

Should maize doubled haploids be induced among F_1 or F_2 plants?

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Abstract Maize (*Zea mays* L.) doubled haploid lines are typically produced from F_1 plants. Studies have suggested that the low frequency of recombinants in doubled haploids may reduce the response to selection. My objective was to determine if, for sustaining long-term response, doubled haploids should be induced in F_1 or F_2 plants during maize inbred development. In simulation experiments, I examined the response to multiple cycles of testcross selection among doubled haploid lines derived from F_1 plants (denoted by DH), doubled haploid lines derived from F_2 plants (DH_{F_2}), and recombinant inbred (RI) lines derived by single-seed descent. For a trait controlled by 100 or more quantitative trait loci (QTL), the cumulative responses to selection were up to 4–6% larger among DH_{F_2} lines than among DH lines. The cumulative responses were up to 5–8% larger among RI lines than among DH lines. The QTL become unlinked as the number of QTL in a finite genome decreases, and the responses among RI, DH, and DH_{F_2} lines were equal or nearly equal when only 20 QTL controlled the trait. Metabolic-flux epistasis reduced the differences in the response among RI, DH, and DH_{F_2} lines. Overall, the results indicated that doubled haploids should be induced from F_2 plants rather than from F_1 plants. If year-round nurseries are used and new F_1 crosses for inbred development are initially created on a speculative basis, the development of

doubled haploids from F_2 rather than F_1 plants should not cause a delay in inbred development.

Introduction

Technology for creating maize (*Zea mays* L.) doubled haploids reduces the time required to produce inbreds. Whereas six or more generations of selfing are needed to produce recombinant inbreds (RI) from F_1 plants, doubled haploids are produced in only two generations. The routine production of maize doubled haploids has been made possible by the development of inducer lines that, when used in crosses, lead to a high frequency of haploids (e.g., >8%, Röber et al. 2005; Seitz 2005). Putative haploids are identified through morphological markers and haploid seedlings are treated with colchicine to double their chromosomes so that their selfed seeds are diploid (Chase 1951; Bordes et al. 1997; Melchinger et al. 2005; Seitz 2005).

A key question in applying doubled haploid technology in maize breeding is the generation during which haploids should be induced. Production of doubled haploids will be the quickest if haploids are induced among F_1 plants. In practice, F_1 -derived doubled haploids (denoted by DH) are typically produced in maize (Röber et al. 2005; Melchinger et al. 2005; Smith et al. 2008). Studies have shown, however, that the reduced recombination in DH lines may decrease the response to a single cycle (Riggs and Snape 1977) or to multiple cycles (Jannink and Abadie 1999) of selection.

Because DH lines are produced after only one meiosis, the number of underlying crossover events is expected to be less among DH lines than among RI lines. Likewise, the number of entire chromosomes or the size of chromosome segments that are passed intact from a parental

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inbred are expected to be larger among DH lines than among RI lines. In maize, Murigneux et al. (1993) found that the percentage of recombinants was twice higher among RI lines than among DH lines. Smith et al. (2008) found an average of 15 recombinations per RI genome versus ten recombinations per DH genome. The results of both Murigneux et al. (1993) and Smith et al. (2008) were consistent with theory indicating that the ratio between recombination frequencies among RI and DH lines approaches 2.0 when loci are tightly linked and decreases to 1.0 when loci are unlinked (Haldane and Waddington 1931). Furthermore, Smith et al. (2008) found that the percentage of lines with four or more intact parental chromosomes ($N=10$ chromosomes in maize) was 37% among DH lines and 13% among RI lines.

Maize breeders typically develop new and improved inbreds from crosses among the most elite inbreds (Bauman 1981; Mikel and Dudley 2006; Nelson et al. 2008). Suppose two DH lines both inherit an intact copy of chromosome 1 from the same parental inbred. If the two DH lines are crossed to form a new F_1 for inbred development, all of the alleles on chromosome 1 from the other parent become lost. The limited recombination in DH lines, coupled with selection of only a few improved inbreds per cross, may therefore effectively lead to bottlenecks that would reduce the selection gain when DH lines are used continuously in selection (Jannink and Abadie 1999). On the other hand, the limited recombination in DH lines could preserve superior epistatic combinations of alleles (Mayr 1954, p 165) that may have accumulated in elite germplasm.

