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Crafting for a better MAGIC: systematic design and test for multiparental advanced generation inter-cross population

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Short running title

MAGIC population design

Abstract

Multiparental advanced generation inter-cross (MAGIC) populations are valuable crop resources with a wide array of research uses including genetic mapping of complex traits, management of genetic resources and breeding of new varieties. Multiple founders are crossed to create a rich mosaic of highly recombined founder genomes in the MAGIC recombinant inbred lines (RILs). Many variations of MAGIC population designs exist; however, a large proportion of the currently available populations have been created empirically and based on similar designs. In our evaluations of five MAGIC populations, we found that the choice of designs has a large impact on the recombination landscape in the RILs. The most popular design used in many MAGIC populations has been shown to have a bias in recombinant haplotypes and low level of unique recombinant haplotypes, and therefore is not recommended. To address this problem and provide a remedy for the future, we have developed the “magicdesign” R package for creating and testing any MAGIC population design via simulation. A Shiny app version of the package is available as well. Our “magicdesign” package provides a unifying tool and a framework for creativity and innovation in MAGIC population designs. For example, using this package, we demonstrate that MAGIC population designs can be found which are very effective in creating haplotype diversity without the requirement for very large crossing programs. Further, we show that interspersing cycles of crossing with cycles of selfing is effective in increasing haplotype diversity. These approaches are applicable in species which are hard to cross or in which resources are limited.

Keywords: Multiparental advanced generation inter-cross (MAGIC), Multiparental population (MPP), Population design, Quantitative genetics, Genetic diversity

Introduction

The multiparental advanced generation inter-cross (MAGIC) population was initially proposed in crops by Mackay and Powell (2007) as a highly recombined population derived from multiple founders. The MAGIC term is largely relevant in plants, however the concept was derived from the mapping approach using genetically heterogeneous stock in mice (Mott et al. 2000) and is very close to the Collaborative Cross (CC) population in mice (Churchill et al. 2004). The first MAGIC population was produced using 19 founders in *Arabidopsis thaliana* (Kover et al. 2009). The MAGIC pedigree described by Cavanagh et al. (2008) has served as a foundation for the design of many MAGIC populations in subsequent years. Briefly, the MAGIC pedigree shows a single funnel going from 8 founders to a recombinant inbred line (RIL). Starting with 8 founders labelled as A to H, two-way crosses are made as (A × B), (C × D), (E × F) and (G × H). Next, four-way crosses are made as ((A × B) × (C × D)) and ((E × F) × (G × H)). Lastly, eight-way crosses are made as (((A × B) × (C × D)) × ((E × F) × (G × H))) followed by several generations of selfing. Using this crossing scheme, the end of the funnel is a RIL with its genome composed of contributions from all 8 founders. Alternatively, a MAGIC population design may involve multiple funnels like the elite wheat MAGIC population by Mackay et al. (2014). Regardless of the designs, MAGIC RILs have diverse recombination landscape and rich mosaics of founder genomes (Scott et al. 2020).

Over the years, MAGIC populations have been used in various studies with great success. MAGIC populations are popular choices in mapping quantitative trait loci (QTLs) due to their high mapping power and resolution (Valdar et al. 2006), for examples resistance QTLs in bread wheat (Stadlmeier et al. 2019), cold tolerance QTLs in maize (Yi et al. 2020) and high-throughput phenotype QTLs in rice (Ogawa

et al. 2021). In addition to single trait analyses, multivariate analyses (multi-trait or multi-environment) have been demonstrated in MAGIC populations (Scutari et al. 2014, Verbyla et al. 2014). Diouf et al. (2020) used a tomato MAGIC population to dissect the underlying genetic-by-environment (G × E) and plasticity for climate adaptation traits. Aside from QTL mapping, MAGIC populations are valuable resources for genomic selection owing to their properties of highly recombined genomes and large population size (Scott et al. 2021). Following that, there are opportunities for using MAGIC RILs in breeding new varieties (Bandillo et al. 2013, Li et al. 2013). With large numbers of founders, MAGIC populations also provide a dynamic asset for the management of genetic resources (Thépot et al. 2015) and may be used to improve our understanding of crop adaptation (Scott et al. 2021). Given their longevity with a broad array of uses, MAGIC populations are an invaluable community resource for creative and impactful research.

Considering the importance of MAGIC populations in crop research, several previous studies have explored their designs. Ladejobi et al. (2016) investigated a genetic diversity-based approach in founder selection and compared the distributions of recombinant haplotypes within small interval for several MAGIC population designs. Zheng et al. (2018) calculated recombination densities in several MPP designs and showed that higher recombination densities can be achieved by increasing the number of crossing generations. Similar work in mice also showed that additional crossing and maintenance generations increase the number of recombinations in RILs (Valdar et al. 2006). Other simulation studies that compared several MPP designs suggest that MAGIC-like populations are better for generating high number of recombinations, and smaller non-recombining genomic bin region (Rockman and Kruglyak 2008, Klasen et al. 2012). The development of MAGIC

100 populations has been summarized recently in reviews by Huang et al. (2015),
101 Arrones et al. (2020) and Scott et al. (2020).

102 Many of the past works have resulted in software for simulating and
103 identifying recombinations, as well as calculating QTL mapping power and
104 resolution. However, there is none dedicated for the design and test of novel MAGIC
105 crossing schemes. The “GA” R package and GeneDrop software (Ladejobi et al.
106 2016) can be used to calculate founder genetic diversity and simulate
107 recombinations, but neither is capable of producing a MAGIC pedigree. The RABBIT
108 software (Zheng et al. 2015), similar to its counterparts HAPPY (Mott 2008),
109 “mpMap” R package (Huang et al. 2011), and “qtl2” R package (Broman 2019), can
110 be used to determine founder genotypes and thus identify recombination breakpoints
111 in MAGIC, but it still does not create a MAGIC pedigree. Given that MAGIC has
112 applications beyond QTL mapping, it would be beneficial to explore novel crossing
113 schemes that require minimum effort to construct populations suited for wide array of
114 uses.

115 Here, we have sought to understand the relationship between MAGIC
116 population designs and population recombination landscape. We selected and
117 analyzed five MAGIC populations with publicly available marker genotypes for the
118 founders and RILs, genetic map positions and pedigrees. The selected populations
119 comprise the UK wheat 8-founder (Mackay et al. 2014), German wheat 8-founder
120 (Sannemann et al. 2018), cowpea 8-founder (Huynh et al. 2018), tomato 8-founder
121 (Pascual et al. 2015) and UK wheat 16-founder (Scott et al. 2021) MAGIC
122 populations. These MAGIC populations were created from different designs. We
123 contrasted the observed recombinant haplotypes to expected (simulated)
124 recombinant haplotypes in each population. A comparable cross-population analysis

125 can be challenging due to many variables like genome sizes, marker genotyping
126 platforms and numbers of founders. Fortunately, there are two elite wheat 8-founder
127 MAGIC populations (Mackay et al. 2014, Sannemann et al. 2018) that were
128 genotyped with the same 90k SNP array (Wang et al. 2015), which allowed us to
129 directly compare the two populations in greater depth. We found that the
130 recombination landscape varies across designs and that this variation is consistent
131 across species.

