4 (Fig. S1).

Figure 4 also shows the differences between breeding programs were smaller when $G \times Y$ variance was 10. At this level of $G \times Y$ variance, the best performing two-part

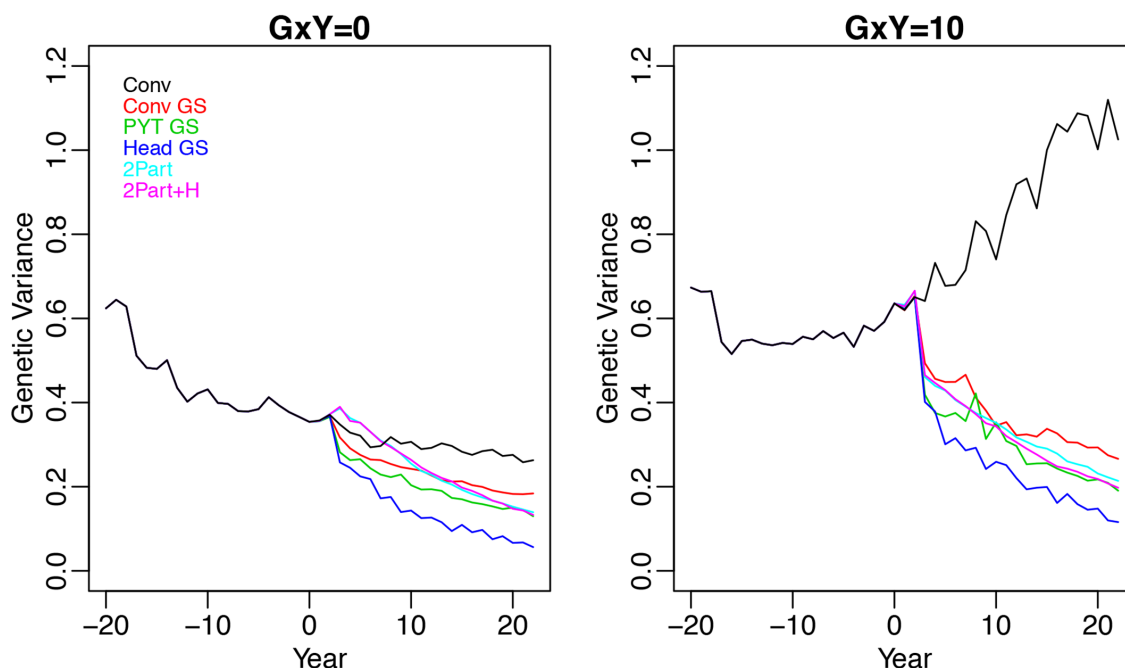


Fig. 5. Genetic variance for all breeding programs when genotype-by-year ($G \times Y$) variance is 0 and 10. Genetic variance is expressed as the genetic variance among headrows in each year of the simulation. Each line represents the overall mean for all 10 replicates. Conv, conventional breeding program; Conv GS, conventional program with genomic selection; PYT GS, preliminary yield trial genomic selection program; Head GS, headrow genomic selection program; 2Part, two-part program with PYT genomic selection; 2Part+H, two-part program with headrow genomic selection.

program, 2Part+H, generated 2.36 (95% CI [2.08, 2.67]) times the genetic gain of the conventional breeding program. The 2Part+H program also produced 1.31 (95% CI [1.20, 1.43]) times the genetic gain of the Head GS program. When $G \times Y$ variance was 0, these values were 2.47 (95% CI [2.36, 2.59]) and 1.46 (95% CI [1.36, 1.57]), respectively.

Figure 4 also shows that genetic gain is increased in the conventional program by adding genomic selection without changing cycle time. This is shown by comparing genetic gain in the Conv program with genetic gain in the Conv GS program. When $G \times Y$ variance was 0, the Conv GS program produced 1.21 (95% CI [1.18, 1.25]) times the genetic gain of the Conv program. When $G \times Y$ variance was 10, this value increased to 1.39 (95% CI [1.23, 1.58]) times the genetic gain.

Variability in yearly genetic gain was greater with more $G \times Y$ variance. This is shown by the lines for individual replicates in Fig. 4. All breeding programs showed fairly consistent year-to-year genetic gain in each replicate when $G \times Y$ variance was 0. When $G \times Y$ variance was 10, year-to-year genetic gain was less consistent within replicates. This is shown by the larger fluctuations in mean genetic value between successive years for individual replicates. For both levels of $G \times Y$ variance, the two-part programs produced the most consistent year-to-year genetic gains.

The trends for genetic gain in EYT entries matched the trends for headrow entries from Year 6 onward (Figs. S2 and Fig. S3). All breeding programs using genomic selection showed approximately the same amount of

genetic gain prior to Year 6, and this genetic gain was greater than the genetic gain in the conventional breeding program. Year 6 was the first year in which EYT entries were derived from crosses that used parents selected by genomic selection. Thus differences in genetic gain from Years 1 to 5 reflect the difference between using genomic selection or phenotypic selection on existing germplasm.

