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Interval-specific congenic strains (ISCS): an experimental design for mapping a QTL into a 1-centimorgan interval

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Abstract. A general experimental design that allows mapping of a quantitative trait locus (QTL) into a 1-cM interval is presented. The design consists of a series of strains, termed "interval-specific congenic strains (ISCS)". Each ISCS is recombinant at a specific 1-cM sub-interval out of an ordered set of sub-intervals, which together comprise a wider interval, to which a QTL was previously mapped. It is shown that a specific and previously detected QTL of moderate or even small effect can be accurately mapped into a 1-cM interval in a program involving a total of no more than 1000 individuals. Consequently, ISCS can serve as the ultimate genetic mapping procedure before the application of physical mapping tools for positional cloning of a QTL.

Introduction

Many traits of human biological and economical significance are quantitative in nature. Because of the multiplicity of factors involved in trait variation, genetic analysis of quantitative traits by classical Mendelian methods is not possible. The advent of methods for uncovering protein and DNA polymorphisms has allowed procedures for quantitative trait loci (QTL) mapping through linkage to genetic markers to be extended to numerous experimental and agricultural species (Flint et al. 1995; Hilbert et al. 1991; Nienhuis et al. 1987; Paterson et al. 1988; Weller et al. 1988). To this end, various statistical methods have been developed to exploit the genotypic and phenotypic information in a mapping population to a maximal extent (Weller 1986; Jensen 1989; Keightley and Bulfield 1993; Lander and Botstein 1989; Knapp et al. 1990; Haley and Knott 1992; Darvasi et al. 1993, Darvasi and Soller 1994; Jansen 1993); and several approaches have been developed in order to increase mapping accuracy, among them the use of multiple markers (Jansen and Stam 1994; Zeng 1993, 1994), multiple trait analysis (Korol et al. 1995; Changjian and Zeng 1995), and advanced intercross lines (AIL) (Darvasi and Soller 1995). However, even with these methods, it is not feasible to map a QTL of moderate effect to within a confidence interval that is sufficiently narrow (say 1 cM) to provide a basis for positional cloning.

Drosophila geneticists have introduced a procedure that may be termed "genetic chromosome dissection" (GCD) for QTL mapping. In this procedure, genetic markers are used to construct chromosomes recombinant for known specific regions, and QTL are assigned to specific chromosomal regions according to the quantitative effects exhibited by lines specific for the various recombinant chromosomes (Breese and Mather 1957; Davies 1971; Shrimpton and Robertson 1988a, 1988b). With the advent of DNA level polymorphisms, GCD methods have become generally applicable to experimental and agricultural populations. This was first proposed and demonstrated by Paterson and associates (1990), in which GCD mapping was successfully applied to mapping QTL in an interspecific cross in tomato. Eshed and Zamir (1995) further produced a set of introgression lines (IL) for fine QTL mapping. Through this process a QTL affecting fruit mass in tomato was mapped to an interval of 3.2 cM. Additionally, advanced backcrosses (the AB-QTL design) has been suggested and used as an efficient mapping population, taking advantage of the reduction in genetic variation (Tanksley and Nelson 1996; Tanksley et al. 1996). In the present study, the above concepts are integrated to present a formalized design under the GCD framework, for fine QTL mapping. The design is based on systematic marker-directed construction of a series of congenic strains (for example, Frankel et al. 1995; Morel et al. 1996; Yui et al. 1996), each with a recombination point in a specific interval out of a series of small tandem intervals covering a chromosomal region of interest. These strains are termed "interval-specific congenic strains" (ISCS). Given the current ability to saturate specific chromosomal regions with markers to almost any desired density (Tanksley et al. 1995), it is shown that an appropriately design small set of ISCS can readily provide a 1-cM resolution for QTL mapping.

Theory and results

Assumptions and notations. Let $Q_1, Q_2, \ldots, Q_n$ represent the n QTL previously identified as differentiating the two inbred strains. It is assumed that (i) Q_1 to Q_n explain most of the genetic variation in the trait of interest (a slight modification appropriate when this assumption does not hold will be discussed); (ii) estimates of gene effect are available for each of the Q_i; (iii) each Q_i has already been mapped, with high confidence, into an interval i with length of L_i cM; (iv) each interval i has been, or can be, saturated with markers, $M_{i,0}, M_{i,1}, \ldots, M_{i,Li}$, at a 1-cM marker spacing.