132 Following our results, we have created the “magicdesign” package in R (R
133 Core Team 2021) for the purpose of creating and testing different MAGIC population
134 designs. Three major steps are involved in the package pipeline: design creation,
135 population simulation and comparative analysis. Users can create a design by either
136 specifying input variables or providing a custom pedigree. Once a design is created,
137 “magicdesign” converts it into a crossing scheme from the founders to final RILs and
138 simulates a population based on the crossing scheme. After multiple iterations of
139 simulation, distributions of recombinant haplotypes and founder genomes are
140 summarized. Results from one or more designs can be combined and compared
141 visually in plots. In addition, “magicdesign” produces a pedigree in both text and plot
142 formats that can be used as a guide to support crossing work in practice. Aside from
143 the described roles, “magicdesign” serves as a tool to advance the use of MAGIC in
144 future multiparental populations.

145

Materials and Methods

Evaluation of MAGIC population designs

We surveyed all available MAGIC populations that have been published to date (including pre-prints) and identified five populations with publicly available marker data. These five MAGIC populations include wheat with 8 UK elite founders (Mackay et al. 2014), wheat with 8 German elite founders (Sannemann et al. 2018), cowpea with 8 founders (Huynh et al. 2018), tomato with 8 founders (Pascual et al. 2015) and wheat with 16 UK diverse founders (Scott et al. 2021). These populations are referred to as wheat-UK8, wheat-DE8, cowpea, tomato and wheat-UK16, respectively (Table 1). These datasets were chosen because the marker data for the founders and recombinant inbred lines (RILs), genetic map positions, and pedigree are publicly available. The wheat-UK16 population is an exception as it has founder dosages to compensate for the lack of genetic map positions. Links to the source datasets are listed in the Data Availability section. Other publicly available datasets like the wheat with 8 German founders (Stadlmeier et al. 2018), wheat with 8 Australian founders (Shah et al. 2019), rice with 8 founders (Raghavan et al. 2017), maize with 9 founders (Dell'Acqua et al. 2015) and *Arabidopsis* with 19 founders (Kover et al. 2009) are excluded because at least one component of the data needed for our purpose is not present.

All five chosen MAGIC populations vary in numbers of RILs and marker density (Table 1). The original wheat-UK8 dataset is made of 643 RILs and 18,599 markers while the original wheat-DE8 dataset is made of 910 RILs and 7,579 markers. To maintain a fair comparison between these two populations, we kept only 5,138 markers that are common between wheat-UK8 and wheat-DE8. In wheat-DE8,

missing data were previously imputed to numerical mean (twice the allele frequency). These imputed marker data cannot be used in "qtl2" (Broman et al. 2019) for calculating founder genotype probabilities, so we reverted the imputed marker data by converting any non-integer marker data to missing. The cowpea dataset is made of 305 RILs and 32,114 markers after removing 16 markers in the original dataset where the marker data is missing in at least one founder. The tomato dataset is made of 238 RILs and 1,345 markers. The wheat-UK16 dataset is made of 504 RILs and 1,065,178 markers.

Identification of recombinant haplotypes in MAGIC populations

To identify recombinant haplotypes, the biallelic marker data in the RILs need to be converted into founder genotypes. For each dataset except wheat-UK16, we determined the founder genotypes in each RIL using the "qtl2" package (Broman et al. 2019) in R (R Core Team 2021). We first calculated the founder genotype probabilities using *calc.genoprob* function with error probability of 0.01 (1%) and Haldane map function. Next, we inferred the founder genotypes from the probabilities using *maxmarg* function with minimum probability of 0.5001. We chose a slightly higher threshold than the previously used minimum probability of 0.5 by Gardner et al. (2016). Since the genotype probabilities for all founders at each RIL's marker sum to 1, the threshold of 0.5001 eliminates the risk of the *maxmarg* function picking a founder genotype at random when there are two or more with the same probability above the threshold. For the wheat-UK16 dataset, the founder genotype dosages are readily available. These founder genotype dosages were calculated from STITCH (Davies et al. 2016), which is a different software but uses the same underlying hidden Markov model (HMM) as "qtl2". We inferred the founder

194 genotypes in the wheat-UK16 dataset using an equivalent threshold of 1 because
195 the estimated genotype dosages sum to 2. Markers without any founder genotype
196 probabilities above the threshold were set to missing.

197 Using the inferred founder genotypes in each dataset, we identified the
198 recombinant haplotypes at each breakpoint. The recombinant haplotype is a
199 combination of flanking founder genotypes at each breakpoint. For example, 1_5 is
200 the recombinant haplotype at a breakpoint where the two flanking founder genotypes
201 are founder 1 and 5. For a population with n founders, there are $n^2 - n$ individual
202 recombinant haplotypes. Therefore, in an 8-founder MAGIC population, there are 56
203 individual recombinant haplotypes (1_2, 1_3, …, 8_6, 8_7). In order to summarize
204 the results, we summed the counts of each individual recombinant haplotype in
205 every RIL, and averaging the counts across all RILs to obtain the mean counts of
206 individual recombinant haplotypes.

207 As a control, we simulated a similar MAGIC population based on the original
208 pedigree for each dataset and calculated the true counts of recombinant haplotypes.
209 We first derived an approximated crossing scheme from the pedigree. Since the
210 information on replicates is not always present in the pedigree, we assumed that no
211 replicates and considered all funnels to be independent. Next, we used the
212 “AlphaSimR” package (Gaynor et al. 2021) in R (R Core Team 2021) to simulate the
213 MAGIC populations for a total of 100 iterations. “AlphaSimR” is a package designed
214 for simulating plant and animal breeding programs, and we used a fraction of its
215 functionality to simulate crosses and recombinations. In order to keep track of
216 founder genotypes, we expanded each marker into $n - 1$ markers with the same
217 exact genetic map position to prevent recombination among these markers. Using
218 $n = 4$ founders as example, the founders are coded as 000, 100, 010 and 001

across the three expanded markers. This expanded marker system tracked the true founder genotypes from the start to the end of simulation, and therefore allowed us to calculate the true counts of recombinant haplotypes using the same method described in the previous paragraph. In all datasets except for wheat-UK16, we used the same genetic map positions as the actual datasets. In wheat-UK16, we used equally-spaced markers at 0.5 cM since the genetic map positions were not available for this dataset.

We included an additional control using a hybrid approach of actual and simulated datasets. Specifically, we converted the founder genotypes in simulated RILs into biallelic marker data and inferred the founder genotypes using the same procedures as we did in the actual datasets. This approach was applied to all four MAGIC datasets except wheat-UK16. The counts of recombinant haplotypes identified from this approach provide an upper limit to the inferred number of founder genotypes using "qtl2" (Broman et al. 2019). There is a caveat, however, that since our simulation is based on an approximated crossing scheme, the outcomes of this approach may not precisely represent the upper limit.