Genetic Variance

The change in genetic variance for headrow entries over time involved interactions between breeding program design, level of $G \times Y$ variance, and simulation year. This is shown in Fig. 5, which plots mean genetic variance of headrow entries over time. The first plot shows the change in genetic variance during the burn-in phase and for each future breeding program when $G \times Y$ variance equals 0. The second plot shows the same breeding programs when $G \times Y$ variance equals 10. The occurrence of an interaction is evident in the differences between the two plots.

Figure 5 shows that all breeding programs lose genetic variance over time when $G \times Y$ variance is 0. However, the rate of loss differs between breeding programs. The Conv GS, PYT GS, and Head GS programs show a large initial drop in genetic variance relative to the conventional program followed by a more gradual decrease over time. In contrast, the two-part programs show an initial increase in genetic variance followed by a more rapid decrease in genetic variance over time.

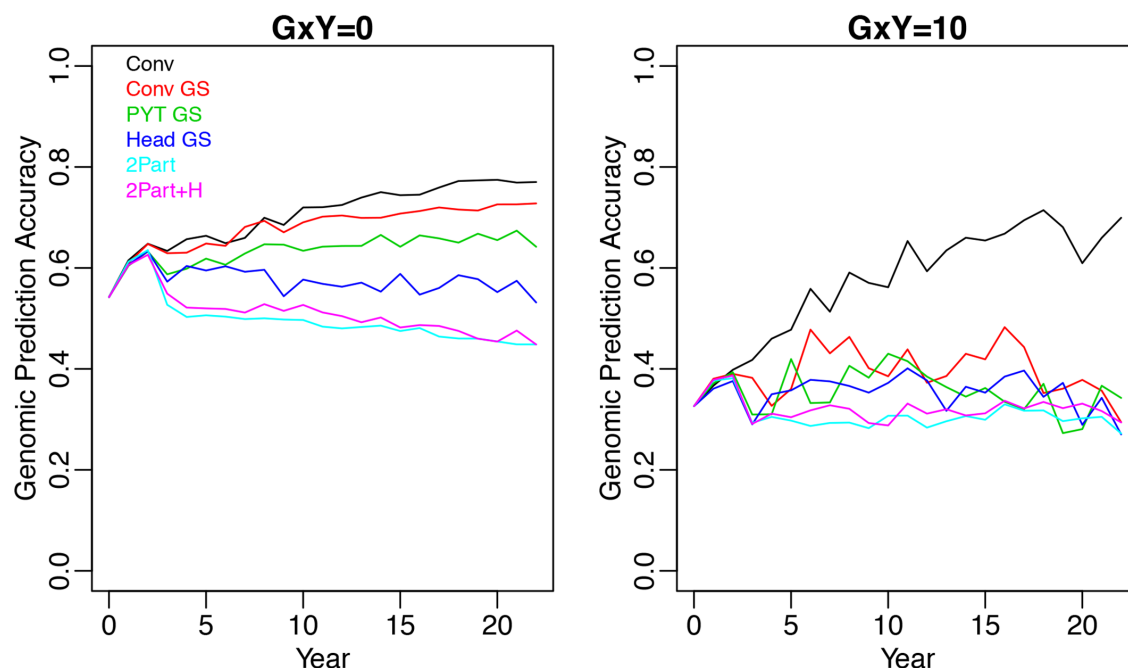


Fig. 6. Genomic prediction accuracy for all breeding programs when genotype-by-year ($G \times Y$) variance is 0 and 10. Genomic prediction accuracy is expressed as the correlation between true and genomic predicted genetic values of headrow entries over time. Conv, conventional breeding program; Conv GS, conventional program with genomic selection; PYT GS, preliminary yield trial genomic selection program; Head GS, headrow genomic selection program; 2Part, two-part program with PYT genomic selection; 2Part+H, two-part program with headrow genomic selection.

Figure 5 also shows that the conventional breeding program leads to increased genetic variance over time when $G \times Y$ variance is 10. However, the breeding programs using genomic selection showed decreasing genetic variance over time. Additionally, the initial decrease in genetic variance observed immediately after the implementation of genomic selection was greater when $G \times Y$ variance was 10 than when it was 0.

Genetic variance in the 2Part+H program remained higher than the Head GS program and lower than the conventional program. When $G \times Y$ variance was 0, the 2Part+H program had 2.73 (95% CI [1.96, 3.79]) times the genetic variance of the Head GS program and 0.53 (95% CI [0.49, 0.58]) times that of the conventional program in Year 22. When $G \times Y$ variance was 10, these values were 1.97 (95% CI [1.48, 2.62]) and 0.25 (95% CI [0.17, 0.37]), respectively.

The changes in genetic variance for $G \times Y$ variances of 2 and 4 were intermediate between those seen in Fig. 5 (Fig. S5). Genetic variance in the conventional program stayed approximately constant when $G \times Y$ variance was 2 and gradually increased when $G \times Y$ variance was 4.

