A strategy to exploit surrogate sire technology in livestock breeding programs

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Abstract

Background

In this work, we performed simulations to develop and test a strategy for exploiting surrogate sire technology in livestock breeding programs.

Methods

One hundred replicates of various scenarios were performed. Scenarios followed a common overall structure but differed in the strategy used to identify elite donors and how these donors were used in the product development part.

Results

The results of this study showed that using surrogate sire technology would significantly increase the genetic merit of commercial sires, by as much as 6.5 to 9.2 years' worth of genetic gain compared to a conventional breeding program. The simulations suggested that an identification strategy involving three-stages, the first of which uses genomic selection while the subsequent stages use progeny testing was the most effective strategy of all of those tested.

Conclusions

The use of one or a handful of elite donors to generate the production animals would be very different to current practice. While the results demonstrate the great potential of surrogate sire technology there are considerable risks but also other opportunities. Practical implementation of surrogate sire technology would need to account for these.

Background

In this study, we performed simulations to develop a strategy for exploiting surrogate sire technology [1] in livestock breeding programs. Surrogate sire technology allows the creation of males that lack their own germline cells, but have transplanted spermatogonial stem cells from other “elite donor” males. With this technology, a single male could give rise to huge numbers of progeny, potentially as much as all the production level animals in a particular time period. Using a single male to generate many or even all production animals offers many advantages. Firstly, it would reduce the genetic lag between the elite nucleus animals and the production animals. Secondly, it could enable better matching of specific management plans to the genetics. Thirdly, as we outline in the discussion it could enable elite donors to be selected for combining ability. The latter could increase production on farm and increase investment and innovation in breeding by enabling a greater ability to protect intellectual property.

Typically, livestock breeding programs are implicitly or explicitly organized in pyramid structures (Figure 1). At the top layer of the pyramid is the nucleus population. Nucleus populations are improved using recurrent selection and nowadays most selection decisions are made using genomic based testing rather than traditional phenotype based testing strategies [2–6]. In the middle of the pyramid is the multiplier layer. Multiplier herds multiply the genetics from the nucleus and sometimes cross purebred lines to produce F_1 (two-way) crosses. The base of the pyramid is the commercial layer. It is made up of commercial herds, and contains the majority of animals. The commercial producers often make a final cross between the terminal line sires and the maternal line dams.

The need to generate huge numbers of production animals in the commercial layer and the limited number of progeny that any male can produce means that large numbers of nucleus animals must contribute genetics to the subsequent layers and that one to several generations are required to undertake the multiplication process. These factors gives rise to a genetic lag, a difference in genetic mean between the nucleus and commercial layers, equivalent to several years genetic gain [7] (e.g., 4 years in a conventional pig breeding program). Surrogate sire technology would allow a single elite nucleus male to produce very large numbers of progeny, by donating spermatogonial stem cells to its surrogates [1]. This could shorten the lag between the nucleus, multiplication and commercial layers. It could also increase the genetic merit in multiplication and commercial layers, because these animals could descend from the best male or best few males in the nucleus as opposed to many of the nucleus males.

Using surrogate sire technology in this way would require that animal breeding programs be restructured so that the best males be identified and tested to become donors of spermatogonial stem cells. It would require that this process of identification and testing takes place in a sufficiently small amount of time so that the extra genetic gain at the commercial level would not be significantly reduced by the extra time required by the breeders to identify, test donors and generate surrogate sires.

Plant breeding programs that produce clonally propagated individuals or inbred or hybrid lines (Figure 1) are conceptually similar to what a restructured animal breeding program that identifies elite donors might look like. These types of plant breeding programs seek: (i) to identify the best individual, or a handful of

individuals, from a population of individuals; and (ii) to disseminate this individual very widely at the commercial layer [8]. To identify the best individual, plant breeders typically use multiple stage testing and selection. As the breeding program progresses through these stages the number of individuals being tested is reduced and the precision of these tests increases. The small numbers of individuals in the final stages are intensively tested in large replicated experiments that are repeated across several environments and years. This ensures that the final individual released by the breeding program is very well characterized and carries a minimal risk of having major undetected weakness. This is necessary because this individual will have a huge footprint in the commercial layer. Similar levels of evaluation would be needed in livestock if elite donors were to have equally large footprints on the commercial layer.

The objective of this study was to develop a strategy for exploiting surrogate sire technology in livestock breeding programs. This strategy involved a subtle, but important, reorganisation in the animal breeding programs so that they combine components of traditional animal and plant breeding programs. The reorganization is similar to the two-part breeding program design that has recently proposed for the incorporation of genomic selection into plant breeding programs [9]. The reorganization involves an explicit partitioning of animal breeding programs into a population improvement component and a product development component. The population improvement component would be similar to the currently used recurrent selection component in many animal breeding nucleus populations that use genomic selection. The product development component would be similar to traditional plant breeding programs and would involve a number of stages of testing to identify the elite donors. The product development component could make use of testing for combining ability, if that was appropriate for the particular species of interest.

Several alternative versions of the reorganized breeding program were compared to different variants of a conventional breeding using simulation. The alternative versions varied: (i) the number of stages of testing; (ii) the accuracy of genomic prediction at the first stage of testing; (iii) the numbers of individuals tested at subsequent stages; and (iv) the number of progeny used to test the individuals at each stage. The results showed that using surrogate sire technology would significantly increase the genetic merit of commercial sires, by as much as between 6.5 and 9.20 years' worth of genetic gain compared to different variants of a conventional breeding program. The simulations suggested that an identification strategy involving three-stages, the first of which uses genomic selection while the subsequent stages use progeny testing was the most effective strategy of all of those tested. The use of one or a handful of elite donors to generate the production animals would be very different to current practice. While the results demonstrate the great potential of surrogate sire technology there are considerable risks and these are discussed.

Methods

Simulation was used to evaluate the impact of a strategy for exploiting surrogate sire technology in livestock breeding programs. One hundred replicates of various scenarios were performed. Scenarios followed a common overall structure (Fig. 2) but differed in the strategy used to identify elite donors and how these donors were used in the product development part.

Conceptually, the simulation scheme was divided into historical and future phases. The historical phase represented historical evolution and recent historical animal breeding efforts up to the present day, under the assumption that livestock populations have evolved for tens of thousands of years, followed by 22 recent generations of modern animal breeding with selection on genomic breeding values in a nucleus population. The future phase represented 20 future generations of modern animal breeding, with selection on genomic breeding values in a nucleus population that subsequently supplied genetic improvement to multiplication and commercial layers. The historical animal breeding generations were denoted -21 to 0 and the future animal breeding generations were denoted 1 to 20. The multiplier and commercial layers were not explicitly simulated but were instead represented with the average genetic merit of nucleus males that would give rise to multiplication and commercial animals while accounting for the time lag. Specifically, we only focused on a breeding program that produced terminal males.

Simulations involved the five following steps:

(i) Generating whole-genome sequence data,

(ii) Generating quantitative trait variants (**QTV**) affecting phenotypes,

(iii) Generating pedigree structures for a typical livestock population,

(iv) Performing selection and dissemination to the commercial layer, and

(v) Testing the effect of a strategy for exploiting surrogate sire technology to do the dissemination.

Results are presented as the mean of one hundred replicates for each scenario and encompass the genetic merit of nucleus males that would give rise to commercial animals at a given time point.

Whole-genome sequence data and historical evolution

Sequence data were generated using the Markovian Coalescent Simulator (MaCS) [10] and AlphaSim [11] for 400 base haplotypes for each of ten 10 chromosomes in the genome. Chromosomes (each 100 cM long and comprising 10^8 base pairs) were simulated using a per site mutation rate of 2.5×10^{-8}, a per site recombination rate of 1.0×10^{-8}, and an effective population size (N_e) that varied over time in accordance with estimates for the Holstein cattle population [12–15] as follows: N_e was set to 100 in the final generation of the coalescent simulation, to N_e = 1256, 1000 years ago, to N_e=4350, 10,000 years ago, and to N_e=43,500, 100,000 years ago, with linear changes in between these time-points. The resulting sequences had approximately 540,000 segregating sites.

Quantitative trait variants

A quantitative trait was simulated by randomly sampling 10,000 QTV from the segregating sequence sites in the base population, with the restriction that 1000 QTV were sampled from each of the 10 chromosomes. For these QTV, the allele substitution effect was randomly sampled from a normal distribution with a mean of 0 and standard deviation of 0.01 (1.0 divided by the square root of the number of QTV).

The effects of the QTV were in turn used to compute true breeding values (TBV) for a trait.

To simulate selection using genomic selection, without the full computational burden and complexity of simulating training sets and then calculating genomic breeding values using best linear unbiased prediction, pseudo genomic breeding values (**GEBV**) were calculated as TBVs with certain levels of noise added to them to render them representative of genomic breeding values with different levels of accuracy. Pseudo GEBV with accuracy of 0.50, 0.70 and 0.90 were used.