Production of the ISCS. An F_2 population is produced from the two parental strains. Each F_2 individual is genotyped at n pairs of markers, $M_{i,0}$ and $M_{i,Li}$ ($i = 1 \ldots n$), flanking each of the n, QTL-containing intervals. Individuals with a recombination event detected at one or more of the n intervals are retained; all others are discarded. Recombinant individuals at interval i are also genotyped with all $M_{i,0}$ to $M_{i,Li}$ markers, to locate the point of recombination within a specific $M_{i,j}$–M_{j+1} interval. F_2 individuals will be progressively generated and genotyped until at least one recombinant is obtained within each of the L_i intervals for each of the n QTL. From this point, each QTL will be treated independently, and a specific set of ISCS will be developed to map each QTL. Since we now consider only one specific interval, the subscript notation "i" from here on is omitted from all symbols.

Producing an ISCS requires retaining a specific recombinant donor interval in the chromosomal region of interest, while removing all other donor chromosomal material that has an effect on the quantitative trait of interest. This is achieved by (i) recurrent backcrossing of the recombinant interval into the recipient genome, and

(ii) simultaneous marker-assisted selection against all other donor chromosomal intervals known to contain other QTL. Specifically, to initiate the j-th ISCS for mapping a specific QTL, at least one individual with a recombination at the M_j–M_{j+1} interval is backcrossed to one of the parental strains (the recurrent strain). Since j ranges from 0 to L-1, L strains are initiated in each QTL-specific mapping set of ISCS. At the j-th ISCS, the markers M_j and M_{j+1} serve as the selecting markers for the desired recombinant interval and are genotyped. Individuals that do not retain the M_j–M_{j+1} recombinant chromosome are discarded. Individuals that retain the desired recombinant chromosome are now examined with respect to donor alleles flanking all other QTL-containing chromosomal regions. Individuals carrying a minimum number of flanking donor marker alleles are retained and utilized as donor parents in a further round of backcrossing and selection. This procedure is repeated for a total of two or three backcross generations, depending on the number of QTL-containing regions that must be monitored. At the end of this procedure, individuals are obtained that are partially heterozygous at the chromosomal region of interest (with a recombination at a specific M_j–M_{j+1} interval), but are homozygous for recurrent parent QTL affecting the trait of interest at all other chromosomal regions.

One generation of brother-sister matings (or selfing in plants) is now carried out and homozygotes for the M_j–M_{j+1} recombinant donor-interval are selected for further matting. This will establish the j-th ISCS, homozygous for a single recombinant donor interval, and otherwise homozygous for recurrent parent QTL. At this point, it is also appropriate to genotype each strain with all M_1 to M_L markers to verify that only a single recombinant point is present within the entire M_0–M_L interval.

Mapping the QTL with the ISCS. The j-th ISCS is constructed to carry a recombinant haplotype at the M_j–M_{j+1} interval. That is, the j-th ISCS will carry either the donor marker allele or the recurrent parent marker allele at the M_j marker, and the alternative marker allele at the M_{j+1} marker. It will also carry either the donor or the recurrent parent allele at the QTL. The marker status will be known through the production of the ISCS, while the QTL status of the j-th ISCS can be determined by phenotyping a set of N individuals from that strain.

According to the marker and QTL status, the j-th ISCS will necessarily locate the QTL to either the M_0–M_{j+1} or M_j–M_L intervals. Consider now the case where the true QTL location is at the interval M_j–M_{j+1}. The j − 1 ISCS will necessarily locate the QTL at the right-hand interval M_{j-1}–M_L, while the j + 1 ISCS will necessarily locate the QTL at the left-hand interval M_0–M_{j+2}. Depending on exact QTL and recombination point location, the j-th ISCS will locate the QTL, either at the left-hand interval M_0–M_{j+1} or the right-hand interval M_j–M_L. The QTL can now be located according to the overlap of the left-hand or right-hand intervals determined by the j-th ISCS and either the right-hand interval determined by the j − 1 ISCS or the left-hand interval determined by the j + 1 ISCS. In either case (that is, considering the j-th ISCS and the j − 1 ISCS, or considering the j-th ISCS and the j + 1 ISCS), the overlapped region will be 2 cM wide defined by the intervals M_{j-1}–M_{j+1} or M_j–M_{j+2}. Actually, however, the two critical ISCS map the QTL to an interval smaller than 2 cM. This smaller interval is defined by the two points of recombination within the M_j–M_{j+1} and M_{j+1}–M_{j+2} (or M_{j-1}–M_j and M_j–M_{j+1}) intervals and has an expected length of 1 cM. Genotyping additional markers within the M_j–M_{j+2} (or M_{j-1}–M_{j+1}) interval will locate more precisely the limits of the expected 1-cM interval that contains the QTL. A schematic example of this procedure is presented in Fig. 1.

