

1 **Clonal breeding strategies to harness heterosis: insights from stochastic simulation**

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18

19 **Abstract**

20 To produce genetic gain, hybrid crop breeding can change the additive as well as dominance genetic
21 value of populations, which can lead to utilization of heterosis. A common hybrid breeding strategy is
22 reciprocal recurrent selection (RRS), in which parents of hybrids are typically recycled within pools based
23 on general combining ability (GCA). However, the relative performance of RRS and other possible
24 breeding strategies have not been thoroughly compared. RRS can have relatively increased costs and
25 longer cycle lengths which reduce genetic gain, but these are sometimes outweighed by its ability to
26 harness heterosis due to dominance and increase genetic gain. Here, we used stochastic simulation to
27 compare gain per unit cost of various clonal breeding strategies with different amounts of population
28 inbreeding depression and heterosis due to dominance, relative cycle lengths, time horizons, estimation
29 methods, selection intensities, and ploidy levels. In diploids with phenotypic selection at high intensity,
30 whether RRS was the optimal breeding strategy depended on the initial population heterosis. However, in
31 diploids with rapid cycling genomic selection at high intensity, RRS was the optimal breeding strategy
32 after 50 years over almost all amounts of initial population heterosis under the study assumptions. RRS
33 required more population heterosis to outperform other strategies as its relative cycle length increased and
34 as selection intensity decreased. Use of diploid fully inbred parents vs. outbred parents with RRS
35 typically did not affect genetic gain. In autopolyploids, RRS typically was not beneficial regardless of the
36 amount of population inbreeding depression.

37

38 **Key Message**

39 Reciprocal recurrent selection sometimes increases genetic gain per unit cost in clonal diploids with
40 heterosis due to dominance, but it typically does not benefit autopolyploids.

41

42 Introduction

43 Hybrid breeding may achieve genetic gain by changing the additive as well as dominance genetic
44 value of populations over breeding cycles. Hybrid breeding strategies are widely used in diploid, inbred-
45 hybrid crops such as maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* L.), but an assessment of these
46 strategies' genetic gain per unit cost over a wide range of dominance genetic architectures has not yet
47 been conducted (Duvick, 2005; Longin et al., 2012). Additionally, breeding strategies to cost-effectively
48 utilize dominance in clonal breeding programs, particularly autopolyploids, have not been fully explored
49 or widely initiated (Diaz et al., 2021; Ceballos et al., 2020; Darkwa et al., 2020; Batte et al., 2020;
50 Lindhout et al., 2018). Dominance has been observed via inbreeding depression and heterosis in
51 economically important traits of various clonal species, such as fresh yield in diploid cassava (*Manihot*
52 *esculenta*) and autohexaploid sweetpotato (*Ipomoea batatas*; Ceballos et al., 2015; Diaz et al., 2021).
53 However, clonal crops have differences from inbred-hybrid crops which could affect the optimal breeding
54 strategy to achieve genetic gain when heterosis due to dominance is present. Therefore, we compare
55 breeding strategies in model clonal crop breeding programs by stochastic simulation with various genetic
56 architectures of heterosis due to dominance.

57 The first consideration in clonal hybrid breeding is that clonal crops may be diploid, as are
58 cassava and white yam (*Dioscorea rotundata*), but are often various degrees of autopolyploid, as in potato
59 (*Solanum tuberosum*), sweetpotato, sugarcane (*Saccharum* spp.), and banana (*Musa* spp.). The
60 quantitative genetics of autopolyploids are an active area of research, and the increased transmissibility of
61 dominance value in autopolyploids with random mating compared to diploids suggests that breeding
62 strategies to harness heterosis due to dominance may differ between diploids and autopolyploids
63 (Amadeu et al., 2020). The second consideration is that the multiplication ratio of clonal crops may be
64 low; for example, maize typically produces around 200 seeds per cross (200:1), but white yam currently
65 produces around 4 to 8 propagules per plant (4:1 to 8:1; Aighewi et al., 2015). Therefore, hybrid breeding
66 strategies which require two stages of crossing may face penalties in species with low multiplication
67 ratios due to the additional time needed for multiplication. Finally, clonal crop genotypes can be routinely
68 reproduced identically by asexual reproduction rather than inbreeding to full homozygosity (McKey et al.,
69 2010). Many clonal crops are difficult or impossible to self and display severe inbreeding depression;
70 some populations lose viability even without complete homozygosity (Lebot, 2019). It has long been
71 recognized that hybrid breeding does not require fully inbred parental genotypes to harness heterosis;
72 rather, fully inbred parents are required to identically reproduce hybrid genotypes in inbred-hybrid crops
73 that cannot be clonally propagated (Schnell, 1961; Lamkey & Edwards, 1999). However, occasional
74 concern that clonal breeding would benefit from fully inbred lines remains (Ceballos et al., 2015; Powell
75 et al., 2020).

76 The key reason to pursue a hybrid breeding strategy is to utilize heterosis and avoid inbreeding
77 depression due to dominance while also increasing additive value. The mean additive value of traits can
78 be increased by increasing the frequency of favorable alleles, but for traits with both additive and
79 dominance gene action, there is a breeding opportunity to increase mean total genetic value by also
80 maintaining or increasing frequency of heterozygous genotypes. Fundamentally, dominance value (d)
81 refers to deviation of heterozygote genetic value from mean homozygote value at a locus (Falconer &
82 Mackay, 1996). For evolutionary reasons, dominance may tend to be positive in the direction of fitness—
83 i.e., across loci which exhibit dominance, heterozygote value is often greater than mean homozygote
84 value on average (Lynch & Walsh, 1998; Manna et al., 2011; Yang et al., 2017).

85 In traits of crops that do not exhibit dominance, selection on individual value with random mating
86 increases the mean genetic value of populations, because the frequency of favorable alleles can be
87 increased without regard for their transitory allocation into homozygous or heterozygous genotypes in the
88 next breeding cycle (Hallauer & Darrah, 1985). Each unit of increase in the frequency of a favorable
89 allele produces linear increase in mean genetic value. In traits with adequate dominance, the allocation of

90 alleles into heterozygous genotypes nonlinearily affects mean genetic value (Schnell, 1961). Maintaining
91 or increasing the frequency of heterozygous genotypes that exhibit dominance increases mean genetic
92 value because these heterozygous genotypes have disproportionately higher values than the less fit, lower-
93 value homozygous genotype (Wei & Van der Steen, 1991). At a locus with dominance, the lower-value
94 homozygous genotype is often referred to as a deleterious recessive genotype, and the decrease in
95 population fitness due to deleterious recessive loci is sometimes called genetic load (Fisher, 1935; Muller,
96 1950; Falconer & Mackay, 1996). Ultimately, fixing the favorable homozygous genotype leads to higher
97 mean genetic value than maintaining heterozygous genotypes in absence of complete dominance or
98 overdominance—so maximizing heterosis is suboptimal with incomplete dominance—but if the
99 favorable homozygote is not fixed it is prudent to avoid the deleterious recessive state (Rembe et al.,
100 2019). Even in absence of true overdominance, linkage disequilibrium of dominant alleles in breeding
101 populations can lead to pseudooverdominance (Jones, 1917). If the haplotypes are not broken over the
102 breeding time horizon, they prevent stacking of favorable alleles and effectively behave as an
103 overdominant locus (Bingham, 1998; Werner et al., 2020).

104 The biologically dominant gene action of individual alleles of complex traits leads to population-
105 wide heterosis and inbreeding depression (Hallauer et al., 2010; Lamkey & Edwards, 1999; Labroo et al.,
106 2021). Here, we borrow from the framework of heterosis and inbreeding depression presented by
107 Falconer & Mackay (1996) and Lamkey & Edwards (1999). As defined by Falconer & Mackay (1996),
108 inbreeding depression is the difference in value between any population at Hardy-Weinberg equilibrium
109 (P_{HWE}) and the population if fully inbred (homozygous; P_I), or $P_I - P_{HWE}$. Heterosis can then be
110 considered the opposite of inbreeding depression due to dominance, $P_{HWE} - P_I$. Lamkey & Edwards
111 (1999) further partition heterosis into values which are relevant to RRS programs. Panmictic heterosis is
112 the difference in the inter-pool hybrid value (P_{F_1}) to the mean of the intra-pool genotypes at Hardy-
113 Weinberg equilibrium ($P_{A_{HWE}}$, $P_{B_{HWE}}$), or $P_{F_1} - \frac{1}{2}(P_{A_{HWE}} + P_{B_{HWE}})$. Baseline heterosis refers to the
114 difference in value of the intra-pool genotypes at Hardy-Weinberg equilibrium to the value of the intra-
115 pool genotypes if fully inbred to homozygosity (P_{A_I} , P_{B_I}), or $\frac{1}{2}[(P_{A_{HWE}} - P_{A_I}) + (P_{B_{HWE}} - P_{B_I})]$.
116 Inbred-midparent heterosis is the sum of panmictic and baseline heterosis. Lamkey & Edwards (1999)
117 specifically define inbreeding depression as the reversal of baseline heterosis, but here we consider the
118 more general definition of Falconer & Mackay (1996). We acknowledge that heterosis due to epistasis is
119 possible, and that heterosis due to epistasis is not the reversal of inbreeding depression, but we do not
120 consider epistasis in this study (Lynch, 1991; Lynch & Walsh, 1998).

121 As stated, increasing favorable allele frequencies can increase the additive value of populations.
122 Recurrent selection (RS) is a breeding strategy which increases the frequency of favorable alleles
123 (Hallauer et al., 2010). In RS, a single pool of genotypes is formed. The genotypes are evaluated, and the
124 best genotypes are selected. The selected genotypes are then randomly intermated to restart the breeding
125 cycle, which concentrates favorable alleles in the next generation. However, with random mating in a
126 single pool, it is challenging to increase the frequency of heterozygotes beyond 0.5, because Hardy-
127 Weinberg equilibrium is nearly constantly restored by random mating relative to the previous generation
128 (Falconer & Mackay, 1996). Therefore, when traits have appreciable dominance, reciprocal recurrent
129 selection (RRS) can be a viable alternative strategy to RS (Comstock et al., 1949; Hallauer et al., 2010).
130 In RRS, germplasm is split into at least two pools. Within each pool, intra-pool genotypes may or may not
131 be fully inbred. Intra-pool genotypes also may or may not be evaluated for their per-se performance.
132 Next, the intra-pool genotypes are crossed to genotypes of the opposing pool to form single-cross inter-
133 pool F_1 hybrids; typically, a sample of intra-pool genotypes is used because the number of all possible
134 crosses becomes impractically large. The inter-pool hybrids are evaluated. Then, intra-pool parents of
135 hybrids are usually selected based on estimates of their average inter-pool performance in F_1 hybrids, or
136 general combining ability (GCA; Comstock et al., 1949; Schnell, 1961). The two pools remain strictly
137 separated with no mixing of pools during recycling, and over breeding cycles, this process leads to the

138 formation of heterotic pools (Duvick et al., 2004). Heterotic pools arise because selection on GCA not
139 only increases the frequency of favorable alleles, but also drives and drifts apart the frequencies of alleles
140 between pools, particularly those which exhibit dominance (Rembe et al., 2019). Upon inter-pool
141 crossing, this difference in allele frequency produces an excess of heterozygous genotypes in the F_1
142 hybrids compared to the frequency of heterozygous genotypes in the parent pools (Lamkey & Edwards,
143 1999). The excess of heterozygosity leads to population-wide heterosis as excess dominance value is
144 expressed in the inter-pool hybrids over the intra-pool parents. This panmictic heterosis occurs regardless
145 of whether the intra-pool genotypes are fully inbred. If the intra-pool genotypes are inbred, upon inter-
146 pool crossing both panmictic heterosis and baseline heterosis are observed in their hybrids, as
147 heterozygosity exceeds not only the diverged pools if they were outbred but also the fully inbred lines.

