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Simulation of the distribution of parental strains' genomes in RC strains of mice

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Abstract. Recombinant Congenic strains (RC strains) were developed to facilitate mapping of genes influencing complex traits controlled by multiple genes. They were produced by inbreeding of the progeny derived from a second backcross from a common 'donor' inbred strain to a common 'background' inbred strain. Each RC strain contains a random subset of approximately 12.5% of genes from the donor strain and 87.5% of genes from the background strain. In this way the genetic control of a complex disease may be dissected into its individual components. We simulated the production of the RC strains to study to what extent they have to be characterized in order to obtain sufficient information about the distribution of the parental strains' genomes in these strains and to acquire insight into parameters influencing their effectiveness in mapping quantitative trait loci (QTLs). The donor strain genome in the RC strains is fragmented into many segments. Genetic characterization of these strains with one polymorphic marker per 3.3 centiMorgans (cM) is needed to detect 95% of the donor strain genome. The probability of a donor strain segment being located entirely in between two markers of background strain origin that are 3 cM apart (and hence escaping detection) is 0.003. Although the donor strain genome in the RC strains is split into many segments, the largest part still occurs in relatively long stretches that are mostly concentrated in fewer than 13 autosomes, the median being 9 autosomes. Thus, in mapping QTLs, the use of RC strains facilitates the detection of linkage.

Introduction

The understanding of the genetics of many diseases that are caused by an alteration in a single gene is advanced and has resulted in the widespread practice of genetic counseling and attempts at gene therapy. In contrast, the multigenically controlled diseases such as cancer and atherosclerosis are more common than the single-gene diseases but remain poorly understood. Even in the mouse, where inbred strains and experimental models of human diseases are available, the analysis of multigenic traits is hampered by the complexity of the genetic system.

Recombinant Congenic (RC) strains have been proposed as a genetic tool for the analysis of multigenic traits, such as tumor susceptibility (Demant and Hart 1986). The advantage of the RC strain system is that a complex phenomenon controlled by multiple genes can be efficiently analyzed by separating non-linked genes that control a trait into different strains having largely the same genetic background. In this way, we should be able to identify the particular genes and study them individually. A series of RC strains is produced by crossing two standard inbred strains, one of which serves as a background strain, the other as a donor strain. Two generations of backcrossing to the background strain, followed by brother-sister mating, produce a series of new homozygous strains (the RC strains) each of which carries a random fraction of only about 12.5% of the genome from the donor strain and 87.5% of the genome from the common background strain.

Three series of RC strains of mice were produced: BALB/c-c-STS/Dem (CcS/Dem), C3H-c-C57BL/10/Dem (HcB/Dem), and O20-c-B10.O20/Dem (OcB/Dem). In these series of RC strains, the chromosomal segments derived from their respective donor and background strains were identified by typing them for a large number of markers (Moen et al. 1991; Groot et al. 1992, 1996; Stassen et al. 1996). The proportion of the alleles of background and donor strain origin in each of the three series corresponds to the expected ratio of 7:1.

To map a quantitative trait locus (QTL) of interest, one has to determine which genetic marker correlates with the quantitative trait studied using a backcross or an F_2 cross between an RC strain and the background strain. Initially, the CcS/Dem series was used to study colon tumor susceptibility (Moen et al. 1991, 1996b), for which several genes have been mapped: *Scc1, -2, -3, -4, -5;* Susceptibility to *c*olon *c*ancer *1, 2, 3, 4,* and *5* on mouse Chromosomes (Chrs) 2, 2, 1, 17, and 18, respectively (Moen et al. 1992, 1996a; van Wezel et al. 1996). Additionally, these CcS/Dem strains were used to study immunological reactivity (Lipoldová et al. 1995; Holan et al. 1996) and susceptibility to radiation-induced apoptosis in thymus, which revealed three new loci (*Rapop1, -2,* and *-3* on mouse Chrs 16, 19, and 3, respectively; Mori et al. 1995a, 1995b). The OcB/Dem strains were used to study lung tumor susceptibility (Fijneman et al. 1994) and were instrumental in mapping of the susceptibility genes *Sluc1, -2, -3* and *-4* (Fijneman et al. 1996).