Determination of unique or identical recombinant haplotypes

Following the identified recombinant haplotypes in wheat-UK8 and wheat-DE8, we classified these recombinant haplotypes into unique or identical groups based on their positional overlaps within an interval. Recombinant haplotypes of the same founder pairs are considered identical if they fall within the same interval, otherwise unique if they do not overlap. The intervals are arranged in non-overlapping bins of approximately 1 or 10 cM from the start to end of a chromosome. Identical recombinant haplotypes exist due to replications of cross progeny in the

MAGIC population. While this classification cannot distinguish between identical and independent recombinant haplotypes within the interval, the probability of independent recombinant haplotypes is low and assumed equal between wheat-UK8 and wheat-DE8. Therefore, the results from this comparison can elucidate the effect of MAGIC population designs on the proportions of unique against identical recombinant haplotypes.

Minimum probability in calling founder genotypes

The minimum probability used in calling founder genotypes determines the power in identifying the correct founder genotypes in the RILs. We selected 10 thresholds ranging from 0.1 to 1.0 with an increment of 0.1. We applied each threshold to the *maxmarg* function in "qtl2" (Broman et al. 2019) in simulated populations based on wheat-UK8 and wheat-DE8. These simulated populations are similar to the previously described hybrid approach where the simulated founder genotypes in RILs are converted to biallelic markers prior to calculating genotype probabilities. For each threshold, we computed the proportions of correct, incorrect or missing founder genotypes by comparing the inferred to true founder genotypes. The ideal threshold should have a high proportion of correct founder genotypes with low proportions of incorrect and missing founder genotypes.

Marker density in MAGIC population

We used the proportion of recombinant haplotype recovered (PRHR) to quantify the recombinations in MAGIC population that is captured in the marker data. We calculated the empirical PRHR in all five datasets as the counts of recombinant haplotypes in the actual dataset divided by the counts in the simulated dataset. Since the marker density is not constant along the genomes in actual datasets, we

sought to determine a clearer relationship between PRHR and marker density via simulation. We simulated a single chromosome of 200 cM with 8 founders crossed using the same design as wheat-UK8. The founder alleles were simulated based on the correlations among founders in wheat-UK8 using the *rmvbin* function in "bindata" package (Leisch et al. 2021) in R (R Core Team 2021). We simulated a total of 4,000 markers that are equally spaced at 0.05 cM. To test lower marker densities, we thinned the same simulated marker data to 0.10, 0.20, 0.40, 0.80, 1.60, 3.20, 6.40, 12.80 cM respectively. For each marker density, we inferred the founder genotypes in the RILs using "qtl2" (Broman et al. 2019) at a probability threshold of 0.5001 and calculated the counts of recombinant haplotypes. Lastly, we obtained the PRHR by taking the counts of recombinant haplotypes for each marker density divided by the true counts.

Results

Classifications of MAGIC population designs

Variations in the MAGIC population designs can be described by the number of founders and the crossing scheme (Figure 1A). It is convenient to first consider two classes of designs based on the number of founders: power of two (P2) and non-power of two (NP2). As the names suggest, the P2 class has $n = 2^i$ founders for any $i > 1$ whereas the NP2 class has $n \neq 2^i$ and $n > 2$ founders. P2 designs are generally easier to implement in practice because the numbers of individuals in a funnel are halved in every crossing generation. For either P2 or NP2 classes, the crossing scheme can be structured, unstructured and semi-structured (Figure 1A). A structured design involves strictly defined crosses among the founders and intermediates such that the crossing scheme can be further classified into full, partial

balanced, partial unbalanced or basic designs. These designs are elaborated further in subsequent paragraphs. On the other hand, an unstructured design involves random crosses among the founders and intermediates, while a semi-structured design is a combination of structured and unstructured designs. Additional features of a structured design include: (1) precise tracing of the ancestry of each RIL back to its progenitors, (2) number of crossing generations is equal to $log_2 n$ rounded up to the nearest integer. These features may not hold true in unstructured or semi-structured designs.

Within a structured design, there are two primary types based on the number of funnels: full and partial (Figure 1A). A full P2 design has $n!/2^{n-1}$ funnels while a partial P2 design has one or more funnels but less than $n!/2^{n-1}$ funnels. The numerator is the total number of permutations of 1 to n founders, and the denominator is the total number of equivalent permutations by the MAGIC definition. Directions of crosses are disregarded in defining a funnel. The denominator can be described as $2^{n-1} = \prod_{x=2^i}^{n} 2^{n/x}$ for $i > 0$. In an example with four founders, 1234, 1243, 2134, 2143, 3412, 3421, 4312 and 4321 are all equivalent funnels. For simplicity, ((1 × 2) × (3 × 4)) is written as 1234. Full and partial types exist in NP2 designs although the number of funnels in a full design cannot be generalized similarly (Table S1). From a practical perspective, a full P2 design is achievable for four or eight founders but not for 16 or more founders as the number of required funnels becomes unmanageable.

Within a partial design, the funnels can be chosen in either a balanced or an unbalanced way (Figure 1A). A balanced design has an equal number of founders among the funnels and equal frequency of founder pairs at each crossing

generation. In a four-founder MAGIC design, 1234, 1324 and 1423 form a set of three balanced funnels. First, each founder occurs thrice in the set of funnels. Second, each founder is paired once with another founder in the two-way crosses, and twice in the four-way crosses. For example, founder 1 meets founder 2 once in the two-way cross (first funnel) and twice in the four-way cross (second and third funnels). Coincidentally, since $n = 4$ and $n!/2^{n-1} = 4!/2^3 = 3$, the set of three balanced funnels is equivalent to a full design for four founders. Unlike the partial balanced design where the number of funnels is restricted to set rules, the partial unbalanced design is formed by funnels chosen randomly. Differences between balanced and unbalanced designs are explored in a later section. Additionally, we coin the special case of partial unbalanced design with one funnel as a basic design. Examples of all of the designs are shown in Figure S1.

Based on our survey of 48 MAGIC populations in 15 crop species that have been described in either published or pre-print literature to date, there are 39 P2 and 9 NP2 designs (Figure 1B and Table S2). The numbers of founders range from four to 60, with 4 and 8 founders as the predominant numbers. Despite the ease of handling required for crosses based on a full design with 4 founders, all 10 of the populations were created using a basic design. Of the 26 MAGIC populations with 8 founders, there are 16 basic designs, nine partial designs and one semi-structured design. The popularity of the basic design can be ascribed to Cavanagh et al. (2008), who provided an illustrated pedigree of a basic design. We refrained from classifying the partial designs into balanced and unbalanced designs due to the lack of pedigree information in many MAGIC populations. Regardless of the number of founders, there has not been any MAGIC population created with a full design. There are several 8-founder populations that came close to a full design. The bread wheat

MAGIC population by Mackay et al. (2014) had 210 out of 315 required funnels for a full design. The maize MAGIC population by Dell'Acqua et al. (2015) had mixed funnels from pooling different four-way individuals and had to introduce an additional founder due to a failed two-way cross. The three bread wheat MAGIC populations by Shah et al. (2019) came closest to a full design with 311 to 313 funnels.

