

REVIEW ARTICLE

Multiparent intercross populations in analysis of quantitative traits

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Abstract

Most traits of interest to medical, agricultural and animal scientists show continuous variation and complex mode of inheritance. DNA-based markers are being deployed to analyse such complex traits, that are known as quantitative trait loci (QTL). In conventional QTL analysis, F₂, backcross populations, recombinant inbred lines, backcross inbred lines and double haploids from biparental crosses are commonly used. Introgression lines and near isogenic lines are also being used for QTL analysis. However, such populations have major limitations like predominantly relying on the recombination events taking place in the F₁ generation and mapping of only the allelic pairs present in the two parents. The second generation mapping resources like association mapping, nested association mapping and multiparent intercross populations potentially address the major limitations of available mapping resources. The potential of multiparent intercross populations in gene mapping has been discussed here. In such populations both linkage and association analysis can be conducted without encountering the limitations of structured populations. In such populations, larger genetic variation in the germplasm is accessed and various allelic and cytoplasmic interactions are assessed. For all practical purposes, across crop species, use of eight founders and a fixed population of 1000 individuals are most appropriate. Limitations with multiparent intercross populations are that they require longer time and more resource to be generated and they are likely to show extensive segregation for developmental traits, limiting their use in the analysis of complex traits. However, multiparent intercross population resources are likely to bring a paradigm shift towards QTL analysis in plant species.

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Introduction

The establishment of the first linkage map by Sturtevant (1913) led to a continuing interest among geneticists to identify genes and to localize them on genetic maps. During the initial period, the focus was on morphological traits showing discrete mode of inheritance. However, majority of the traits of interest to medical, agricultural and animal scientists show continuous variation. Such traits are known as quantitative traits. With the understanding of the inheritance of such complex traits through multiple factors or polygenes, biologists realized the challenges ahead to handle such traits. Karl Sax first associated morphological markers with quantitative traits (Sax 1923). Gelderman (1975) coined the term quantitative trait locus (QTL) to denote 'a region of the genome that is associated with an effect on a quantitative trait'. Using morphological traits alone, identification of such loci was very difficult as they show high genotype ×

environment and epistatic interactions and morphological traits represent only 5% of the whole genome, besides being stage-dependent in expression.

Discovery of DNA structure by Watson and Crick (1953), and subsequent discoveries in the field of molecular biology have brought a paradigm shift in the way genes and genetic maps are viewed and constructed. New array of DNA-based markers like restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeat (SSR) and single nucleotide polymorphism (SNP) have transformed the linkage analysis, more particularly the QTL studies. The principles and various methodologies of QTL analysis have been reviewed by various authors (Gupta 2002; Mackay 2004; Flint *et al.* 2005) and are not discussed here. However, it must be stressed that all these methodologies rely on meiotic recombination among markers and detection of the recombinants in the segregating mapping populations. For such analyses, large population with accurate genotyping and phenotyping are required (Keurentjes *et al.* 2011).

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Conventional mapping resources

In early QTL studies, the F₂ and backcross (BC) populations were most commonly used. However, such populations are not fixable due to their inherent heterozygous genetic constitution. This restricts their wide utility in QTL analysis. To address these limitations, biologists nowadays are extensively using recombinant inbred lines (RIL), backcross inbred lines (BIL) and double haploids (DH) (Rakshit *et al.* 2002; Collard *et al.* 2005). Among these resources, RIL, derived by single seed descent from F₂ individuals from hybrids between two distinct homozygotes, are most commonly used in QTL analysis (Keurentjes *et al.* 2011). To avoid complications of segregation of multiple loci (like epistasis) in QTL analysis, introgression lines (IL) and near isogenic lines (NILs) are also in use. In these, small chromosomal regions from donor parents are introduced in a recurrent parental background. Advantage of NIL over RIL is that often it allows detection of minor QTL, which are missed in RILs (Keurentjes *et al.* 2007). Seeds of these populations can be retained indefinitely, making such populations potentially immortal (Huang *et al.* 2011). However, all these populations are derivative of biparental crosses. Such populations have two major limitations. Firstly, they principally rely on the recombination events taking place in F₁ generation and not enough time is available to shuffle the genome in small fragments. As a result, the QTL get placed on a large chromosomal region (Li *et al.* 2010). To circumvent this limitation, Darvasi and Soller (1995) introduced the concept of advanced intercross lines (AICL), which is produced by randomly and sequentially intercrossing a population derived from a biparental cross. Repeated crossing reduces the linkage disequilibrium (LD) and increases the precision of location of the QTL. Second limitation of biparental cross-derived mapping populations including AIC is that it allows mapping of only the allelic pairs present in the two parents. Thus, the whole genetic variation cannot be exploited in such studies. Goffinet and Gerber (2000) suggested combining QTL results from different independent analyses through meta-analysis, while Li *et al.* (2005) have suggested methods to collate data generated from independent biparental studies. Recently Bentsink *et al.* (2010) identified 11 QTL for seed dormancy in *Arabidopsis* by simultaneously analysing six RIL populations. However, none of these techniques can examine epistasis and interactions of QTL with genetic background, which underline quantitative traits.