Until now, the distribution of the lengths of the donor strain segments in the RC strains has not been established precisely. There are two extreme possibilities: (a) the genetic material of donor strain origin is divided into many short segments on most or all chromosomes, or (b) it is concentrated into a limited number of long segments on a few chromosomes whereas the rest of the genome contains only genetic material of the background strain. Obviously, the shorter the donor strain segments in the RC strains are the higher the number of genetic markers for which they have to be typed to detect most of the donor strain's contribution. Moreover, once linkage has been found, the fine mapping of a QTL is much easier in the second case, because the QTL and the marker to which the linkage has been found are more likely to be located on the same donor strain-derived segment. It is, therefore, of great

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convenience for the fine mapping of a QTL to know for an RC strain the probability that two linked markers originating from the same parental strain are separated by one or more segments of the other parental strain.

Finally, as the efficiency of detecting linkage is higher when the number of segregating chromosomes is smaller (Lander and Schork 1994), it is relevant to establish how many of the autosomes in an RC strain actually carry any genetic material of donor strain origin.

Although some of these questions can be solved by theoretical statistical analyses, it is simple to perform numerical simulations, especially since the development of such simulations is facilitated by recent advances in software engineering technology. With the introduction of ''object-oriented'' design and efficient computer languages that support object-oriented techniques (Cox 1986; Stroustrup 1991) it has become possible to create computer programs that use ''software objects'' mimicking ''biological objects'' rather closely in terms of the information they contain and the operations that they perform. The rather direct correspondence between the simulated process and the simulating computer program simplifies understanding of the computer program and reduces the probability of conceptual errors. This approach also permits further study, such as simulation of the segregation of QTLs etc.

Here we report the construction of an ''object-oriented'' program for the mouse genetics that has been tailored to simulate the construction of RC strains. Using this program, we simulated the construction of 15,000 RC strains (that is, 15,000 brother-sister *pairs* of mouse genotypes in each generation including the 20^{th} inbred generation). The resulting database of genotypes provides information about the expected distribution of the genetic material from the background and donor strain the genomes of the RC strains.

Materials and methods

Object-oriented simulation of RC strains. The creation of the RC strains simulation program was divided into two phases. First, object types and their associated operations were defined and implemented for each genetically relevant concept. Second, a simulation program was constructed in terms of these objects performing their characteristic operations. The key object types defined for our simulation are *chromosome, chromosome pair, haploid* and *diploid.*

Chromosomes are represented by ordered lists of value pairs **[type, length].** The value pairs encode continuous regions of genomic material originating from the same parental strain or ''type'' with a particular length expressed in centiMorgan (cM). The lists are ordered in such a way that the first segment in the list is located at the centromere (all mouse chromosomes are acrocentric). The distance between the centromere and subsequent segments in the list is calculated by summing the lengths of preceding segments. *Chromosome pairs* (the software objects) are simply pairs of ordered segment lists of *chromosomes* (which in this context represent the individual chromosomes as derived from the second meiotic division). Thus, for example, a 100-cM chromosome pair **''CHR''** on which all loci are homozygous, except for the small region between cM 24.5 and cM 26.022, is represented by the following pair of ordered segment lists, **''chr a''** and **''chr b'':**

chr a: {C, 20}, {S, 12.022}, {C, 40.978}, {S, 7.0}, {C, 20}.
chr b: {C, 20}, {S, 4.5}, {C, 1.522}, {S, 6.0}, {C, 40.978}, {S, 7.0}, {C, 20}.[1]

A *haploid* is the representation of a single set of chromosomes in a gamete. A *diploid* is built from two *haploids* and thus contains the representation of a double set of chromosomes. A *diploid* can perform a meiosis operation to produce a new *haploid.* Two such new *haploids* can be joined to create a new *diploid.* A multiplication operator for *diploids* is defined as follows. When two parental *diploids* are multiplied they both perform *meioses* and the resulting *haploids* are joined into an offspring *diploid.* This offspring *diploid* is the result of the multiplication. Once these types and their operations are implemented the construction of a simulation program for a particular breeding scheme is fairly simple. Figure 1 (see appendix) shows how the top level procedure of a program, *Make_RC_strain,* is implemented in terms of multiplying *diploids* to simulate a particular breeding scheme of (back)crossing and subsequent inbreeding.