Genomic Prediction Accuracy

Genomic prediction accuracy over time also showed an interaction between breeding program and level of $G \times Y$ variance. This is shown in Fig. 6, which plots correlations between the genetic values for headrow entries and their

genomic predicted values. The first plot shows all breeding programs when $G \times Y$ variance was 0, and the second plot shows those programs when $G \times Y$ variance was 10. Genomic prediction accuracies start higher when $G \times Y$ variance was 0. In subsequent years, genomic prediction accuracy in the conventional program increased, while it slightly increased or slightly decreased for the other breeding programs depending on the breeding program and the level of $G \times Y$ variance.

Genomic prediction accuracy in the two-part breeding programs was also measured in the population improvement stage. This is shown in Fig. 7, which plots the correlation between genetic values and genomic predicted values of parental candidates for each cycle of crossing. There were two cycles per year. Each cycle is plotted at half-year increments in Fig. 7. The first plot shows the accuracy when $G \times Y$ variance was 0, and the second plot shows the accuracy when $G \times Y$ variance was 10. Both plots show accuracy decreasing rapidly in the first couple of cycles and then holding approximately steady in subsequent years. Both plots show yearly oscillations corresponding with the annual updating of the training population.

DISCUSSION

The results highlight four main points for discussion: (i) the impact of the different breeding strategies on genetic gain; (ii) the impact of the different breeding strategies on changes in genetic variance across time; (iii) the impact of the different breeding strategies on genomic prediction

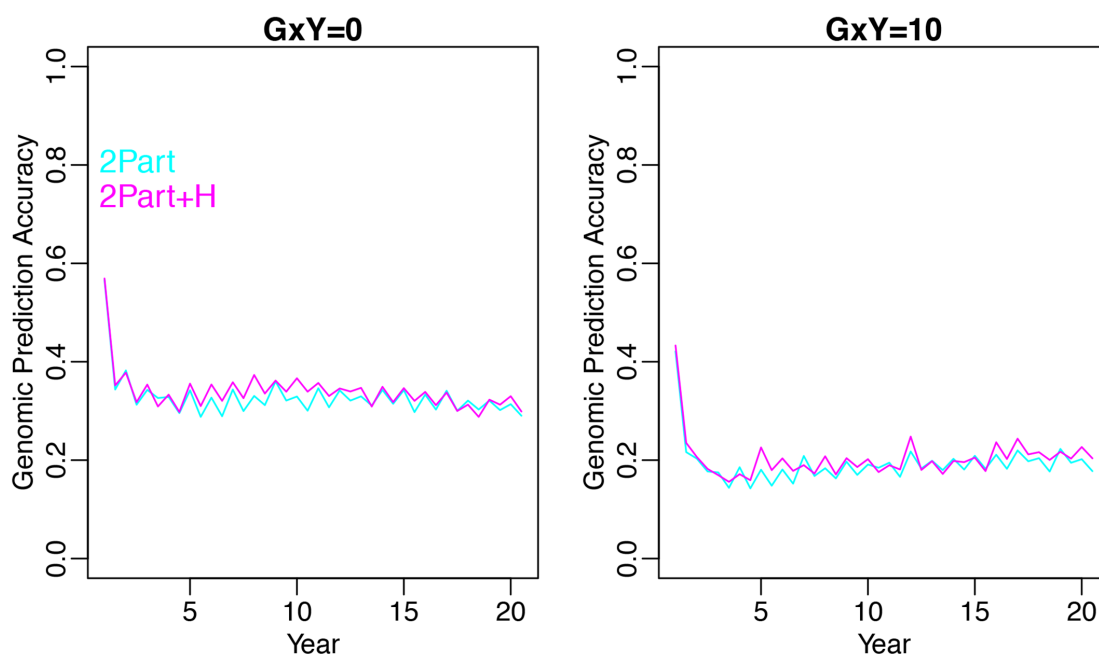


Fig. 7. Genomic prediction accuracy in the population improvement part of the two-part programs when genotype-by-year ($G \times Y$) variance is 0 and 10. Genomic prediction accuracy is expressed as the correlation between true and genomic predicted genetic values of all population improvement plants over time. 2Part, two-part program with PYT genomic selection; 2Part+H, two-part program with headrow genomic selection.

accuracy; (iv) limitations of the simulations that were undertaken; and (v) practical implementation of the two-part strategy in real breeding programs.

Impact of the Different Breeding Strategies on Genetic Gain

Breeding programs using the two-part strategy produced the most genetic gain, and all programs using genomic selection produced more genetic gain than the Conv program. These results were consistent across both the different levels of simulated $G \times Y$ variance and the years of the breeding programs. The programs using the two-part genomic selection strategy produced between 2.36 and 2.47 times more genetic gain than the Conv program. The two-part programs also produced between 1.31 and 1.46 times more genetic gain than the best performing breeding program using a more standard genomic selection strategy. All breeding programs using genomic selection produced more genetic gain than the Conv program. These results suggest that: (i) using genomic selection improved the rate of genetic gain in breeding programs; and (ii) the two-part strategy was the best strategy for implementing genomic selection.