Second generation mapping resources

The second generation mapping resources propose to address many of the limitations associated with conventional mapping populations. In recent past, potentiality of association mapping in QTL analysis has been emphasized by various authors (Gupta *et al.* 2005; Rafalski 2010; Nordborg and

Weigel 2008). Two approaches are followed in association mapping: candidate gene based and genomewide association (GWA) mapping. In both approaches, maximum trait differences available in the germplasm are statistically associated with specific genotypes for common SNPs or SNP haplotypes. However, association mapping is generally influenced by predominantly unknown population structure, often leading to spurious associations (Hirschhorn and Daly 2005). On the other hand, compensating for population structure may remove true positives (Keurentjes *et al.* 2011). The situation becomes much more complicated in crop plants where high-density-consensus maps are not available. These two factors strongly restrict the widespread adoption of such analyses. Another limitation of GWA is that rare alleles, even those having large effects, may remain undetected as is the case with the *CRY2* allele from Cvi (Cape Verde Islands) accession of *Arabidopsis* (Atwell *et al.* 2010; Brachi *et al.* 2010). In recent past, to circumvent these limitations, nested association mapping (NAM) populations have been established in maize (Yu *et al.* 2008) and *Arabidopsis* (Bentsink *et al.* 2010; Brachi *et al.* 2010). In making such populations, a central parent is crossed with other diverse parents in star design (Huang *et al.* 2011). For example in maize the NAM population has been generated by pooling 200 RIL from 25 families, each being developed by intercrossing 25 diverse maize inbred lines to one common parent, B73 (Yu *et al.* 2008; Buckler *et al.* 2009; McMullen *et al.* 2009). Such populations help in fine mapping of QTL. However, interactions of QTL with genetic background cannot be examined in such population as one parent is common in all component subpopulations. Consequently, Cavanagh *et al.* (2008) proposed a multiparent advanced generation intercross (MAGIC) population to address the major limitations of available mapping resources, and this concept was also utilized in *Arabidopsis* multiparent recombinant inbred line (AMPRIL) population as an additional resource for dissecting the genetics of natural varieties (Huang *et al.* 2011).

Multiparent intercross population, a multi-utility mapping resource

Advanced intercrosses (AIC) are proposed to address the major limitation of lower resolution of biparental crosses. Yalchin *et al.* (2005) extended AIC in mice by involving multiple parents (heterogeneous stocks) in the crossing scheme. Consequently, they could map a QTL explaining 10% of the phenotypic variation for anxiety in mice to a 4.8-Mb region. Prior to this, in mice, a Complex Trait Consortium was created involving 94 different laboratories across 13 countries to analyse complex traits (Churchill *et al.* 2004). The concept of eight-way 'funnel' breeding scheme of Complex Trait Consortium in mice was extended to crop plants by the name MAGIC population by Cavanagh *et al.* (2008). Under this scheme as many diverse founder