Pedigree structure, gamete inheritance, and recombination rates

After generating whole-genome sequence data and QTV, a pedigree of 42 generations for the nucleus population was simulated. Each generation included 1,000 (**SmallScenario**) or 5,000 (**BigScenario**) individuals of equal sex ratio. The different numbers of individuals were used to quantify any impact of nucleus population size on the benefit of surrogate sire technology. All females (500 for the SmallScenario or 2,500 for the BigScenario) and 50 males, selected by truncation selection on GEBV, were used as the parents of each generation. In the first generation of the recent historical animal breeding population (i.e. generation -22), the chromosomes of each individual were sampled from the 400 base haplotypes. In later generations (i.e., generations -21 to 20), the chromosomes of each individual were sampled from parental chromosomes with possible recombination events. A recombination rate of 1 Morgan per chromosome was used, resulting in a 10 Morgan genome. Crossovers were simulated to occur without interference.

Scenarios

Two different strategies were used to identify males from the nucleus who,

through the use of conventional multiplication or surrogate sire technology, would give rise to commercial animals. The first strategy, which represents a conventional strategy, involved selecting the top 50, 200, or 500 males in both the SmallScenario and the BigScenario based on GEBV. The second strategy was inspired by the multi-stage testing strategies that are common in plant breeding. Here two-stage and three-stage testing strategies were evaluated. In the two-stage strategy a subset of potential elite donors was first selected based on GEBV (**S0**) and the best of these were then progeny tested (**S1**). Based on these test results the best individual or set of individuals was used as elite donors of spermatogonial stem cells to surrogate sires. In the three-stage strategy a subset of potential elite donors was first selected based on GEBV (**S0**), the best of these were then progeny tested (**S1**), and finally the best of these were further progeny tested with even more resources per individual (**S2**). Thus, just as is the case with plant breeding programs, as the testing progressed through these stages the number of individuals being tested was reduced and the precision with which they were tested increased. And as with plant breeding programs, the small numbers of individuals that were tested in the final stages were very intensively tested.

To quantify the impact of different amounts of testing resources and different distributions of these resources across the different stages of testing different accuracies of GEBV at S0 were simulated, different numbers of elite donor candidates tested and different numbers of progeny for testing each elite donor candidate at S1 and S2 were simulated. At S0 GEBV with accuracy of 0.50, 0.70 and 0.90 were used. To ensure that each breeding program had the same costs a total of 14,000 progeny were assumed to be available for the progeny testing stages. When this involved a single stage of progeny testing (S1 only) the 14,000 progeny were used to test 14 elite

donor candidates each tested with 1,000 progeny, 28 elite donor candidates each tested with 500 progeny, 56 elite donor candidates each tested with 250 progeny, 112 elite donor candidates each tested with 125 progeny, 224 elite donor candidates each tested with 63 progeny or 448 elite donor candidates each tested with 31 progeny.

When the multi-stage testing strategy involved two-stages of progeny testing (S1 and S2) either 2,000, 4,000 or 6,000 of the progeny were assigned to S1 testing with the remaining 12,000, 10,000 or 8,000 being assigned to S2. At S1 either 100, 200 or 400 elite donor candidates were tested. Thus, when 2,000 progeny were used at S1 the 100 elite donor candidates were each tested with 20 progeny, the 200 elite donor candidates were each tested with 10 progeny, and the 400 elite donor candidates were each tested with 5 progeny. When 4,000 progeny were used at S1 the 100 elite donor candidates were each tested with 40 progeny, the 200 elite donor candidates were each tested with 20 progeny, and the 400 elite donor candidates were each tested with 10 progeny. When 6,000 progeny were used at S1 the 100 elite donor candidates were each tested with 60 progeny, the 200 elite donor candidates were each tested with 30 progeny, and the 400 elite donor candidates were each tested with 15 progeny.

At S2 either 10 or 20 elite donor candidates that had been advanced from S1 were tested. When 12,000 progeny remained to be used at S2 the 10 elite donor candidates were each tested with 1,200 progeny and the 20 elite donor candidates were each tested with 600 progeny. When 10,000 progeny remained to be used at S2 the 10 elite donor candidates were each tested with 1,000 progeny and the 20 elite donor candidates were each tested with 500 progeny. When 8,000 progeny remained

to be used at S2 the 10 elite donor candidates were each tested with 800 progeny and the 20 elite donor candidates were each tested with 400 progeny.

From each of these testing strategies either 1 or 5 elite donors were to donate spermatogonial stem cells to surrogate sires used in the commercial layer. It was assumed that producing a sufficient number of surrogate sires from these donors to meet the needs of the commercial layer would take one further year. In practice, this period could be used to perform even more intense testing on the elite donors.

The time taken to transfer germplasm from the nucleus to the commercial layer was assumed to be 4 years for the conventional breeding program design that did not involve the use of surrogate sire technology, 5.5 years for the multi-stage testing strategy with two-stages of testing and 6.5 years for the multi-stage testing strategy with three-stages of testing. However, in order to enable comparison, the genetic merit of sires that would be used in the commercial layer but produced by either the conventional or the surrogate sire technology we needed to adjust the expected genetic merit of the sires from the conventional strategy to account for the extra genetic lag invoked by the multiplication process. The elite donors to be used as surrogates sires would not need to be multiplied and thus would not have their genetic merit diluted in the way that those who follow a conventional multiplication process would have, because the females in the multiplier layer would be of lower genetic merit than that of the sires entering it. To account for this, we calculated a dilution factor as follows:

$$D = \sum_{i=1}^{k=6} \Delta G_{t-i} * (w)^{i}$$

Where D is the dilution factor, ΔG is the average genetic gain in generation t and w is the relationship coefficient between the animal and his maternal male ancestor in the generation i. k was fixed to 6 meaning that for every correction we went back 6 generations with w ranging from 0.5 in $t-1$ generation to 0.015625 in the $t-6$ generation. This dilution factor added an equivalent of ~1.04 years of extra time to the conventional multiplication process.

All of these different factors gave 96 different scenarios for each level of GEBV accuracy assumed. The steps and the resources used at each step are shown in Figure 2.

Comparison of different scenarios

To ensure that sufficient numbers of generations had been traversed to allow the dilution factor to be calculated we chose to present the results in terms of the genetic merit of terminal sires used in the commercial layer emerging from generation 11 and each subsequent generation calculated in units of the standard deviation of TBV of the nucleus animals in the base generation ($\sigma_{TBV_{base}}$) as $(\overline{TBV_{curr}} - \overline{TBV_{base}})/\sigma_{TBV_{base}}$, where $\overline{TBV_{curr}}$ is the mean TBV of the nucleus males chosen n years earlier that gave rise to sires of the commercial population and $\overline{TBV_{base}}$ is the mean TBV of nucleus animals in the base generation. Generation 0 was used as the base generation to observe the genetic improvement since the start of the future generations of animal breeding. Calculating the genetic merit in the commercial layer in this way allowed the different strategies to be compared in absolute terms and at specific time points in terms of the number of years it takes the conventional breeding program when selecting the top 50 males to deliver the same

level of genetic merit to the commercial layer.

Results

Using surrogate sire technology was effective for increasing the genetic merit of terminal sires used in the commercial layer. When animals in the commercial layer were progeny of surrogate sires their genetic merit was as much as 6.5 to 9.20 years-worth of genetic gain higher than those that were progeny of sires emerging from different variants of a conventional multiplication process. In both the SmallScenario and the BigScenario the most effective strategy for identifying donors involved three-stages of selection in which genomic selection was used at the first stage, 100 donors were tested each with 60 progeny at the second stage and 20 donors were tested each with 400 progeny at the third stage (see Table1 for details). The benefit of surrogate sire technology was greatest when the accuracy of GEBV was lowest and when the conventional strategy required large proportions of the nucleus males to be used in the commercial layer.

In what follows the results are divided into 4 sub sections for ease of presentation: (i) comparison of conventional and the best performing strategies to identify elite donors for surrogate sires; (ii) comparison of different variants of a two-stage testing strategy to identify elite donors for surrogate sires; (iii) comparison of different variants of a three-stage testing strategy to identify elite donors for surrogate sires; and (iv) comparison of two and three-stage testing strategies to identify elite donors for surrogate sires.

Comparison of conventional and the best performing strategies to identify elite donors for surrogate sires

Figure 4 and Figure S1 shows the average genetic merit of commercial sires derived from the best performing strategy to identify elite donors and the conventional strategy that did not use surrogate sires plotted against time, for three different GEBV accuracies (0.5, 0.7 and 0.9) for both the SmallScenario and the BigScenario respectively. We compared the surrogate sires strategy with three different conventional strategies that either selected the top 50, 200, or 500 males to be advanced to the multiplier layer. At all points in time and for all three GEBV accuracies commercial sires derived from the best performing strategy to identify elite donors had a higher genetic merit than those derived from the conventional strategy and the benefit was greater when more males were advanced to the multiplier layer for the conventional strategy. The benefit of using surrogate sire technology decreased as the accuracy of GEBV increased. Across time the difference between using the conventional strategy and using surrogate sire technology was almost constant. These trends were common between the SmallScenario and the BigScenario, although with differences in magnitude.