Empirical evaluation of two bread wheat MAGIC populations

Our evaluation on two bread wheat MAGIC populations derived from distinct sets of 8 elite founders shows that the distributions of recombinant haplotypes differ for each MAGIC design (Figure 2 and Figure S2). We used the wheat-UK8 and wheat-DE8 populations, in which wheat-UK8 is an example of a partial design while wheat-DE8 is an example of a basic design (Table S2). To maintain our cross-population comparison as fair as possible, we reduced the original wheat-UK8 and wheat-DE8 datasets to smaller subsets with common markers (Figure 2), although the same analysis was performed on the original datasets too (Figure S2). The subsets include all 643 RILs in wheat-UK8 and 910 RILs in wheat-DE8, and 5,138 common markers arranged in the same genetic map positions as Gardner et al. (2016). This genetic map is chosen over the original genetic map in the wheat 90k array (Wang et al. 2014) because of higher map quality.

The distribution of all recombinant haplotypes is less skewed in wheat-UK8 than in wheat-DE8 (Figure 2). In wheat-UK8, none of the recombinant haplotype appears more frequently than others (Figure 2A). In any given RIL, there are 0.879 ± 0.227 (mean ± standard deviation) individual recombinant haplotypes. In wheat-DE8, eight recombinant haplotypes appear about twice as frequently as the others (Figure 2B). There are 1.910 ± 0.313 of these eight recombinant haplotypes (1_2, 2_1, 3_4,

364 4_3, 5_6, 6_5, 7_8 and 8_7) instead of 0.845 ± 0.150 of the other recombinant
365 haplotypes. In addition, the mean count of recombinant haplotype is approximately
366 normally distributed in wheat-UK8 (Figure 2C) but is skewed to the right in wheat-
367 DE8 (Figure 2D). The eight skewed recombinant haplotypes match with all of the
368 founder pairs in two-way crosses in wheat-DE8. This is not a coincidence because
369 two-way crosses have the largest founder genomes to recombine. With every
370 generation of crosses, the founder genomes are halved and so there are fewer
371 recombinations between any two founders. Examples of the detrimental
372 consequences of the skew in recombinant haplotypes are: (1) reduction in QTL
373 mapping power and resolution when the pairs of founders with higher skew carry the
374 same haplotypes surrounding the causative QTL, (2) limited novel haplotypes for
375 breeding use.

376 While wheat-UK8 has a slightly lower number of recombinant haplotypes per
377 RIL than wheat-DE8 in both reduced (Table 2) and full (Table S3 and S4) datasets,
378 the proportion of unique recombinant haplotypes is higher in wheat-UK8 than in
379 wheat-DE8 (Figure 3 and Figure S3). Due to the imprecision of inferred
380 recombination breakpoints, we defined recombinant haplotypes with breakpoints
381 within any non-overlapping intervals as identical. We chose the intervals to be 1 cM
382 and 10 cM wide. With the interval width set to 1 cM, there are 17,786 distinct
383 recombinant haplotypes distributed among 643 RILs in wheat-UK8, which is
384 equivalent to 27.66 distinct recombinant haplotypes per RIL. Similarly, there are
385 17,643 distinct recombinant haplotypes distributed among 910 RILs in wheat-DE8,
386 which is equivalent to 19.39 distinct recombinant haplotypes per RIL. When the
387 interval is set to 10 cM, the counts and proportions of unique recombinant
388 haplotypes decrease and the differences between wheat-UK8 and wheat-DE8 holds

(Figure S3). There are many practical implications of having more unique recombinant haplotypes: (1) increased mapping power and resolution, (2) increased options of novel haplotypes for breeding, and (3) minimized redundancy of the same recombinant haplotypes. For any pair of founders that share a haplotype carrying linked QTLs, all recombinations between the two founders within the region are non-informative. Such haplotype can be broken down by recombinations between other pairs of founders, which is achievable by having more unique recombinant haplotypes. This is useful to avoid mapping ghost QTLs For example, a previously identified *AOP2/AOP3* locus in *Arabidopsis* (Atwell et al. 2010, Kerwin et al. 2011) was recently re-mapped to two other linked loci, *NDX1* and *GA1* (Sasaki et al. 2021).

Empirical evaluation of three other MAGIC populations

While not directly comparable, the relationship between MAGIC population designs and the distributions of recombinant haplotypes in three other datasets remains consistent. Similar to wheat-DE8, the cowpea and tomato MAGIC populations were created from a basic design and thus have a skewed distribution of recombinant haplotypes (Figure 4). The recombinant haplotypes from two-way founder pairs are higher than the other recombinant haplotypes. In cowpea, the two-way recombinant haplotypes are 0.936 ± 0.171 (mean ± standard deviation) per RIL while the other recombinant haplotypes are 0.384 ± 0.123 per RIL. In tomato, the two-way recombinant haplotypes are 0.907 ± 0.095 per RIL and the other recombinant haplotypes are 0.416 ± 0.116 per RIL. On the other hand, wheat-UK16 was created from a partial balanced design and does not have any skew in its distribution of recombinant haplotypes (Figure S4). The recombinant haplotypes are 0.878 ± 0.102 per RIL.

Minimum probability for calling founder genotype

The minimum probability for calling founder genotype is important for the identification of recombinant haplotypes, and our simulation results suggest that the range of 0.4 to 0.6 gives a good balance of correct, incorrect and missing founder genotype calls (Figure 5A and 5B). This range is in accordance to the threshold of 0.5 used in Gardner et al. (2016). The results are similar between simulated wheat-UK8 and wheat-DE8 populations, so only results from the simulated wheat-UK8 population are elaborated here. At a minimum probability of 0.4, the correct, incorrect and missing founder calls are 69%, 16% and 15% of the total markers, respectively. At a minimum probability of 0.5, the rates are 64%, 11% and 25%. At a minimum probability of 0.6, the rates are 58%, 6% and 36%. As the minimum probability increases, the rates of correct and incorrect founder calls decrease while the missing rate increases. In order to avoid the issue of having two or more founder probabilities above the threshold, the minimum probability can be set to 0.5 or higher. Since the simulations are based on the available diversity among the wheat-UK8 and wheat-DE8 founders, the appropriate range of minimum probability for calling founder genotype may vary in other populations.

Marker density in MAGIC population

In all five analyzed datasets, the proportion of recombinant haplotypes recovered (PRHR) is higher in populations genotyped at higher marker density (Table 1). PRHR is computed by taking the number of recombinant haplotypes in actual dataset divided by the true number of recombinant haplotypes in simulated dataset. Therefore, high PRHR ensures that fine-scale recombinations are captured and increases QTL mapping resolution. In wheat-UK8 and wheat-DE8 with an

437 average marker distance of 1.024 cM, the PRHR is approximately one-third (Figure
438 2). Even though the tomato population is genotyped at a lower marker density with
439 an average marker distance of 1.603 cM, the PRHR is higher than in the two wheat
440 populations (Figure 4B). This is likely because the markers on the wheat D-genome
441 are generally sparse due to its low diversity (Akhunov et al. 2010). The cowpea
442 population is genotyped at a high marker density with an average marker distance of
443 0.030 cM, in which the PRHR is approximately two-thirds (Figure 4A). Lastly, wheat-
444 UK16 is genotyped at the highest marker density of all analyzed datasets with an
445 average marker distance of 0.005 cM, and it has the highest PRHR of almost 80%
446 (Figure S4). Marker density is an important factor in identifying fine-scale
447 recombination breakpoints in MAGIC populations.

