Mammalian Genome 7, 545–548 (1996).

Mammalian Genome

Genomic characterization of two introgression strains (B6.Cb$_4$i$_5$) for the analysis of QTLs

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Received: 17 October 1995 / Accepted: 13 March 1996

The effect of an individual locus that affects complex traits is difficult to detect because it is responsible for only a fraction of the total environmental, genetic, and interaction variation, and its contribution is therefore usually masked. In 1983, to diminish the above-mentioned difficulties, we began the development of a novel approach to QTL mapping, by providing the same standardized genetic background for each QTL that affects a particular trait, and by distributing the QTLs individually in recombinant quasi-congenic lines (Vadasz 1990; Vadasz et al. 1987, 1994a, 1994b). We assumed that the relatively low genetic signal of the distributed QTLs could be amplified in these lines by measuring a number of isogenic animals.

To test this hypothesis, we constructed a series of inbred quasi-congenic recombinant QTL introgression strains by repeated backcross-intercross cycles with concomitant selection for the extreme expressions of a quantitative trait, and fixation of the genes by strict brother-sister mating (Vadasz et al., 1994a). In the present pilot study, in order to assess the amount and distribution of the introduced donor genome, we tested the B6 background strain, the BALB/cJ (C) donor strain, and two strains of the B6.Cb$_4$i$_5$ series, which had been developed by four backcross-intercross cycles with concomitant selection for high midbrain tyrosine hydroxylase (TH) activity, followed by at least 19 generations of brother-sister matings. Currently, not including sublines, we have 14 strains of the B6.Cb$_4$i$_5$ series ($F_{n>20}$).

We present evidence that the strains can be considered quasi-congenic, inbred, and recombinant, and that the method has the potential for QTL mapping of both the differential trait and other quantitative traits affected by passenger genes. Also, we present phenotypic data with respect to the trait being selected for, and demonstrate that the two tested introgression strains have higher TH/MES than the background strain.

Microsatellite markers were selected from the Whitehead Institute/MIT database (Dietrich et al. 1992; Whitehead Institute/MIT 1994) on the basis of previously detected allelic differences between the B6 background and the C donor strain. A 10-cM (average) resolution map was created for two representative QTL introgression strains by testing 169 polymorphic markers in the B6.Cb$_4$i$_5$α-12A (α-12A) and B6.Cb$_4$i$_5$β-13 (β-13) strains and in their B6 background partner strain. PCR products were analyzed on 6% Nusieve agarose gels (Love et al. 1990). Because in the β-13 strain no PCR product was obtained for the most distal marker *D19Mit6*, we could not estimate the length of a segment on Chromosome (Chr) 19 marked by three consecutively positioned C-type alleles (*D19Mit37*, *D19Mit36*, *D19Mit34*). No heterozygous loci were found in the QTL introgression strains. The mapping results indicated that the maximum length of an introgressed donor segment, estimated by the distances of flanking background type markers from the centromere, varied from 3.3 cM to 22.5 cM on the Whitehead Institute/MIT map. The segments are shorter than the estimated average length of an introgressed chromosome segment that carries the differential locus after four backcrosses with concomitant selection for one donor-strain gene (39 cM; Ln $= 200 \times (1–2^{-n})/n$; $n_1 = F_1$; Flaherty 1981), presumably because (i) during the development of the b$_4$i$_5$ series each backcross was followed by intercrosses (Vadasz et al. 1994a), and (ii) there were additional chances for recombination during fixation (Taylor 1978). Assuming one differential locus, the theoretically expected proportion of the fixed, nonselected, nonlinked donor genes was estimated as 3.0%, while the proportion of the linked donor genes is about 1.2% in inbred QTL introgression strains of the b$_4$i$_5$ series. Our results suggest that the total of the introduced donor genome carried by seven segments in each strain was about 73.7 cM (4.6%) in the α-12A strain, and (assuming that the problematic *D19Mit6* marker was of B6-type) was about 117.3 cM (7.3%) in the β-13 strain. The α-12A strain carried C-type chromosome segments on Chrs 2, 8, 9 (2 segments), 13 (2 segments), and 18. The beta-13 strain carried C-type chromosome segments on Chrs 1, 2, 7 (2 segments), 15, 18, and 19.

If the length of an introgressed chromosome segment after four backcrosses is estimated as 39 cM, and if during inbreeding four crossovers per 100 cM occurred (Taylor 1978), then the introgressed segment had a 1.5 chance on the average for meiotic recombination before fixation. Therefore, if the length of the differential segment is approximately 26 cM after fixation, and there are two unlinked QTLs with major effects (Vadasz et al. 1994a), the total length of the donor material (including the nonselected, nonlinked donor genes estimated as 3% of 1600 cM) would be about 100 cM.