148 Despite the widespread popularity of RRS (e.g. in maize breeding), additional assessment of its
149 efficiency is needed to inform decision-making in diverse crops. In absence of heterosis, or even with low
150 amounts of heterosis, RRS is thought to be less efficient than RS in improving mean genetic value of
151 breeding populations because RRS usually requires a longer cycle length (L ; Longin et al., 2014). RRS
152 also usually has higher costs per genotype generated than RS because RRS requires maintaining separate
153 pools of germplasm and evaluating both intra- and inter-pool material (Longin et al., 2014). However, in
154 the presence of adequate dominance, RRS is thought to be more efficient in producing genetic gain than
155 RS because RRS prevents expression of deleterious homozygous recessive states in F_1 hybrids by
156 increasing the frequency their heterozygous genotypes. In other words, RRS harnesses and exploits
157 heterosis due to dominance, which partly entails avoiding inbreeding depression due to dominance.

158 To avoid the challenges of RRS while still making some use of heterosis, animal breeders have
159 developed intermediate strategies (Leroy et al., 2016; Swan & Kinghorn, 1992). Of these strategies, the
160 most relevant to challenges in plant breeding may be terminal crossing (Leroy et al., 2016). Terminal
161 crossing can be thought of as RS within two pools, which are subsequently crossed to obtain panmictic
162 heterosis via drift. In terminal crossing, germplasm is divided into two pools. Within each pool, intra-pool
163 genotypes are evaluated for per-se performance. Then, intra-pool genotypes are “terminally” crossed to
164 the opposing pool to form single-cross inter-pool F_1 hybrids, and the inter-pool hybrids are evaluated for
165 use as products. However, intra-pool parents are selected and recycled as parents using estimates of their
166 intra-pool per-se performance rather than their GCA. As in RRS, the two pools remain strictly separated
167 during recycling. Terminal crossing has a shorter cycle length than RRS because parents can be recycled
168 without waiting for their hybrid progeny phenotypes, and terminal crossing can be logically simpler
169 than RRS because testcrossing is not necessary. As mentioned, terminal crossing can also exploit some
170 panmictic heterosis because allele frequencies within pools come to diverge by drift. However, terminal
171 crossing builds less panmictic heterosis than RRS when dominance is present because it relies on drift
172 and does not actively select for divergence between pools as would GCA.

173 The use of genomic selection (GS) to decrease cycle length can increase the competitiveness of
174 RRS compared to other strategies, especially to establish new hybrid breeding programs (Kinghorn et al.,
175 2010; Rembe et al., 2019). Reciprocal recurrent genomic selection can achieve cycle lengths equal to one-
176 pool recurrent genomic selection and two-pool terminal crossing with genomic selection because parents
177 can be recycled on estimates of their value using their relatives’ phenotypes in a genomic prediction
178 model rather than estimates using the parent’s phenotypes (Kinghorn et al., 2010; Powell et al., 2020).
179 Therefore, in all strategies, parents can be recycled as soon as they can be genotyped and predicted
180 accurately rather than as soon as they can be phenotyped accurately, which is the case with phenotypic
181 selection (PS).

182 A recently developed strategy to address dominance is cross performance, particularly genomic
183 prediction of cross performance (Werner et al., 2020; Wolfe et al., 2021). In genomic prediction of cross
184 performance, a single pool of genotypes is formed. The genotypes and phenotypes are evaluated and used
185 to generate a genomic prediction model which typically includes both additive and dominance effects.

186 Then, the predicted effects are used to calculate the mean performance of all possible crosses in the pool,
187 and the best crosses are selected. Finally, the selected crosses are made to restart the breeding cycle. Key
188 concepts with cross performance are that mating is non-random in a single pool and that the parental
189 selection units are the crosses rather than the individuals. Non-random mating allows combinations of
190 alleles within a locus (i.e. genotypes) to be “cut-and-paste” from parents into progeny, so more
191 heterozygosity and thus more dominance value is maintained than with random mating. In the presence of
192 dominance, genomic prediction of cross performance has been demonstrated to outperform selection on
193 genomic estimated breeding value with random mating in a single pool (Werner et al., 2020). However,
194 the various possible genomics-assisted hybrid breeding strategies have not been compared previously.

195 Finally, the long-term benefit and short-term cost of controlling the inbreeding rate in breeding
196 populations is well understood, particularly with use of pedigree selection or GS (Woolliams et al., 2015).
197 However, it is unknown whether the relative performance of hybrid breeding strategies reacts to different
198 degrees of inbreeding control. We contend that inbreeding control can be viewed as a method to manage
199 inbreeding depression in a population, as demonstrated by Fernández et al. (2021). The relative
200 efficiencies of various breeding strategies to address inbreeding depression may differ depending on the
201 inbreeding rate, which is explored indirectly here via the selection intensity. Inbreeding is caused by
202 selection and drift over breeding cycles, which lead to overrepresentation of homozygous genotypes in
203 breeding generations compared to the base population at Hardy-Weinberg equilibrium. Even if
204 populations are at Hardy-Weinberg equilibrium in terms of genotype frequencies, and thus not inbred *per*
205 *se*, they may still be inbred relative to the base population. Inbreeding due to concentration or fixation of
206 favorable alleles, which can increase overall genetic value, is desirable. However, inbreeding due to drift
207 can increase the frequency of unfavorable alleles and their homozygotes inadvertently. Inbreeding control
208 attempts to limit inbreeding due to drift and thus can prevent inbreeding depression. This is because
209 inbreeding control prevents random loss of heterozygosity which decreases mean genetic value in the
210 presence of directional dominance. Of course, inbreeding control also limits drift of allele frequencies in
211 favorable directions, which often leads to short-term costs. Inbreeding control also informs long-term
212 comparisons of breeding strategies. In its absence, different strategies may completely deplete genetic
213 variance at different timepoints, with no further gain, and long-term comparison is simply a record of
214 these different timepoints. The optimal or acceptable inbreeding rate fundamentally depends on the time
215 horizon of a breeding pipeline (Moeinizade et al., 2019). Different hybrid breeding strategies may have
216 different performance at different time horizons, so inbreeding control may be needed to prevent
217 exhaustion of genetic variance and reveal these differences.

218 In summary, several possible breeding strategies to improve traits with heterosis and inbreeding
219 depression due to dominance exist. We shall now proceed to their comparison. We consider how various
220 amounts of inbreeding depression and heterosis in a population affect breeding strategy efficiencies
221 across ploidies. We test phenotypic strategy efficiencies for species with a juvenility period (i.e. delayed
222 flowering) and low multiplication ratio. We explore the impact of intra-pool evaluation in RRS programs,
223 as well as the impact of intra-pool doubled haploid development.

224 Materials and Methods

225 Stochastic simulations were conducted in the R 4.0.4 computing environment with the package
226 AlphaSimR 1.0.1 on the International Maize and Wheat Improvement Center High-Performance
227 Computing Cluster and the University of Wisconsin-Madison Center For High Throughput Computing (R
228 Core Team, 2021; Gaynor et al., 2021). The general procedure was that 180 starting populations with
229 different genetic architectures were simulated, then combinations of breeding strategies, selection
230 intensities, and estimation methods were applied to each population for 100 breeding cycles in ten
231 replicates (Fig. 1). The responses were then measured with variously assumed cycle lengths.

232 *Genetic architecture simulation*

233 The following steps were common to all scenarios. A genome with haploid $n = 10$ chromosomes
234 was simulated using the AlphaSimR *runMacs()* command, which calls the Markovian Coalescent
235 Simulator of Chen et al. (2009). The “*GENERIC*” species history was used, which implied starting
236 effective population size (N_e) of $100 * \text{ploidy} / 2$, following the scaling recommendations of Arnold et al.
237 (2012), and a mutation rate of $2.5 * 10^{-8}$ mutations per base pair. Following the genome simulation, a
238 founder population of 100 non-inbred hermaphroditic individuals was drawn. A single AD trait with
239 additive and dominance effects was simulated with a starting mean genetic value of zero and additive
240 genetic variance of one using the *addTraitAD()* command. The *useVarA* option was set to *TRUE*, so the
241 starting additive genetic variance in the base population was one for all scenarios, but the dominance
242 variance and thus total genetic variance varied depending on the dominance parameters. Although some
243 types of epistasis can contribute to inbreeding depression and heterosis, epistasis was not considered in
244 this study. We also did not consider environment or genotype x environment effects to reduce the
245 complexity of the study. We assumed no historic population split, which could affect the relative
246 efficiency of the strategies (Lamkey & Edwards, 1999).

247 To create trait genetic architectures for each scenario, all combinations of the following factors
248 and their levels were simulated: number of quantitative trait loci (QTL) per chromosome, *nQtlPerChr*, of
249 100, 1000, or 5000; mean dominance degree, *meanDD*, of 0, 0.5, 1, 1.5, or 10; variance of the dominance
250 degrees, *varDD*, of 0, 0.2, 1, or 10; and, ploidy of 2, 4, or 6 (Fig. 1; Supplemental File 1). The methods of
251 simulating allelic effects in AlphaSimR are described in the vignette “*Traits in AlphaSimR*”; as such,
252 polyploid values were assigned assuming digenic dominance interactions only (Gaynor, 2021; Gallais,
253 2003). Varying the number of QTL, mean dominance degree, variance of the dominance degrees, and
254 ploidy led to 180 populations ($3 * 5 * 4 * 3$) with varied amounts of initial population heterosis (H_0) as
255 well as varied starting dominance and total variance, all of which were recorded (Supplemental Fig. 1;
256 Gaynor et al., 2018).

257 H_0 was the difference in the starting population at Hardy-Weinberg equilibrium from the starting
258 population if fully inbred to homozygosity; it was divided by the starting genetic standard deviation to
259 allow comparison across populations with traits at different scales. This measure of heterosis is not named
260 in the framework of Lamkey & Edwards (1999), but it corresponds to the reversal of inbreeding
261 depression as defined by Falconer & Mackay (1996). With all else equal, the amount of H_0 increases as
262 the mean dominance degree and the square root of the number of QTL increase and decreases as the
263 variance of the dominance degrees increases; however, the effect of the variance of the dominance
264 degrees is relatively smaller (Supplemental Fig. 1; Gaynor et al., 2018). We did not control linkage
265 disequilibrium, which also affects H_0 , so simulating populations with identical parameters as in this study
266 may lead to slightly different H_0 as their linkage disequilibrium varies (Gaynor et al., 2018). Occasional
267 negative H_0 was observed in architectures with *meanDD* = 0 and *varDD* > 0 due to random sampling of
268 dominance degrees, which sometimes led to negative directional dominance in the starting population and
269 higher mean values of inbred than outbred genotypes. Each single trait modeled can be interpreted as
270 representing an index of quantitative traits.