lines as the investigator wish may be deployed to create the mapping population. If n founder lines are taken, they need to be intercrossed for $n/2$ generations till all the founders are combined with equal proportions. Once the intercrossing is over RILs may be derived from them upon selfing (figure 1). Major limitation of this tool is that with increase in founder size the intercrossing cycles also proportionately increases. In species like *Arabidopsis* with short generation period higher number of founders may be used. However, for all practical purposes across crop species use of eight founders as described in figure 1 is most appropriate. At G_2 the double crosses segregate, thus replicated crosses involving more recombinant plants are required. At G_3 the progenies of these crosses are again intercrossed to effect 8-way intercrossing. From next generation (G_4) the recombinant progenies are advanced by selfing for 6–7 generations to obtain MAGIC RIL. At each intercrossing cycle hybridity of each plant to be intercrossed may be confirmed through genotyping. High-throughput SNP genotyping platforms and genotype by sequence approaches are of much use in this regard (Davey *et al.* 2011). As an alternative economical method, SSR markers may also be employed. In a simulation study it has been demonstrated that a fixed population of 1000 MAGIC individuals is adequate to map a single additive locus that accounts for 5% of the phenotypic variation to within 0.96 cM distance (Valder *et al.* 2006). It was also shown that,

with 500 lines, sufficient resolution may be obtained even in presence of high epistasis. Thus a population size of 1000 is a highly reliable resource. There are more recent reports of fine mapping of several known QTL and identification of novel QTL for germination and bolting time in *Arabidopsis*, using only 527 MAGIC lines derived from 19 diverse founders (Kover *et al.* 2009). Further, the potential of the technique has been demonstrated by studying flowering time candidate genes in 275 *Arabidopsis* MAGIC lines (Ehrenreich *et al.* 2009). With preliminary success of these studies now MAGIC resources are being created in many plant species including *Arabidopsis* (Kover *et al.* 2009), wheat (http://www.niab.com/pages/id/93/MAGIC_Populations_in_Wheat), rice (Bandillo *et al.* 2010; Leung *et al.* 2011) etc.

A variant of MAGIC strategy has recently been reported by Huang *et al.* (2011). They developed AMPRIL population to include eight *Arabidopsis* founders in the population. They crossed the founders to produce F_1 hybrids, which were then crossed in diallel fashion (figure 2) to generate six connected

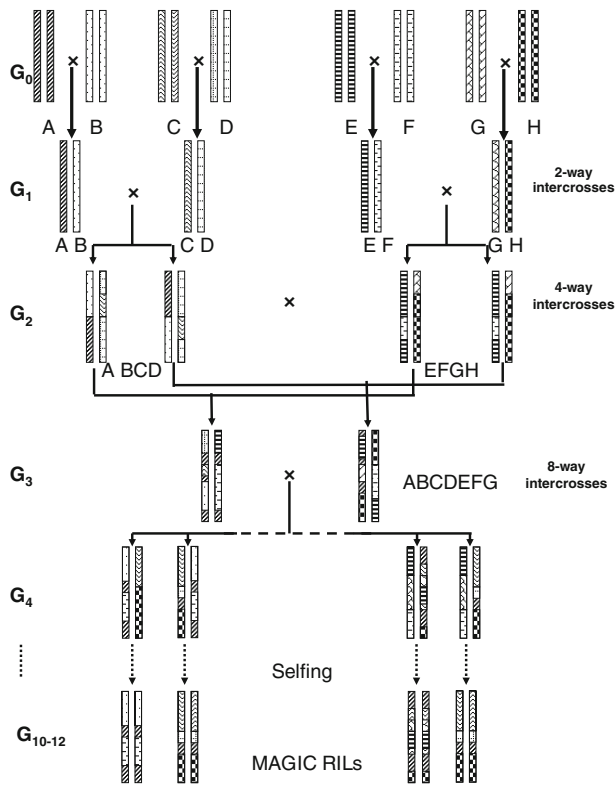
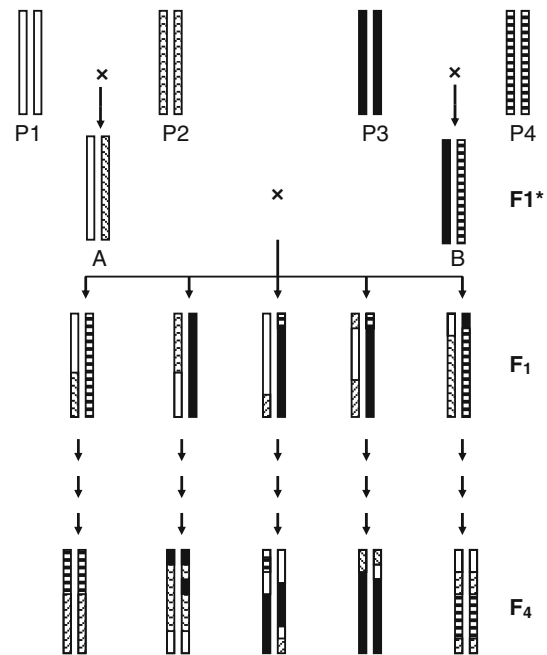


Figure 1. Funnel breeding scheme for MAGIC population development (modified from Cavanagh *et al.* 2008).