448 Under ideal conditions, where the markers are evenly spaced, a marker
449 distance of 0.20 cM or less between two adjacent markers is sufficient to achieve a
450 PRHR of at least 90% (Figure 5C). We tested recombinant haplotype recovery rates
451 for markers that are evenly spaced across 0.05, 0.10, 0.20, 0.40, 0.80, 1.60, 3.20,
452 6.40 and 12.80 cM. At the smallest tested distance of 0.05 cM, approximately 97% of
453 the true recombinant haplotypes can be recovered. As the distance increases, the
454 recovery rate decreases. At the largest tested distance of 12.80 cM, approximately
455 11% of the true recombinant haplotypes can be recovered. These results are more
456 optimistic than the actual results (Table 1). In practice, more markers are required to
457 achieve the same recovery rate for any given marker density because markers are
458 not evenly distributed across the whole genome. In addition, the discrepancy
459 between simulated and actual results can also be attributed to marker quality. For
460 example, the markers on the wheat D-genomes are generally sparser than on the
461 others.

magicdesign: a tool to create and test MAGIC population designs

Given that MAGIC population construction requires a lot of time and effort, and that design choices can impact population attributes, there is a need for a “free trial” before committing to create a MAGIC population. Here, we introduce an R package called “magicdesign”, which is specifically made for creating and testing various MAGIC population designs via simulation. Alternatively, we also provide a user-friendly Shiny app version called “magicdesignee” which implements the “magicdesign” R package in its back-end. Therefore, minimal R knowledge is required for users to use “magicdesignee”.

Briefly, the “magicdesign” package workflow can be described as: (1) design creation, (2) population simulation, and (3) comparative analysis. In the design creation step, the package creates a crossing scheme that spans from the founders to the final RILs based on user inputs. In the population simulation step, the package simulates a MAGIC RIL population constructed from the crossing scheme, and repeats over multiple iterations. At this point, the first two steps may be repeated for other MAGIC population designs. Finally, in the comparative analysis step, the package extracts information from previously tested designs and summarizes the results illustratively. Additional details on each step are described in subsequent sections.

Design creation

In a structured design, the design creation step takes various user inputs to create a crossing scheme. The major inputs include number of founders, number of funnels or funnel sets, and a balanced design indicator. Based on how these inputs are specified, one of the structured designs (Full, Partial Balanced, Partial

Unbalanced, Basic) as shown in Figure 1A is created. As defined previously, a balanced design has an equal number of founders among the funnels and equal frequency of founder pairs at each crossing generation. This design creation step works for either power of 2 (P2) or non-power of 2 (NP2) number of founders. Currently, the allowed range of number of founders is any integer between 3 and 128. The allowed number of funnels or funnel sets varies according to the number of founders and the balanced design indicator, and the full list is provided in Table S1.

Finding a balanced design requires more computation power than finding an unbalanced design. This is because the balanced design requires many funnel permutations to be evaluated while the unbalanced design randomly sample the required number of funnel permutations. To reduce the computational burden, we have identified alternative methods that are less computationally intensive. In the case of 8 founders, we have searched through all 315^7 possible combinations and identified 720 partial balanced funnel sets. There are 7 funnels to make a minimum partial balanced funnel set and any of the 315 funnels from a full design can be chosen to fill each of the 7 funnels in a funnel set. Furthermore, each of the partial balanced funnel set can be combined with another non-overlapping partial balanced funnel set to form a larger partial balanced funnel set. In the case of 16 founders, the number of possible combinations is very large and so we opted for a different approach. To start, we obtained the 15 funnels from Scott et al. (2021), which is a partial balanced set for 16 founders. We searched through all 3^{15} possible permutations of eight- and sixteen-way crosses in these funnels and identified 7,776 partial balanced funnel sets. More partial balanced funnel sets could be found by searching through all 315^{15} possible permutations of four-way crosses, however, that was beyond our available computational capacity. Unlike the case of 8 founders,

these funnel sets do overlap and thus cannot be combined to form a larger set. Instead, by randomly swapping the founders from a starting partial balanced funnel set, a non-overlapping partial balanced funnel set can be created and merged to form a larger set. For other numbers of founders between 4 and 16, the balanced design is created based on a nested incomplete block design (NIBD) generated using the “blocksdesign” package (Edmondson 2020; Edmondson 2021). A MAGIC funnel is analogous to a NIBD as the founders in two-way crosses (experimental block of two plots) are nested within four-way crosses, founders in four-way crosses are nested within eight-way crosses, and so on. Currently, a balanced design in “magicdesign” is limited to 16 or less founders as there is not yet an efficient method for larger number of founders.

In addition, “magicdesign” provides options to further modify the MAGIC population design by specifying the number of replicates, number of selfing generations, and an additional crossing indicator. The number of replicates determines how many seeds from a cross are retained. This can help to increase the haplotype diversity in the MAGIC population when the seeds are not genetically identical. In the case of inbred founders, replicates of two-ways individuals are all identical but not replicates of four-ways (or higher) individuals. The number of selfing generations determine how many generations of selfing are required after each cross. Typically, the selfing step is only applied after the last crossing generation as a way to reduce heterozygosity in the RILs. However, selfing prior to that may be beneficial in increasing recombinant haplotypes. Lastly, the additional crossing indicator allows for an extra crossing generation to further increase recombinant haplotypes. This is similar to the approach taken by Stadlmeier et al. (2018) and Shah et al. (2019).

Alternatively, any MAGIC population design that is not available directly in "magicdesign" can be created by supplying a complete pedigree. The only requirement for the pedigree is that it must detail all crosses involved from the founders to the final RILs. This option provides a greater flexibility to accommodate for semi-structured or unstructured designs. Furthermore, it is also possible to modify a design created from "magicdesign" and provide the pedigree of the modified design.

Population simulation

Once a MAGIC population design is created, "magicdesign" simulates a population based on the design and other user inputs. The major inputs include distance between markers, chromosome genetic lengths, number of simulations and recombinant haplotype interval size. The simulation step will create evenly-spaced markers based on the distance between markers and chromosome genetic lengths. All founders are considered unique and so each of these markers is used to encode for the founder genotypes. The desired number of simulations is selected. In addition, the recombinant haplotype interval size determines the distance between two markers to look for recombinant haplotypes.