271 *Breeding scenarios*

272 Each simulation was initiated by drawing 40 individuals from the same founder population with a
273 given genetic architecture for each of ten replicates. In other words, founder populations were not varied
274 within genetic architectures, and stochasticity within architectures was only due to Mendelian sampling
275 and (at times) random phenotypic error. As such, there was more stochasticity across genetic
276 architectures—which used different founder populations and traits—than within genetic architectures. For
277 simulations with two pools, the 40 individuals were randomly split into two pools of 20 (Cowling et al.,

278 2020). Then, a combination of strategy, selection intensity, and estimation method was applied for 100
279 cycles. Responses were subsequently interpreted with variously assumed cycle lengths. A scenario was
280 defined as a combination of strategy, estimation method, selection intensity, and assumed relative cycle
281 lengths (Fig. 1). Most combinations of the following were assessed: a strategy of One-Pool Breeding
282 Value, One-Pool Predicted Cross Performance, Two-Pool Breeding Value, Two-Pool GCA, or Two-Pool
283 Breeding Value + GCA; an estimation method of phenotypic value, genomic estimated value, or none
284 (true value); and, high or low selection intensity (Fig. 1). For ploidy = 2 only, we considered two
285 additional selection strategies to address inbred-hybrid crops: Two-Pool Doubled Haploid GCA and Two-
286 Pool Doubled Haploid Breeding Value + True GCA. Two-Pool Breeding Value referred to a terminal
287 crossing program. Scenarios with a phenotypic estimation method and the One-Pool Predicted Cross
288 Performance strategy were not considered; although phenotypic cross performance can be estimated as
289 the mean of the parental phenotypes, this scenario was too computationally intensive with phenotypic
290 program sizes used.

291 Phenotypes in the study referred to single phenotypic values per entry with a fixed error variance
292 and an initial broad-sense heritability of 0.5, which represent replicated phenotypes. The broad sense
293 heritability of the phenotypes subsequently changed with genetic variance over cycles. The phenotypic
294 estimate of value referred to these single phenotypic values, which were used for selection, though for
295 Two-Pool GCA the single phenotypic records were used to calculate GCA.

296 Strategy cycle length was assumed to depend on the estimation method. Strategies which used true
297 values or genomic estimates were assumed to have a cycle length of two, which was considered a realistic
298 rapid-cycling length. Some rapid-cycling GS programs may achieve a one-season cycle length, but this is
299 uncommon due to practical constraints (Gaynor et al., 2017). Phenotypic strategies were considered to
300 have different cycle lengths depending on whether fast or slow multiplication was possible. Scenarios
301 with slow multiplication were also assumed to have slow flowering, as occurs in white yam (A. Amele,
302 pers. comm.). Fast multiplication indicated that adequate material for phenotypic evaluation and crossing
303 was available in the season following crossing, and slow multiplication implied adequate material was
304 available after two seasons following crossing. Doubled haploid production was assumed to require one
305 season. All cycle lengths under all assumed constraints are reported in Table 1.

306 We assumed that a single cohort and breeding stage occurred per season, although typical
307 programs may run multiple cohorts at different stages in parallel per season (Covarrubias-Pazaran et al.,
308 2021). As such, to modify the cycle length, the cycle numbers for a given strategy, estimation method,
309 and intensity were multiplied by the appropriate value. For example, the PS scenarios with fast and slow
310 multiplication were obtained from the same simulations, and fast and slow multiplication cycle lengths
311 were imposed by multiplying the cycle number by the strategy cycle length. We assumed that both
312 phenotypic and genotypic information became available post-flowering. Genotypic information was
313 obtained from a simulated SNP-chip with 1000 markers; the number of markers was not varied across
314 genetic architectures. If genomic estimated values were used, the training set for two-pool programs was
315 comprised of the 2,000 most recently evaluated inter-pool individuals, and the training set for one-pool
316 programs was comprised of the 2,000 most recently evaluated intra-pool individuals. To control resources
317 across strategies, we varied program size by decreasing the number of progeny per cross first, then
318 decreasing the number of crosses if necessary. We assumed that the costs of making crosses and growing
319 out non-evaluated plots were negligible. The cost of evaluation plots was assumed to be equal across
320 strategies. For further comparisons, we defined all costs in terms of evaluation plots. We assumed the cost
321 of generating a doubled haploid line was three times the cost of an evaluation plot. We assumed that the
322 cost of phenotyping an individual was equal to the cost of genotyping an individual. With use of outbred

323 intra-pool parents, genotyping both intra-pool parents and their inter-pool segregating progeny was
324 necessary. In the doubled haploid scenarios, we assumed that both intra- and inter-pool genotypes were
325 genotyped, even though the inter-pool progeny genotypes could be inferred from their doubled haploid
326 parents under the assumed cycle lengths. Scenarios which used true values were identical in size to
327 scenarios with genomic estimated values; cost is not a realistic consideration to obtain true values, and the
328 true value scenarios were used to consider a situation with perfect accuracy.

329 A description of each strategy follows. For conciseness, the program sizes are represented by
330 variables, and the values of variables for each scenario are given in Supplemental Table 1. Parents were
331 randomly mated in the first cycle, and in all subsequent cycles a crossing plan conferring maximum
332 avoidance of inbreeding was used (Kimura & Crow, 1963).

- 333 • One-Pool Breeding Value: The parents are made into x crosses with y progeny per cross, totaling
334 z individuals. The z progeny are phenotyped. Then, 2 individuals per family (cross) are selected
335 using the estimate of value for the scenario strategy. The cycle restarts with the selected
336 individuals. Genomic estimates were made from a directional dominance model fit on the training
337 population of intra-pool genotypes using the *RRBLUP_D()* function (Xiang et al., 2016). Code is
338 in Supplemental Files 2—7.
- 339 • Two-Pool Breeding Value: Within each pool, the parents are made into x crosses with y progeny per
340 cross, totaling z intra-pool progeny per pool. The z intra-pool progeny are phenotyped. From
341 each pool, two individuals are then selected randomly. For both pools, all z intra-pool progeny per
342 pool are crossed to both individuals selected from the opposing pool, and each inter-pool cross
343 produces one progeny, creating w inter-pool progeny. The inter-pool progeny are phenotyped.
344 Within each pool, 2 individuals per family (cross) are selected on the scenario surrogate of intra-
345 pool breeding value. The cycle restarts with the selected individuals. Genomic estimates were
346 made from a directional dominance model fit on the training population of inter-pool genotypes
347 using the *RRBLUP_D()* function. We did not explore use of other models or use of intra-pool
348 information in the training set. Code is in Supplemental Files 8—13.
- 349 • One-Pool Predicted Cross Performance: The parents are made into x crosses with y progeny per
350 cross, totaling z individuals. The z progeny are evaluated. The expected mean progeny value for
351 each possible biparental cross is calculated from the expected genotype distribution for each locus
352 under the assumption that gametes pair independently and that the frequency of these gametes
353 follows a binomial distribution. In the case of autopolyploids, these assumptions are consistent
354 with strict bivalent pairing of chromosomes in meiosis, which is the assumption used in this
355 study. True expected mean progeny value is calculated using true QTL and their effects, whereas
356 genomic estimated expected mean progeny value is using SNP markers and their estimated
357 effects (<https://github.com/gaynor/QuantGenResources/blob/main/CalcCrossMeans.cpp>). To
358 conduct maximum avoidance with cross performance, the pairs of families (crosses) which satisfy
359 a maximum avoidance of inbreeding plan are identified. Within those pairs of families, the values
360 of inter-family crosses of their individual members are calculated. Then the two best crosses from
361 each set of paired families are selected. The cycle restarts with the selected crosses. Genomic
362 estimates were made from a directional dominance model fit on the training population of intra-
363 pool genotypes using the *RRBLUP_D()* function. Code is in Supplemental Files 14—17.
- 364 • Two-Pool GCA: Within each pool, the parents are made into x crosses with y progeny per cross,
365 totaling z intra-pool progeny per pool. From each pool, two individuals are selected randomly.
366 For both pools, all z intra-pool progeny per pool are crossed to both individuals selected from the
367 opposing pool, and each inter-pool cross produces one progeny, creating w inter-pool progeny.
368 The inter-pool progeny are phenotyped. Then, within each pool, 2 individuals per family (cross)

369 are selected as parents on GCA. The cycle restarts with the selected individuals. Genomic
370 estimates of GCA were made from a model with parent-specific allelic additive effects fit on the
371 training population of inter-pool genotypes using the *RRBLUP_GCA()* function. Code is in
372 Supplemental Files 18—23.

- 373 • Two-Pool Breeding Value + GCA: these strategies have the same structure as Two-Pool GCA,
374 except that the intra-pool progeny are evaluated before testcrossing. The top ~75% of individuals
375 per family (cross) are selected on the appropriate estimate of breeding value according to
376 scenario, and only the selected individuals are used in testcrossing. With use of genomic
377 estimated values, intra-pool breeding values were estimated with use of a directional dominance
378 model, *RRBLUP_D()*, on a training set of inter-pool genotypes. Intra-pool GCA were estimated
379 with the same training set but the *RRBLUP_GCA()* model. Code is in Supplemental Files 24—
380 29.
- 381 • Two-Pool Doubled Haploid GCA: these strategies have the same structure as Two-Pool GCA,
382 except that all intra-pool progeny were used to create a single doubled haploid line in the season
383 before testcrossing. Code is in Supplemental Files 30—35.
- 384 • Two-Pool Doubled Haploid Breeding Value + GCA: these strategies had the same structure as
385 Two-Pool GCA, except that all intra-pool progeny were used to create a single doubled haploid
386 line in the season following intra-pool crossing. The intra-pool doubled haploid lines were
387 evaluated before testcrossing and the top ~75% of individuals per family (cross) were selected on
388 the appropriate estimate of breeding value according to scenario, and only these selected
389 individuals were used in testcrossing. With use of genomic estimated values, intra-pool breeding
390 values were estimated with use of a directional dominance model, *RRBLUP_D()*, on a training set
391 of inter-pool genotypes. Intra-pool GCA was estimated with the same training set but the
392 *RRBLUP_GCA()* model. Code is in Supplemental Files 36—41.