From a modeling perspective, the simulation of the meiosis process can be considered the essence of the simulation, since in this procedure we lay down most, if not all, the assumptions of our model. For all pairs of chromosomes in the meioses we assume that crossover events are uncoupled, that is, that the occurrence of one crossover event on a chromosome does not influence the probability of the occurrence of another crossover event, nor does it bias the location of its occurrence in any particular way. A more complex model assuming various degrees of interference may be analyzed in the future. In the absence of particular hypotheses on the coupling of crossover events, we simply assume that in a large sample of meioses, the number crossover events on chromosomes with length μ, in Morgan, is described by a Poisson distribution with mean μ and that locations of crossover events are uniformly distributed over the chromosome, between 0 and μ Morgan.

Thus, in the meiosis procedure we first determine the number of crossover events for a particular chromosome, by generating a Poisson random deviate for a distribution with mean μ equal to the length of the chromosome in Morgan. Subsequently, to determine the locations of these crossover events, we generate random deviates uniformly distributed between 0 and μ. Finally, when all crossover locations have thus been determined and processed, a random deviate uniformly distributed between 0 and 1.0 is generated to determine which of the ''haploids'' is returned to be included in the gamete that is used in further reproduction. Figure 2 (see appendix) shows the C++ code for the part of the diploid's meiosis procedure that embodies the above-mentioned assumptions. The pseudo-random number generators *PoissonRandomDeviate* and *UniformRandomDeviate* are ''packaged'' versions of the random number generators published by Press and associates (1992).

```
Make_RC_Strain ( )
{
    // Reserve storage for 21 generations of diploids
    static diploid brother [21] ;
    static diploid sister [21] ;

    int i ;                                          // "loop counter"
    diploid F1 = background ( ) * donor ( ) ;        // cross
    diploid backcross1 = background ( ) * F1 ;       // 1st. backcross

    // In order to implement "inbreeding with a simple loop, backcross 2 specimen are
    // stored at offset 0 in arrays of diploid "brother" and "sister".
    brother [0] = backcross1 * background ( ) ;      // 2nd. backcross
    sister [0] = backcross1 * background ( ) ;       // 2nd. backcross

    // Inbreeding for 20 generations
    i = 1 ;
    while (i < 21) {
            brother [i] = brother [i-1] * sister [i-1] ;
            sister [i] = brother [i-1] * sister [i-1] ;
            i = i + 1 ;
    }

// Save arrays of diploid "brother" and "sister" to file ... (code not shown)
}
```

Fig. 1. Top-level procedure of the computer simulation program 'MAKE_RC_Strain,' which simulates the breeding scheme of the RC strains. This procedure is implemented in terms of multiplying diploids (see also Fig. 2) to simulate the breeding of (back)crossing and subsequent inbreeding.

[1]This is an example for the CcS/Dem series of RC strains in which C has been used to denote the background parental strain, in this case the mouse inbred strain BALB/cHeA, and S has been used to denote the parental donor strain STS/A.

```
chromosome diploid::meiosis(chromosome chr a, chromosome chr b)
{
    float mu = chra.length_cM() / 100.0;
    int numberofcrossovers = PoissonRandomDeviate( mu );
    float *crossoverlocation = new float[numberofcrossovers];

    int count = 0;
    while ( count < numberofcrossovers ) {
        crossoverlocation[count] = UniformRandomDeviate( 0, mu );
        count = count + 1;
    }

    // Do cross overs between the segment lists chr a and chr b
    // by cutting and pasting them at all the crossover locations
    // just determined (code not included).

    delete [] crossoverlocation;

    if (UniformRandomDeviate( 0, 1.0 ) < 0.5)
        return chr a;
    else
        return chr b;
}
```

Fig. 2. Part of the simulation program 'MAKE_RC_Strain' that shows the meiosis procedure in which the number and location of the crossover events will be determined.

In our simulation program we call the procedure *Make_RC_Strain* (Fig. 1) 15,000 times, to create a database of 15,000 pairs of diploid mouse genotypes per generation, including the 20th generation of inbreeding.