Table 1, enumerates some of the main results than can be observed in Figure 4 and Figure S1. Across all accuracies and scenarios tested the best performing strategy to identify elite donors involved a first screen of all candidates based on a GEBV followed by two-stages of progeny testing on a reduced set of candidates with the outcome being the release of a single donor. The benefit was greater when more males were advanced to the multiplier layer for the conventional strategy. When the accuracy of GEBV was low (0.5) the best strategy involved using 6,000 progenies distributed across the 100 candidates at the first stage of testing followed by using 8,000 progenies distributed across the 20 candidates at the second stage of testing. This process of testing and producing a surrogate sire was assumed to take 2.5

additional years compared to the conventional strategy. After accounting for this extra time and for the dilution factor in the conventional multiplication process, on average across all time points the use of surrogate sire technology with this strategy delivered between 6.50 years' worth of extra genetic gain in commercial sires compared to when 50 sires were advanced to the multiplier layer for the conventional strategy and 9.20 years' worth of extra genetic gain in commercial sires compared to a when 500 sires were advanced to the multiplier layer for the conventional strategy for the SmallScenario. For the BigScenario the equivalent values were between 2.72 and 4.10 years' worth of extra genetic gain in commercial sires.

When GEBV accuracy was higher the optimal distribution of testing resources was slightly different. Instead of testing 100 candidates at the first stage of progeny testing, as was the case when the GEBV accuracy was 0.5, 200 candidates were tested in the best performing scenario. All other parameters were the same as those for when the GEBV accuracy was 0.5. The benefit of using surrogate sire technology decreased with increasing accuracy of GEBV and the magnitude changed significantly between the SmallScenario and the BigScenario.

On average across all time points the SmallScenario delivered between 6.50 and 9.20 years' worth of extra genetic gain in commercial sires when GEBV accuracy was 0.5. When GEBV accuracy was 0.7 these values reduced to between 4.52 and 7.20 years' worth of genetic gain in commercial sires and when GEBV accuracy was 0.9 they further reduced to between 2.40 and 5.00 years worth of genetic gain.

On average across all time points the BigScenario delivered between 2.72 and 4.10 years' worth of extra genetic gain at the commercial level when GEBV accuracy was 0.5. When GEBV accuracy was 0.7 these values reduced to between 2.10 and

3.50 years' worth of genetic gain in commercial sires and when GEBV accuracy was 0.9 the impact of using surrogates sire technology delivered on average to between 1.20 and 2.50 years' worth of genetic gain.

The differences in the SmallScenario and the BigScenario were due to the different proportions of candidate males that give rise to commercial sires. In the SmallScenario 10% to 100% contribute while the in the BigScenario 2% to 20% contribute.

For simplicity of presentation and based on the consistency of trends described above, in the following sections we only present comparisons to the conventional strategy in which 50 males were advanced from the nucleus to the multiplier layer.

Comparison of different variants of a two-stage testing strategy

Tables 2 and 3 show the performance of different two-stage testing strategies. Performance is measured as the average years' worth of extra genetic gain at the commercial level delivered by surrogate sire technology compared to a conventional strategy for both the SmallScenario (Table 2) and the BigScenario (Table 3). Consistent with the results reported in the previous section the benefit of surrogate sires was always lower when the accuracy of GEBV was higher. In some situations, the benefit was minimal. In all cases, there was an intermediate optimum for the numbers of candidates tested. Selecting 5 candidates to become elite donors was always worse than selecting one candidate. This behaviour was observed in both the SmallScenario and the BigScenario although with some interesting differences. The BigScenario showed a general shrinkage of years' worth of genetic gain if compared

to the SmallScenario, resulting in a general increase in the number of scenarios that showed only a small benefit from use the surrogate sires technology (See tables S1 and S2).

At all levels of GEBV accuracy the best or joint best of the two-stage testing strategies involved a screen of candidates based on genomic breeding values followed by a single stage of progeny testing in which 112 candidates were tested, each with 125 progeny, followed by the choice of a single donor. Using this strategy on the SmallScenario gave 5.26, 3.58, or 2.20 years worth of extra genetic gain in commercial sires (Table 2) whereas applying it to the BigScenario gave 2.54, 1.97 or 1.07 years worth of extra genetic gain in commercial sires and when GEBV accuracy was 0.5, 0.7 or 0.9 respectively (Table 3).

Just as for the case of selecting one elite donor of spermatogonial cells for surrogate sires, when selecting five elite donors of spermatogonial cells for surrogate sires, testing 112 candidates, each with 125 progeny, gave the highest benefit with 3.62, 2.55 and 1.24 years worth of extra genetic gain in SmallScenario (Table 2) and 2.00, 1.10 and 0.95 in BigScenario (Table 2).

Comparison of different variants of a three-stage testing strategy

Tables 4 and 5 shows the performance of different three-stage testing strategies when either 1 or 5 sires were selected to become an elite donor. By varying several parameters we tested 216 (108 for the SmallScenario and 108 for the BigScenario) different strategies of three-stage testing when the total resources available for progeny testing were kept constant across strategies and equal to that of the two-stage testing strategies. The parameters were the split of resources between

the first and second stages of progeny testing, the number of donor candidates and number of progeny used to test each donor candidate at each of the stages of progeny testing, the accuracy of GEBV for the first screening of donor candidates and the number of donor candidates finally chosen for production of surrogate sires.

Three-stage testing gave a greater benefit than two-stage testing. As for two-stage testing, using one elite donor for surrogate sires gave a greater benefit than using five elite donors and the benefit of surrogate sire technology was greater when the accuracy of GEBV was lower. A total of 14,000 progenies were split across the two stages of progeny testing. Increasing the resources in the first stage of testing gave increased benefit from using surrogate sire technology. For example, with the SmallScenario when the accuracy of GEBV was 0.5 and 6,000 progeny were used in the first progeny test and 8,000 were used in the second progeny test and when one elite donor was used, the best performing strategy gave a benefit of 6.50 years worth of extra genetic gain in commercial sires with surrogate sire technology compared to a conventional strategy where 50 top males were selected using GEBV. This was a greater benefit than the 5.80 years worth of extra genetic gain for the best performing strategy when splitting the progeny 4,000 for the first stage and 10,000 for the second stage which was in turn better than the 5.37 years worth of extra genetic gain for the best performing strategy when splitting the progeny 2,000 for the first stage and 12,000 for the second stage. This trend of greater benefit when more progeny were dedicated to the first stage of progeny testing was observed for almost all strategies that were tested.

For the SmallScenario the difference between testing 100 or 200 donor candidates at the first stage of progeny testing was not consistent. That said, when the

accuracy of GEBV was 0.5 testing 100 candidates at the first stage of progeny testing was usually better than testing 200 and testing 200 candidates was usually better than testing 400. At higher accuracies, there were little differences between testing 100 or 200 candidates at the first stage of progeny testing but both of these sets of strategies were usually better than strategies that tested 400 candidates at the first stage of testing.

In the SmallScenario testing 20 elite donor candidates at the second stage of progeny testing was almost always better than testing 10 candidates. A total of 54 strategies were evaluated for SmallScenario only. In only 6 of these strategies testing 10 candidates was better than testing 20.

Overall for the SmallScenario, when the GEBV accuracy was 0.5, the best performing of different three-stage testing strategies used 6,000 progenies in the first stage of progeny testing to test 100 candidates each with 60 progenies, used 8,000 progenies in the second stage of progeny testing to test 20 candidates each with 400 progeny and selected a single donor for surrogate sires. This strategy test gave a benefit of 6.50 years worth of extra genetic gain in commercial sires compared to a conventional strategy. The same distribution of testing resources was also the joint best when 5, instead of 1, donors were chosen for surrogate sires.

Although all the results presented here refer to the SmallScenario the same trend was observed for the BigScenario with only differences in magnitude of years' worth of genetic gain, specifically a reduction in the benefit observed for surrogate sire technology (See table S1 and S2).

Discussion

The results of this paper suggest that surrogate sire technology could be very beneficial for the dissemination of genetic gain from livestock breeding programs. In summary, our results indicate that benefits of the as much as 6.5 years' worth of genetic gain in commercial sires could be realized with surrogate sire technology. It was best to identify donors for surrogate sires via a three-stage testing strategy involving a first screen based on GEBV followed by two stages of progeny testing. The benefits of surrogate sires were greater when GEBV accuracy was lower. To discuss these results we divide the discussion into four sections: (i) Possible explanations for the observed trends; (ii) Justification and impact of assumptions that were made; (iii) The potential impact of surrogate sire technology on the redesign of animal breeding programs; and (iv) Risks and opportunities of using surrogate sire technology.

Possible explanations for the observed trends

That surrogate sire technology generates such a benefit in terms of years' worth of genetic gain can be explained in the context of the breeders' equation. In any cohort the best few individuals will be some number of standard deviations above the average of the cohort. For example, for the scenario where surrogate sires technology delivered 6.5 years' worth of genetic gain the best individual was 2.69 standard deviations above the mean on average across all years simulated. In contrast, the top 50 individuals were 1.95 standard deviations above the mean on average across all years simulated. Given the breeding program, in this scenario, proceeded at a rate of genetic progress of 0.36 standard deviations per year then choosing the best male as a donor for surrogate sires rather than the top 50 males would produce commercial sires

that are better for more than 8 years' worth of genetic gain. However, given the process of identifying donors for surrogate sires, or the top 50 males in the conventional scheme, does not have perfect accuracy and that it takes time to identify donors and produce surrogate sires (we assumed 3 years) the final result reduces to 6.5 years' worth of genetic gain.