393 *Responses and analysis*

394 The responses reported were as follows:

- 395 • For one-pool scenarios, genetic gain was the mean genetic value at a given timepoint in the intra-
396 pool genotypes following their evaluation (G_t) minus the mean genetic value of the founder
397 population (G_0), or $G_t - G_0$. For the two-pool scenarios, the method was the same except the
398 inter-pool genotypes were used. This allowed comparison of genetic gain in the product pools of
399 both scenarios. Genetic gain was also reported for the intra-pool genotypes in the Two-Pool
400 GCA, Two-Pool Doubled Haploid GCA, Two-Pool Breeding Value + GCA, and Two-Pool
401 Doubled Haploid Breeding Value + GCA scenarios. Genetic gain was divided by the initial
402 population genetic standard deviation.
- 403 • Mean additive value and mean dominance value were reported at a given cycle in the respective
404 product pools for one-pool and two-pool scenarios and scaled to the starting population genetic
405 standard deviation.
- 406 • Inbreeding depression was reported for the product pools of the scenarios as previously described
407 (Falconer & Mackay, 1996).
- 408 • For scenarios with selection on true values, the genomic inbreeding coefficient f was reported for
409 the product pools relative to their initial populations based on a genomic (**G**) additive relationship
410 matrix (Van Raden 2008; Method 1) with allele frequencies from the initial population. For
411 diploids, the mean diagonal of **G** equals $1 + f$ (Powell et al., 2010; Endelman and Jannink, 2012).
412 The more general relationship for ploidy \square is that the mean diagonal of **G** equals $1 + (\square - 1)f$
413 (Gallais 2003). Please note that the inbreeding coefficient was used only to compare inbreeding

414 for identical strategies at high vs. low selection intensity and requires subtlety in interpretation
415 across populations with different levels of homozygosity due to structure.
416 • Panmictic heterosis was reported for the two-pool strategies as previously described (Lamkey &
417 Edwards, 1999).

418 We wish to highlight that the methods used do not permit meaningful comparisons of absolute or
419 scaled values across ploidies. For example, observing that a breeding program for autohexaploids leads to
420 greater mean genetic value than a diploid at a given cycle does not necessarily imply that more gain is
421 possible in autohexaploids.

422 Responses were reported for all scenarios after 15 and 50 years of breeding, at which timepoints
423 genetic variance was non-zero for all scenarios. Genetic variance was later exhausted at different
424 timepoints among scenarios. Responses were also reported for PS at the same cycle numbers (8 and 25) as
425 GS and true scenarios. This was done to demonstrate the effect of using GS as an estimation method,
426 without using it to reduce cycle length, on the relative performance of PS and GS. For clarity, results were
427 grouped by the question of interest. The core strategies to explore the optimal breeding strategy across H_0
428 were One-Pool Breeding Value, One-Pool Cross Performance, Two-Pool Breeding Value, and Two-Pool
429 GCA. The core strategies were also used to explore the optimal estimation method—i.e. genomic
430 estimated or phenotypic—under the experimental assumptions. The non-core strategies, Two-Pool
431 Breeding Value + GCA, Two-Pool GCA, Two-Pool Doubled Haploid GCA, and Two-Pool Doubled
432 Haploid Breeding Value + GCA, were used to assess whether combined selection on intra-pool breeding
433 value and inter-pool GCA increased gain with or without fully inbred intra-pool parents. The non-core
434 strategies were also used to assess whether use of fully inbred diploid intra-pool parents increased the rate
435 of genetic gain.

436 To analyze and plot the results, each response at the timepoint of interest (15 years, 50 years, or 25
437 cycles) for the questions of interest (core or non-core strategies) was linearly modeled in base R as
438 follows:

$$Y_{ijk} = \mu + S_i + H_j + SH_{ij} + \varepsilon_{ijk}$$

439 where Y_{ijk} was the response value for the i^{th} scenario S , the j^{th} H_0 value H , their ij^{th} interaction SH ,
440 and the ijk^{th} error ε of the simulation replicate. The scenario of a response was the combination of
441 strategy, estimation method, selection intensity, and assumed cycle length. All effects were assumed to be
442 fixed, normally distributed, and independently distributed. The coefficient of determination (R^2) value,
443 slope, slope standard error, intercept, and intercept standard error was recorded for each regression
444 (Supplemental File 42). The regressions, the 95% confidence interval of their predicted means, and, at
445 times, raw data points were plotted using the R package ggplot2 (Wickham, 2011). The intersections of
446 the regressions which occurred within the surveyed H_0 values and, when possible, their standard errors
447 were also calculated (Supplemental File 43). The standard errors of the intersections were estimated by
448 maximum likelihood with the R package nlme and used to calculate the 95% confidence interval of the
449 intersection (whuber, 2020; Pinheiro et al., 2017). In accordance with recent guidelines of the statistical
450 community, significance testing was not conducted and confidence intervals were interpreted
451 (Wasserstein & Lazar, 2016; Alexander & Davis, 2022). We assumed regressions could be meaningfully
452 distinguished at a given value of H_0 if their confidence intervals did not overlap.

453 Only selected responses are plotted in the figures and supplementary figures, but plots of all
454 responses for all scenarios in the study are available for reference in Supplemental File 44.

455 **Results**

456 *Genetic gain in the core strategies*

457 The relative performance of the core strategies depended on H_0 , the time horizon, the selection
458 intensity in the program, the relative cycle lengths among strategies, the estimation method, ploidy level,
459 and their interactions. Typically, the comparative advantage of Two-Pool GCA increased with increased
460 H_0 , time horizon, and selection intensity, as well as with use of GS, but it decreased with increased ploidy
461 level or increased cycle length.

462 With use of GS in the clonal diploids, at high intensity Two-Pool GCA was the best strategy after
463 15 years if H_0 was greater than 9.3, and One-Pool Breeding Value or One-Pool Cross Performance was
464 the best strategy if H_0 was lower (Fig. 2). After 50 years, Two-Pool GCA was the best predicted strategy
465 at all positive H_0 values, and its relative advantage increased as H_0 increased (Fig. 2). In contrast, at low
466 intensity, one-pool strategies were always better than Two-Pool GCA after 15 years (Fig. 2). After 50
467 years at low intensity Two-Pool GCA only outperformed One-Pool Breeding Value if H_0 was greater than
468 17.7, a substantially greater amount of H_0 than at high intensity (Fig. 2). High intensity programs had
469 greater genetic gain than low intensity programs on average, but low intensity one pool strategies
470 outperformed high intensity one pool strategies if H_0 was relatively high (Fig. 2). (Of course, two-pool
471 strategies still outperformed the best one-pool strategy over the range at which low intensity one pool
472 strategies outperformed high intensity one pool strategies.)

473 With use of PS and fast multiplication in clonal diploids, Two-Pool GCA was not the best
474 strategy after 15 years at any H_0 value (Supplemental Fig. 2). After 50 years, it required H_0 greater than
475 13.9 to outperform other strategies, and the amount of overperformance was relatively less than with GS
476 (Supplemental Fig. 3). With PS and slow multiplication, Two-Pool GCA never outperformed other
477 strategies over the time horizons surveyed (Supplemental Fig. 2, Supplemental Fig. 3).

478 With use of GS in the clonal autopolyploids, Two-Pool GCA showed fewer advantages than in
479 diploids, and One-Pool Breeding Value or One-Pool Cross Performance were typically better strategies
480 (Fig. 3). At high intensity after 15 years, One-Pool Breeding Value or One-Pool Cross Performance were
481 the best strategies for both autotetraploids and autohexaploids. One-Pool Cross Performance was the
482 better strategy at high H_0 , and One-Pool Breeding Value was the better strategy at low H_0 . After 50 years
483 at high intensity in the autotetraploids, One-Pool Breeding Value or One-Pool Cross Performance
484 provided the most gain if H_0 was less than or equal to 31.0 ± 2.4 ; if H_0 was greater, Two-Pool GCA or
485 Two-Pool Breeding Value provided the most gain, but the advantages were small (Fig. 3). In the
486 autohexaploids, the same strategy pattern was apparent but the intersection occurred at H_0 of 61.7 ± 5.0 .
487 At low selection intensity, One-Pool Breeding Value or One-Pool Cross Performance provided the most
488 gain at both timepoints for both autotetraploids and autohexaploids (Fig. 3).

489 For the clonal diploids, use of the best GS strategy increased genetic gain compared to the best PS
490 strategy with fast multiplication after 50 years (Fig. 3). If GS was not used to reduce cycle length, and all
491 strategies were compared at 25 cycles, then at small values of H_0 , the best PS strategy produced more gain
492 and the best GS strategy produced more gain with greater H_0 (Supplemental Fig. 3). This indicates the
493 dependency of the relative performance of GS and PS on their relative cycle length as well as H_0 . For the
494 clonal autopolyploids, at high intensity the best GS strategy was better than or equal to the best PS
495 strategy (Fig. 3). The advantage of GS decreased as H_0 decreased. At low intensity in autotetraploids, the
496 best GS strategy was indistinguishable from the best PS strategy. At low intensity in autohexaploids, PS
497 outperformed GS if H_0 was low, and vice versa if H_0 was high.

498 Less absolute genetic gain was observed as H_0 increased (Fig. 2—3). Based on the slopes of the
499 regression lines, one-pool strategies were more sensitive to H_0 than two-pool strategies (Supplemental

500 File 42; Fig. 2—3). As genetic gain increased due either to a longer time horizon or higher intensity, the
501 sensitivity of genetic gain to H_0 also increased.

502 *Additive and dominance value in the core strategies*

503 Regardless of ploidy level, strategy, selection intensity, or timepoint, the regression of additive
504 value on H_0 produced a negative slope, while the regression of dominance value on H_0 produced a
505 positive slope (Supplementary File 42; Supplemental Fig. 5—8). If no dominance was simulated, then
506 both dominance value and H_0 were always zero. In general, one-pool strategies produced more additive
507 value than two-pool strategies regardless of ploidy, timepoint, or intensity (Supplemental Fig. 5,
508 Supplemental Fig. 7). In diploids, Two-Pool GCA produced more dominance value than other strategies
509 at high but not low intensity and as timepoint increased, particularly with use of GS (Supplemental Fig.
510 6). In autopolyploids, there was typically little difference in dominance value among strategies
511 (Supplemental Fig. 8).

512 *Inbreeding coefficient with true values for the core strategies*

513 The inbreeding coefficient was recorded for scenarios with an estimation method of none (true
514 values) only. Within a given ploidy level and timepoint, the regression of inbreeding coefficient on H_0 for
515 each strategy differed depending on the selection intensity (Supplemental File 42). After 15 years,
516 regardless of strategy and ploidy, strategies had higher inbreeding coefficients with high selection
517 intensity and lower inbreeding coefficients with low selection intensity across H_0 values (Supplemental
518 Fig. 9). After 50 years, in diploids One-Pool Cross Performance and Two-Pool Breeding Value had
519 higher inbreeding coefficients with high selection intensity and lower inbreeding coefficients with low
520 intensity, but crossover was observed for Two-Pool GCA and One-Pool Breeding Value (Supplemental
521 Fig. 10). For both, high intensity tended to lead to higher inbreeding coefficients when H_0 was smaller,
522 but low intensity led to high inbreeding coefficients with higher H_0 . In autopolyploids, after 50 years all
523 strategies tended to lead to higher inbreeding coefficients under high selection intensity than low selection
524 intensity (Supplemental Fig. 10). The difference in the inbreeding coefficient by intensity was less in
525 autopolyploids than diploids.