The correctness of the simulation, that is, whether its results indeed reflected the built-in assumptions, was checked and confirmed in the following ways:

1. The crossover locations in the databases generated by our simulations are uniformly distributed over the chromosome.
2. The resulting database contained approximately 12.5% of donor strain genome and 87.5% of background strain genome.
3. The recombination frequency in the simulated RC strains corresponds with the theoretically calculated recombination frequency (data not shown).

The databases thus created have been used in a number of analyses concerning the quantitative make-up of the RC strains.

Results

The genomes of the simulated RC strains (Figs. 1 and 2) comprise 87.5% of the background strain and 12.5% of the donor strain genome. We investigated how the 12.5% of donor strain genome is distributed over segments of various lengths. Figures 3 and 4 show the length distribution of continuous chromosomal segments of donor strain origin in the 15,000 simulated RC strains for both a 50-cM and a 114-cM chromosome. The donor DNA is splintered into small pieces: donor strain fragments 3 cM or shorter represent 23% or 25% of all donor strain fragments (for a 114-cM and a 50-cM chromosome, respectively) while fragments 10 cM or shorter represent 56% or 60% of all donor strain fragments (for a 114-cM and a 50-cM chromosome, respectively; Fig. 4). Obviously, in the case of interference, the segments could be longer. Recently, Weeks and colleagues (1994) analyzed the statistical strategies and sample sizes required to detect interference. In the future these approaches may be applied to analyze the RC strain material. The length distribution depends to some extent on the

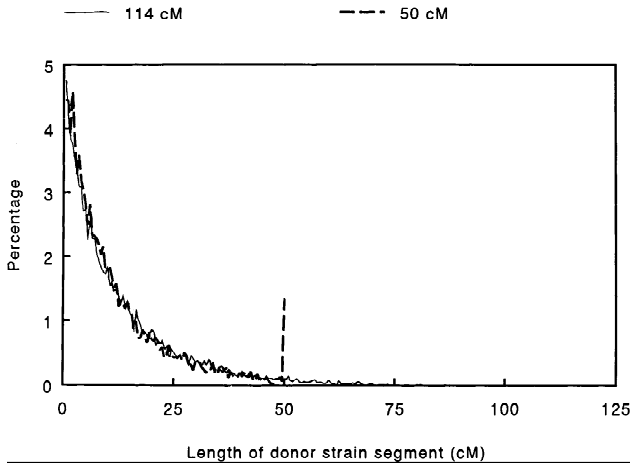

Fig. 3. Length distribution of continuous chromosomal segments inherited from the donor strain in 15,000 simulated RC strains, for both a 50-cM and a 114-cM chromosome (a short and the longest mouse chromosomes, respectively).

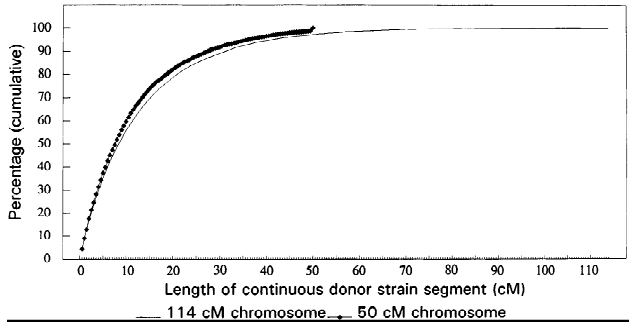

Fig. 4. Cumulative frequency of continuous chromosomal segments of donor strain origin in 15,000 simulated RC strains, for both a 50-cM and a 114-cM chromosome (a short and the longest mouse chromosomes, respectively).

length of the chromosome—in short chromosomes shorter fragments occur. The peak of 1.3% at 50 cM in Fig. 3 shows that about 1.3% of the donor strain segments consist of the intact 50-cM donor strain chromosome.

We have also determined in what percentage of RC strains the 50-cM chromosome of donor strain origin was inherited fully intact. In our simulation we observed a frequency of 1.4% (data not shown), which is equal to the analytically derived frequency, since for an infinite number of RC strains one should expect a frequency of $(e^{-0.5}/2)^3/2 = 1.4\%$.