The benefit of surrogate sires technology depended on the proportion of male candidates that the conventional scheme used to give rise to commercial sires. If the breeding program needed to use a large proportion of its male candidates (e.g., the top 200 or 500) the benefit of surrogate sire technology was greater than if it needed to use a few. Again, this result is entirely consistent with quantitative genetic theory. The average genetic merit of the top 100 or more male candidates will be lower than that of the top 50 candidates. Therefore, if a breeding program needs to deliver 50 sires to the commercial level the benefit of using surrogate sires compared to a conventional strategy will be lower than if it needs to deliver 100 sires. This explains why the benefit of surrogate sires appeared lower for the BigScenario which had 2,500 candidate males per year than for the SmallScenario which had 500 candidate males per year. If both scenarios deliver the same number of sires to the commercial level (e.g., 100 sires) the average difference in genetic merit between the top 100 and the top single individual will be lower for the BigScenario than for the SmallScenario. The same logic also explains why selecting five as opposed to one donor for surrogate sires gave a lower benefit.

The trends observed in terms of differences in the benefit accruing from different strategies to test the benefit of surrogate sires also can be explained in the context of the breeders' equation. When the accuracy of GEBV, which was used to

perform the first stage of testing, was lower the benefit of surrogate sire technology was higher. Under the conventional strategy, the average genetic merit of the nucleus candidates that gave rise to commercial sires will be lower when the GEBV accuracy from the first-stage is lower than when it is higher. With surrogate sires this reduction in genetic merit due to the low accuracy of GEBV for the first screening is compensated by the subsequent progeny testing stages. This is in line with the analysis of Dickerson and Hazel [16], who compared the use of progeny testing as a supplement to earlier culling on own or sibling performance. Their conclusion was that the use of progeny test is warranted when heritability is low in which case accuracy of GEBV from own or sibling phenotypes (or genomic prediction in our study) is low. Genomic selection can be thought of as a light touch first screen, the purpose of which is to identify the top portion of animals which then have a large amount of progeny testing resources focused upon them. The purpose of the subsequent progeny testing stages can be thought of as being to search for the best individual within this group.

This same logic also explains why the three-stage strategy was better than the two-stage strategy. In the three-stage strategy there were two-stages of progeny testing after the initial stage compared to just one stage of progeny testing in the two-stage strategy. In the three-stage strategy the first stage of genomic selection can also be thought of as a light touch second screen, the purpose of which is to identify the top portion of animals (e.g., the top 50%) which then have a large amount of progeny testing resources focused upon them. In the three-stage strategy the first stage of progeny testing serves to use a portion of the overall testing resources to evaluate many candidates relatively inaccurately in order to discard most candidates. In the second stage of progeny testing the remaining candidates can be tested more

intensively and the final candidate can be chosen very precisely. In terms of the breeders' equation the first stage of progeny testing maximizes selection intensity while the second stage maximize selection accuracy. The three-stage strategy appears to address both of these parameters more optimally than the two-stage strategy.

While there is substantial body of literature on multi-stage selection [17–21], we are not aware of literature that addresses joint optimisation of proportion of selected individuals, testing resources (i.e., accuracy), and number of selection stages. However, the observed trends in this study have logical explanations. All the performances observed in the two-stages strategy are expected. Although it is well known that increasing the number of progeny per candidate leads to an increasing progeny test's accuracy [22,23], it is equally well known that the number of candidates to be tested is an extremely important variable and the trade-off between the two is crucial to optimize the selection process. In our simulations, the optimum was reached when 112 candidates were progeny tested, given a fixed number of available resources, in our case 14,000 progeny. This behaviour was consistent across the different levels of GEBV accuracy, although the level of accuracy heavily influenced the amount of extra genetic gain because higher GEBV's accuracy directly translates to a better selection of the animals even before performing the progeny test. These trends are consistent with the long-established use of multiple stages of testing in plant breeding programs[8]. Most plant breeding programs that deliver a single genotype (e.g., inbred line) to the commercial layer use multistage testing strategies to identify this individual. Typically, in these multistage testing strategies a very large number of individuals are imprecisely evaluated at the first stage of testing. At each subsequent stage of testing the number of individuals tested is reduced and the precision with which they are evaluated is increased.

Justification and impact of assumptions that were made

There are a huge range of strategies that could be taken for identification of donors for surrogate sires and we only compared a small subset in this study. We choose the tested range of scenarios because we believe they could demonstrate the properties of surrogate sire technology. They show that in some circumstances surrogate sires can deliver a large benefit and in others small benefit. We chose the three levels of GEBV accuracy as these levels reflect what might be possible in breeding programs of various sizes in the next years. To ensure that all strategies used an equal set of resources we set the total number of progeny involved in progeny testing to 14,000. We chose this number as it was divisible in many ways and thereby enabled several strategies to be compared and because this number was similar the 10,000 progeny that would be used by an animal breeding program that each year tested 100 candidates each with 100 progeny, a scale of progeny testing that was not uncommon in some animal breeding programs before the advent of genomic selection.

In the two-stage testing strategy the total testing resources were distributed across many or few candidates. As expected testing an intermediate to higher number of candidates (i.e., 112 to 224) tested using relatively small numbers of progeny (i.e., 125 to 63) gave higher benefits than testing a few candidates (e.g., 14) with many progeny or a very high number of candidates (448) with very few progeny (31). These trends fit within the expectations of the breeders' equation and occur due to the interplay between selection intensity and selection accuracy. However, when the selected elite donors of spermatogonial cells for surrogate sires are to be used to produce huge numbers of progeny at the commercial level, the risk of a donor

carrying some major defect that was not identified by the testing process must also be minimized. For this reason, it is unlikely that a strategy in which donors are tested with a single stage of progeny testing using a ~200 or less progeny would ever be used by a commercial breeding program.

It was this logic that motivated us in our design of the three-stage testing scheme. Our intuition was that in the first stage of progeny testing many candidates could be evaluated with relatively low accuracy but in the second stage of progeny testing a handful of individuals could be tested with many progeny. We evaluated 10 or 20 candidates and tested them using either 800 or 400 progeny as we felt that this would reflect a level of testing that would give a very high degree of certainty that the selected donors would not carry any major defects that were undetected by the testing process.

A major assumption of this study was the amount of time it took to identify elite donors and then to make surrogate sires. In this study, we assumed that this would take 1.5 years for the two-stage strategy and 2.5 years for the three-stage strategy. This was based on the logic that in pigs it would take ~6 months for a male to reach sexual maturity, a further ~4 months for a successful gestation, and a further ~6 months to collect terminal line phenotypes on progeny. Thus, to perform the first stage progeny testing on an individual would take ~16 months. We assumed that it would take another year to perform a second round of progeny testing and equally one year to produce surrogate sires. In contrast, we assumed that the genetic improvement from the conventional program could be delivered to the commercial population within 3.5 to 4 years in a highly optimized breeding program but that this genetic merit would be diluted to some degree by the genetic merit of females in the

multiplier layer. This resulted in the two-stage strategy taking 5.5 years and the three-stage strategy taking 6.5 years for using surrogate sire technology compared to about ~5.04 years for the conventional strategy (calculated as 4 years in time plus a further ~1.04 years to account for the dilution of genetic merit). It is likely that this time could be shortened or lengthened for both the strategies using surrogate sire technology and the conventional strategy in several ways and depending on the assumed species. The benefit of using surrogate sire technology would change accordingly.

The potential impact of surrogate sires on the redesign of animal breeding programs

Animal breeding programs maximize the genetic merit of commercial animals within the available financial, physical, technical and physiological constraints. Implicitly a breeding program has two objectives: (i) improving the mean of the population; and (ii) developing and delivering a product to the commercial producers. In dairy cattle for example, before the advent of genomic selection, breeders used progeny testing schemes that intensively evaluated relatively small numbers of candidate males and used the best performing of these as both parents to improve the population and as a breeding product to be used by the commercial layer. In doing so dairy cattle breeders maximised selection accuracy but were constrained in their ability to increase selection intensity and decrease generation interval. However, the products used by commercial producers were well tested and therefore an individual producer could rely on using relatively few sires, who together could serve entire geographic regions. The advent of genomic selection changed this paradigm significantly. Under genomic selection progeny testing of small numbers of

candidates has been replaced with a genomic testing of large numbers of candidates. Those with best predictions are used as parents to improve the population and as products sold in teams of sires (i.e., a group of sires sold together rather than a single sire sold on its own). In doing so dairy cattle breeders increased selection intensity and reduced generation interval, but are somewhat constrained in their ability to achieve very high levels of accuracy. Given that each candidate male has not had their merit assessed based on phenotypes of their progeny there is a risk that certain sires are not that good or may carry mutations that are highly detrimental (e.g., a *de-novo* mutation that prevents progeny from lactating) [24–26]. To overcome this risk, breeders recommend that commercial producers use semen of a larger number (i.e., team) of sires and limit their use of any one sire.