Induction of DH lines among F_2 plants would allow an increased amount of recombination in the resulting DH_{F_2} lines without substantially increasing the amount of time needed for generating inbreds. The responses, however, to continuous selection among DH, DH_{F_2} , and RI lines have not been compared. My objective in this study was to determine if, for sustaining long-term response, doubled haploids should be induced in F_1 or F_2 plants in maize inbred development.

Materials and methods

Genetic models, founder inbreds, and Cycle 0 base population

I simulated a trait controlled by $L = 20, 100,$ or 200 quantitative trait loci (QTL), each with four alleles. The sizes of each of the ten maize chromosomes corresponded to those in the Senior et al. (1996) linkage map. The QTL were located at random on the ten chromosomes according to a uniform distribution across the entire genome. The QTL1

had the largest effect, QTL2 had the second largest effect, and the L th QTL had the smallest effect. The QTL effects were defined as the genotypic values when the lines were testcrossed to an unrelated inbred tester.

In the additive model, the magnitudes of QTL effects followed a geometric series (Lande and Thompson 1990) in which the top 10% of the QTL with the largest effects accounted for 34% of the total genetic variance. At the k th QTL, the testcross genotypic values of the four homozygotes were $1.8a^k$ for Q_1Q_1 , $0.6a^k$ for Q_2Q_2 , $-0.6a^k$ for Q_3Q_3 , and $-1.8a^k$ for Q_4Q_4 , where $a = (1 - L)/(1 + L)$ as specified by Lande and Thompson (1990). The genotypic values were scaled in this manner so that half the difference in genotypic values among the four homozygotes had a mean of a^k . Given that testcross means behave in a purely additive manner (Hallauer and Miranda 1989, p 28), dominance was assumed absent and the testcross genotypic value of each of the six heterozygotes was equal to the mean value of the corresponding homozygotes. The testcross genotypic value of a line was equal to the sum of the corresponding testcross genotypic values across the all QTL.

In the epistatic model, the testcross genotypic values were modeled as the flux in a linear metabolic pathway of L enzymes, each coded by a QTL (Kacser and Burns 1981). The flux, in turn, was modeled by adapting the biallelic model of Bost et al. (1999) to a four-allele model at each QTL. First, the mean activity of the k th enzyme was modeled as $m_k = 1 - a^{\nu k}$, where $\nu = 1$ with 20 QTL, $\nu = 3$ with 100 QTL, and $\nu = 4$ with 200 QTL. These values of ν and m_k were chosen so that, at the population level, the sum of enzyme control coefficients (Bost et al. 1999) of the top 10% of QTL with the largest effects was equal to 34% of the sum of enzyme control coefficients across all QTL. Next, the scale factor for the variation in enzyme activity at the k th locus was $b_k = m_k c \sqrt{2}$ (Bost et al. 1999), where $c = 0.15$ was the coefficient of variation. Lastly, the testcross enzyme activities at the k th locus (E_k) were $m_k + 1.8b_k$ for Q_1Q_1 , $m_k + 0.6b_k$ for Q_2Q_2 , $m_k - 0.6b_k$ for Q_3Q_3 , and $m_k - 1.8b_k$ for Q_4Q_4 . Values of E_k were always positive. Dominance for enzyme activity was assumed absent. The testcross genotypic value of a line was then calculated from the corresponding enzyme activities across all QTL as $1/\sum(1/E_k)$ (Bost et al. 1999).