Comparative analysis

After simulating one or more designs, the final step is to compare the design qualities in terms of recombinant haplotype proportions and distribution of founder genomes in the RILs. In general, a good MAGIC population design should yield consistently higher recombinant haplotype proportions as well as an even distribution of founder genomes compared to other designs.

559 To demonstrate comparative analysis with “magicdesign”, the five designs in
560 Table 3 are used as examples. These designs are all applied to a fictitious species
561 with five chromosomes of 1.0, 1.5, 2.0, 2.5, 3.0 Morgans (M) length. All five designs
562 are created based on a MAGIC population of 8 founders. Design 1 is a full design
563 and so it has all 315 funnels. Design 2 and 4 are both partial balanced design with
564 one funnel set (7 funnels), and the only difference between them is that the four-way
565 individuals in design 4 are selfed once before making eight-way crosses. Design 3 is
566 similar to design 2 except it is a partial unbalanced design with 7 funnels. Lastly,
567 design 5 is a basic design with 1 funnel inspired by the design used in Stadlmeier et
568 al. (2018). The numbers of replicates are varied for each design to achieve similar
569 final RIL population size close to 1,000. Aside from design 1 which has the highest
570 number of crosses, the other designs have fairly similar numbers of crosses. Design
571 1, 2 and 3 require 7 generations from founders to RILs, while design 4 and 5 require
572 8 generations because of the additional selfing and crossing generation respectively.

573 First, we investigated the designs’ effects on recombinant haplotypes within a
574 5 cM interval. In term of total recombinant haplotypes, a good design should have
575 high mean with low variance. High mean implies a reduction in linkage disequilibrium
576 (LD) and thus improves QTL mapping resolution (Ladejobi et al. 2016) as well as
577 prediction of marker effects in genomic prediction (GP). Low variance ensures that
578 the proportion of recombinant haplotypes in the created MAGIC population remains
579 close to the simulated mean and minimizes the risk of constructing a poorly
580 recombined MAGIC population. For design 1 to 5 respectively, the means are 0.167,
581 0.167, 0.169, 0.186 and 0.202 while the variances are 0.000158, 0.000244,
582 0.000293, 0.000452 and 0.003000 (Figure 6A). The means are similar in design 1, 2
583 and 3, slightly higher in design 4 and highest in design 5. However, the variances are

584 lowest in design 1, similar in design 2 and 3, slightly larger in design 4, and
585 substantially larger in design 5.

586 In any RIL derived from an 8-founder MAGIC population, there are 56 distinct
587 recombinant haplotypes and a good design should have high mean with low
588 variance. Mean number of unique recombinant haplotypes that approaches the
589 theoretical maximum of $n^2 - n$ is important for maximizing QTL mapping resolution
590 and generating novel haplotypes for breeding new varieties. In a population of
591 MAGIC RILs with high proportion of recombinant haplotypes but low number of
592 unique recombinant haplotypes, the QTL mapping resolution can be poor when the
593 recombinant haplotypes are largely composed of pairs of founders carrying the same
594 causative QTL haplotype. Low variance is beneficial for the same reason as
595 explained in previous paragraph. The means for the number of unique recombinant
596 haplotypes are 52.20, 49.93, 50.29, 47.81 and 34.51 for design 1 to 5 respectively
597 while the variances are 3.31, 4.29, 4.21, 4.68 and 34.78 for design 1 to 5
598 respectively (Figure 6B). The means are highest in design 1, similar in design 2 and
599 3, slightly lower in design 4 and lowest in design 5. The variances follow a similar but
600 reverse trend as the means except for design 5 where the variance is over seven
601 times greater. Equivalently, the coefficients of variation (CVs) are 0.035, 0.041,
602 0.041, 0.045 and 0.171 for design 1 to 5 respectively. That for design 5 is
603 approximately four times that for the other designs.

604 The distributions of individual recombinant haplotype should be consistent
605 across all recombinant haplotypes with minimal variability across simulations in a
606 good design. This metric offers an in-depth view of individual recombinant
607 haplotypes by combining the two previously described metrics. Here, we can identify
608 the individual recombinant haplotypes that deviate from the others, which can be a

609 cause of concern relating to poor QTL mapping resolution and lack of novel
610 recombinant haplotypes for breeding uses. With the exception of design 5, all other
611 designs have similar distributions of individual recombinant haplotype (Figure 6C and
612 Table S5). Similar to wheat-DE8 (Figure 2B), cowpea and tomato (Figure 4), design
613 5 has more two-ways recombinant haplotypes than other recombinant haplotypes.
614 Furthermore, the spreads of two-ways recombinant haplotypes in design 5 are much
615 higher than the others, which imply low consistency.

616 Similar to the previous criterion, the proportions of founder genomes should
617 be consistent across all founders with low variability across simulations in a good
618 design. This is an important metric that highlights the disparity in founder genome
619 distribution. Multiple uses of MAGIC populations are compromised when the
620 disparity is large, for instance, rare QTLs may drop out, GP training model and
621 breeding options become skewed, and valuable diversity is lost in a genetic resource
622 management program. With 8 founders, the expected proportion of each founder
623 genome in a population is 0.125. The proportions are within 0.01 of expectation for
624 all designs except for design 5, which has 5 out of 8 proportions exceeding the range
625 (Figure 7A). On the other hand, the variances are lowest in design 1, slightly higher
626 in design 2, 3 and 4, and highest in design 5 (Figure 7A).

627 In any single chromosome, a RIL can carry tracts of 1 to 8 unique founder
628 genomes and it is generally better to have more unique founder genomes. This
629 metric is similar to the first metric showing the total recombinant haplotypes where
630 higher number of unique founder genome suggests more recombinations. In
631 addition, this metric highlights the relationship between genetic length and the
632 number of unique founder genomes, which demonstrates the advantages of MAGIC
633 in species with many genetically long chromosomes. In the shortest chromosome

634 (chromosome 1), design 1, 2 and 3 frequently produce 3 unique founders, while
635 design 4 and 5 frequently produce 4 unique founders (Figure 7B). In the longest
636 chromosome (chromosome 5), design 1, 2, 3 and 4 frequently produce 6 unique
637 founders while design 5 frequently produces 7 unique founders (Figure 7B).

638 Lastly, a good design should have short non-recombinant segments, which
639 can be achieved by increasing the number of crossing generations from the founders
640 to the RILs. Short non-recombinant segments imply higher QTL mapping resolution
641 and possibly better marker effect prediction in GP. Across all chromosomes, design
642 5 has the most short non-recombinant segments, followed by design 4, and design
643 1, 2 and 3 being undiscernible (Figure 7C).

644 Of all the five designs considered here, each has its own advantages and
645 disadvantages. Design 1, 2 and 3 are highly similar except that design 1 tends to
646 show smaller variability than the other two at the cost of more crossing work
647 required. Design 4 is slightly better than the first three in most occasions, although it
648 is slightly more variable and requires one additional generation. Design 5 is generally
649 poor and should be avoided if possible, although the additional crossing generation
650 helps in increasing the number of unique founders and reducing non-recombinant
651 segment lengths. Of all designs considered, design 4 appears to be the best option if
652 the additional generation is acceptable, otherwise either design 2 or 3 is a good
653 alternative. Across all the metrics used for comparisons in “magicdesign”, there is no
654 observable difference between design 2 (balanced) and 3 (unbalanced).