The fact that the very short segments of donor strain genome occur most frequently does not mean that the largest part of the donor strain genome is present in these short pieces. In Fig. 5 the frequency of each segment (given in Fig. 3) has been multiplied by its length. The result shows that the segments of medium length (5–25 cM) contain 54% of the donor strain genome. The data from Fig. 5 are plotted cumulatively in Fig. 6. These data show that for a chromosome 50 cM long, 80% of all donor strain genes will be presented in segments longer than 9 cM and 50% in segments longer than 19.5 cM. For a chromosome of 114 cM these values are 10.5 cM and 23 cM, respectively.

Subsequently, we have determined what percentage of donor strain genome would be detected in genotyping the RC strains. Therefore, we placed markers randomly on the genomes of the 15,000 RC strains with an average spacing of x markers per centiMorgan and counted the number of donor strain segments that were not detected by any marker. The percentage of donor strain

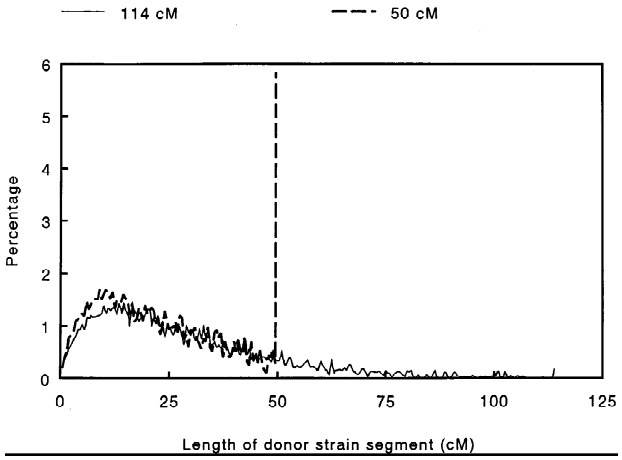

Fig. 5. Percentage of donor strain DNA present in continuous chromosomal segments of different lengths. The length of these donor strain segments is given in cM.

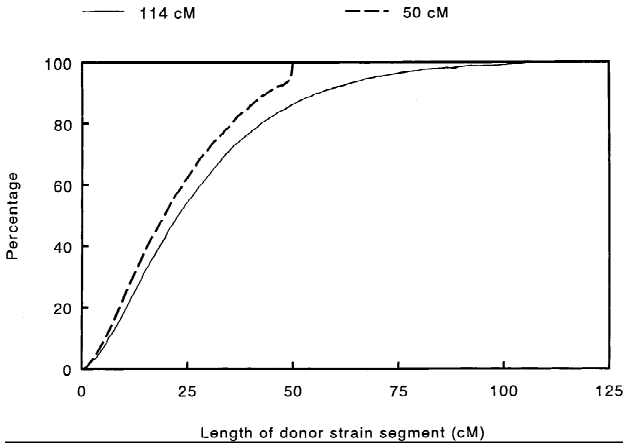

Fig. 6. Cumulative percentage of donor strain DNA in chromosomal segments of different lengths. The length of the continuous chromosomal donor segments is given in cM.

genome that would be detected depends on the marker density and on the length of the chromosome. The higher the marker density is, the higher the percentage of donor strain genome that will be detected on a particular chromosome. On the other hand, with a given marker density, for example one marker each 25 cM, part of the segments smaller than 25 cM will be missed. Figure 6 shows that this fraction of the donor strain segments is smaller in a 114-cM chromosome than in a 50-cM chromosome. Thus, at a given marker density the percentage of donor strain genome that will not be detected in genotyping the RC strains is smaller, the larger the chromosome is. Figure 7 shows the mean percentage of donor strain genome that would be undetected, considering the actual lengths of all 19 autosomes (Evans 1989) as a function of the average marker density. We conclude that an average marker density of 30 markers per Morgan is needed to detect 95% of the total donor strain genome.

The computer-simulated RC strains have also been used to establish the probability of a donor strain segment being inserted between two markers of background strain origin as a function of their distance. This probability increases with the distance between the two markers (Fig. 8). It is rather low (0.8%) if the two background strain markers were 5 cM apart. The probability of DNA of background strain origin to occur between two markers of donor strain origin is higher: 3% if the two donor strain markers are 5 cM apart (Fig. 8).