At a given QTL, each of four founder inbreds (I_1, I_2, I_3, I_4) carried a unique QTL allele. So that the founder inbreds complemented each other, four configurations were considered between founder inbreds and QTL alleles. First, I_1 had Q_1 , I_2 had Q_2 , I_3 had Q_3 , and I_4 had Q_4 at the following QTL: $k = 1, 5, 9, \dots, (L - 3)$. Second, I_1 had Q_4 , I_2 had Q_3 , I_3 had Q_2 , and I_4 had Q_1 at the following QTL: $k = 2, 6, 10, \dots, (L - 2)$. Third, I_1 had Q_3 , I_2 had Q_1 , I_3 had Q_4 , and I_4

had Q_2 at the following QTL: $k = 3, 7, 11, \dots, (L - 1)$. Finally, I_1 had Q_2 , I_2 had Q_4 , I_3 had Q_1 , and I_4 had Q_3 at the following QTL: $k = 4, 8, 12, \dots, L$. Given that the QTL were randomly located in the genome, repulsion and coupling linkages were generated at random.

Cycle 0 initially comprised equal proportions of three double crosses among the founder inbreds: $(I_1 \times I_2) \times (I_3 \times I_4)$; $(I_1 \times I_3) \times (I_2 \times I_4)$; and $(I_1 \times I_4) \times (I_2 \times I_3)$. A total of $N = 500$ or 1,000 DH, DH_{F_2} , and RI lines were then simulated to form Cycle 0. The DH lines were created by simulating meiosis in a Cycle 0 plant (i.e., double cross) and doubling the chromosomes of a random gamete. The Kosambi mapping function was used to relate map distances and recombination frequencies. The DH_{F_2} lines were created after one generation of selfing the Cycle 0 plants. The RI plants were created by single-seed descent with seven generations of selfing.

To estimate the total genetic variance (V_G), which was needed to simulate phenotypic values and calculate standardized responses to selection, a large ($N = 20,000$), random-mated Cycle 0 population was created from equal proportions of the three double crosses. For the additive and epistatic genetic models, testcross genotypic values of S_0 plants were calculated and V_G was obtained as the variance among testcross genotypic values. This large population was not used in selection.

Selection among DH, DH_{F_2} , and RI lines

The overall selection procedure (Fig. 1) involved 15 cycles of inbred development. For each of the three types of inbreds, the best $N_{Sel} = 10$ (for $N = 500$) or 20 (for $N = 1,000$) lines were selected in Cycle 0. Selection was based on testcross phenotypic values, which were obtained by adding a random nongenetic effect to the testcross genotypic value of each line. The nongenetic effects were normally distributed with a mean of zero and a variance of V_E . The V_E corresponded to a heritability, on a testcross-mean basis, of $H = 0.20, 0.50$, or 0.80 in a random-mated Cycle 0 S_0 population. Pairs of the N_{Sel} lines from Cycle 0 were crossed to form 5 (for $N_{Sel} = 10$) or 10 (for $N_{Sel} = 20$) Cycle 1 F_1 's. The crossing scheme among the N_{Sel} lines was as follows: $[1 \times (N_{Sel} - 1)]$, $[2 \times (N_{Sel} - 2)]$, $[3 \times (N_{Sel} - 3)]$, ..., $[(N_{Sel}/2 - 1) \times (N_{Sel}/2 + 1)]$, and $[(N_{Sel}/2) \times (N_{Sel})]$ (Fig. 1). This crossing scheme was used to minimize crosses between related N_{Sel} lines after Cycle 1 (i.e., each F_1 plant in Cycles 2–15 had four unique grandparents).

For each Cycle 1 F_1 , a total of 100 DH, DH_{F_2} , or RI lines were then simulated so that the total population size across all Cycle 1 F_1 's remained constant at $N = 500$ or 1,000 in the next cycle. The selection and recombination procedures in Cycle 0 were repeated in Cycle 1 and in the succeeding cycles until Cycle 15 was obtained.