Exact determination of the marker and QTL status in either of the two ISCS, which define the QTL position, is crucial. Therefore, at these two ISCS, marker and QTL status should be verified.

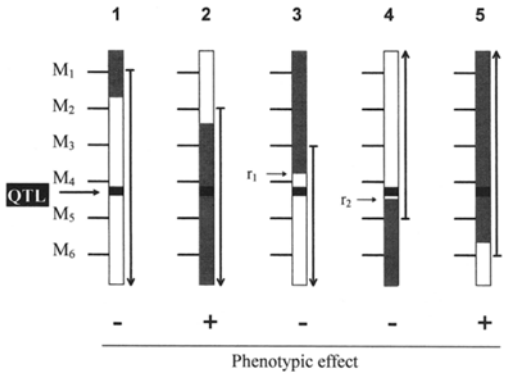

Fig. 1. A schematic example of ISCS mapping for a QTL previously mapped to a 5-cM interval. Dark and white fragments represent donor and recipient genome respectively. Five ISCS are constructed; each locates the QTL according to the phenotypic effect (+, the ISCS retained donor QTL allele; −, the ISCS did not retain donor QTL allele) and the marker information, as indicated by the arrows. In this example, ISCS 3 and 4 locate the QTL to the M_3–M_5 marker interval. Genotyping additional markers within the M_3–M_5 interval can locate the QTL to the smaller r_1–r_2 interval, defined by the two closest points of recombination to either side of the QTL.

Verifying marker status is done, simply, by repeating the genotyping; verifying QTL status is somewhat more laborious and will require phenotyping additional individuals from these two lines to reduce the statistical error of QTL assignment from, say, 0.05 to, say, 10^{-4}.

Mapping linked QTL. The presence of two QTL in a given donor chromosome may have been recognized in the initial mapping exercise, if the QTL were reasonably far apart one from the other (Zeng 1993, 1994). However, if the QTL were closely linked, they would probably have been detected as a single QTL and mapped to a single chromosomal region. If this is the case, some of the ISCS will show the expected effect (they will contain both QTL), some of the ISCS will show no effect, and some of the ISCS will show partial effect. Some of the ISCS showing partial effect will not show any overlapping of donor genome, indicating the presence of two QTL. When evidence for such an effect is found, it will be necessary to increase the number of individuals phenotyped at each ISCS to determine, with higher significance, the corresponding quantitative effect of that strain. In this way, however, QTL separated by as little as 1–2 cM can be independently mapped and their individual effects evaluated.

Number of progeny required in the initial F_2 population. The F_2 sample size required to obtain at least one recombinant in each 1-cM interval depends directly on the total number of 1-cM intervals being screened (denoted m) taken over all QTL being mapped. Thus, m is determined by the number of QTL and by the accuracy with which these QTL have been previously mapped. For example, if 2 QTL are being followed, and these were previously mapped to separate 5-cM intervals, m will equal 10. Since 1/100 is the probability of recombination in a single 1-cM interval, the number of meioses until a recombination is obtained in one of the m intervals follows a geometric distribution with probability parameter $p_0 = m(1/100)$ and expectation $1/p_0$. After a recombinant is identified in one of the m intervals, the additional number of meiosis that must be scanned until a recombinant is obtained in one of the m-1 remaining intervals follows an independent geometric distribution, with probability parameter $p_1 = (m - 1)/100$ and expectation $1/p_1$. This continues, with additional independent geometric distributions for each additional interval having respective probability parameters of $p_i = (m - i)/100$ until $i = m - 1$, which represents the

case of the last interval without recombination. Thus, the expected total number of meioses, I, that must be scanned to provide at least one recombinant within each interval will be the sum of all the expectations for each interval:

$$I = \sum_{i=0}^{m-1} \frac{1}{p_i} = 100 \sum_{i=0}^{m-1} \frac{1}{m-i} = 100 \sum_{i=1}^{m} \frac{1}{i}$$

This represents the sum of the well-known harmonic series and is calculated numerically.