526 *Inbreeding depression with the core selection strategies*

527 Subsequent to the simulation of an initial amount of inbreeding depression, the amount of
528 inbreeding depression in the population potentially could change as allele frequencies changed due to
529 selection and other forces. Regardless of ploidy level, strategy, selection intensity, or timepoint, the
530 regression of population inbreeding depression on H_0 produced a positive slope as expected, given that
531 populations with greater H_0 sustained greater amounts of inbreeding depression regardless of breeding
532 cycle (Supplemental File 42). In general, with comparisons at the same number of cycles, the amount of
533 inbreeding depression for a given ploidy level, estimation method, intensity, and timepoint did not
534 dramatically differ by strategy although some differences were detected (Supplemental Fig. 11—12).
535 Greater reduction of population inbreeding depression was not associated with greater genetic gain.

536 *Panmictic heterosis with the core selection strategies*

537 Panmictic heterosis was zero for the one-pool strategies by definition. For the two-pool strategies,
538 the regression of panmictic heterosis on H_0 produced positive slopes, indicating that the amount of
539 panmictic heterosis strategies built increased with the amount of H_0 regardless of ploidy (Supplemental
540 File 42; Fig. 4). Two-Pool GCA tended to build more panmictic heterosis than Two-Pool Breeding Value,
541 and their relative difference decreased as H_0 decreased. In general, Two-Pool GCA built increasingly

542 more panmictic heterosis than Two-Pool Breeding Value as selection intensity and timepoint increased.
543 However, the difference in panmictic heterosis between Two-Pool GCA and Two-Pool Breeding Value
544 decreased as ploidy level increased.

545 *Breeding Value + GCA strategies*

546 Strategies in which intra-pool evaluation was used to advance genotypes to intra-pool crossing,
547 Two-Pool Breeding Value + GCA and Two-Pool Doubled Haploid Breeding Value + GCA, showed
548 increased genetic gain with PS and unchanged genetic gain with GS compared to strategies without intra-
549 pool evaluation, Two-Pool GCA and Two-Pool Doubled Haploid GCA (Supplemental Fig. 13). The same
550 pattern was observed across ploidies for Two-Pool Breeding Value + GCA and Two-Pool GCA. More
551 interestingly, with selection on GCA, intra-pool genetic value tended to decrease over cycles (compared
552 to the initial intra-pool genotypes) regardless of whether intra-pool evaluation was used at high H_0
553 (Supplemental Fig. 14). However, intra-pool genetic value tended to increase over cycles at low H_0 . Intra-
554 pool evaluation increased intra-pool genetic values compared to its absence with PS and fast
555 multiplication without use of doubled haploids, but intra-pool evaluation had no effect on intra-pool
556 genetic values with GS or with PS and use of doubled haploids (Supplemental Fig. 14).

557 *Doubled Haploid GCA strategies*

558 The use of intra-pool fully inbred lines generally led to unchanged genetic gain after 50 years
559 with GS, but in some cases increased genetic gain with PS. (Supplemental Fig. 13). With PS, Two-Pool
560 Doubled Haploid GCA increased gain compared to Two-Pool GCA but had similar performance to Two-
561 Pool Breeding Value + GCA and Two-Pool Doubled Haploid Breeding Value + GCA (Supplemental Fig.
562 13). Intra-pool fully inbred lines typically had lower mean genetic values than intra-pool outbred clones in
563 both the short and long term (Supplemental Fig. 14). The difference in doubled haploid and outbred intra-
564 pool genotypes was greater as H_0 increased as they suffered additional inbreeding depression
565 (Supplemental Fig. 14). Population inbreeding depression typically did not differ between Two-Pool
566 Doubled Haploid GCA and Two-Pool GCA, nor between Two-Pool Doubled Haploid Breeding Value +
567 GCA and Two-Pool Breeding Value + GCA (Supplemental Fig. 15).

568 **Discussion**

569 Although Two-Pool GCA sometimes provided substantially greater rates of genetic gain per unit
570 cost than other strategies in clonal diploids, its relative performance depended on heterosis and inbreeding
571 depression due to dominance in the trait population, the time horizon, the selection intensity in the
572 program, the relative achievable cycle lengths among strategies, the estimation method, ploidy level, and
573 their interactions. The use of GS rather than PS drastically increased the competitiveness of Two-Pool
574 GCA, indicating that GS unlocks novel opportunities to utilize heterosis. Increased selection intensity
575 increased the relative performance of Two-Pool GCA to other strategies, perhaps indicating that Two-
576 Pool GCA is more competitive at higher inbreeding rates. In typical diploid programs with high selection
577 intensities, if Two-Pool GCA could achieve equal cycle lengths as other strategies, then Two-Pool GCA
578 tended to increase the rate of genetic gain per unit cost at lower amounts of H_0 than if Two-Pool GCA
579 required a longer cycle length. However, in autopolyploids, Two-Pool GCA usually did not increase the
580 rate of genetic gain compared to One-Pool Breeding Value or One-Pool Cross Performance.
581 Autopolyploid Two-Pool GCA tended to provide an advantage in genetic gain at higher values of H_0 than
582 in diploids, if at all, and the amount of relative increase was less than in diploids. As in other studies, the
583 use of GS tended to increase gain compared to PS likely due to increased accuracy, faster inbreeding, and
584 decreased cycle length across H_0 ; use of GS to reduce of the cycle length was a determining factor in

585 whether it outperformed PS at the heritabilities used (Powell et al., 2020; Gaynor et al., 2017; Heslot et
586 al., 2015; Heffner et al., 2010; Longin et al., 2015).

587 *Clonal diploids*

588 In clonal diploids, Two-Pool GCA appeared to outperform other strategies in some conditions
589 because of its exceptional ability to increase the dominance value of F_1 hybrid populations, as well as the
590 additive value. Fundamentally, this is because use of Two-Pool GCA can increase not only the frequency
591 of favorable alleles but also the frequency of heterozygote genotypes relative to Hardy-Weinberg
592 equilibrium in F_1 hybrids of two pools, leading to panmictic heterosis (Lamkey & Edwards, 1999). The
593 latter is achieved by selection on GCA, which differs from breeding values in a single pool because
594 dominance value (d) is weighted by allele frequencies in the opposite pool (Schnell, 1965; Rembe et al.,
595 2019). Selection on GCA drives apart allele frequencies between pools, which results in a sustained
596 increase in heterozygosity and therefore dominance value in the F_1 hybrids. Although both additive and
597 dominance value are transmissible with selection on breeding value and random mating in a single pool,
598 the frequency of heterozygotes is limited by Hardy-Weinberg equilibrium, which is overcome by non-
599 random mating in two pools (Hardy, 1908; Weinberg, 1908). Reducing population heterosis (inbreeding
600 depression) was neither required nor a strategic advantage to make genetic gain, and at longer time
601 horizons genetic variance was exhausted due to drift and selection well before any changes in population
602 heterosis or inbreeding depression were observed. Generally, the advantages of Two-Pool GCA in clonal
603 diploids increase as:

- 604 • the amount of H_0 due to dominance increases, because ability to increase dominance value
605 becomes relatively more important
- 606 • the time horizon increases, because formation of heterotic pools with diverged allele frequencies
607 requires selection over breeding cycles
- 608 • its relative cycle length to the other strategies decreases, because cycle length directly impacts the
609 rate of genetic gain, and Two-Pool GCA has a necessarily longer cycle length than the other
610 strategies with PS but not GS
- 611 • the selection intensity increases, perhaps because higher selection intensities lead to more
612 inbreeding which lead to greater reductions in heterozygosity due to selection and drift which are
613 better alleviated by GCA compared to other strategies, or because higher selection intensities
614 more rapidly drove apart allele frequencies between pools
- 615 • its relative cost to the other strategies decreases; however, we did not investigate different levels
616 of relative cost among strategies because this was demonstrated by Longin et al. (2014) and its
617 particulars are highly program-specific.

618 The amount of trait population heterosis can be estimated experimentally in breeding populations,
619 but it is typically unknown. Better methods and increased effort to estimate heterosis in breeding
620 programs would be useful to inform decision-making. However, for clonal diploids which can utilize
621 rapid-cycling GS, the benefit of Two-Pool GCA was robust to H_0 under the study assumptions. Two-Pool
622 GCA provided the most gain over most H_0 values and timepoints surveyed, and if H_0 was relatively low
623 Two-Pool GCA only modestly decreased gain in the short term. Programs for which Two-Pool GCA is
624 relatively more expensive than assumed here may require more H_0 to reap its benefit. In contrast to GS,
625 moving to Two-Pool GCA without adequate population heterosis or time presented a risk of decreased
626 genetic gain for phenotypic programs. Interestingly, clonal crops using PS with a low multiplication ratio
627 never benefited from Two-Pool GCA over the time horizons in the study, highlighting this consideration
628 for clonal species and the usefulness of efforts to increase the multiplication ratio (Aighewi et al., 2015).

629 It would be useful to confirm the optimal GS strategies for programs with low multiplication ratios,
630 particularly with multiple cohorts running in parallel per season. Please see Supplemental File 5 for
631 discussion of Two-Pool Breeding Value and One-Pool Cross Performance in diploids, which may be
632 useful for programs which cannot transition to Two-Pool GCA.

633 In applied diploid inbred-hybrid RRS programs of seed crops, intra-pool genotypes are often first
634 selected as parents of hybrids on their per-se value (Lee & Tracy, 2009). In clonal crops with relatively
635 lower multiplication ratios, increased performance of intra-pool parents may not drastically increase
636 hybrid propagule or seed production, so it was unclear whether resource allocation to intra-pool
637 evaluation is efficient. For the costs and proportions of individuals advanced assumed in the study, we
638 observed that a round of intra-pool advancement on breeding value before intra-pool recycling on GCA
639 typically increased genetic gain with PS or did not change the rate of genetic gain with GS in the inter-
640 pool hybrids. Intra-pool evaluation led to a shift from dominance to additive gain compared to forgoing
641 intra-pool evaluation. As such, breeders likely have some flexibility in whether to conduct intra-pool
642 evaluation. For example, with multiple traits, it is common to cull intra-pool parents for markers and
643 highly heritable traits; unless negative genetic correlations are present in the trait index, this decision
644 likely would not decrease genetic gain for inter-pool traits, assuming it does not increase cycle length. For
645 the GS scenarios here, it was likely suboptimal to predict intra-pool breeding values from a training set of
646 inter-pool individuals, and predicting intra-pool breeding values from intra-pool individuals may increase
647 genetic gain.