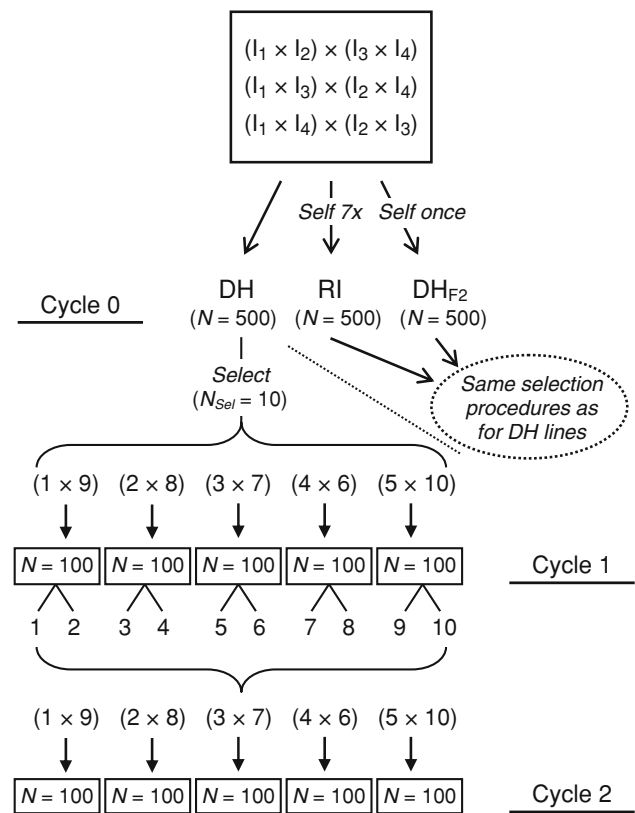


Fig. 1 Development of DH, RI, and DH_{F_2} populations from four founder inbreds (I_1, I_2, I_3 , and I_4), illustrated with a total number of lines equal to 500 and the $N_{Sel} = 10$ best lines selected in each cycle

Data analysis

Each simulation experiment comprised a combination of the type of inbred (DH, DH_{F_2} , or RI), number of QTL, genetic model (additive or metabolic flux), N and N_{Sel} , and H . Each simulation experiment was repeated 1,000 times and the results were averaged across repeats. Each repeat differed in the location of QTL, the large Cycle 0 population generated to estimate V_G , the genotypes of the lines generated, and their phenotypic values.

For each cycle, standardized responses to selection were obtained by subtracting the mean of Cycle 0 from the mean of the particular cycle and dividing the difference by the square root of V_G . The statistical significance ($P = 0.05$) of differences in selection response was determined with z tests, using the variances of the selection response across the 1,000 repeats of an experiment.

Results

Across cycles of selection for testcross performance, the ratio between the response among RI lines and the response

among DH lines ($R_{RI:DH}$) was largest with 200 QTL, intermediate with 100 QTL, and smallest with 20 QTL. Consider the additive model, a heritability of $H = 0.50$, and the best $N_{Sel} = 20$ lines being selected in each cycle. When 200 QTL controlled the trait, $R_{RI:DH}$ was 105% in Cycle 5 and increased to a maximum of 108% in Cycle 15 (Fig. 2). These $R_{RI:DH}$ values corresponded to selection responses that were 0.37–1.11 genetic standard deviations higher among RI lines than among DH lines ($LSD_{0.05} = 0.07\text{--}0.10$; results not shown). When the number of QTL decreased from 200 to 100, $R_{RI:DH}$ decreased to 103% in Cycle 5 and 105% in Cycle 15. The $R_{RI:DH}$ was 100–101% across different cycles when 20 QTL controlled the trait.

Differences were small between the selection responses among RI and DH_{F2} lines. With 200 QTL, $H = 0.50$, and $N_{Sel} = 20$, the ratio between the response among RI lines and the response among DH_{F2} lines ($R_{RI:DH_{F2}}$) in the additive model was less than 103% across cycles (Fig. 2). These $R_{RI:DH_{F2}}$ values corresponded to selection responses that were 0.11–0.36 genetic standard deviation higher among RI lines than among DH_{F2} lines (significant at $P = 0.05$). The values of $R_{RI:DH_{F2}}$ decreased when fewer QTL controlled the trait.

The responses were larger among DH_{F2} lines than among DH lines. With 200 QTL, $H = 0.50$, and $N_{Sel} = 20$, the ratio between the response among DH_{F2} lines and the response among DH lines ($R_{DH_{F2}:DH}$) in the additive model was less than 103% in Cycle 5 and about 106% in Cycle 15 (Fig. 2). These $R_{DH_{F2}:DH}$ values corresponded to selection responses that were 0.26–0.74 genetic standard deviation

higher among DH_{F2} lines than among DH lines (significant at $P = 0.05$). As with $R_{RI:DH}$ and $R_{RI:DH_{F2}}$, $R_{DH_{F2}:DH}$ decreased when fewer QTL controlled the trait. When the number of QTL decreased from 200 to 20, the $R_{DH_{F2}:DH}$ values under the additive model corresponded to selection responses (across cycles) that were only 0.05–0.09 genetic standard deviation higher among DH_{F2} lines than among DH lines ($LSD_{0.05} = 0.06\text{--}0.07$).