	A	B	C	D
A		×	×	×
B	×		×	×
C	×	×		×
D	×	×	×	

Figure 2. Construction of AMPRIL population. Four founder accessions (P1, P2, P3 and P4) are crossed to produce two hybrids (A and B) and one four-way cross. The resulting population is selfed for three generations (F_1 to F_4). For development of AMPRIL population in same scheme eight founder accessions (P1 to P8) led to four hybrids: A, P1 × P2; B, P3 × P4; C, P5 × P6; D, P7 × P8. These hybrids were crossed in diallel scheme (adapted from Huang *et al.* 2011).

four-way crosses. The resulting offspring were selfed for three generations and genotyped in F₄, and phenotyped in F₅ generation. Following this strategy, they identified four QTL for flowering time. However, they failed to detect number of QTL that were detected in biparental populations involving separately the founders of the AMPRIL population.

In multiparent recombinant inbred populations both linkage and association analyses can be conducted without encountering the limitations of structured populations (Brachi et al. 2010). However, the statistical complexity in analysis of such population is very high as compared to biparental crosses (table 1). A statistical package, HAPPY, specifically to analyse heterogeneous stocks including MAGIC has been reported (<http://spud.well.ox.ac.uk/arabidopsis>). This program does not require any pedigree information. It generally uses analysis of variance *F* statistics to test for linkage. Since MAGIC population has an extent of kinship among the RIL, the program uses linear mixed effects model (empirical Bayes) and hierarchical Bayes QTL mapping in analysing the data. Huang et al. (2011) adapted a mixed model methodology to test main effect of QTL and their interaction among themselves and with different genetic background. Huang and George (2011) have recently developed a versatile computational platform, R/mpMap to address the complex statistical need of multiparent cross derived mapping populations. They have implemented interval mapping in the platform using haplotype probabilities. It has interfaces with earlier mapping platforms, R/qrtl (Broman et al. 2003) and R/happy (Mott et al. 2000), thus becoming a useful resource.

To compare utility of different types of populations for mapping, Broman (2005) calculated the number of crossovers that accumulate in offspring population (i.e. number of informative crossovers) in different mapping populations. For backcross population, or a double haploid population, the expected number of informative crossover per morgan

distance for a single offspring (γ) is 1. In case of two-way and four-way RILs, γ is 2 and 3, respectively. For MAGIC population, $\gamma = 6$ (Kover et al. 2009), while for AMPRIL it is 3.625. Thus, MAGIC gives maximum number of informative crossovers among different mapping populations. Darvasi and Soller (1997) suggested a measure of expected total number of informative crossover per centimorgan in a population as a measure of precision of QTL location. For AMPRIL, Huang et al. (2011) calculated this value as 19 per cM, while for MAGIC and NAM populations, it is 32 per cM and 100 per cM respectively. Thus MPRIL population has wide advantages to map QTL effectively.

Further comparison between the most commonly used mapping resources are presented in table 1. Since diverse founders are used in MAGIC and AMPRIL populations larger genetic variation in the germplasm is accessed, which is not possible in biparental populations. With use of more parents in the population various allelic and cytoplasmic interactions are also accessed, and assessed in such second generation mapping populations. It is highly probable to obtain useful MAGIC RIL having agronomic superiority due to desirable allelic recombination, which may directly be used in the breeding programme. During MAGIC or similar population development, seeds at any generation may be retained and advanced to develop RIL, which will allow both coarse and fine mapping. In a large MAGIC population with >1000 RIL it is also possible to assess epistatic as well as G×E interactions to understand complex traits like yield, quality traits, abiotic stress tolerance etc. Keurentjes et al. (2011) have made a comparison between the ability of detection of QTL for flowering time in *Arabidopsis* using different mapping populations. They pointed out detection of many QTL at similar positions using various populations. However, the number of alleles detected was much lower in multiparent populations. Huang et al. (2011) attributed this to the complexity of the genetic interaction leading to

Table 1. Comparison between biparental linkage analysis, association mapping and MAGIC.