648 Interestingly, the effect of recycling on GCA on intra-pool mean value over cycles depended on
649 H_0 : it tended to decrease intra-pool value as H_0 increased but increase intra-pool value as H_0 decreased. In
650 absence of dominance, intra-pool breeding value is equal to GCA, so intra-pool genotypes selected for
651 GCA are nearly the same as those which would be selected on breeding value at low H_0 (Rembe et al.,
652 2019). This likely led to increases in intra-pool genetic value. As dominance increases, and as allele
653 frequencies differ between pools, the values of intra-pool breeding value and GCA diverge. At high H_0 ,
654 selection on GCA led the parental pools to suffer inbreeding depression as they were driven to
655 homozygous states, thus decreasing their value over breeding cycles. Conducting intra-pool advancement
656 on breeding value sometimes slightly increased intra-pool parents' value compared to forgoing intra-pool
657 evaluation. However, at the proportion of individuals advanced (75%), intra-pool selection did not
658 prevent decrease in intra-pool value when population heterosis was high. In practice, if population
659 heterosis is high and it is necessary to maintain or increase intra-pool value with Two-Pool GCA, it may
660 be necessary to select intra-pool parents more stringently on their breeding values or even to recycle intra-
661 pool parents on an index of intra-pool breeding value and GCA (Longin et al., 2006).

662 Another concern in clonal diploids is whether RRS programs benefit from using fully inbred
663 parents, as is done in other species. We did not observe substantial increases in genetic gain with use of
664 inbred parents in RRS, especially with intra-pool evaluation. With all else equal, it is expected that
665 inbreeding depression (loss of baseline heterosis) suffered in the intra-pool parents is fully reversed in the
666 inter-pool hybrids, as well as the addition of the panmictic heterosis value, so intra-pool inbreeding is
667 unnecessary to harness heterosis. The cost and time to generate inbred lines are likely higher than
668 assumed in our study, given that doubled haploid technologies do not exist for most clonal species.
669 Furthermore, the simulated inbred line values may correspond to total non-viability in some species or
670 populations, especially those with high population inbreeding depression. It has been proposed that use of
671 inbred parents could enable seed systems in clonal crops and reduce the cost of propagation, the time and
672 cost required to transport clones across national borders, and the spread of disease (McKey et al., 2010;
673 Ceballos et al., 2015). These are worthy considerations that are considered externalities in the current

674 study, but they are completely independent of the use of RRS and could equally be availed in one-pool
675 strategies. Programs considering line development should thoroughly assess their germplasm's tolerance
676 of full inbreeding as well as the tradeoffs in time and resources needed for line development.

677 *Clonal autopolyploids*

678 In contrast to clonal diploids, Two-Pool GCA typically did not outperform other strategies in
679 clonal autopolyploids. Instead, One-Pool Breeding Value or One-Pool Cross Performance was the safest
680 option depending on H_0 . A larger range of H_0 values were considered in autopolyploids than diploids;
681 RRS did not benefit autopolyploids at the same and some greater amounts of H_0 which benefited diploids.
682 This is likely because autopolyploids inherit multiple chromosome copies per gamete, and therefore
683 autopolyploids sustain greater heterozygosity across all gametes, genotypes, and matings at segregating
684 loci even in response to selection on One-Pool Breeding Value (Supplemental Fig. 16; Bartlett &
685 Haldane, 1934; Bever & Felber, 1992). The relative advantage of Two-Pool GCA in diploids is due to its
686 ability to increase heterozygosity of inter-pool populations at loci with dominance. Because the frequency
687 of heterozygotes compared to homozygotes at segregating loci in autopolyploid populations is already
688 relatively high compared to diploids, there is not only less value to be gained by increasing heterozygote
689 frequency with Two-Pool GCA but also less value lost to the smaller increase in deleterious recessive
690 homozygote frequency under selection on One-Pool Breeding Value (Supplemental Fig. 16,
691 Supplemental Table 2). Though this study considered clonal species, these conclusions should be
692 applicable to non-clonal autopolyploids.

693 Consistent with this hypothesis, the relative overperformance of one-pool strategies compared to
694 Two-Pool GCA was greater in autohexaploids than autotetraploids: autohexaploids inherit more
695 chromosome copies per gamete (3) than autotetraploids (2), leading to greater heterozygosity at
696 segregating loci. We expect that the relative genetic gain per unit cost of Two-Pool GCA to One-Pool
697 Breeding Value would be further reduced at higher autopolyploidies. Another line of support for this
698 hypothesis was that the relative performance of Two-Pool GCA to other strategies increased with GS at
699 high intensity. High-intensity GS likely increased inbreeding and genetic drift compared to low-intensity
700 GS or high-intensity PS, so the ability of Two-Pool GCA to relieve homozygosity became more
701 important. However, One-Pool Cross Performance was similarly capable of relieving inbreeding in this
702 situation and is less logically demanding. Finally, Two-Pool GCA built more panmictic heterosis than
703 Two-Pool Breeding Value, but the difference was less in autopolyploids than diploids. This indicates
704 breeding for heterosis with GCA was less effective in autopolyploids, since it more narrowly
705 outperformed incurrence of heterosis due to drift.

706 It is possible that further increasing the inbreeding rate in autopolyploids (e.g. by reducing the
707 number of parents or using truncation selection without inbreeding control) could increase the relative
708 performance of Two-Pool GCA to other strategies, but this would not necessarily increase genetic gain.
709 However, further investigation of strategy relative performance over additional inbreeding rates is
710 warranted. Tangentially, the accuracy of autopolyploid genomic estimates tended to be similar to diploids
711 at low H_0 , but increasingly lower than diploids at high H_0 , suggesting that allelic effects may be harder to
712 predict in autopolyploids than diploids as dominance increases. This is sensible because more dominance
713 effects are present in autopolyploids per phenotypic observation. However, it did not seem to be the main
714 cause of the decreased advantage of Two-Pool GCA in the autopolyploids, which also appeared with use
715 of true values. It may be worth noting that the lack of advantage to selection on Two-Pool GCA only
716 applies to autopolyploids, not to allopolyploids for which chromosome copies are not independently
717 assorted.

718 The lack of advantages of Two-Pool GCA in autopolyploids does not imply that autopolyploids
719 cannot or do not exhibit heterosis. Selection on Two-Pool GCA or Two-Pool Breeding Value led to clear
720 panmictic heterosis in the autopolyploids simulated in the study. Empirical evidence of panmictic
721 heterosis in autohexaploid sweetpotato, for example, is readily available for fresh root yield (Diaz et al.,
722 2021). The point is that even if autopolyploids exhibit heterosis or inbreeding depression, RRS did not
723 provide increased gain per unit cost compared to RS on breeding value in a single, merged pool under the
724 study assumptions. In the case of sweetpotato, two pools exhibiting panmictic heterosis emerged when a
725 single breeding population was split into two locations (M. Andrade, pers. comm.). Over approximately
726 twenty years, the pools were selected separately by truncation (W. Gruneberg, pers. comm.), and
727 therefore allele frequencies likely came to diverge between pools due to selection and drift. Reunion of
728 the pools then led to population-level panmictic heterosis in the F₁ hybrids (Diaz et al., 2021). The
729 existence of panmictic heterosis in autohexaploids does not imply that Two-Pool GCA or Two-Pool
730 Breeding Value is the optimal breeding strategy for autohexaploids. The observed panmictic heterosis in
731 sweetpotato could also be availed by intermating the two pools and conducting RS on breeding value in
732 the single, merged pool. However, further comparisons of strategy efficiencies with pre-existing diverged
733 pools would be informative in both diploids and autopolyploids.

734 The relatively decreased homozygosity of autopolyploids compared to diploids with selection on
735 breeding value does not imply that autopolyploids suffer less inbreeding depression than diploids in the
736 event that they do experience homozygosity of unfavorable alleles. This misconception may arise from
737 failure to differentiate the inbreeding rate and inbreeding depression value. Autopolyploids in fact may
738 experience more inbreeding depression in response to increased homozygosity than diploids, which can
739 be observed in simulated autopolyploids produced by chromosome doubling with digenic dominance.
740 Although few comparable estimates of inbreeding depression in real data are available, one such dataset is
741 that of Yao et al. (2020), which compared genetically matched diploid and autotetraploid maize. In a
742 selfing series of each, Yao et al. observed similar inbreeding depression in the diploids and
743 autotetraploids at the same selfing generation (2020). Since autotetraploids are less inbred than diploids at
744 a given selfing generation, their similar inbreeding depression suggest that autotetraploid inbreeding
745 depression was more severe per unit increase in homozygosity. Of course, it cannot be concluded that the
746 maize autotetraploids used experienced only inbreeding depression due to digenic dominance, and the
747 inbreeding depression observed could be due to loss of higher-order dominance interactions as well.

748 *Assumptions, limitations, and future research directions*

749 The conclusions of this study depend on the assumptions made and parameters used. Further
750 exploration of these factors is welcomed, and we encourage breeding programs to simulate and optimize
751 their specific situation when information is readily available. Exploration of ranges of values is helpful to
752 explore factors which affect the relative performance of breeding strategies, but once identified, the
753 number of real-world constraints on breeding programs is much smaller than all possible constraints on
754 breeding programs.

755 The breeding schemes used are not optimal but are rather a baseline for comparison of population
756 improvement methods. For example, we did not optimize accuracy within the breeding strategies and
757 estimation methods, which may require different designs for optimal accuracy. Particularly, testcrossing
758 is necessary with phenotypic Two-Pool GCA but is suboptimal for genomic estimated Two-Pool GCA
759 (Fristche-Neto et al., 2017; Seye et al., 2020). We did not optimize tester choice or number and simply
760 used two random testers. With GS and Two-Pool Breeding Value, prediction of intra-pool genotypes
761 from an inter-pool training set was suboptimal compared to use of intra-pool training genotypes, which
762 has been demonstrated in prediction of purebred animals from crossbreds (Wei & Van der Werf, 1994;

763 Moghaddar et al., 2014; Hidalgo et al., 2016). However, to address the lack of optimization of accuracy,
764 we simulated all scenarios with true values to control accuracy across strategies and did not observe
765 radically different trends of the breeding strategies with respect to population heterosis. The scenarios
766 with true values have controlled accuracy but less genetic drift than GS scenarios, because true values are
767 like using phenotypes with broad-sense heritabilities of one (Daetwyler et al., 2007; Sonesson et al.,
768 2012).

769 We did not optimize each scenario to a given time horizon. The number of parents used were
770 certainly not optimal for the time horizons explored, because unused genetic variance remained for all
771 scenarios. It is possible that different strategies could produce different amounts of gain at optimal
772 intensities for the times considered, and it may be that this also varies by genetic architecture. Somewhat
773 arbitrarily, we also assumed a fixed number of parents per strategy rather than a fixed number of parents
774 per pool.

775 We did not fully explore all possible genetic architectures, particularly those including epistasis
776 or higher-order autopolyploid dominance. We note that positive directional dominance could arise from
777 selection and was not necessarily present in the starting population for situations when Two-Pool GCA to
778 presented advantages over one-pool strategies—e.g., with an initial mean dominance degree of zero and
779 non-zero variance of dominance degrees (Falconer & Mackay, 1996; Varona et al., 2018). We did not
780 consider environment or genotype x environment effects, which may affect the relative performance of
781 GS and PS and depletion of genetic variance. We assumed a fixed marker density and genome size. We
782 assumed biallelic loci. We do not expect that multiallelic loci in autopolyploids would likely lead to
783 increased advantages of Two-Pool GCA, because with linkage disequilibrium haplotypes of biallelic loci
784 effectively behave as a single multiallelic locus. We did not vary the probability of autopolyploid
785 multivalents.