Each F_2 individual provides two informative meioses with respect to a possible recombination event within any 1-cM interval. Thus, the expected number of F_2 progeny that will provide at least one recombination at each 1-cM interval is simply 1/2. A single F_2 individual showing recombination in more than one of the m intervals is crossed twice or more, to originate two or more independent ISCS. This may provide some reduction in the number of F_2 individuals required.

Figure 2 presents the expected number of F_2 individuals that are required to obtain at least one recombinant within each of the m intervals. For example, if one QTL, previously mapped to a 10-cM interval, is being mapped, then m = 10 and the expected number of F_2 progeny screened to obtain a recombinant in each of the m intervals is approximately 150. If 10 QTL, each previously mapped to an independent 10-cM interval, are being mapped, m = 10 and the expected number of F_2 progeny screened to identify all 100 desired recombinants is only 260. This is almost a sixfold reduction relative to the 1500 that would have been required had screening been carried out for each QTL in a separate experiment. It clearly follows from this that at this stage significant savings can be obtained by dealing with all QTL simultaneously.

Number of individuals required within a single ISCS. The number of individuals required to determine which QTL allele is present in a given strain depends on the standardized allele substitution effect, d, of the QTL. Since in an ISCS the genetic variance is close to 0, the standardized difference between the expectation of the two homozygous QTL genotypes, 2δ, is approximated by:

$$2\delta = \frac{2d}{\sqrt{1-h^2}}$$

where h^2 is the broad-sense heritability of the trait. Thus, the number of individuals, N_r, replicated from a specific ISCS required to determine the QTL genotype with a confidence level, α, is:

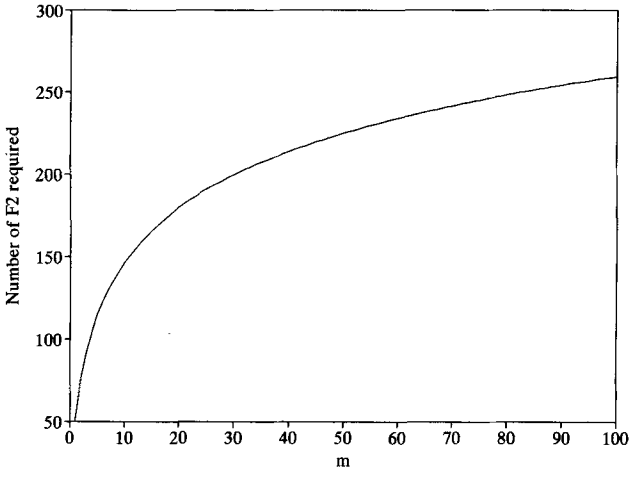

Fig. 2. The number of F_2 population required to identify m (1 cM wide) recombinant intervals.

$$N_r = \left(\frac{Z_{1-\alpha}}{\delta}\right)^2$$

where $Z_{1-\alpha}$ is the truncation point of a standard normal distribution at a level of 1 − α. It should be noted that the two alternative hypotheses (that is, the donor QTL was retained versus the donor QTL was not retained) are symmetrical, and thus α represents both type I and type II errors. The expression for N_r is exact when d is known and is a close approximation when the estimate of d has a small standard error. Otherwise, N_r should be increased to maintain the desired type I and type II errors.

Table 1 presents the number of individuals required within each strain to determine the presence or absence of the relevant QTL. Two significance levels are presented: 0.05, which is appropriate for the initial tests, and 10^{-4}, which is appropriate for retesting the two lines found to determine the QTL location.

Discussion

The ISCS design can serve as an efficient means for mapping a QTL into an interval that will be small enough (on the order of 1 cM) to proceed with positional cloning of the gene. The procedure does not require an excessive amount of rearing, phenotyping, or genotyping. For example, if a QTL has been previously mapped to a 10-cM interval, then about 150 F_2 individuals will be initially required to identify suitable recombinants, and 10 ISCS will be produced. The production of each strain, as here described, will require a very small number of individuals, usually not more than 40. Each strain will be genotyped, even for small effects, again for no more than 40 individuals. Then, the two lines determining the QTL location will be retested with usually not more than 100 additional individuals. Thus, the total number of individuals required in this case will be about 1000. This will allow a mapping resolution of 1 cM. Mapping accuracy in a standard F_2 population, defined as the 95% confidence interval with a dense genetic map, can be estimated by use of the Darvasi and Soller (1997) formula: CI = $1500/Nd^2$ (where, CI = 95% confidence interval; N = population size; and d = standardized gene effect). Thus, achieving the 1-cM mapping resolution in an F_2 population for a large effect (d = 0.5) or a moderate effect (d = 0.25) will require 6000 or 24,000 individuals, respectively. Furthermore, an F_2 or backcross population provides mapping resolution as an estimated 95% confidence interval, which may be biased owing to statistical assumptions, whereas ISCS provides a marker-defined interval with practically 100% confidence of containing the gene. Thus, ISCS presents a means of mapping a selected number of QTL into a 1-cM interval, which is unachievable in practice with standard F_2 or similar populations. It should be noted, however, that in a backcross or F_2 population, all QTL are mapped with the same population, whereas in ISCS each set of strains will locate only one QTL.