655

Discussion

Ease of design appears as a major factor in driving the design choices in currently available MAGIC populations. These MAGIC populations are predominantly made of 4 or 8 founders crossed using a basic design (Figure 1B). There are several possible explanations to the choice popularity. First, P2 designs are easier to handle than NP2 designs since the individuals in every funnel are halved at every crossing generation. Besides, 4 and 8 founders are effectively the lowest numbers of founders available in P2 designs, and higher numbers of founders require more generations of crossing and may increase the design complexity. Of all the explored designs, the basic design likely requires the least amount of effort in population construction. The only other option that may rival a basic design is the unstructured design with random mating, which often relies on segregation of male sterility loci. Unfortunately, this system is not always readily available in every species and may restrict the founder choices.

Choice of MAGIC population design plays a critical role in determining the recombination landscape in the RILs. In the comparison between wheat-UK8 and wheat-DE8, we identified a bias in individual recombinant haplotypes in the basic design but not the partial design (Figure 2). The bias resulted in more two-way recombinant haplotypes than other recombinant haplotypes. The bias might be exacerbated if the pairs of founders in two-ways are genetically more similar than others, which can happen if the founders stratify into two or more groups. It is possible to avoid pairing the founders of the same groups in two-ways if the grouping is known. For example, Pascual et al. (2015) made the two-way crosses by crossing tomato founders with large fruits to founders with small fruits, and Ogawa et al. (2018) followed similarly by crossing *indica* rice founders to *japonica* rice founders.

681 This countermeasure is only possible if the numbers of founders are equal between
682 groups, but not if the founders cannot be subdivided equally like the barley
683 (Sannemann et al. 2015), cowpea (Huynh et al. 2018) and wheat (Stadlmeier et al.
684 2018) MAGIC populations. Besides, the stratification may be incomplete due to other
685 traits that are not considered, for example, flowering time and nutrition qualities in
686 tomatoes.

687 In addition to the bias, the basic design also resulted in a lower proportion of
688 unique recombinant haplotypes than the partial design (Figure 3). Since a basic
689 design always has less funnels than any other designs, high replication of cross
690 progeny is required to bring the number of RILs up. In general, replicates reduce the
691 amount of crossing work required in prior generation by keeping more than one
692 progeny from a single cross to advance. The recombination landscape in these
693 replicated individuals is non-independent because any prior recombinations are
694 passed down from their parents. The detriments from replication can be minimized
695 by replicating in earlier generations as subsequent crosses will reduce the non-
696 independence among replicates. In a MAGIC population with 8 inbred founders, the
697 earliest meaningful replication would be the four-way individuals. However, replicates
698 prior to the final crosses do increase the amount of downstream crossing work, and
699 so it is important to consider the trade-offs between available work resources and
700 uniqueness of recombinant haplotypes.

701 High marker density is needed to capture the highly recombined genomes of
702 MAGIC RILs. We used the proportion of recombinant haplotypes recovered (PRHR)
703 as a measure of how well the markers capture recombinant haplotypes. PRHRs in
704 the five analyzed datasets correlate well with the marker density. Even with the high
705 marker density in wheat-UK16, the PRHR is only 0.799 (Table 1), which suggests

706 that one-fifth of the recombinant haplotypes is still missing. Some explanations
707 include the sparser marker density in the D-genomes, uneven marker density along
708 the genomes and segregation distortions of the founder genomes. To generalize the
709 relationship between marker density and recombinant haplotypes further, our
710 simulation results showed that marker distance of 0.80 cM or less is sufficient to
711 recover over three-quarters of the recombinant haplotypes. Despite the results from
712 actual datasets being less optimistic than the results from simulation, the importance
713 of high marker density in MAGIC populations still holds.

714 Given that the advantages and disadvantages of different MAGIC population
715 designs are largely unexplored, the “magicdesign” package serves as an important
716 tool to create and test different designs. Specifically, “magicdesign” provides the
717 opportunity to evaluate the options before committing to years of effort in
718 constructing MAGIC populations. In our examples, an additional selfing generation
719 offers a simple path to improvement (Figure 6 and 7), especially in inbreeding
720 species. When used in combination with speed breeding (Watson et al. 2018), the
721 additional time due to selfing can be minimized. In addition, “magicdesign” also acts
722 as a bridging tool for researchers who are new to MAGIC populations by providing a
723 starting point to creating a MAGIC population. The opportunity to create and test
724 different designs will encourage innovation in MAGIC population designs rather than
725 relying on previously used designs.

726 While the initial version of “magicdesign” package involves simulations with
727 relatively simple parameters, we intend to expand the package scopes to cover
728 broader biological aspects that are relevant to MAGIC. For example, the
729 recombination landscape is generally perceived to be uneven (Petes 2001) and it will
730 be useful to consider recombination hot and cold spots. Gene density varies along

731 the genome and may need to be accounted for in MAGIC simulation, although non-
732 coding and unannotated regions cannot be discounted too given their biological
733 importance (Jiang 2015). Founder diversity was previously shown to be important in
734 MAGIC population design (Ladejobi et al. 2016). Currently, this feature is not
735 available in "magicdesign" and will be considered as a priority for subsequent
736 versions. Overall, "magicdesign" is a valuable resource for unifying the process of
737 creating and testing MAGIC population designs, and providing the flexibility for
738 additional features to be included in future updates as the package grows with users'
739 feedback and research demands.

740 **Data Availability**

741 The MAGIC datasets used in this work were sourced from
742 www.niab.com/research/agricultural-crop-research/resources/niab-magic-population-
743 resources (wheat-UK8), doi.org/10.1186/s12864-018-4915-3 (wheat-DE8),
744 doi.org/10.1111/tpj.13827 (cowpea), doi.org/10.1111/pbi.12282 (tomato) and
745 mtweb.cs.ucl.ac.uk/mus/www/MAGICdiverse/ (wheat-UK16). Links to source
746 datasets without DOI have been archived at web.archive.org on April 13, 2021.

747 The "magicdesign" package and its installation instructions are available for
748 download at github.com/cjyang-sruc/magicdesign. Detailed instructions are available
749 at cjyang-sruc.github.io/magicdesign_vignette. The Shiny app "magicdesignee" can
750 be found at magicdesign.shinyapps.io/magicdesignee/. R scripts used in all analyses
751 can be found at cjyang-sruc.github.io/files/magicdesign_analysis.R.

752

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Figures and Tables

Figure 1. Classifications of MAGIC population designs.

[**A**] Flowchart of classifying MAGIC population designs based on their crossing schemes. [**B**] Distribution of MAGIC population designs in all 48 surveyed populations.

Figure 2. Distributions of recombinant haplotypes in two wheat MAGIC populations.