Improved selection accuracy and/or decreased cycle time underpinned the increased genetic gain observed in breeding programs that used genomic selection. The use of genomic selection in the Conv GS program did not decrease cycle time compared with the conventional program without genomic selection. Therefore, the increased genetic gain observed in the Conv GS program was solely

the result of increased accuracy of parent selection. The other breeding program strategies using genomic selection involved some degree of shortening of breeding cycle time. This shortening of the breeding cycle contributed to the increased genetic gain that they delivered, together with increases in accuracy of parent selection in some cases.

In the two-part programs, the shortening of breeding cycle time was the major driver of increased genetic gain. This can be observed by comparing the two-part programs to the Head GS breeding program, which used genomic selection beginning in the headrow stage to select parents of the next breeding cycle. The two-part programs generated more genetic gain than the Head GS program but had lower accuracy of parent selection. The accuracy of parent selection is shown in Fig. 7 for the two-part programs and in Fig. 6 for the headrow stage of the Head GS program. Following the first cycle of selection, the accuracy of parent selection in the two-part program dropped to less than 0.4 when $G \times Y$ variance was 0 and to about 0.2 when $G \times Y$ variance was 10. In comparison, the accuracy of parent selection in the Head GS program was approximately 0.6 when $G \times Y$ variance was 0 and 0.4 when $G \times Y$ variance was 10. This indicates the Head GS program more accurately selected parents than either of the two-part programs, but it did not produce as much genetic gain. Thus, although the accuracy of parent selection in the two, two-part programs was lower than that of the Head GS program, the two-part programs could deliver more genetic gain per unit of time because they had 2 more cycles of selection per unit time than the Head

GS program (2 per year for the two-part versus 0.5 per year for the Head GS program).

The rankings for the different breeding programs based on genetic gain delivered provides further evidence for the critical importance of reduction of cycle time for increasing genetic gain. There was a perfect rank correlation between the cycle time of a breeding program and the genetic gain that it produced across all the scenarios that we tested. These findings are consistent with the expectation that the greatest benefit of genomic selection for genetic gain in crops will come from decreased cycle time (Heffner et al., 2009), as has been predicted and observed in genomic selection of dairy cattle (Schaeffer, 2006; García-Ruiz et al., 2016).

Genetic gain in the two-part strategy could be further increased by adding more cycles per year and thus further decreasing cycle time. In the present study the two-part strategies only involved two breeding cycles per year. Depending on the biology of a particular species, many more breeding cycles per year may be possible. For example, seven cycles per year have been achieved in spring wheat using “speed breeding” techniques (Lee Hickey, personal communication). While we expect that additional breeding cycles per year would increase the genetic gain, this increase may not be linear because of two factors. The first relates to an expected decrease in the accuracy of selection and the second relates to a rapid loss of genetic variance from the population.

The accuracy of genomic prediction, and therefore selection, will decrease in each additional cycle added to the two-part strategy. This is due to plants in each additional cycle being less related to the training population. It is well known that genomic prediction accuracy decreases when relationships between the individuals in the training set and the selection candidates decrease (Habier et al., 2007; Clark et al., 2012; Hickey et al., 2014). In the present study this trend can be observed in Fig. 7, where a cyclical pattern exists for the genomic selection accuracies. The cyclical pattern arises because the genomic selection training set is only updated once per year in the two-part strategies, but there are two breeding cycles. The individuals in the second breeding cycle in a given year, which have lower selection accuracy than those in the first breeding cycle, are one generation more removed to the training set than those in the first breeding cycle. The cyclical loss of accuracy would be exacerbated if the number of breeding cycles per year increased. One way to mitigate this problem would be to assemble huge training sets of hundreds of thousands of individuals. With such training sets the accuracy of genomic selection is less dependent on relatedness between training individuals and selection candidates. While assembling such data sets may be possible in large breeding programs, further research is needed to develop solutions for breeding programs without this capacity.

Increasing the number of cycles in the two-part strategy could increase short-term genetic gain but may result in decreased long-term genetic gain. This behavior is dependent on how the cost of adding additional cycles is offset. A simple way of offsetting this cost would be to reduce the number of plants genotyped in each cycle while keeping selection intensity the same. This method for offsetting cost could reduce long-term genetic gain, because it would decrease effective population size. Populations with smaller effective population sizes are prone to more rapid loss of genetic variance due to drift (Charlesworth, 2009). As genetic variance decreases, the ability to make subsequent genetic gain also decreases. Thus long-term genetic gain in the two-part strategy depends on how genetic variance is maintained. Optimal contribution theory (Woolliams et al., 2015) and introgression of new genetic variation are well established solutions for averting decline of genetic variance in breeding programs, and further research is needed to determine their roles in two-part breeding strategies.