Recombinant congenic strains (RCS) are produced by a series of backcrosses to a recurrent parent followed by inbreeding (Wehrhahn and Allard 1965; Demant and Hart 1986). ISCS are similar to RCS in that polygenic effects are dissected to different strains. In contrast to RCS, however, ISCS (i) are designed to be recom-

Table 1. Number of individuals required to determine QTL status in a given strain.

Standardized gene effect (d)	Heritability	Significance level 0.05	Significance level 10^{-4}
0.25	0.2	41.6	188.2
	0.5	32.5	147.0
	0.8	15.6	70.6
0.5	0.2	10.4	47.0
	0.5	8.1	36.8
	0.8	3.9	17.6

binant at a specific series of chromosomal locations; (ii) carry only specific QTL-containing chromosomal regions; and (iii) are inbred only to the extent required.

The ISCS can be produced sequentially. In the first stage, fewer strains are produced with a wider interval than the 1-cM intervals suggested here. Then in the second stage, 1-cM intervals are produced in smaller regions, as determined in the first stage. This can significantly reduce the number of strains required. Nevertheless, the simultaneous production of the entire set of ISCS saves time. It also serves as an internal error control. That is, if one ISCS provides erroneous information, there are good chances that it will be in conflict with the information provided by other strains and thus will be detected.

ISCS are most appropriate for species with a relatively short generation cycle and a dense genetic map. The mouse is, therefore, an obvious candidate on both counts for the application of ISCS. In cases where a dense marker map is not available, the ISCS required to provide a 1-cM mapping resolution can be produced by the two-stage procedure. At the first stage, wider intervals are used with existing markers. The production of new markers in the region of interest is now required to proceed with the second stage. It should be noted, however, that a dense marker map at the region of interest will be required for fine mapping regardless of the method applied.

In case the detected QTL do not explain most of the trait variation, an additional step should be included in the production of ISCS; namely, further backcrossing, before the homozygotization step, should be carried out to eliminate residual donor alleles at chromosomal regions, other than those carrying detected QTL. Alternatively, as outlined by Jacob and colleagues (1991), all the additional backcrossing can be carried out in one line only. That is, produce one congenic strain carrying the donor QTL-containing chromosomal region to serve as parental lines for the production of the ISCS.

The analysis of linked QTL in a standard mapping population is complex (Lander and Botstein 1989). Even with the implementation of sophisticated statistical methods specifically designed for this purpose, the identification of closely linked QTL does not seem possible with realistic sample sizes (Zeng 1993, 1994). ISCS, however, may be an important experimental resource for disentangling tightly linked QTL (that is, separated by only 1–2 cM).

As here presented, the ISCS are initiated from an F_2 population. An F_2 is preferred over a backcross, for example, since in F_2 the chances of obtaining a recombinant offspring are twice as great as in a backcross. If, however, at the initial stage of the experiment, other sorts of intercrossed populations between the two lines of interest are available, these can be used for the screening of recombinants. Thus, existing backcross populations can be used. Other population designs, when available, may have particular advantages. In particular, (i) with recombinant inbred strains (RIS), the points of recombination in each line are known, so that individuals with recombinations at the desired locations can be used directly; (ii) with advanced intercross lines (AIL) (Darvasi and Soller 1995), a significantly higher proportion of recombination is present over the entire genome, thus fewer individuals will be scanned to find specific recombination sites; (iii) with introgression lines (IL; Eshed and Zamir 1995), any individual with an introgressed chromosomal region has already been purified of all other donor genome and can serve directly for production of recombinant F_2 in the desired chromosomal region. In any of the above cases, the identified recombinant individual will be backcrossed to the recurrent parent (in IL a further intercross will be necessary to identify recombination points), and the ISCS will be produced as previously described.

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