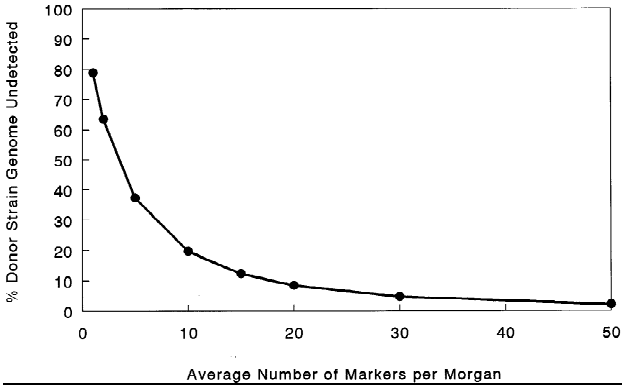

Fig. 7. Percentage of donor strain genome that might be missed in genotyping the RC strains as a function of the marker density. The marker density is given as the number of markers per Morgan. Autosome lengths were adopted from Evans (1989).

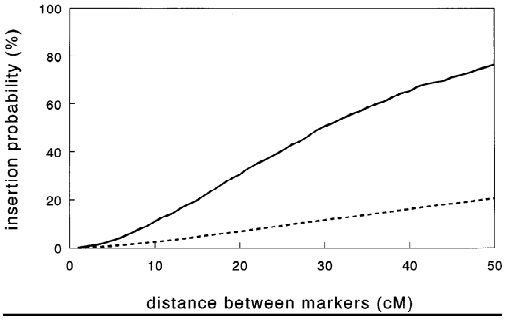

Fig. 8. The probability of a donor strain segment located between two markers of the background strain (- - -) and vice versa (—) as it depends on the distance (cM) between the markers.

We also examined in what percentage of the RC strains two donor strain markers occurred together as a function of their distance. The theoretically calculated frequency of this linkage is

$$y = 1 - [7x/4*(1 + 6x)]$$

[equation (1) in Demant and Hart 1986], where x is the recombination probability at a single meiosis, according to Haldane and Waddington (1931), assuming no interference:

$$x = (1 - e^{-2*d})/2$$

where d is the genetic distance between the two loci in Morgans. The frequency of linkage in the simulated RC strains corresponded with this theoretically calculated frequency (data not shown).

Finally, we determined how many autosomes with at least one segment from the donor strain genome are present in an RC strain (Fig. 9). To this end, the actual lengths of all 19 autosomes were used (Evans 1989). We enumerated autosomes with at least one donor strain segment. The simulation revealed that there are no strains in which there are less than 4 out of 19 autosomes with at least one very small donor strain segment. In 98% of the strains there are at most 13 autosomes carrying a segment of donor strain origin. The median is at 9 autosomes. However, in genotyping experiments, the ability to detect the actual number of autosomes carrying donor strain segments will be hampered by a limited marker density. Nevertheless, we would like to compare these simulation data with data resulting from our genotyping experiments. To this end, we simulated a situation where the genome coverage by polymorphic markers is not dense by ''being blind for''; that is, not counting segments smaller than 5 cM. These values were compared with the actual results of typing the CcS/Dem strains for 326 markers (Groot et al. 1996) that amount to

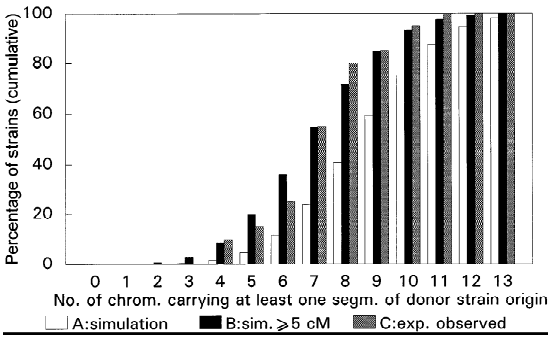

Fig. 9. Percentage of strains carrying a given number of chromosomes with at least one segment of donor strain origin. The open boxes (A) show the simulation data, considering all chromosomes with at least one donor strain segment. The black boxes (B) give the simulation data considering all chromosomes with at least one donor strain segment longer than 5 cM. The dashed boxes (C) show the experimentally observed data from the CcS/Dem series of RC strains.

having approximately 1 marker per 5 cM (Fig. 9). The simulation data, in which all autosomes with at least one donor strain segment longer than 5 cM were taken into account, were similar to the experimentally observed data obtained from the CcS/Dem strains. The number of autosomes with at least one donor strain segment did not exceed 11 in 98% of the strains and was often lower: the median was 7.