Breeding strategies that used genomic selection had more stable genetic gain in the presence of $G \times Y$. This is shown by looking at the lines for individual replicates in Fig. 4 when $G \times Y$ variance equals 10. These lines show fairly consistent yearly increases for all the breeding programs using genomic selection, with the programs using the two-part strategy having the most consistent increases. In contrast, the genetic gain for the Conv program was more prone to large fluctuations. These differences arise because genomic selection breeding programs use genomic predictions that are based on several years of historic phenotypic information. In contrast the conventional breeding program makes selection decisions using only a single year or at most a few years of phenotypic information. This use of historic phenotypic information with genomic selection could provide breeding programs with greater robustness of genetic gain in years with extreme weather. The greater robustness would allow the two-part strategy to deliver more consistent genetic gain than existing breeding program.

Impact of the Different Breeding Strategies on Changes in Genetic Variance across Time

The trends for genetic variance were less straightforward than those for genetic gain, because they varied according to the level of $G \times Y$ variance. All breeding programs that used genomic selection displayed a decrease in genetic variance over time, regardless of the level of $G \times Y$ variance simulated. However, the trend for change in genetic variance for the conventional program depended on the level of $G \times Y$ variance. It displayed a consistent loss in genetic variance when there was no $G \times Y$ variance and displayed a consistent gain in genetic variance when $G \times Y$ variance was high.

In breeding programs using genomic selection, the most significant decreases in genetic variance occurred

immediately after the implementation of genomic selection. This initial loss in genetic variance is mostly due to increased selection accuracy in early stages of a breeding program. Increased selection accuracy results in the Bulmer effect, which decreases genetic variance under directional selection due to the buildup of negative linkage-disequilibrium between the causal loci (Bulmer, 1971). Further changes in genetic variance depend on multiple factors that differ between breeding programs. These factors include effective population size, selection intensity, selection accuracy, and the number of cycles per year.

The breeding programs using the two-part strategy differed from other breeding programs using genomic selection in that they showed a small initial increase in genetic variance. This initial increase in genetic variance was due to using a cycle of random mating to convert the fully inbred parents to outbred parents. Afterward, genetic variance decreased according to the factors mentioned above.

Most of the factors resulting in the loss of genetic variance can be manipulated in a two-part breeding program with relative ease. For example, the number of selection candidates per cycle and/or the number of parents per cycle can be changed to vary both effective population size and selection intensity. Differences in these values between the two-part breeding programs and the headrow genomic selection program may explain why the two-part breeding programs maintained more genetic variance. The two-part breeding programs used more parents per cycle (200 vs. 50) and fewer selection candidates per cycle (3000 vs. 6870 to 6920). It is likely these values could be optimized for both long- and short-term genetic gain. Future studies are needed to identify optimal values.

The maintenance, and in some cases increase, of genetic variance in the Conv program when $G \times Y$ variance was high can be explained by balancing selection. Balancing selection that occurs in the presence of genotype-by-environment interaction involves fluctuating allelic effects between environments, resulting in different individuals being selected in different environments. This fluctuating of the breeding goal results in genetic variation being maintained (Gillespie and Turelli, 1989). Allelic effects in our simulation fluctuate each year due to our model for $G \times Y$. This results in no single doubled haploid line being ideal in all years. The conventional breeding program thus favors different doubled haploid lines in each year of the simulation. Multiple years of phenotypic selection favors doubled haploid lines with high average performance over its years of evaluation. In contrast, the genomic selection model selects doubled haploid lines on the basis of its average SNP effects in the training population. If these average SNP effects don't change, genomic selection will always favor a single genotype and, thus, will not maintain genetic variance in the same way as phenotypic selection in the presence of $G \times Y$.

If maintenance of genetic variance by $G \times Y$ is important to breeding programs, then the failure to observe this in genomic selection breeding programs is noteworthy. It suggests that genomic selection could result in rapid depletion of genetic variance that may hamper long-term potential. During the course of this simulation, no noticeable affect from the loss of genetic variance was observed on long-term selection gain. This indicates that the loss of genetic variance was either unimportant or was offset by other benefits of using genomic selection.

Impact of the Different Breeding Strategies on Genomic Selection Accuracy

Genomic prediction accuracy over time varied across breeding programs. These variations ranged from an increase in accuracy over time in the conventional breeding program to a decrease in accuracy over time in the two-part breeding programs. These differences are due to the many factors that affect the accuracy of genomic predictions, such as effective population size, training population size, the genetic distance between the training and prediction populations, and the trait's genetic architecture (Daetwyler et al., 2008; Goddard, 2009; Pszczola et al., 2012; Hickey et al., 2014).

Training population size and the genetic distance between the training and prediction populations are likely responsible for the observed differences in genomic prediction accuracy. The training population grew in size each year of the simulation at an equal rate for all breeding programs. In isolation, the increasing training population size is expected to increase genomic selection accuracy. This mechanism explains how some of the breeding programs experienced an increase in genomic prediction accuracy over time. The other breeding programs showed either no change or a decrease in genomic prediction accuracy over time. This may be explained by the contravening effect of increasing genetic distance between the training and prediction populations. This effect is expected to decrease genomic prediction accuracy and is introduced by selection. This can explain why the rate of genetic gain for breeding programs observed in Fig. 4 was approximately inversely related to genomic prediction accuracy in Fig. 6.