A strategy for using surrogate sire technology would need to exploit aspects of both genomic selection and progeny testing. Genomic selection could be used to drive the population improvement and, as demonstrated in the present paper, to identify a subset of candidates that could enter a progeny testing scheme. The role of the progeny testing component would be to ensure that the surrogate sires that are released to the commercial sector are not significantly worse than it is predicted to be based on GEBV and that they do not carry detrimental mutations. As demonstrated by the results of the present study a multi-stage strategy to progeny testing was a more efficient use of resources than a single-stage strategy that was commonly used in by animal breeding programs in the past. Multi-stage testing has a long history of use in plant breeding programs which also have a long history delivering products to commercial producers in a way that is highly analogous to what surrogate sires would enable for animal producers.

The majority of commercial producers for all of the major stable crops (maize, wheat, rice) use inbred lines or hybrids between a pair of inbred lines at the commercial level. These inbred or hybrid lines can be grown on huge areas. Plant and animal breeding program designs have diverged somewhat over the years owing to differences in biology, economics, and technical possibilities. Surrogate sire technology, combined with genomic selection, could result in a coalescence of designs across plant and animal breeding. One such design that could apply to both is the two-part scheme recently proposed by Gaynor et al. (2017)[9]. In this scheme, rapid recurrent selection based on genomic breeding values is used to increase the mean of the population, while a multi-stage testing strategy is used to periodically extract, test, and develop a product from the population. The population improvement component resembles the traditional nucleus layer of animal breeding programs, while the product development component resembles the traditional multi-stage testing to derive inbred or hybrid lines of plant breeding programs. The latter could also be seen as an improved multiplication layer of animal breeding programs that exploit breed complementarity to deliver a commercial product.

In the present work, we focused on the use of surrogate sires to serve as sires of commercial individuals (e.g., a terminal sire in a pig population). To do this, donors for surrogate sires were chosen based on their general combining ability. The strategy could also be extended so that specific combining ability was exploited to produce a relatively homogenous set of females from a maternal line that are crossed with single terminal male (via surrogate sires). Use of specific combining ability is widespread in hybrid crops where it exploits complementarity of pairs of individuals and heterosis generated by the “nicking together” of specific pairs of individuals. An extension of the strategy for the use of surrogate technology that was proposed in the present paper

to enable it to exploit specific combining ability could involve extending the multi-stage testing to include a later set of stages where specific crosses are progeny tested. Because in livestock the parents of individuals are outbred (compared to crops where they are inbred) a tiered strategy may be needed in the maternal line(s) which homogenizes the haplotypes that are present in dams released from the maternal lines. For example, using a single surrogate sire, grandsire and great-grandsire on the maternal population would result in a pool of females that carried one of two haplotypes for 87.5% (0.5 + 0.25 + 0.125) of their genome. The terminal surrogate sire would be chosen for what could be termed pseudo specific combining ability that nicked well with these haplotypes.

Risks and opportunities of using surrogate sire technology

Surrogate sires present risk and opportunities to commercial production. The most obvious risk relates to the genetic homogeneity of commercial animals if a single surrogate sire, or a set of very closely related surrogate sires were used. If a disease emerged that this homogenous group of animals was susceptible to it could have major detrimental impacts on commercial production. Having such large groups of homogeneous animals would also increase the selection pressure on disease pathogens to evolve pathogenicity to the group. Plant breeders and commercial crop growers have extensive experience in managing the potential to have genetic homogeneity across large segments of the production area. They have developed strategies to minimize the risk of disease outbreaks and other failures such as crop rotation, using multiple varieties on a farm, creating varietal blends consisting of multiple genotypes, and taking holistic strategies to pathogen management [27]. Aside

from rotation, which may be difficult to use in practice, these strategies would likely have important roles in ensuring the effectiveness of surrogate sire technology in livestock.

The most obvious opportunity emanating from surrogate sire technology also relates to the genetic homogeneity of commercial animals and can also draw on practices that are well established in crop production. In crops, management plans (e.g., https://catalog.extension.oregonstate.edu/em9004) are supplied to a farmer alongside the seed. These plans are specifically tailored to the genotype of the variety based on extensive sets of field trials. They include recommendations for target market, expected performance, optimum sowing date, seeding rate, soil type and water, fertilizer, pesticide and fungicide requirements. These management plans complement the genetics of the variety and increase the benefit obtained from the genetic potential in a generic environment. Similar management plans could be developed for surrogate sires and the benefits would be similarly expected to exceed the benefit (e.g., 6.5 year's worth of genetic gain) that was observed in the present study for the genetics alone. The phenotype data collected to development of the management plans would also serve to further test and validate a particular surrogate sire.

Another obvious opportunity emanating from surrogate sire technology that also relates to the genetic homogeneity is the potential for increasing the homogeneity of product. In livestock production, uniformity of end product is an important topic. In meat animals, for instance, uniformity has economic benefits because excessive variability in carcass weight or conformation is penalized by slaughterhouses [28,29]. A genetically homogeneous commercial population, achieved through the use of

surrogate sire technology, could aid product uniformity. If this was to be achieved most of this increase in uniformity would need to emanate from matching very specific management plans to the homogenous genetics because homogeneous genetics in itself has limited ability to increase phenotypic homogeneity. Van Vleck [30] showed that in the context of cloned animals, if heritability is 25%, then the phenotypic standard deviation among clones would be 87% of that of uncloned animals and only if heritability is 100% will clone mates have complete uniformity.

Compared to conventional multiplication surrogate sire technology enables shorter lag between nucleus and production animals and requires a smaller number of parents contributing to commercial animals. This offers several advantages including the ability to rapidly change the entire genetics at the production level. This could be used to rapidly respond to sudden changes in requirements such as pressure from a new disease or the emergence of a new market for the product that has specific requirements (e.g., marbling levels in meat).

Surrogate sire technology would be costly to implement in practice because it would require capacity in advanced molecular biology and infrastructure for progeny testing to maintained. However, it presents other opportunities through which costs can be saved. For example, multiplier populations to produce terminal sires would not need to be maintained.

Finally, surrogate sire technology either by the release of a handful of individuals from the nucleus or through the release of individuals selected for pseudo specific combining ability presents breeding programs with an enhanced opportunity to protect its intellectual property. This protection of intellectual property would the breeding companies incentive to invest in breeding programs thus avoiding critical

market failure, which increases benefits to farmers. Typically breeding companies share the benefits two-thirds to the farmers and one third to the breeding company which more than offsets the cost of purchase of the genetics by a produced. The most spectacular example of the benefits of cross-breeding are seen in Maize which has seen a 6-fold increase in productivity since hybrid breeding was introduced in the 1930's [31].

Conclusions

The results of this study showed that using surrogate sire technology would significantly increase the genetic merit of commercial sires, by as much as 6.5 to 9.20 years' worth of genetic gain compared to a conventional breeding program. The simulations suggested that an identification strategy involving three-stages, the first of which uses genomic selection while the subsequent stages use progeny testing was the most effective strategy of all of those tested. The use of one or a handful of elite donors to generate the production animals would be very different to current practice. While the results demonstrate the great potential of surrogate sire technology there are considerable risks but also other opportunities. Practical implementation of surrogate sire technology would need to account for these.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

JMH conceived the study. JMH, MB, and PG designed the study. PG performed the analysis. JMH and PG wrote the first draft of the manuscript. MG, WOH, GG, JJ, AJM and RCG helped to interpret the results and refine the manuscript. All authors read and approved the final manuscript.

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Figures

Figure 1. Example animal (on the left) and plant (on the right) breeding schemes.

Figure 2. Map of the scenarios used in the study.

Figure 3. Timeline of the different approaches to identify and disseminate genetic improvement.

Figure 4. Average genetic merit of commercial sires derived from the best performing strategy to identify elite donors and the conventional strategy (top 50, 200 and 500 sires) that did not use surrogate sire technology for SmallScenario (a and b) and BigScenario (c and d) plotted against time when accuracies of genomic selection was 0.5 or 0.7.

Tables

Table 1. Best performant scenarios across all the simulations performed for 3 different levels of GEBV (0.5,0.7 and 0.9).

Table 2. Performance of the scenarios tested using the two-stage testing strategy across 3 different levels of GEBV (0.5,0.7 and 0.9) for SmallScenario.

Table 3. Performance of the scenarios tested using the two-stage testing strategy across 3 different levels of GEBV (0.5,0.7 and 0.9) for BigScenario.

Table 4. Performance of the scenarios tested using the three-stage testing strategy across 3 different levels of GEBV (0.5,0.7 and 0.9) when 1 sire was selected to become surrogate sire for SmallScenario.

Table 5. Performance of the scenarios tested using the three-stage testing strategy across 3 different levels of GEBV (0.5,0.7 and 0.9) when 5 sires were selected to become surrogate sires for SmallScenario.