Properties	Biparental	Association	MAGIC	AMPRIL
Founder parents	2	≥100	≥8	8
Crossing requirement	Yes	No	Yes	Yes
Time to establish	Moderate	Low	Long	Long
Population size	~200	~100	~1000	~500
Suitability for coarse mapping	Yes	No	Yes	Yes
Suitability for fine mapping	No	Yes	Yes	Moderate
Amount of genotyping required	Low	High	High	High
Amount of phenotyping required	Low	High	High	High
Relevance of population structure	No	Yes	No	No
Statistical complexity	Low	High	High	High
Use of germplasm variation	Low	High	High	High
Practical utility	Low	High	High	Moderate
Relevance over time	Low	High	High	High

Modified from Cavanagh et al. (2008)

dilution of QTL detection power. They hypothesized that due to comparable allelic effects among founder relatives, few QTL remained undetected in their study. Secondly, they also suggested that segregation of QTL with different effects in different subpopulations needed to be accommodated in the QTL model. In this regard, the recent analysis environment R/mpMap developed by Huang and George (2011) may be helpful.

Another limitation of MAGIC or similar multiparent intercross populations is that they are likely to show extensive segregation for developmental traits, like maturity and plant height. Segregation for such traits may influence the overall performance for complex traits like yield or drought tolerance. Thus, it may limit their utility in analysis of such traits (Varshney *et al.* 2009; Gupta *et al.* 2010). A careful selection of parents can address this limitation. Other limitation of such mapping resources is that development of such population needs more time and resources than traditional mapping populations (table 1). Large scale phenotyping of such a population is another limitation which may be addressed using high throughput phenotyping methods. However, such facilities are not readily available with the breeders, limiting wider applicability of such next generation mapping resources.

Conclusions

MAGIC and similar populations combine the advantages of linkage analysis and association studies. Thus, they have immense potential in augmenting the QTL analysis. With availability of genome sequence in several crops including rice, sorghum and maize, and initiation of sequencing projects in many more, MAGIC populations will be an ideal resource to generate high-density maps using germplasm of direct relevance to the breeders. With the availability of next generation sequencing and advanced statistical analysis platforms such analysis will be further practical and affordable. Since such populations can be tested across environments, and by various investigators, there is an urgent need to initiate MAGIC population development in a consortium manner taking clue from Complex Trait Consortium in mice.

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Appendix

Glossary of terminology used.

Terminology	Definition
Advanced intercross lines (AIL)	AIL are randomly and sequentially intercrossed population that initially originated from a cross between two inbred lines
Association mapping	Association mapping, also known as linkage disequilibrium mapping, is a method of mapping QTL that takes advantage of historic linkage disequilibrium to link phenotypes (observable characteristics) to genotypes by establishing statistical association
Backcross population	Segregating population derived by back crossing F ₁ hybrid to one of its parents
Backcross inbred lines (BIL)	Inbred lines derived from backcross populations through repeated selfing
Double haploids (DH)	Individuals obtained by chromosome doubling of haploids
F ₂ populations	Progenies obtained by selfing of or intermating among F ₁ hybrids
G×E interactions	A condition where the relative differences among phenotypes and/or their ranking vary according to environmental conditions
Genetic or linkage maps	A linear or circular diagram that shows the relative positions of genes on a chromosome as determined by linkage analysis
Heterogeneous	Organisms with dissimilar traits
Intergenic interactions (epistasis)	A situation of effect of one gene being influenced by the alleles of another gene
Markers	A phenotypic trait or a DNA sequence which can be placed on linkage maps
Multiparent advanced generation intercross (MAGIC) population	Segregating population derived through intercrossing multiple parents (≥8)
Nested association mapping (NAM) population	A population developed by pooling equal number of progenies from large number of crosses involving one common parent
Polygenes	One among many genes involved in quantitative inheritance
Quantitative trait loci (QTL)	Region of the genome that is associated with an effect on a quantitative trait
Recombinant inbred lines (RIL)	Fixed population developed by selfing each F ₂ individuals from a biparental cross

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