Metabolic-flux epistasis slightly reduced the differences among the responses with RI, DH, and DH_{F2} lines. With 200 QTL, $H = 0.50$, and $N_{Sel} = 20$, the $R_{DH_{F2}:DH}$ in Cycle 15 decreased from 106% in the additive model to 104% in the epistatic model (Fig. 2). The $R_{DH_{F2}:DH}$ values in Cycles 5–15 in the epistatic model corresponded to selection responses that were 0.10–0.36 genetic standard deviation higher among DH_{F2} lines than among DH lines (significant at $P = 0.05$). With 20 QTL, where $R_{DH_{F2}:DH}$ was barely greater than 100% in the additive model, $R_{DH_{F2}:DH}$ remained 100–102% in the epistatic model.

While increases in H and N_{Sel} led to increases in the response to selection, both H and N_{Sel} had little effect on $R_{RI:DH}$, $R_{RI:DH_{F2}}$, and $R_{DH_{F2}:DH}$. Consider the additive genetic model with 100 QTL. The $R_{DH_{F2}:DH}$ in Cycle 15 was 103–104% when N_{Sel} was 20 and when H varied from 0.20 to 0.80 (Fig. 3a). When N_{Sel} decreased from 20 to 10, the $R_{DH_{F2}:DH}$ in Cycle 15 were likewise 103–104% regardless of H (Fig. 3b).

The variation in the response increased as the number of cycles of selection increased and as H and N_{Sel} decreased. Again consider the additive genetic model with 100 QTL.

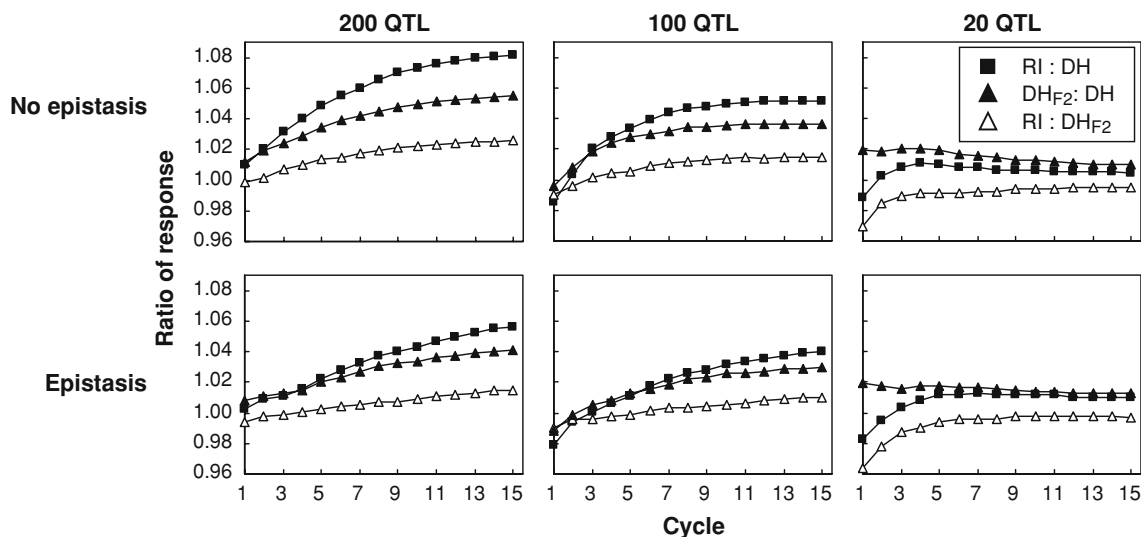


Fig. 2 Ratio between selection response among RI lines and DH lines ($R_{RI:DH}$, solid squares), ratio between selection response among DH_{F2} lines and DH lines ($R_{DH_{F2}:DH}$, solid triangles), and ratio between selection response among RI lines and DH_{F2} lines ($R_{RI:DH_{F2}}$, open triangles)

for different genetic models. Responses are for a trait with a heritability of $H = 0.50$ and with $N_{Sel} = 20$ lines selected out of 1,000 lines in each cycle