Supplementary material

Figure S1. Average genetic merit of commercial sires derived from the best performing strategy to identify elite donors and the conventional strategy (top 50, 200 and 500 sires) that did not use surrogate sire technology for SmallScenario (a and b) and BigScenario (c and d) plotted against time when accuracy of genomic selection was 0.9.

Table S1. Performance of the scenarios tested using the three-stage testing strategy across 3 different levels of GEBV (0.5,0.7 and 0.9) when 1 sire was selected to become surrogate sire for the BigScenario.

Table S2. Performance of the scenarios tested using the three-stage testing strategy across 3 different levels of GEBV (0.5,0.7 and 0.9) when 5 sires were selected to become surrogate sires for the BigScenario.

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Figure 1 Typical animal (on the left) and plant (on the right) breeding schemes

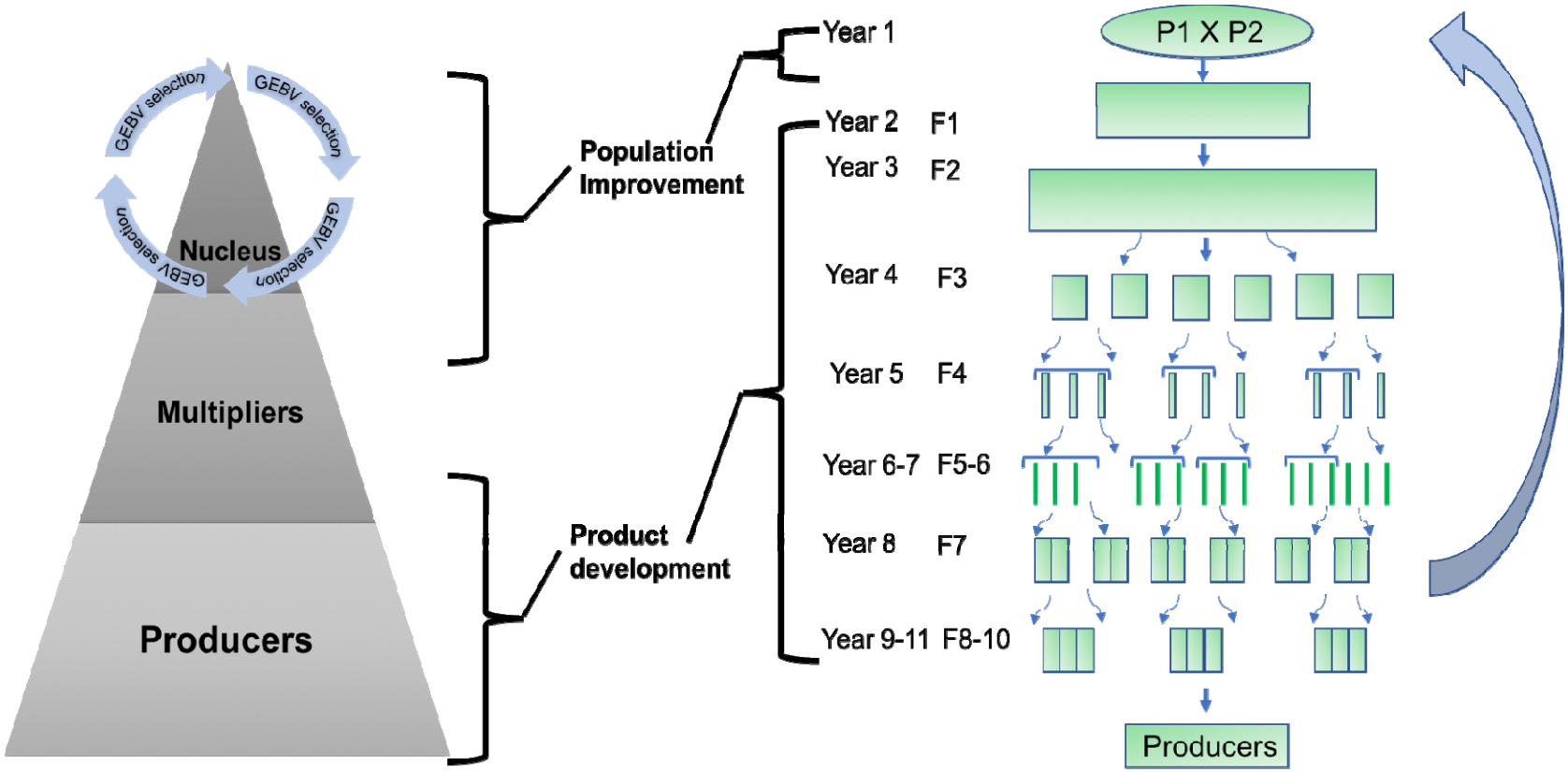

Figure 2 Mind map of the scenarios used in the study

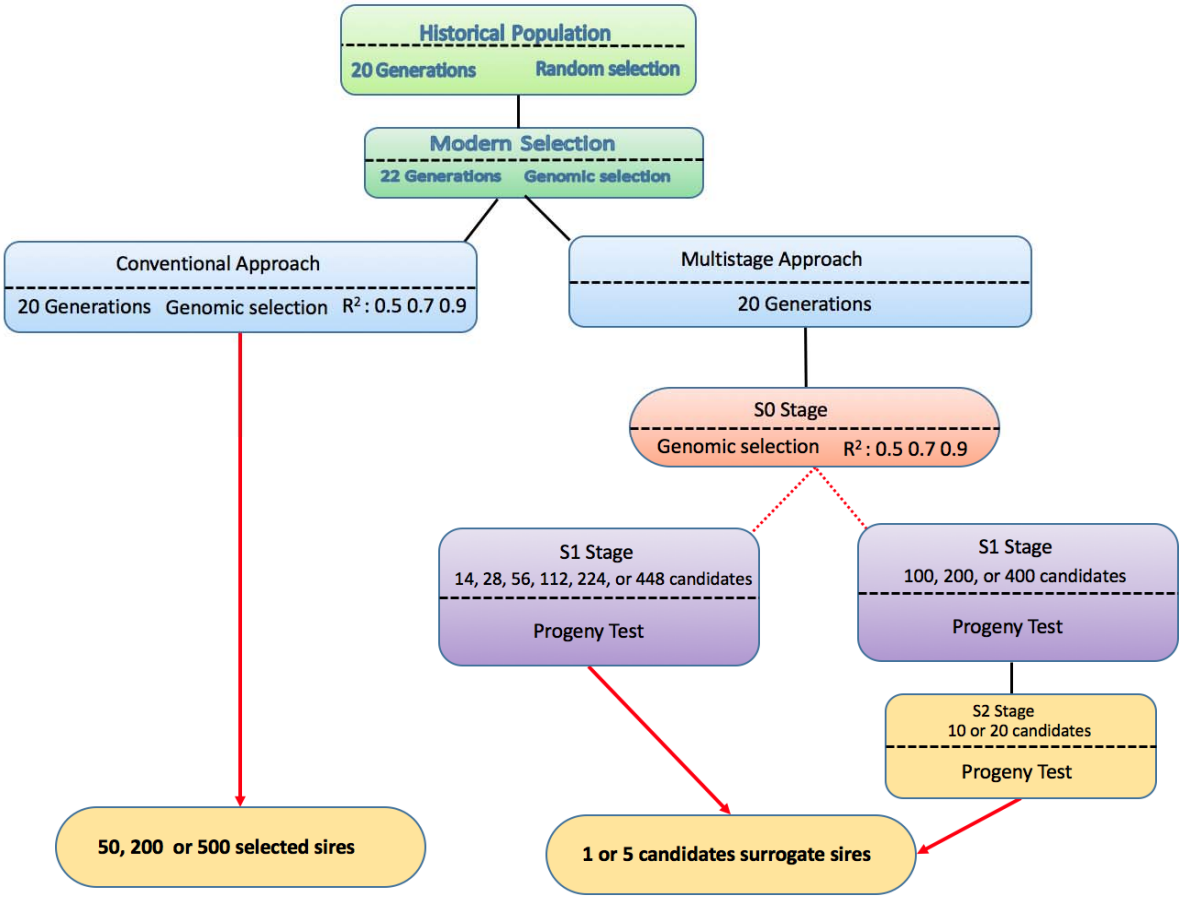

Figure 3 Timeline of the different approaches to identify and disseminate genetic improvement