Limitations of the Simulations That Were Undertaken

Because of certain assumptions in our simulation methodology, the simulations conducted in this paper did not model the full complexity of actual breeding programs. In this section we discuss the limitations and impact of a few of these assumptions: (i) assumptions that impact genomic selection accuracy; (ii) assumptions about the reproduction of the simulated species; (iii) assumptions about the making of crosses; and (iv) assumptions about the complexity of the breeding goal.

Assumptions That Impact Genomic Selection Accuracy

The genomic prediction accuracies observed in these simulations are likely higher than those in real-world conditions. This was because of conditions in the simulation that favored high genomic selection accuracy such as: (i) molecular markers with no genotyping errors; (ii) genetic control of the trait that did not involve epistasis; and (iii) breeding programs that did not use germplasm exchange.

The accuracy of genomic selection affects the performance of the simulated breeding programs. These effects should affect all breeding programs using genomic selection similarly, so we don't expect the relative performance of breeding programs using genomic selection to change much under real conditions, which suggests that the two-part strategies should still outperform other genomic selection strategies. However, the relative performance of the conventional program to the programs using genomic selection could change. If this were to occur, the two-part strategies should still outperform the conventional breeding program because of the magnitude of difference observed in the simulation, provided that a sufficiently high-quality genomic selection training population is used.

Assumptions About the Reproduction of the Simulated Species

Assumptions about the reproductive ability of the simulated species affect the performance of the two-part program as it seeks to push the species to its biological limits in terms of breeding cycles per year to maximize genetic gain. In this paper we chose a conservative two cycles of crossing per year to guard against overestimating the benefit of the two-part program. More cycles per year could easily be performed with some species, especially those that do not require vernalization. Doing so could result in increased genetic gain if the accuracy of genomic selection and the utilization of genetic variance were both managed properly as previously discussed.

Assumptions About the Making of Crosses

The other important assumption about the simulated species involved how crossing was performed. It was assumed that maturity differences needed for crossing were easily obtained by growing female and male plants in separate greenhouses. However, crosses between early-flowering males and late-flowering females, or late males and early females, may be missed owing to asynchrony. It was also assumed that a hybridizing agent could be used for crossing at relatively little cost; if this is not possible, then more laborious manual crosses would have to be performed. This would also cost more and, thus, require other components of the breeding program to be reduced if the cost was to remain equal. That said, this should only have a minor effect on the performance of the two-part program, as the

overall cost of crossing is expected to be small relative to the total cost of the breeding program. Benefits gained by controlling parental pairing may even be more advantageous than cost savings gained from using a hybridizing agent.

Assumptions About the Complexity of the Breeding Goal

The breeding program examined in this simulation only considered grain yield. Real-world breeding programs must also consider additional traits relating to agronomic performance, disease resistance, and end-use quality. Genomic selection in the two-part strategy would have to account for all the traits or at least ensure that sufficient variation for these traits is maintained for selection of favorable genotypes in the product development section. Genomic selection has been used for some of these traits in wheat (Rutkoski et al., 2011; Battenfield et al., 2016), and it should be possible to use it for more. The challenge is deciding how to use all these predictions in a coherent way. Wheat and many other crops lack widely accepted selection indices where the relative weights placed on each trait that needs to be selected up on are clearly defined. Developing a selection index is likely necessary for the two-part strategy to be useful. The modified versions of the two-part strategy discussed below may not need a selection index.

Practical Implementation of the Two-Part Strategy

The two-part strategy as examined in this study shows great promise for use in plant breeding programs. However, breeders are likely to be hesitant to change their programs completely until it is proven in practice. This could be accomplished by using a modified version of the two-part strategy as a portion of a traditional breeding program design. This would be accomplished by using the population improvement component for prebreeding, to rapidly produce improved breeding lines. These lines are then introduced as parents in a conventional breeding program's crossing block. The prebreeding strategy could be used to produce high-yield lines, as in the present simulation, or used to develop parents with a narrow focus, such as improved disease resistance or specific quality attributes, the main limitation on breeding objective being the availability of phenotypic data to train the genomic selection model that drives the program. The prebreeding program would require a sufficiently large population size to ensure consistent performance, and further research is needed to determine this size.

Modifications can be made to the two-part strategy to allow for outside germplasm to be introduced. The population improvement sections of the two-part programs in this paper were modeled as closed systems only for the sake of simplicity of simulation. Closed systems are not a requirement of the two-part strategy. Outside germplasm can be introduced to the population improvement section

in one or more cycles of crossing. If this germplasm is used as males, their alleles would be interspersed within the population. This would effectively integrate the outside germplasm into the population improvement section. Optimal contribution selection (Woolliams et al., 2015) or a similar approach could be used to ensure that the newly introduced diversity is not quickly eliminated through genomic selection prior to its inclusion in the training population (Gorjanc et al., 2016). The optimal contribution selection could also be used to maintain more genetic variance, while maximizing genetic gain to achieve greater long-term genetic gain (Cowling et al., 2016).