Assuming that the 169 microsatellite markers are randomly distributed in the genome of 1600 cM, the proportion (P) of the genome lying within ±10 cM from the markers is 88% ($P = 1 - e^{-2Nd/D}$; Jacob et al. 1991). The above estimates indicate that about 12% of the donor segments have not been detected, and that about 240 markers will be needed to ensure that 95% of the genome will lie within ±10 cM of a marker.

A detailed summary of the genotyping results of the informative polymorphic markers is provided in Fig. 1 and Fig. 2. Two loci were of identical C-type in the two strains: *D2Nds1* (50 cM from the centromere, on a maximum 3.3 cM long segment) and *D18Mit107* (26.2 cM from the centromere, on a maximum 9 cM [α-12A] and on a maximum 12.4 cM [β-13] long segment). If the probability of detecting a fixed, nonselected, nonlinked donor gene in one of the b$_4$i$_5$ strains is about 3.05% ($p = 0.0305$), then their joint probability of occurrence is $p = 0.00093$, while the probability of retaining in two strains the same passenger gene residing 10 cM from the differential gene is $p > 0.3$ (Flaherty 1981). Thus, it is possible that a maximum 3.3-cM region about 50 cM from the

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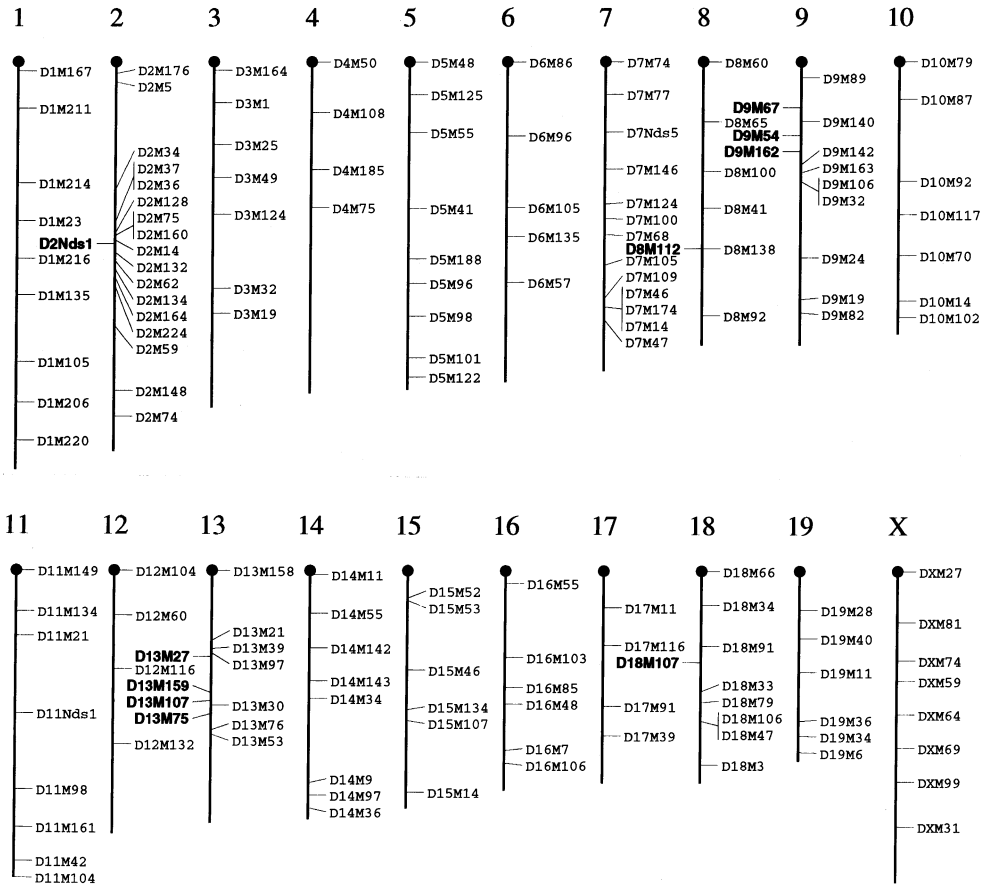

Fig. 1. Microsatellite marker map of B6.Cb$_4$i$_5$α-12A. Marker loci whose α-12A alleles were **C-like** are depicted to the **left** of each chromosome (in bold type); those whose alleles are B6-like are on the right. The locus symbols for the Mit markers have been abbreviated for greater legibility.

centromere on Chr 2 and a maximum 12.4 cM region about 26 cM from the centromere on Chr 18 carry candidate genes that affect mesencephalic TH activity (TH/MES). Introgression of a complex phenotype usually implies the possibility of introgression of several genes, thus raising the question of what the probability is that two differential loci are carried by the same chromosome segment, limiting the resolving power of an RQI system. Based on the hypothesis that the expression of a complex phenotype does not require chromosome-specific organization of the genes (that is, it is not necessary that all contributing genes are carried by the same chromosome in a certain order), we estimated that the probability that k randomly distributed differential loci are carried by the same chromosome segment of L cM, in a genome of D cM, is $p = (L/D)^k$. Because our previous studies (Vadasz et al. 1994a) indicated two unlinked loci, we estimated that the probability that any one 3.3-cM (or 2.4-cM) region would contain two loci influencing TH/MES is $p = 0.000004$ (or $p = 0.00006$). These results suggest that it is likely that differential loci are carried by separately detectable recombinant chromosome segments.