[**A**] Plot shows mean count of each recombinant haplotype in a single RIL in wheat-UK8. The boxplot shows mean count from true founder genotypes (100 simulated iterations). The red and blue points show mean count from inferred founder genotypes. [**B**] Plot shows mean count of each recombinant haplotype in a single RIL in wheat-DE8. [**C**] Histogram of the mean count in wheat-UK8. [**D**] Histogram of the mean count in wheat-DE8.

Figure 3. Distributions of unique and identical recombinant haplotypes in two wheat MAGIC populations.

Recombinant haplotypes are considered identical if they are of the same founder pairs and present in the same 1 cM interval, otherwise unique. [**A**] Counts of the number of identical recombinant haplotypes in wheat-UK8. The left most point is the count of unique recombinant haplotypes. [**B**] Counts of the number of identical recombinant haplotypes in wheat-DE8. [**C**] Proportions of unique and non-unique (identical) recombinant haplotypes in wheat-UK8 and wheat-DE8.

Figure 4. Distributions of recombinant haplotypes in cowpea and tomato MAGIC populations.

[**A**] Plot shows mean count of each recombinant haplotype in a single RIL in cowpea. The boxplot shows mean count from true founder genotypes (100 simulated iterations). The red and blue points show mean count from inferred founder genotypes. [**B**] Plot shows mean count of each recombinant haplotype in a single RIL in tomato.

Figure 5. Ideal threshold for inferring founder genotypes and marker density in MAGIC population.

[**A**] Proportions of correct, incorrect and missing founder genotypes inferred at different minimum probability (minprob) in simulated wheat-UK8 population. [**B**] Proportions of correct, incorrect and missing founder genotypes inferred at different minprob in simulated wheat-DE8 population. [**C**] Proportions of recombinant haplotypes recovered (PRHR) at different marker density along a simulated chromosome of 200 cM. The marker density is adjusted by having markers equally spaced at 0.05, 0.10, 0.20, 0.40, 0.80, 1.60, 3.20, 6.40 and 12.80 cM apart.

Figure 6. Distributions of recombinant haplotypes in five MAGIC population designs.

Recombinant haplotypes are evaluated within a 5 cM interval over 100 iterations of simulation. [**A**] Proportions of total recombinant haplotypes. [**B**] Number of unique recombinant haplotypes. [**C**] Proportions of six chosen recombinant haplotypes. Complete results are available in Table S5.

Figure 7. Distributions of founder genomes in five MAGIC population designs.

Founder genomes are evaluated from 100 iterations of simulation. [**A**] Proportions of each founder genome in the MAGIC RILs. [**B**] Proportions of the MAGIC RILs carrying tracts of 1 to 8 unique founder genomes in each chromosome. [**C**] Mean count of non-recombinant segment length in each RIL's chromosome.

Table 1. Summary of five analyzed MAGIC populations.

The wheat-UK8 and wheat-DE8 datasets have been reduced to share the same markers and maps for comparison. The proportion of recombinant haplotypes recovered (PRHR) is calculated as number of recombinant haplotypes in actual dataset divided by number of recombinant haplotypes in simulated dataset. PRHR is shown as mean ± standard deviation.

dataset	n	genome (cM)	marker	distance (cM/marker)	PRHR	reference
wheat-UK8	8	5,262	5,138	1.024	0.296 ± 0.094	Mackay et al. (2014)
wheat-DE8	8	5,262	5,138	1.024	0.331 ± 0.058	Sannemann et al. (2018)
cowpea	8	979	32,114	0.030	0.674 ± 0.207	Huynh et al. (2018)
tomato	8	2,156	1,345	1.603	0.410 ± 0.106	Pascual et al. (2015)
wheat-UK16	16	5,262	1,065,178	0.005	0.799 ± 0.092	Scott et al. (2021)

Table 2. Number of informative recombinations in wheat-UK8 and wheat-DE8.

The number of informative recombinations (NR) is calculated for both simulated and actual wheat-UK8 and wheat-DE8 datasets. Note: recombinant inbred line (RIL), Morgan (M).

Chr	NR/RIL				NR/RIL/M			
	wheat-UK8		wheat-DE8		wheat-UK8		wheat-DE8	
	sim	actual	sim	actual	sim	actual	sim	actual
1A	7.21	1.91	7.21	2.35	3.13	0.83	3.13	1.02
1B	11.77	3.57	11.76	4.31	3.44	1.04	3.44	1.26
1D	3.74	0.68	3.73	0.99	2.92	0.53	2.92	0.78
2A	8.37	3.67	8.37	3.17	3.32	1.45	3.32	1.26
2B	12.43	2.63	12.43	3.61	3.34	0.71	3.34	0.97
2D	4.97	1.69	4.96	1.42	2.71	0.92	2.71	0.77
3A	10.21	4.50	10.19	4.16	3.37	1.48	3.36	1.37
3B	9.89	3.35	9.90	4.18	3.51	1.19	3.51	1.48
3D	4.45	0.61	4.44	0.45	2.29	0.32	2.28	0.23
4A	6.95	2.51	6.96	2.11	3.30	1.19	3.30	1.00
4B	7.55	2.21	7.58	2.23	3.40	0.99	3.41	1.01
4D	2.95	0.51	2.96	0.43	2.75	0.47	2.75	0.40
5A	10.23	4.15	10.23	4.47	3.26	1.32	3.26	1.43
5B	10.97	2.59	10.96	3.25	3.53	0.83	3.53	1.05
5D	4.76	1.33	4.75	1.52	2.39	0.67	2.39	0.76
6A	9.70	3.38	9.68	4.48	3.48	1.21	3.47	1.61
6B	8.85	1.97	8.84	2.46	3.40	0.76	3.40	0.95
6D	3.92	0.43	3.93	1.14	1.82	0.20	1.83	0.53
7A	13.53	4.21	13.50	5.07	3.53	1.10	3.52	1.32
7B	9.28	2.64	9.27	2.97	3.23	0.92	3.23	1.03
7D	4.40	0.74	4.40	1.05	2.36	0.40	2.35	0.56
All	166.10	49.24	166.05	55.84	3.16	0.94	3.16	1.06

964 **Table 3. Five MAGIC population designs tested in magicdesign.**

965 The numbers of replicates, selfing generations and crosses are listed separately for
966 each generation. For example, in design 1, the eight-way individuals are replicated
967 three times and then selfed for four generations. The total number of crosses is
968 shown in parentheses.

	Design 1	Design 2	Design 3	Design 4	Design 5
Founders	8	8	8	8	8
Type	Full	Partial balanced	Partial unbalanced	Partial balanced	Basic
Replicates	1, 1, 3	1, 9, 15	1, 9, 15	1, 9, 15	1, 4, 4, 15
Selfing	0, 0, 4	0, 0, 4	0, 0, 4	0, 1, 4	0, 0, 0, 4
Crosses	28, 210, 315 (553)	28, 14, 63 (105)	19, 13, 63 (95)	28, 14, 63 (105)	4, 2, 4, 64 (74)
Generations	7	7	7	8	8
RIL	945	945	945	945	960
Funnel	315	7	7	7	1

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