786 We assessed H_0 as a predictor of various responses. H_0 appeared to explain the variance of
787 responses among strategies well, but it is possible that its components—mean dominance degree, the
788 variance of the dominance degrees, and the square root of the number of QTL—could reveal different
789 patterns of strategy performance if used as predictors rather than H_0 . We plotted genetic gain of the core
790 strategies with use of true values after 50 years with use of each component as a predictor of responses
791 with both other components held constant in all possible combinations (Supplemental Fig. 17—25). In
792 general, we observed similar patterns as with use of H_0 for mean dominance degree and the square root of
793 the number of QTL, with the relative performance of Two-Pool GCA increasing as each of these
794 increased. The relative performance of Two-Pool GCA increased as mean dominance degree increased
795 regardless of whether incomplete dominance, complete dominance, or overdominance was simulated;
796 notably, overdominance did not decrease the relative advantage of Two-Pool GCA (Rembe et al., 2019).
797 However, for the variance of dominance degrees, if the mean dominance degree was low then advantage
798 of Two-Pool GCA increased as the variance of dominance degrees increased, even though the variance of
799 dominance degrees has an inverse relationship with H_0 . This seemed to be because selection on GCA led
800 to directional dominance in the breeding population when loci with positive dominance degrees were
801 present. This trend reversed to expectation as mean dominance degree and the number of QTL increased.

802 With use of maximum avoidance at high vs. low intensity, there were necessarily more full
803 siblings per family at high vs. low intensity. Availability of additional full siblings at high intensity may
804 have increased the accuracy of prediction of dominance values (Misztal et al., 1998), which could affect
805 the relative performance of Two-Pool GCA. However, the difference in relative performance between
806 Two-Pool GCA and other strategies at high vs. low intensity was also apparent with use of true values at
807 perfect accuracy, indicating the influence of the inbreeding rate.

808 Although we completely disregarded product development strategies or prediction of inter-pool
809 crosses in addition to GCA for RRGS, we presume that population improvement strategies which
810 produce populations with higher means and similar distributions will lead to extraction of higher-value
811 products with all else, such as product evaluation strategy, equal. Allocation of resources among stages
812 was not explored.

813 The study considered plausible values for the cost of phenotyping, genotyping, and phenotyping
814 to genotyping among strategies, but these may differ among applied programs. Particularly, the cost of
815 two-pool vs. one-pool breeding depends strongly on crop biology. We assumed that the cost of controlled
816 inter-pool crossing was negligible, which may not be the case in some crops.

817 Multiple frameworks to model dominance in polyploids are available; here, only digenic
818 dominance is considered, while other frameworks allow for additional intra-locus interactions (Gallais,
819 2003). It does not seem likely that other valuations of various possible heterozygotes or inclusion of
820 additional intra-locus interactions would change the relative performances of the strategies presented
821 here, because the superfluity of Two-Pool GCA seems to arise from the increased frequency of
822 heterozygotes in autopolyploids regardless of their valuation. However, further study may reveal
823 unexpected results.

824 We note that heterosis in autopolyploids is not maximized with single crosses among two
825 diverged pools, i.e. heterosis is progressive (Groose et al., 1989; Washburn & Birchler, 2014; Washburn
826 et al., 2019; Labroo et al., 2021). Autopolyploid heterosis due to dominance is progressive because
827 autopolyploids have fewer parents than inherited gametes. If allele frequencies diverge randomly across
828 the genome among parents, additional heterosis occurs by making multi-parental crosses because
829 additional heterozygosity can be stacked into the progeny genome. We do not expect that utilization of
830 progressive heterosis in autopolyploids would change the relative performance of the strategies because
831 the additional heterosis is likely relatively small compared to the potential additional time needed to make
832 additional crosses as well as the resources needed to maintain additional pools. However, testing this
833 hypothesis is warranted. We note that progressive heterosis due to digenic dominance can be observed by
834 the simulation methods of the study
835 (https://github.com/gaynor/AlphaSimR_Examples/blob/master/misc/ProgressiveHeterosis.R).

836 As mentioned repeatedly, comparisons of gain across ploidies from simulation should not be
837 made because they are not guaranteed to reflect biological reality. Real data, which are likely population-
838 specific, would be needed. For example, we assume that the minimum homozygote and maximum
839 heterozygote value are the same in diploids and polyploids, but there is evidence that this is unrealistic in
840 some populations because polyploid populations produced by colchicine doubling sometimes have higher
841 mean values than their diploid progenitors (Sattler et al., 2016). For example, in the case of potato, our
842 findings strongly suggest that Two-Pool GCA is not likely to be the optimal breeding strategy for
843 autotetraploid potato, whereas Two-Pool GCA is likely to be the optimal breeding strategy for diploid
844 potato if GS is used or H_0 is adequate. However, we cannot determine from simulation alone whether
845 overall genetic gain is likely to be higher in autotetraploid or diploid potato.

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1094 *Competing Interests*

1095 The authors have no relevant financial or non-financial interests to disclose.

1096 *Author Contribution Statement*

1097 The authors confirm contribution to the paper as follows: study conception: GCP, JBE, RCG;
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1114 *Data availability*

1115 All code and results generated for the current study are available in the Supplementary Information. Raw
1116 data are available at <https://doi.org/10.7910/DVN/7RVFL8>. The initial simulated populations used are
1117 available upon request.

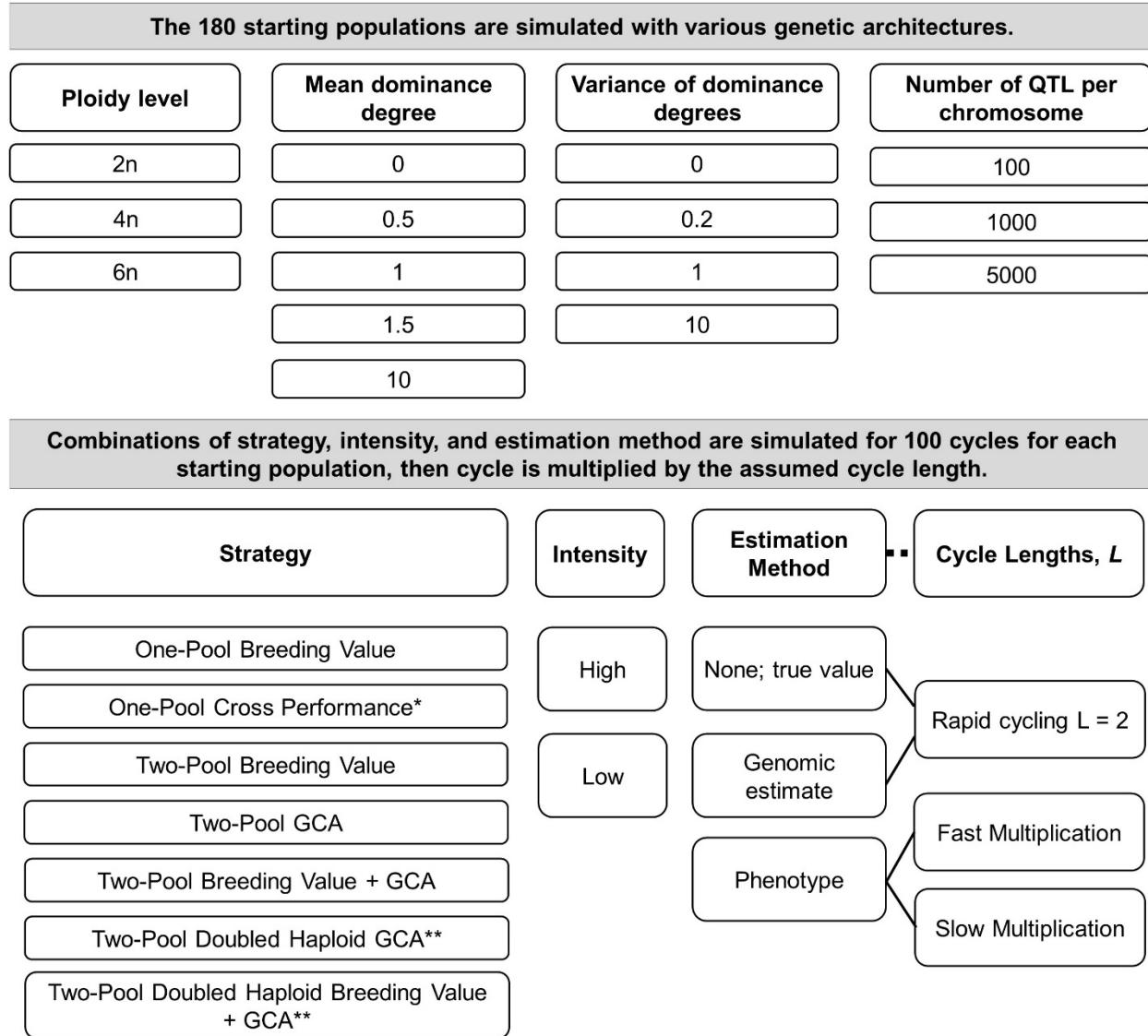


Figure 1. Overview of the study methods. Populations at three ploidy levels with varied amounts of population heterosis were generated by simulating all combinations of the ploidy level, number of QTL, mean dominance degrees, and variance of dominance degrees shown. Linkage disequilibrium was not controlled. For further details of these parameters' relationship to inbreeding depression and heterosis, please see Gaynor et al., 2018. After simulating the 180 starting populations, a combination of breeding strategy, selection intensity, and estimation method was run on each population, except that strategies with doubled haploids were only run for ploidy = 2 (**). Because multiple cohorts per cycle were not simulated, cycle length was varied by multiplying cycle number by the appropriate value and not by running an independent simulation (dashed line). The combination of strategy, intensity, estimation method, and cycle lengths defined a scenario. All combinations of the scenario factors were assessed, except that the cycle lengths depended on the estimation method (solid lines) and a phenotypic estimate of One-Pool Cross Performance was not considered (*). Cycle lengths (L) by strategy and estimation method are given in Table 1.