Discussion

The RC strains have been proven to be useful for mapping loci involved in multigenic control of quantitative traits such as colon tumor susceptibility (Moen et al. 1991, 1992, 1996a; van Wezel et al. 1996), susceptibility to radiation-induced apoptosis in thymus (Mori et al. 1995a, 1995b), and susceptibility to lung cancer (Fijneman et al. 1996). To map a gene of interest, one has to establish linkage between the quantitative trait studied and a donor strain allele of a known marker, using a backcross or F_2-cross between an RC strain and the background strain, as was shown for the genes *Scc1*, *Scc2* (Moen et al. 1992, 1996a); *Scc3*, *-4*, and *-5* (van Wezel et al. 1996); *Rapop1*, *-2*, and *-3* (Mori et al. 1995a, 1995b); and *Sluc1*, *-2*, *-3*, and *-4* (Fijneman et al. 1996).

One aspect is the assessment of the optimal level of genetic characterization of the RC strains. It is important to known which parts of the genome of the RC strains are inherited from the donor strain, because for the establishment of linkage one has to find a correlation between the studied trait and a donor strain allele of a known marker. If the 12.5% contribution of the donor strain consisted completely of segments as large as an entire chromosome, then one marker per chromosome should suffice to characterize the RC strains. In reality, chromosome crossover reduces the length of the continuous segments of donor strain DNA. Considering this, the genetic map of the RC strains with polymorphic markers must be so dense that the probability to detect all donor strain segments is high. As shown in Fig. 7, 91.6% and 95% of the donor strain genome will be detected when an average marker density of 20 markers per Morgan and 30 markers per Morgan, respectively, is used. Furthermore, as shown in Fig. 8, there is a probability of 0.8% and 0.3% of the presence of a donor strain segment between two markers of background strain origin 5 cM or 3 cM apart, respectively. Hence the marker density on the present genetic map of the CcS/Dem RC strains with polymorphic markers at 5-cM intervals should be increased in order to be able to find nearly any gene.

To establish linkage does not require that both the mapped gene and the marker are located on the same segment of donor strain origin, because establishment of linkage of two genes depends only on the frequency of recombination between them and not on the genetic composition of the segment between the genes. In the case of relatively long donor strain segments, the gene of interest and the linked marker are more likely to be located on the same donor strain segment. Therefore, the longer the donor strain segments are, the easier the subsequent fine mapping of the gene is. Consequently, the information about the length of segments of donor strain origin is useful for assessment of the general suitability of the RC strain system for gene identification.

Lander and Schork (1994) pointed out that the correct assessment of evidence of linkage to a trait depends, among others, on the number of segregating chromosomes. The number of chromosomes segregating in crosses between an RC strain and the background strain is smaller than in crosses between inbred strains, where all autosomes are segregating, because in crosses with RC strains only those chromosomes carrying a segment of donor strain origin have to be considered. Even with a complete coverage of the genome, the number of chromosomes in an RC strain with at least one donor strain segment does not exceed 13 in 98% of the RC strains and is often smaller (the median is 9). Hence, the probability of a false indication of linkage with the RC strain system is smaller.

In conclusion, the computer simulation of the RC strain system demonstrates the suitability of the RC strains for mapping QTLs of interest: with the RC strain system, the false-positive rate in declaring linkage is smaller, and once linkage has been established the QTL is likely to be located on a relatively long segment of donor strain origin (⩾9 cM) that facilitates the fine mapping of the responsible gene.

The programming methodology developed here should be of use for the simulation of various breeding schemes. Its object orientation simplifies its utilization in different contexts. Moreover, it opens the possibility of a direct correspondence between the language of the geneticist and the language of the programmer. For instance, the terminology A*B used by the geneticist to indicate the crossing of strains A and B occurs as A*B in the computer program. The greatly increased speed of desktop computers and the object-oriented programming methodology facilitate the use of advanced simulations so as to optimize breeding strategies.

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