The population improvement component doesn't need to rely exclusively on genomic selection. Phenotypic selection could be implemented using "speed breeding" protocols currently used in rapid cycling for disease resistance (Dinglasan et al., 2016; Riaz et al., 2016). Further testing is needed to determine the benefit of this approach relative to a pure genomic selection approach. It is likely that the benefit will depend on the particular trait and availability of sufficiently accurate phenotype evaluations or predictions.

The strength of the two-part strategy is that it combines multiple breeding philosophies within a flexible framework. The population improvement component manages the breeding program's germplasm, using continuous cycles of recurrent selection to achieve the shortest possible cycle time and the maximum genetic gain. This structure is very similar to that of animal breeding programs and would benefit from explicitly defined long-term breeding goals, with the use of common techniques such as optimal contribution selection for managing genetic variance and multiple trait analysis with selection indices for improving many traits simultaneously. The product development component of the strategy makes use of the best aspects of current plant breeding programs. It retains the design of traditional plant breeding programs, giving breeders the flexibility to use any standard breeding strategy. Breeders can thereby make use of the existing approaches that have been developed for dealing with their species' biology and their specific breeding objectives.

Since all programs examined in this paper were constrained to equal operating costs, two conclusions can be drawn from the results: (i) implementing genomic selection in breeding programs increases the rate of genetic gain, and (ii) the two-part strategy is the most cost-effective approach for implementing genomic selection. The whole breeding program simulation approach used in this paper allowed for modeling the complex interactions that arise in different program designs. The particular programs modeled in this study were generalized representations of true programs. However, the approaches used in this study could be repeated with real-world breeding program designs and costs to measure cost-effectiveness, or other aspects, of genomic selection strategies.

Conflict of Interest Disclosure

The authors declare that there is no conflict of interest.

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REFERENCES

- Akdemir, D., and O.U. Godfrey. 2015. EMMREML: Fitting mixed models with known covariance structures. R package version 3.1. <http://CRAN.R-project.org/package=EMMREML> (accessed 30 June 2017).
- Battenfield, S.D., C. Guzmán, R.C. Gaynor, R.P. Singh, R.J. Peña, S. Dreisigacker, A.K. Fritz, and J.A. Poland. 2016. Genomic selection for processing and end-use quality traits in the CIM-MYT spring bread wheat breeding program. *Plant Genome* 9(2): 0. doi:10.3835/plantgenome2016.01.0005
- Bernardo, R. 2009. Genomewide selection for rapid introgression of exotic germplasm in maize. *Crop Sci.* 49(2):419. doi:10.2135/cropsci2008.08.0452
- Bernardo, R. 2010. Genomewide selection with minimal crossing in self-pollinated crops. *Crop Sci.* 50(2):624. doi:10.2135/cropsci2009.05.0250
- Bernardo, R. 2014. *Essentials of plant breeding*. Stemma Press, Woodbury, MN.
- Bernardo, R., and J. Yu. 2007. Prospects for genomewide selection for quantitative traits in maize. *Crop Sci.* 47(3):1082. doi:10.2135/cropsci2006.11.0690
- Bulmer, M.G. 1971. The effect of selection on genetic variability. *Am. Nat.* 105(943):201–211. doi:10.1086/282718
- Charlesworth, B. 2009. Effective population size and patterns of molecular evolution and variation. *Nat. Rev. Genet.* 10(3):195–205. doi:10.1038/nrg2526
- Chen, G.K., P. Marjoram, and J.D. Wall. 2009. Fast and flexible simulation of DNA sequence data. *Genome Res.* 19(1):136–142. doi:10.1101/gr.083634.108
- Clark, S.A., J.M. Hickey, H.D. Daetwyler, and J.H. van der Werf. 2012. The importance of information on relatives for the prediction of genomic breeding values and the implications for the makeup of reference data sets in livestock breeding schemes. *Genet. Sel. Evol.* 44(1):4. doi:10.1186/1297-9686-44-4
- Cowling, W.A., L. Li, K.H.M. Siddique, M. Henryon, P. Berg, R.G. Banks, and B.P. Kinghorn. 2016. Evolving gene banks: Improving diverse populations of crop and exotic germplasm with optimal contribution selection. *J. Exp. Bot.* <https://academic.oup.com/jxb/article/doi/10.1093/jxb/erw406/2457456/Evolving-gene-banks-improving-diverse-populations> (accessed 8 May 2017).