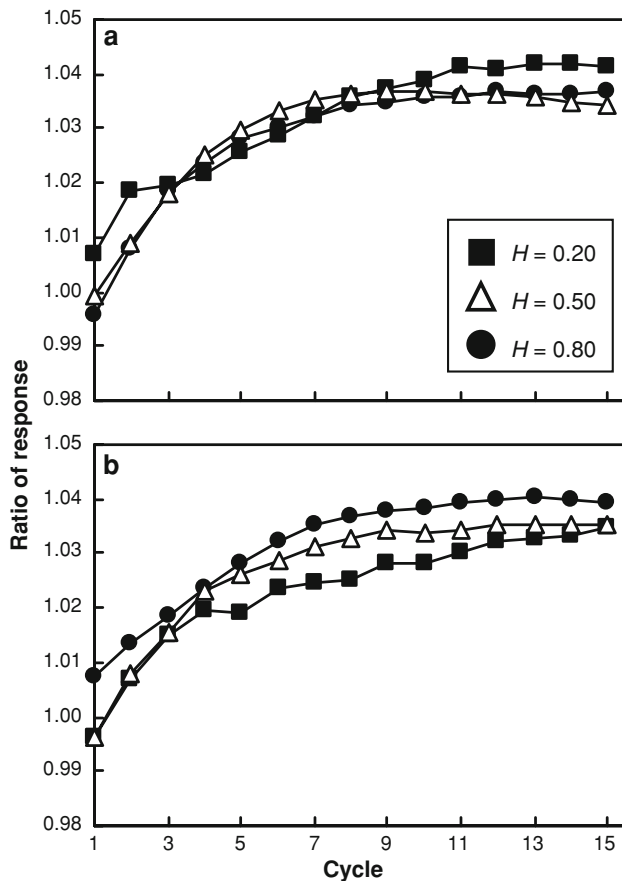


Fig. 3 Ratio between selection response among DH_{F_2} lines and DH lines ($R_{DH_{F_2}:DH}$) for the additive genetic model with 100 QTL controlling a trait and with a heritability of $H = 0.20$ (solid squares), 0.50 (open triangles), and 0.80 (solid circles). Responses are for **a** $N_{sel} = 20$ out of 1,000 lines and **b** $N_{sel} = 10$ out of 5,000 lines selected in each cycle

The standard deviation (in units of the genetic standard deviation) of the responses among DH_{F_2} testcrosses was 0.65 in Cycle 1 with $H = 0.20$ and $N_{sel} = 20$. With N_{sel} constant (20), this standard deviation increased to 0.74 in Cycle 15 ($H = 0.20$) and decreased to 0.59 with $H = 0.80$ (Cycle 1). When N_{sel} decreased from 20 to 10, the standard deviation of the response increased from 0.65 (Cycle 1, $H = 0.20$) to 0.70 (Cycle 1, $H = 0.20$).

Discussion

Factors affecting selection response among DH, DH_{F_2} , and RI lines

The strength of linkage among QTL was the key factor that affected the relative responses to selection among RI, DH_{F_2} , and DH lines. For a trait controlled by 100 or more QTL, the cumulative responses to testcross selection were up to

4–6% larger among DH_{F_2} lines than among DH lines. The cumulative responses were up to 5–8% larger among RI lines than among DH lines. Jannink and Abadie (1999) likewise found higher responses among RI lines than among DH lines for a genetic model involving 30 QTL, recombination frequencies of 0.05 or 0.15, and no epistasis. With a finite genome, the strength of linkage among QTL is largely determined by the number of QTL. If the size of the maize linkage map is 1,749 cM (Senior et al. 1996) and 200 randomly located QTL control the trait, the mean distance between adjacent QTL is about 9 cM. But if only 20 QTL control the trait, the QTL would on average be unlinked with a mean distance of 87 cM between adjacent QTL.