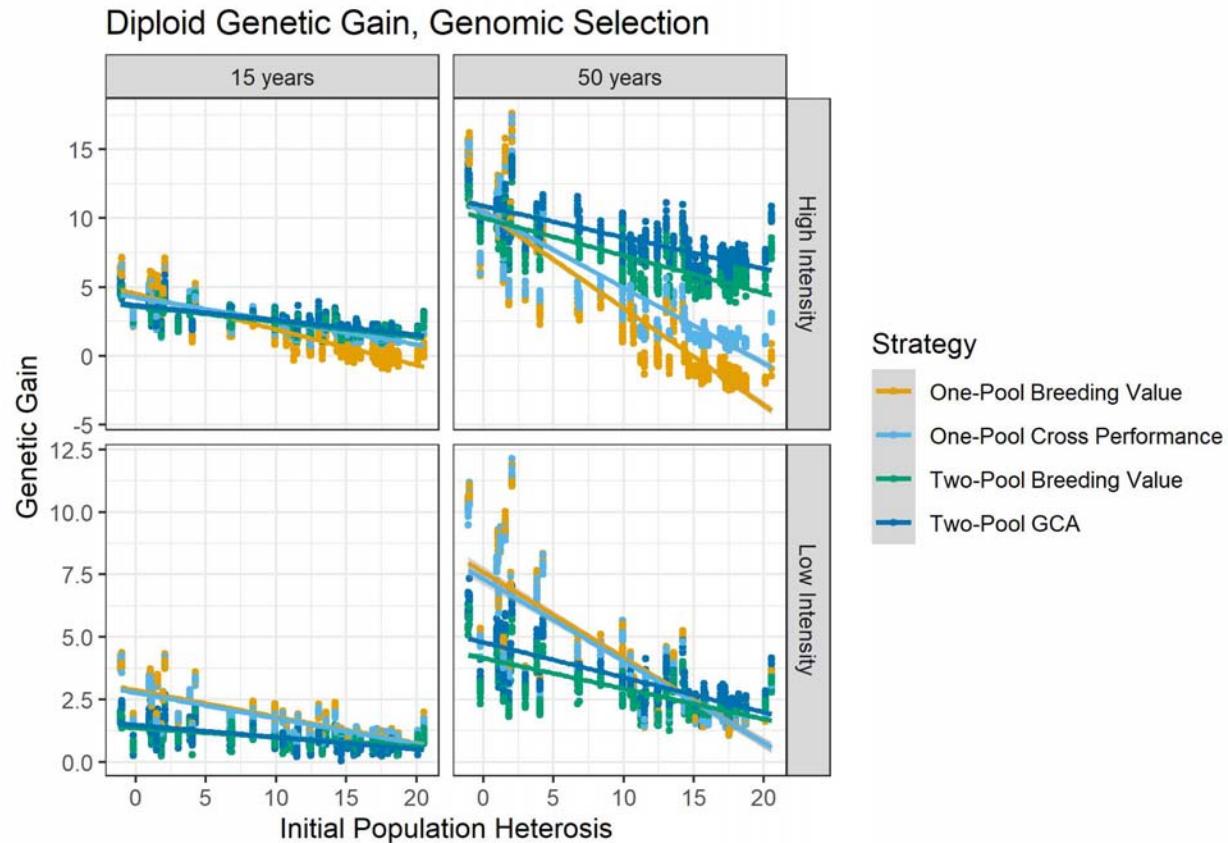


Figure 2. Genetic gain in diploids after 15 and 50 years with use of GS regressed on breeding scenario, initial population heterosis, H_0 , and their interaction. Colored lines indicate regressions by breeding strategy with GS and cycle length 2, and grey bands indicate the standard error of the predicted means. Dots indicate raw data points and dot color indicates strategy as in the lines. At high intensity after 15 years, the differences among strategies were marginal, and after 50 years Two-Pool GCA provided the most gain over almost all H_0 values. At low intensity, two-pool strategies required more H_0 and time to outperform the one-pool strategies than at high intensity.

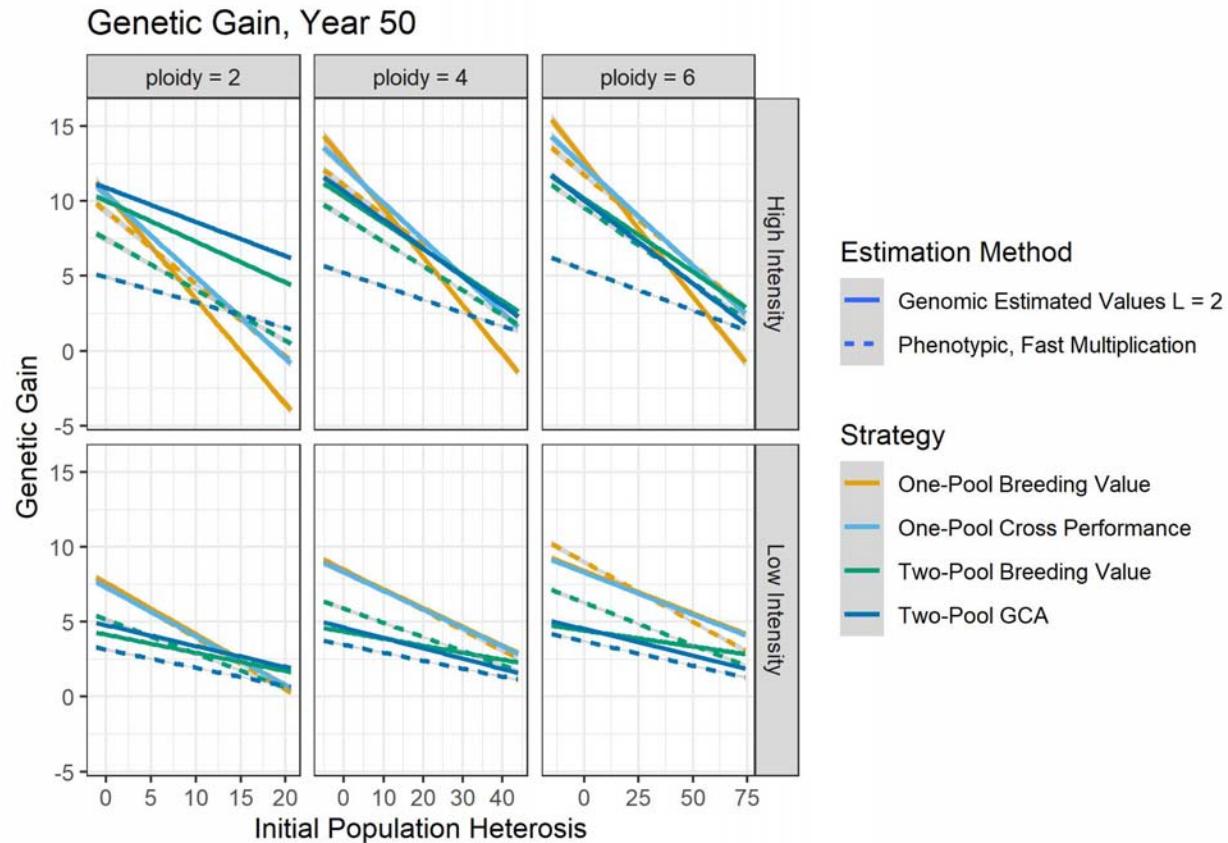


Figure 3. Genetic gain for each ploidy level after 50 years of breeding with use of genomic and phenotypic selection and various strategies as a function of H_0 , breeding scenario, and their interaction. Line color indicates strategy, and grey bands indicated the standard error of the predicted mean. Line type indicates estimation method with the accompanying set of cycle lengths (L). In clonal diploids at high intensity, genomic selection on Two-Pool GCA is the best strategy regardless of H_0 , but this advantage is not apparent in the autopolyploids. Instead, the autopolyploids tend to benefit from one-pool strategies. Use of GS typically increases or does not change genetic gain at high intensity, particularly for diploids. It is not appropriate to compare amounts of genetic gain across ploidy levels.

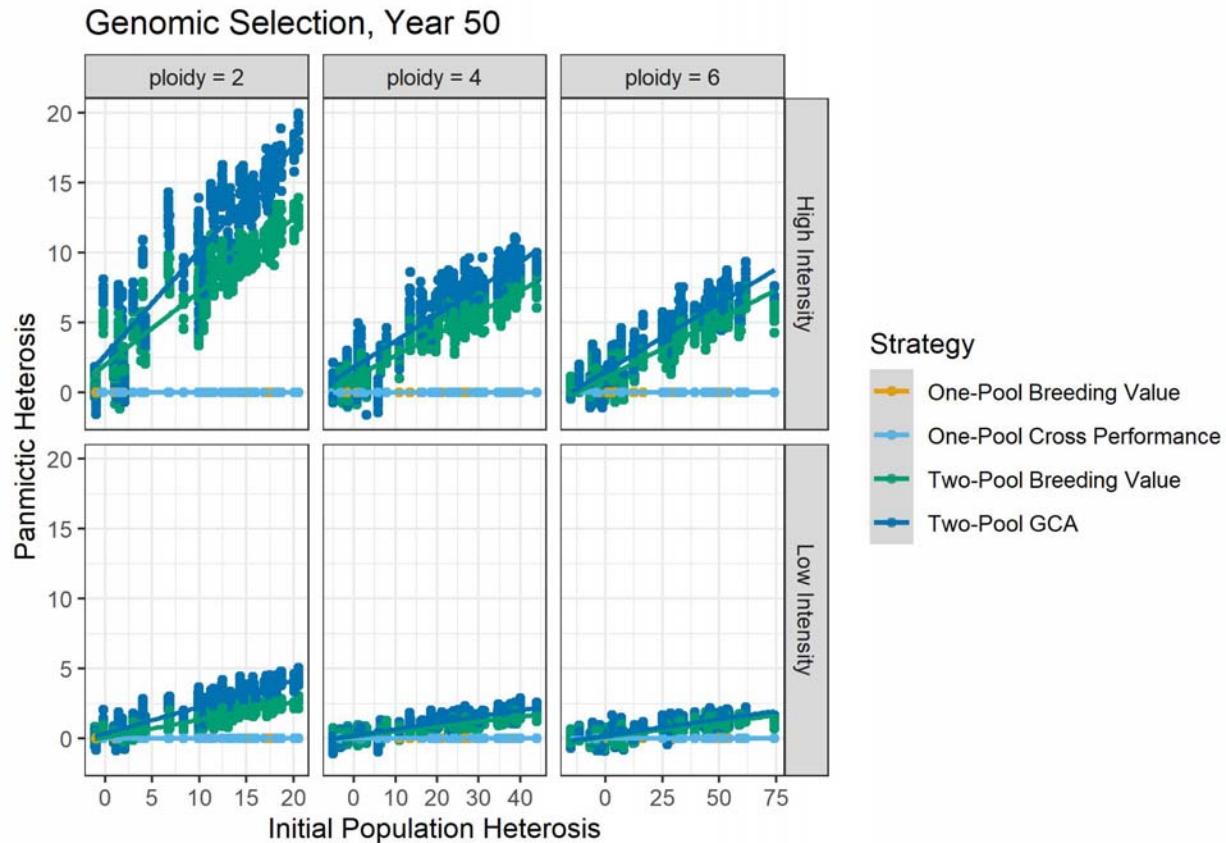


Figure 4. Panmictic heterosis for each ploidy as a function of initial population heterosis, H_0 , after 50 years of breeding with each strategy and use of genomic selection with cycle length of 2. Colored lines indicate strategy and grey bands indicate the standard error of their predicted means. Colored dots indicate the corresponding strategy raw data points. Two-Pool GCA tended to build more panmictic heterosis than Two-Pool Breeding Value, especially in diploids at high intensity, because Two-Pool GCA leads to increased divergence of allele frequencies between pools by selection. Two-Pool Breeding Value builds panmictic heterosis primarily by drift, and one-pool strategies do not build panmictic heterosis. Two-pool strategies lead to clear panmictic heterosis in autopolyploids even though neither two-pool strategy was optimal in terms of genetic gain. Comparisons of absolute values across ploidies are not likely to be biologically relevant.