- Daetwyler, H.D., B. Villanueva, and J.A. Woolliams. 2008. Accuracy of predicting the genetic risk of disease using a genome-wide approach. *PLoS One* 3(10):E3395. doi:10.1371/journal.pone.0003395
- Dinglasan, E., I.D. Godwin, M.Y. Mortlock, and L.T. Hickey. 2016. Resistance to yellow spot in wheat grown under accelerated growth conditions. *Euphytica* 209(3):693–707. doi:10.1007/s10681-016-1660-z
- Eddelbuettel, D., and C. Sanderson. 2014. RcppArmadillo: Accelerating R with high-performance C++ linear algebra. *Comput. Stat. Data Anal.* 71:1054–1063. doi:10.1016/j.csda.2013.02.005
- Faux, A.-M., G. Gorjanc, R.C. Gaynor, M. Battagin, S.M. Edwards, D.L. Wilson, S.J. Hearne, and J.M. Hickey. 2016. AlphaSim—Software for the simulation of breeding programs. *Plant Genome* 9(3). doi: 10.3835/plantgenome2016.02.0013
- Forster, B.P., E. Heberle-Bors, K.J. Kasha, and A. Touraev. 2007. The resurgence of haploids in higher plants. *Trends Plant Sci.* 12(8):368–375. doi:10.1016/j.tplants.2007.06.007
- García-Ruiz, A., J.B. Cole, P.M. VanRaden, G.R. Wiggans, F.J. Ruiz-López, and C.P.V. Tassell. 2016. Changes in genetic selection differentials and generation intervals in US Holstein dairy cattle as a result of genomic selection. *Proc. Natl. Acad. Sci. USA* 113(28):E3995–E4004. doi:10.1073/pnas.1519061113
- Gillespie, J.H., and M. Turelli. 1989. Genotype-environment interactions and the maintenance of polygenic variation. *Genetics* 121(1):129–138.
- Goddard, M. 2009. Genomic selection: Prediction of accuracy and maximisation of long term response. *Genetica (The Hague)* 136(2):245–257.
- Gorjanc, G., J. Jenko, S.J. Hearne, and J.M. Hickey. 2016. Initiating maize pre-breeding programs using genomic selection to harness polygenic variation from landrace populations. *BMC Genomics* 17:30. doi:10.1186/s12864-015-2345-z
- Habier, D., R.L. Fernando, and J.C.M. Dekkers. 2007. The impact of genetic relationship information on genome-assisted breeding values. *Genetics* 177(4):2389–2397.
- Heffner, E.L., M.E. Sorrells, and J.-L. Jannink. 2009. Genomic selection for crop improvement. *Crop Sci.* 49(1):1. doi:10.2135/cropsci2008.08.0512
- Hickey, J.M., S. Dreisigacker, J. Crossa, S. Hearne, R. Babu, B.M. Prasanna, M. Grondona, A. Zambelli, V.S. Windhausen, K. Mathews, and G. Gorjanc. 2014. Evaluation of genomic selection training population designs and genotyping strategies in plant breeding programs using simulation. *Crop Sci.* 54(4):1476–1488. doi:10.2135/cropsci2013.03.0195
- Hoerl, A.E., and R.W. Kennard. 1976. Ridge regression iterative estimation of the biasing parameter. *Commun. Stat. Theory Methods* 5(1):77–88. doi:10.1080/03610927608827333
- Meuwissen, T.H.E., B.J. Hayes, and M.E. Goddard. 2001. Prediction of total genetic value using genome-wide dense marker maps. *Genetics* 157(4):1819–1829.
- Peng, J.H., D. Sun, and E. Nevo. 2011. Domestication evolution, genetics and genomics in wheat. *Mol. Breed.* 28(3):281–301. doi:10.1007/s11032-011-9608-4
- Pszczola, M., T. Strabel, H.A. Mulder, and M.P.L. Calus. 2012. Reliability of direct genomic values for animals with different relationships within and to the reference population. *J. Dairy Sci.* 95(1):389–400. doi:10.3168/jds.2011-4338
- R Development Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Ramsey, F.L., and D.W. Schafer. 2002. The statistical sleuth: A course in methods of data analysis. 2nd ed. Duxbury Press, Pacific Grove, CA.
- Riaz, A., S. Periyannan, E. Aitken, and L. Hickey. 2016. A rapid phenotyping method for adult plant resistance to leaf rust in wheat. *Plant Methods* 12:17. doi:10.1186/s13007-016-0117-7
- Rutkoski, J.E., E.L. Heffner, and M.E. Sorrells. 2011. Genomic selection for durable stem rust resistance in wheat. *Euphytica* 179(1):161–173. doi:10.1007/s10681-010-0301-1
- Schaeffer, L.R. 2006. Strategy for applying genome-wide selection in dairy cattle. *J. Anim. Breed. Genet.* 123(4):218–223. doi:10.1111/j.1439-0388.2006.00595.x
- Thuillet, A.-C., T. Bataillon, S. Poirier, S. Santoni, and J.L. David. 2005. Estimation of long-term effective population sizes through the history of durum wheat using microsatellite data. *Genetics* 169(3):1589–1599. doi:10.1534/genetics.104.029553
- Woolliams, J.A., P. Berg, B.S. Dagnachew, and T.H.E. Meuwissen. 2015. Genetic contributions and their optimization. *J. Anim. Breed. Genet.* 132(2):89–99. doi:10.1111/jbg.12148