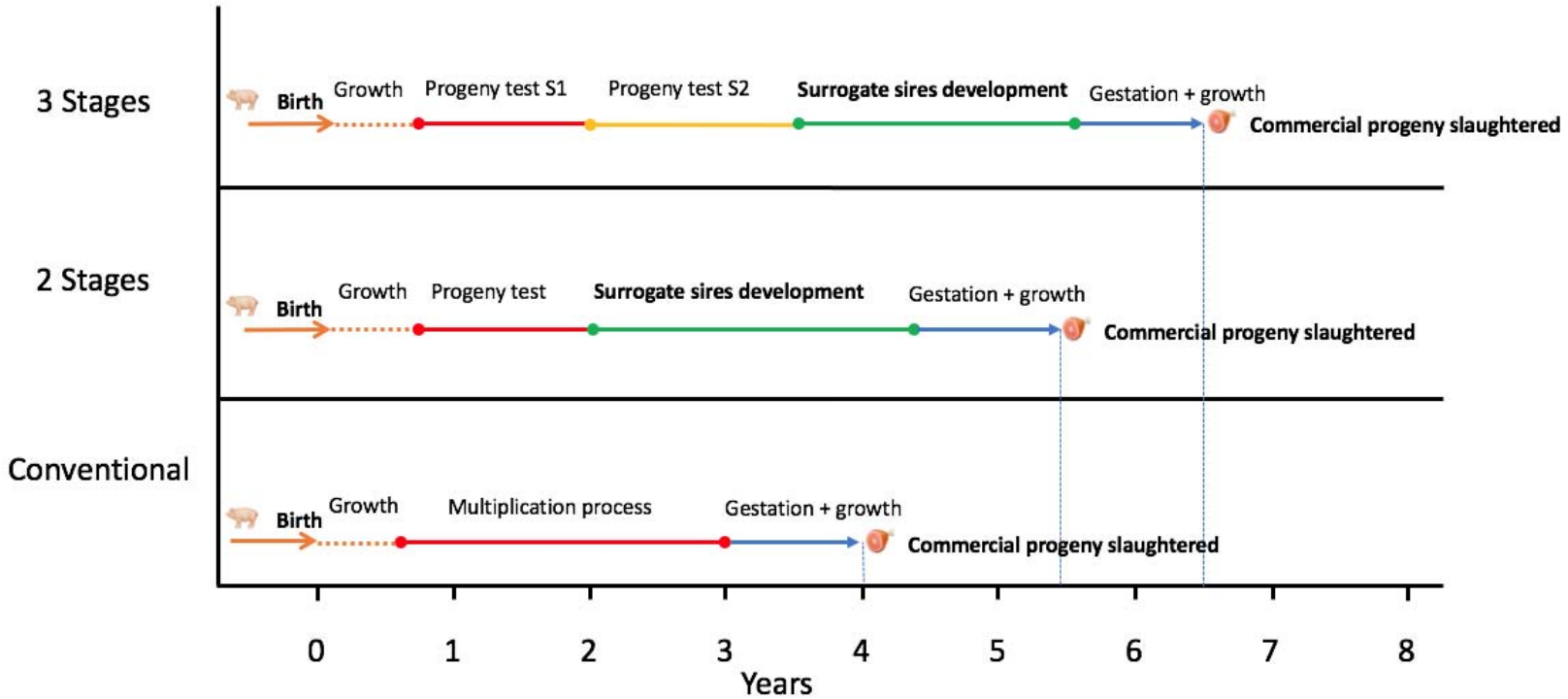

Figure 4 Average genetic merit of commercial sires derived from the best performing strategy to identify elite donors and the conventional strategy (top 50, 200 and 500 sires) that did not use surrogate sire technology for SmallScenario (a and b) and BigScenario (c and d) plotted against time

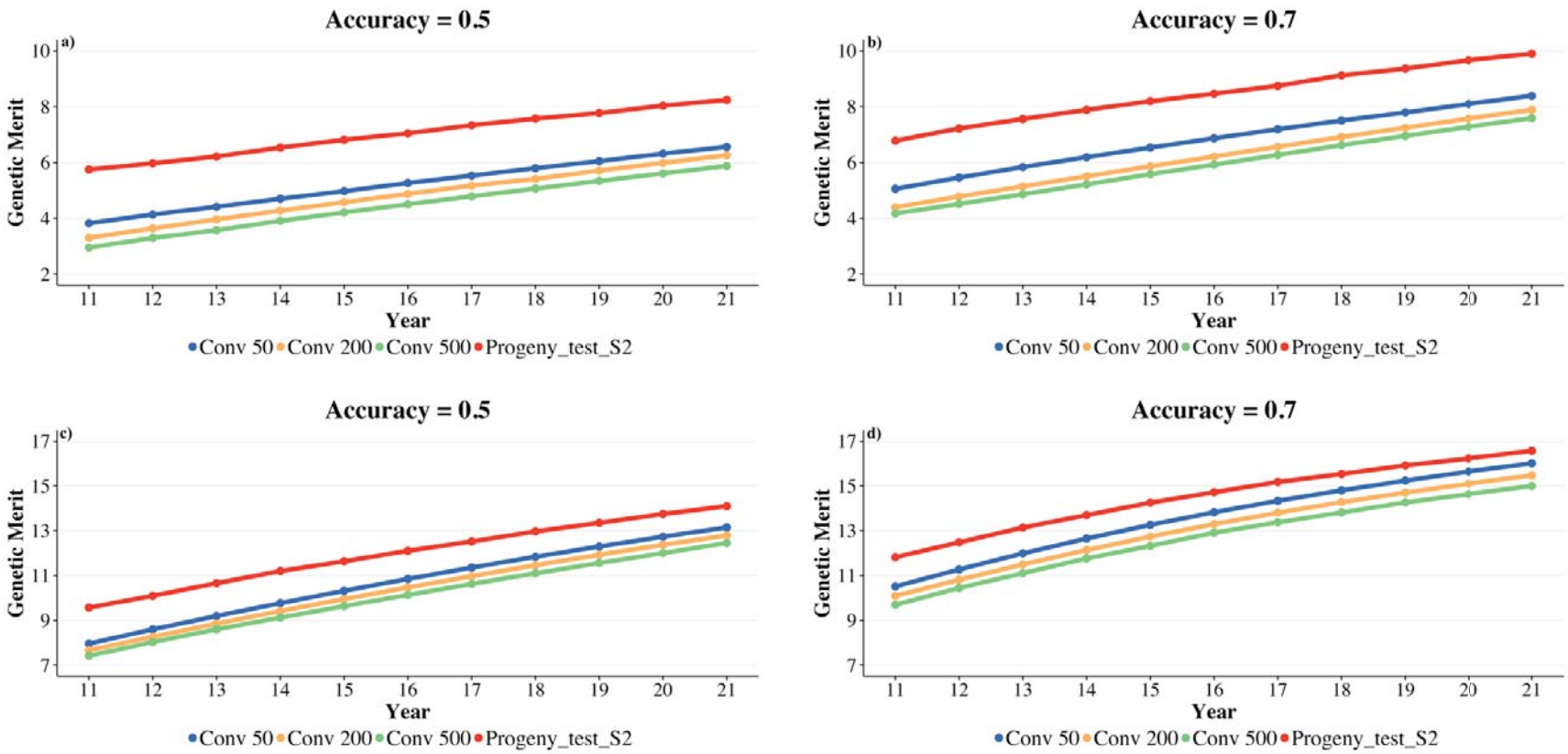

Figure S1 Average genetic merit of commercial sires derived from the best performing strategy to identify elite donors and the conventional strategy (top 50, 200 and 500 sires) that did not use surrogate sire technology for SmallScenario (a) and BigScenario(b) plotted against time

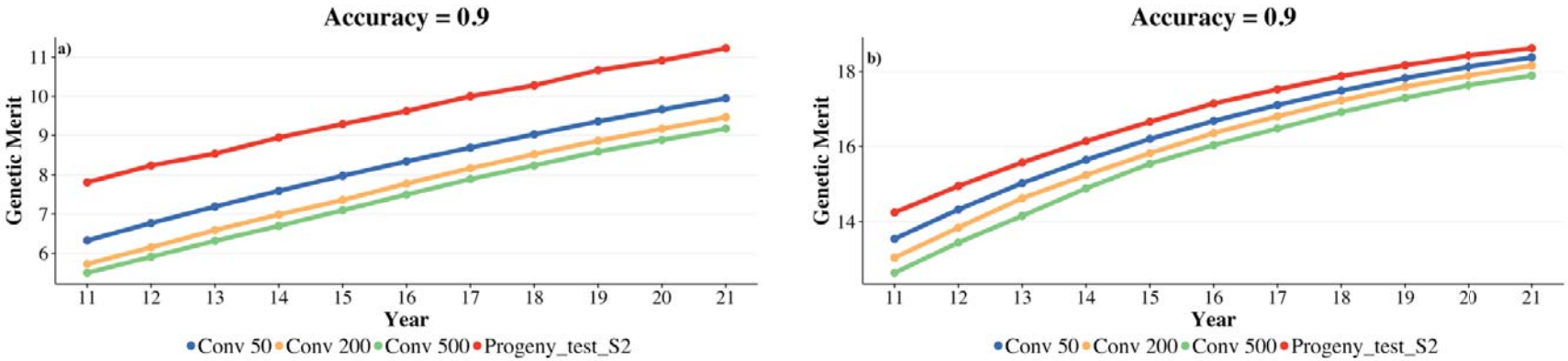

Table 1. Best performing strategies for identifying elite donors for 3 different levels of accuracy