What is the biological significance of mapping genes that affect TH/MES? TH is the first, and the rate-limiting enzyme in the biosynthesis of dopamine, a catecholamine neurotransmitter. In our introgression studies (Vadasz et al. 1987, 1994a, 1994b), TH activity was used as an index of the number of dopamine neuron cell bodies in the mesencephalon (Ross et al. 1986; Baker et al. 1980). Because dopamine plays a central role in brain reward mechanisms, motor activity, etc., we hoped to create a system of quasi-congenic strains by which one could map some of the elusive QTLs that affect the mesotelencephalic dopamine system, and one can study on the same genetic background the neurobiological phenotypes in which dopamine has been implicated. It was also hoped that mapping of dopamine system-specific QTLs in the mouse would provide new perspective in studies on neuropsychiatric disorders in which dopamine is suspected to play a significant role, such as substance abuse, schizophrenia, hyperactivity with attention deficit, and Parkinson's disease.

In the 12 generations of selection for high TH/MES (B6.C series), the range of mean TH/MES was from 3.1 to 3.6 nmol DOPA/MES per hour ($n = 45$–90 in each replicate line), while the coefficient of variation (CV%) ranged from 9.7% to 19.6% (cf. Vadasz et al. 1994a; a more detailed report is in preparation). Recently, at the end of the introgression experiments we tested the progenitor strains again for TH/MES. The results (mean ± S.E.M.) confirmed several previous comparisons indicating a C(3.50 ± .07) > B6(3.00 ± .1) > I(2.48 ± .06) rank order in TH/MES (Vadasz et al. 1994b). In the present study we used inbred strains of the quasi-congenic B6.Cb$_4$i$_5$ series that were derived after four backcrosses from the B6.Cb$_4$i$_5$ generation by strict brother-sister matings for at least 20 generations ($F_{n>20}$). We tested the two introgression strains, which were characterized for microsatellite markers (Figs. 1 and 2), for TH/MES. We found significantly higher TH/MES (means ± SEM) in the B6.Cb$_4$i$_5$α-12A (3.39 ± 0.06; $F_{1,18} = 10.02$, $p = 0.0053$) and B6.Cb$_4$i$_5$β-13 (3.48 ± 0.06; $F_{1,18} = 15.17$, $p = 0.0011$) strains in comparison with their background strain B6 (3.00 ± 0.11; $n = 10$ in each strain, one-way ANOVA).

Although the phenotypic data seem to support the suggestion that introgressed regions on Chrs 2 and 18 might contain the selected QTLs, we consider these preliminary results indicative only of the power of the recombinant introgression system, because reliable identification of QTLs requires a map of higher resolution with more evenly distributed markers, and genotypic and phenotypic characterization of a larger set of QTL introgression strains.