The number of QTL controlling quantitative traits is generally unknown. Nevertheless, traits such as kernel oil and protein in maize are likely controlled by more than 50 QTL (Dudley 1977; Laurie et al. 2004). Furthermore, maize breeders select for multiple traits at a time (Bauman 1981; Hallauer 1990). If a trait such as kernel oil is controlled by at least 50 QTL, then selecting for multiple complex traits such as grain yield, moisture, and stalk and root lodging strongly suggests that more than 100 QTL underlie the genetic gain in maize inbred development. Also, regardless of the number of QTL, linkage among QTL may be tight if the QTL are clustered in families of genes that are similar in structure and function (Li et al. 2007; Onishi et al. 2007).

The smaller advantage of RI lines over DH and DH_{F_2} lines under the epistatic model than under the additive model suggested that the increased frequency of recombinants in RI lines may have been partially offset by the disruption of favorable allele combinations found in each parent. Riggs and Snape (1977) found that complementary gene action increases the genetic variance among DH lines when coupling linkages are present and decreases the genetic variance among DH lines when repulsion linkages are present. The current study involved a random mixture of coupling and repulsion linkages and metabolic-flux epistasis, which leads to an L-shaped distribution of gene effects that is consistent with empirical estimates of QTL effects from mapping experiments (Bost et al. 1999). However, the nature of epistatic interactions in maize remains unknown particularly in elite inbreds for which continuous selection may have led to coadapted gene complexes (Mayr 1954, p 165) that comprise favorable combinations of epistatic genes.

Empirical experiments, perhaps with elite versus older germplasm, are therefore needed to determine the possible influence of epistasis on the usefulness of RI versus DH or DH_{F_2} lines. Furthermore, the RI, DH, and DH_{F_2} lines were obtained at random in this study. Molecular markers, however, may be used to select RI, DH, or DH_{F_2} lines that have either increased frequencies of recombinants or decreased

frequencies of recombinants (Smith et al. 2008). This procedure would then allow the subsequent phenotypic evaluation of a subset of lines in which either the break up of repulsion linkages or preservation of favorable gene complexes has been enhanced.

Repeated generations of selfing prior to inducing doubled haploids will lead to an increased number of recombinants to the extent that selection responses among DH_{F_3} lines (i.e., derived from F_3 plants) or DH_{F_4} lines may be greater than the selection response among DH_{F_2} lines. However, delaying the induction of doubled haploids until the F_3 or F_4 generation is likely unfeasible for two reasons. First, inducing doubled haploids after the F_2 generation will further increase the time required for inbred development and could defeat the main purpose of producing doubled haploids in the first place. Second, the opportunity for increased recombination due to an additional meiosis quickly declines with each additional selfing generation.

In particular, linkage affects the gametic output of double heterozygotes only. An *Aabb* single heterozygote, for example, will produce equal frequencies of *Ab* and *ab* gametes regardless of whether or not the two loci are linked. With 100 QTL and a linkage map of 1,749 cM (Senior et al. 1996), the mean recombination rate between adjacent QTL is $r = 0.18$ based on the Kosambi mapping function. The frequency of double heterozygotes then decreases by $[(1/2 - r)^2 + 1/4]$ with each selfing generation (Karlin 1968). This means that in the cross between two inbreds, the frequencies of double heterozygotes with $r = 0.18$ are 100% in the F_1 , 35% in the F_2 , 12% in the F_3 , and 4% in the F_4 . With 200 QTL and $r = 0.09$, the frequencies of double heterozygotes are 100% in the F_1 , 42% in the F_2 , 17% in the F_3 , and 7% in the F_4 . The frequency of double heterozygotes in which a crossover event will lead to a recombinant genotype therefore drastically decreases after the F_2 generation.