Accuracy GEBV	Sires tested S1	Sires tested S2	Sires selected*	Resources**	Conv50 $\bar{X}_{YGG}$***	Conv200 $\bar{X}_{YGG}$***	Conv500 $\bar{X}_{YGG}$***
Small Scenario							
0.5	100	20	1	6000S1/8000S2	6.50	7.50	9.20
0.7	200	20	1	6000S1/8000S2	4.52	6.50	7.20
0.9	200	20	1	6000S1/8000S2	2.40	4.50	5.00
Big Scenario							
0.5	100	20	1	6000S1/8000S2	2.72	3.50	4.10
0.7	200	20	1	6000S1/8000S2	2.10	2.50	3.50
0.9	200	20	1	6000S1/8000S2	1.20	1.70	2.50

**Number of sires selected to become elite donors*
***Total number of progeny allocated in the first progeny test (S1) and in the second progeny test (S2) respectively*
****Average Years' worth of genetic gain (YGG) calculated across 20 generations of future breeding*

Table 2 Best performing of the two-stage strategies for identifying elite donors for 3 different levels of accuracy for SmallScenario

Sire Tested	Progeny/Sire	Sires selected	$YGG_{0.5}$***	$YGG_{0.7}$***	$YGG_{0.9}$***
14	1000	1	4.12	3.0	1.80
28	500	1	4.72	3.0	1.20
56	250	1	5.11	3.50	2.20
112	125	1	5.26	3.58	2.20
224	63	1	4.80	2.77	1.30
448	31	1	3.81	2.06	1.11
14	1000	5	2.90	1.90	0.2
28	500	5	3.10	2.06	0.5
56	250	5	3.60	2.40	1.10
112	125	5	3.62	2.55	1.24
224	63	5	3.41	1.90	0.33
448	31	5	2.76	1.63	0.23

**** Average years' worth of genetic gain over 20 generations of future selection starting from a GEBV with accuracy 0.5, 0.7 and 0.9 respectively*

Table 3 Best performing of the two-stage strategies for identifying elite donors for 3 different levels of accuracy for BigScenario

Sire Tested	Progeny/Sire	Sires selected	$YGG_{0.5}$***	$YGG_{0.7}$***	$YGG_{0.9}$***
14	1000	1	2.27	1.74	0.72
28	500	1	2.35	1.9	0.80
56	250	1	2.48	1.95	0.99
112	125	1	2.54	1.97	1.07
224	63	1	2.05	1.82	0.85
448	31	1	1.86	1.50	0.39
14	1000	5	1.73	1.21	0.49
28	500	5	1.73	1.25	0.69
56	250	5	1.94	1.08	0.95
112	125	5	2.00	1.10	0.95
224	63	5	1.79	1.04	0.48
448	31	5	1.02	0.77	0.33

**** Average years' worth of genetic gain over 20 generations of future selection starting from a GEBV with accuracy 0.5, 0.7 and 0.9 respectively*

Table 4 Best performing of the three-stage strategies for identifying elite donors for 3 different levels of accuracy for SmallScenario when 1 sire was selected to become an elite donor

Resources*	Sire Tested S1	Progeny/Sire S1	Sire Tested S2	Progeny/Sire S2	$YGG_{0.5}$***	$YGG_{0.7}$***	$YGG_{0.9}$***
2000S1/12000S2	100	20	10	1200	5.28	3.51	2.20
	100		20	600	5.37	3.62	2.37
	200	10	10	1200	4.91	3.25	2.22
	200		20	600	5.12	3.32	2.12
	400	5	10	1200	4.52	3.68	2.05
	400		20	600	4.72	2.67	1.40
4000S1/10000S2	100	40	10	1000	5.53	3.57	2.18
	100		20	500	5.80	4.01	2.31
	200	20	10	1000	5.30	3.46	2.43
	200		20	500	5.37	3.78	2.32
	400	10	10	1000	4.31	3.29	1.64
	400		20	500	4.52	3.49	1.38
6000S1/8000S2	100	60	10	800	5.92	4.13	2.01
	100		20	400	6.50	4.18	2.16
	200	30	10	800	5.27	4.21	2.06
	200		20	400	5.71	4.50	2.40
	400	15	10	800	5.01	3.40	1.62
	400		20	400	5.78	3.51	1.22

**Number of total progeny allocated in the first progeny test (S1) and in the second progeny test (S2) respectively*
**** Average years' worth of genetic gain over 20 generations of future selection starting from a GEBV with accuracy 0.5, 0.7 and 0.9 respectively*

Table 5 Best performing of the three-stage strategies for identifying elite donors for 3 different levels of accuracy for SmallScenario when 5 sires were selected to become an elite donor

Resources*	Sire Tested S1	Progeny/Sire S1	Sire Tested S2	Progeny/Sire S2	$YGG_{0.5}$***	$YGG_{0.7}$***	$YGG_{0.9}$***
2000S1/12000S2	100	20	10	1200	4.14	2.14	1.12
	100		20	600	4.44	2.17	1.20
	200	10	10	1200	3.04	2.11	1.20
	200		20	600	3.68	2.51	1.26
	400	5	10	1200	2.15	1.50	1.00
	400		20	600	2.19	1.43	1.05
4000S1/10000S2	100	40	10	1000	4.41	2.45	1.29
	100		20	500	4.53	2.51	1.23
	200	20	10	1000	4.10	2.24	1.10
	200		20	500	4.12	2.71	1.25
	400	10	10	1000	4.16	1.69	1.05
	400		20	500	4.18	2.02	1.17
6000S1/8000S2	100	60	10	800	4.55	3.09	1.64
	100		20	400	5.05	3.19	1.77
	200	30	10	800	4.60	2.12	1.30
	200		20	400	5.05	2.25	1.38
	400	15	10	800	4.09	1.67	1.16
	400		20	400	4.61	2.23	1.25

**Number of total progeny allocated in the first progeny test (S1) and in the second progeny test (S2) respectively*
**** Average years' worth of genetic gain over 20 generations of future selection starting from a GEBV with accuracy 0.5, 0.7 and 0.9 respectively*

Table S1 Best performing of the three-stage strategies for identifying elite donors for 3 different levels of accuracy for BigScenario when 1 sire was selected to become an elite donor.

Resources*	Sire Tested S1	Progeny/Sire S1	Sire Tested S2	Progeny/Sire S2	$YGG_{0.5}$***	$YGG_{0.7}$***	$YGG_{0.9}$***
2000S1/12000S2	100	20	10	1200	2.21	1.53	0.90
	100		20	600	2.31	1.70	0.92
	200	10	10	1200	2.11	1.55	1.0
	200		20	600	2.16	1.11	0.88
	400	5	10	1200	2.24	1.31	0.90
	400		20	600	2.28	1.50	0.77
4000S1/10000S2	100	40	10	1000	2.21	1.58	0.83
	100		20	500	2.14	1.65	0.85
	200	20	10	1000	2.11	2.07	0.91
	200		20	500	2.20	2.09	0.97
	400	10	10	1000	2.32	1.67	0.89
	400		20	500	2.33	1.99	0.93
6000S1/8000S2	100	60	10	800	2.53	2.02	0.99
	100		20	400	2.72	2.08	1.11
	200	30	10	800	2.44	1.90	1.12
	200		20	400	2.63	2.10	1.20
	400	15	10	800	2.33	1.95	0.91
	400		20	400	2.39	1.95	0.90

**Number of total progeny allocated in the first progeny test (S1) and in the second progeny test (S2) respectively*
**** Average years' worth of genetic gain over 20 generations of future selection starting from a GEBV with accuracy 0.5, 0.7 and 0.9 respectively*

Table S2 Best performing of the three-stage strategies for identifying elite donors for 3 different levels of accuracy for BigScenario when 5 sire was selected to become an elite donor

Resources*	Sire Tested S1	Progeny/Sire S1	Sire Tested S2	Progeny/Sire S2	$YGG_{0.5}$***	$YGG_{0.7}$***	$YGG_{0.9}$***
2000S1/12000S2	100	20	10	1200	1.66	1.19	0.65
	100		20	600	1.65	1.19	0.78
	200	10	10	1200	1.56	1.25	0.63
	200		20	600	1.50	0.98	0.34
	400	5	10	1200	1.58	1.12	0.70
	400		20	600	1.64	1.18	0.77
4000S1/10000S2	100	40	10	1000	1.69	1.27	0.66
	100		20	500	1.72	1.22	0.59
	200	20	10	1000	1.55	1.25	0.43
	200		20	500	1.63	1.37	0.57
	400	10	10	1000	1.32	1.25	0.31
	400		20	500	1.45	1.35	0.44
6000S1/8000S2	100	60	10	800	1.80	1.10	0.37
	100		20	400	1.85	1.37	0.67
	200	30	10	800	1.64	1.15	0.46
	200		20	400	1.63	1.19	0.50
	400	15	10	800	1.72	1.19	071
	400		20	400	1.60	1.23	0.88

**Number of total progeny allocated in the first progeny test (S1) and in the second progeny test (S2) respectively*
*** Values below 0.1 years were set to 0*
**** Average years' worth of genetic gain over 20 generations of future selection starting from a GEBV with accuracy 0.5, 0.7 and 0.9 respectively*