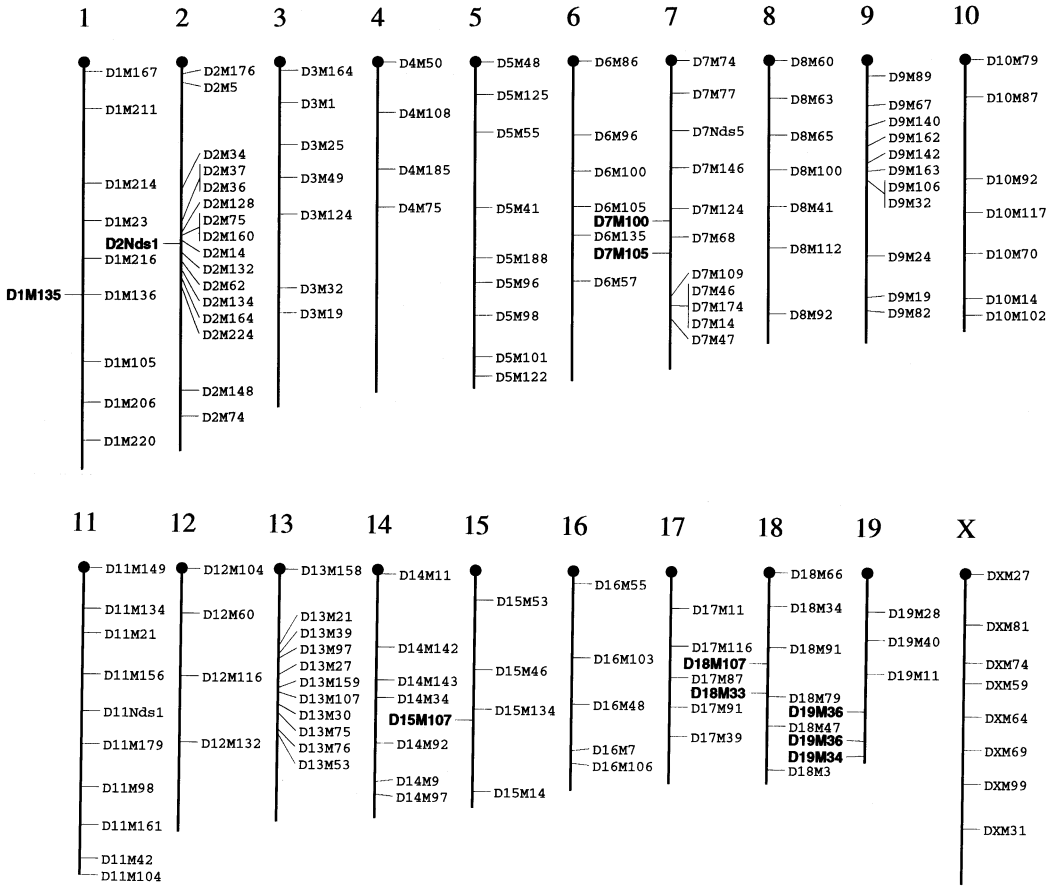

Fig. 2. Microsatellite marker map of B6.Cb$_4$i$_5$β-13. For more information see Fig. 1.

In addition, there are several concerns to be addressed. For example, further experiments are needed to test the possibility that there is some meiotic or premeiotic selection against B6 alleles of loci residing on the introgressed chromosome segments. Because we have previously measured TH/MES in the CXB RI strains (Vadasz et al. 1982), establishing the inheritance of these alleles in the RI strains might shed some light on this issue. Also, the relative position and absolute distances between microsatellite markers may be very different in RQI systems from that in the map generated by analysis of the B6 × *Mus castaneus* intersubspecific cross (Whitehead Institute/MIT 1994), because (i) in intersubspecific crosses the frequency of meiotic crossovers tend to be somewhat lower than that observed between inbred laboratory strains; (ii) according to the 1995 Chromosome Committee Reports, several discrepancies in gene order have arisen involving *DMit* markers (for example, Chr 13); (iii) the fixation (inbreeding) phase in the development of the RQI strains is somewhat comparable to fixation of loci in the development of a RI system. In the RI system there is a fourfold amplification of the linkage map (Taylor 1978).

In the B6.Cb$_4$i$_5$α-12A and B6.Cb$_4$i$_5$β-13 strains we did not find discrepancies in gene order between the 1995 Chromosome Committee consensus map for *DMit* markers, and the map generated by analysis of the B6 × *Mus castaneus* intersubspecific cross (Whitehead Institute/MIT 1994). However, the introgressed chromosome segments were either smaller or larger in size on the Whitehead Institute/MIT map. In the following, we list the segment sizes (in cM) estimated by using the Whitehead Institute/MIT and the consensus map, respectively: Chr 1 (18.4 vs. 20.4), Chr. 2 (3.3 vs. 13), Chr. 7 (7.8 vs. 10.9, and 16.6 vs. 5), Chr. 9 (13.9 vs. 17, and 10.2 vs. 6), Chr. 15 (22.5 vs. 16.5), Chr. 18 [12.4 (β-13) and 9 (α-12A) vs. 18 and 15]. Similar sizes were found on Chr. 8 (18.1 vs 18.1) and Chr. 18 (3.4 vs. 31), while data on the consensus map were not available yet for Chr. 13, and no map positions were assigned to our *DMit* markers for Chr. 19 at the time of comparison. Taken together, the comparison indicates that about 60% of the introgressed chromosome segments were somewhat smaller on the Whitehead Institute/MIT map, and variations between maps have to be taken into account. However, the observed map differences do not change the basic conclusions of this report.

Acknowledgments. Development of the recombinant QTL introgression strains was supported by a grant from the National Institute of Health to Cs. Vadász. Mapping was supported by The Harry Frank Guggenheim Foundation. TH/MES data for B6.Cb$_4$i$_5$α-12A, B6.Cb$_4$i$_5$β-13, and B6 were obtained in collaboration with Harriet Baker, whose contribution is gratefully acknowledged. We thank Abel Lajtha for helpful comments on an earlier version of this report. Preliminary results of these studies were reported at the Workshop in Mouse Molecular Neurogenetics, The Jackson Laboratory, Bar Harbor, Maine (1994), and at the 25th Annual Meeting of the Behavior Genetics Association, Richmond, Virginia (1995).

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