Choice of generation for inducing doubled haploids

Overall, the results suggested that DH_{F_2} lines are an ideal compromise between DH lines and RI lines. Selection responses among DH_{F_2} lines were only $\leq 3\%$ lower than the responses among RI lines and were up to 6% higher than the responses among DH lines. The variation in selection responses indicated that the advantage of DH_{F_2} over DH lines may not always be realized in each biparental cross. The advantage of DH_{F_2} over DH lines should be realized, however, when averaged across the large numbers of biparental crosses that are evaluated each year in large (e.g., commercial) breeding programs for major crops. While this simulation study used maize as a model species, the results were consistent with expectations from quantitative genetic

theory and should be generally applicable to other species for which doubled haploids can be routinely generated.

Selection is meaningless among the homogeneous F_1 plants obtained by crossing two inbreds, and the advantage of DH_{F_2} over DH lines may further increase if selection is practiced among the F_2 plants from which DH_{F_2} lines are produced. Selection among F_2 plants may be based on plant type and disease and insect resistance in a nursery (Bauman 1981; Hallauer 1990) or on marker-trait associations determined from prior information (Johnson 2001; Eathington et al. 2007; Bernardo 2008). Simulation studies have also suggested that selection gains may be higher if testcrosses of F_2 plants are first evaluated in yield trials and doubled haploids are subsequently developed only from the F_2 plants (or their F_3 families) with the best performance (Longin et al. 2007).

This study focused on the response after a fixed number of cycles of testcross selection rather than on the gain per unit time with RI, DH, and DH_{F_2} lines. As previously mentioned, doubled haploid technology allows the development of DH lines in only two generations and DH_{F_2} lines in three generations. Compared with RI lines, the reduction in the time required per cycle due to the use of DH or DH_{F_2} lines will vary according to how year-round nurseries or greenhouses are used in maize inbred development. But the percentage of reduction in the time per cycle with DH or DH_{F_2} lines will be much greater than the 5–8% increase in selection response due to the continuous use of RI lines. In other words, while the long-term per cycle response is expected to be greater with RI lines, the response per unit time will be much greater with DH or DH_{F_2} lines.

If year-round nurseries or greenhouses are used and new F_1 crosses for inbred development are created on a speculative basis, the use of DH_{F_2} lines instead of DH lines should not cause a delay in inbred development. Suppose that experimental inbreds are evaluated in hybrid combination during the summer of year 1 (Table 1). The breeder then waits for the yield trial data to decide which experimental inbreds should be used as parents of new F_1 crosses for inbred development. The use of year-round nurseries or greenhouses will allow the testcross evaluation of DH lines in the summer of year 3. In contrast, waiting for the yield trial data before making F_1 crosses for inbred development will delay the testcross evaluation of DH_{F_2} lines until year 4. This delay will be avoided if, during the summer of year 1, the breeder creates a larger set of speculative F_1 crosses involving experimental inbreds at the same time that hybrids of the experimental inbreds are evaluated in yield trials (Table 1). When the yield trial data become available, the best F_1 crosses can then be selected and selfed to obtain F_2 plants from which DH_{F_2} lines can be generated. In this scheme, the testcrosses of the DH_{F_2} lines can be evaluated in year 3. For

Table 1 Timeline for development and evaluation of DH and DH_{F2} maize lines

Season	DH lines	DH _{F2} lines
Summer, year 1	Yield trials	Yield trials; create speculative F ₁ crosses
November–February	Create F ₁	Select F ₁ crosses and self to obtain F ₂ plants
March–June	Cross F ₁ plants to haploid inducer	Cross F ₂ plants to haploid inducer
July–November	Germinate putative haploid kernels Treat seedlings with colchicine	Germinate putative haploid kernels Treat seedlings with colchicine
December–March	Self resulting plants to obtain DH seeds Cross DH lines to tester	Self resulting plants to obtain DH _{F2} seeds Cross DH _{F2} lines to tester
Summer, year 3	Yield trials of DH testcrosses	Yield trials of DH _{F2} testcrosses

DH lines, creating speculative F₁ crosses in year 1 will not speed up the breeding process because the yield trials of DH testcrosses will still have to be conducted in year 3. The use of year-round nurseries and the creation of speculative crosses in breeding with DH_{F2} lines will therefore prevent any delay in the inbred development.

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