Zoological Studies **37**(1): 31-38 (1998)

Zoological Studies

Alcohol Preference Genetics in the House Mouse

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(Accepted October 21, 1997)

Rachel L. Derr, Jeremy L. Peirce and Lee M. Silver (1998) Alcohol preference genetics in the house mouse. *Zoological Studies* **37**(1): 31-38. Various strains of inbred mice show strain-specific and widely varying levels of alcohol consumption. Most strains consume moderate levels of ethanol; however, C57BL/6 mice consume ethanol at very high levels and DBA/2 mice consume ethanol at very low levels. We have mapped several loci, 1 located on chromosome 2 and 1 on chromosome 11, which are involved in this phenotype. We outline here the progress of this research and our strategy for higher-resolution mapping and analysis of the loci discovered.

Key words: Ethanol, C57BL/6, DBA/2, Mice, 2-bottle choice.

Over the past several decades, hundreds of studies involving twins, adoption, and half-siblings have confirmed the role of heredity in the development of alcoholism in human males and females (Kaij 1960, Shuckit et al. 1972, Cadoret et al. 1980, Kendler et al. 1992) These investigations estimate that 1st-degree relatives of alcoholics have a 3-7 times greater risk of becoming alcoholic themselves (Gilligan et al. 1987). Many linkage analyses and association studies have aimed at identifying the specific genetic factors that lead to this predisposition. Little progress has been made toward this end, however, due to the fact that alcoholism is such a complex multifactorial trait. The inheritance of alcoholism is polygenic; it is determined by several quantitative trait loci (QTL) which contribute to the phenotype in additive, or possibly interactive ways. While the aggregate influence of a set of QTLs may be large, the individual QTLs are difficult to map because each accounts for only a small part of the trait variance (Crabbe et al. 1994). The genetic dissection of alcoholism is additionally confounded by genetic heterogeneity, incomplete penetrance, and strong environmental influences that allow a predisposing allele to be present in some nonalcoholics as well as absent in some alcoholics (Begleiter and Kissih 1995).

Although no significant positive allelic associations have yet been found, a strong negative association between alcoholism and certain alleles for genes important in alcohol metabolism has been observed. Specifically, a low-activity allele for aldehyde dehydrogenase (ALDH2*2) and 2 high-activity alleles for alcohol dehydrogenase (ADH2*2 and ADH3*1) are present in much lower frequencies in alcoholics than in nonalcoholics (Thomasson et al. 1993). For example, in Taiwan, ALDH2*2 is 4 times more frequent in alcoholics than in nonalcoholics (Harada et al. 1982). The fact that all 3 of these gene forms lead to the accumulation of toxic levels of acetaldehyde in the liver, which causes a very unpleasant flush reaction in the carrier after only a couple of drinks, provides a probable explanation for the uncommon observance of these alleles in alcoholics.

Because the complexity of alcoholism greatly limits the power of human studies to identify the predisposing genes, experimental animal models must be used to extend the reach of genetic analyses. Animal models allow for the examination of larger numbers and more generations of subjects, the arrangement of informative matings, making measurements not possible on humans, and the greater homogenization of both the environment and the genetics involved (Silver 1995). The important disadvantage, however, of employing an

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animal model to a behavior like alcoholism is the impossibility of finding an animal that exhibits the whole spectrum of alcoholic behaviors displayed by humans (Hesselbrock 1995).

The C57BL/6 (B6) inbred mouse strain, nevertheless, can be viewed as a good animal model because it displays many of the individual features of alcoholism, as seen in assays that probe neurological sensitivity, dependence, withdrawal, and consumption with or without the availability of competing fluids (Begleiter and Kissih 1995). Over 35 years ago, McClearn and Rodgers first showed that B6 mice consume unusually large amounts of alcohol at highly reproducible and strain-specific levels when given a choice between 10% ethanol and water. While most inbred strains, such as 129/ReJ, C3H/HeSnJ, BALB/cJ, and an A/J-related strain, consume 10% ethanol in moderate amounts representing 0.15 to 0.25 of the total liquid intake, the B6 mice exhibit extreme alcohol preference with alcohol intake fractions over 0.75. In contrast, the DBA/2J strain of mice display extreme alcohol avoidance with intake fractions of 0.07 or less (McClearn and Rodgers 1959 1961, Rodgers and McClearn 1962). Interestingly, B6 preference and DBA avoidance behaviors have also been observed in choice-tests involving many other drugs, like cocaine, morphine, nicotine, opiates, and amphetamine (Alexander et al. 1993, Meliska et al. 1995).

All earlier attempts to map QTLs for alcohol-related traits, including alcohol preference, took advantage of the BXD set of 26 recombinant inbred (RI) strains (Plomin and McClearn 1993, Phillips et al. 1994, Rodriguez et al. 1995). Although using RI strains opens opportunities to make comparisons across time and across studies, the statistical power of the approach is severely limited by the small number of RI strains available (26 for the BXD RI series). Indeed, according to a newly recommended set of QTL threshold values none of the previous alcohol-preference QTL findings are significant or even suggestive, and it is doubtful that the BXD RI set can ever prove sufficient for mapping QTLs (Lander and Kruglyak 1995).

Our study is the 1st that uses a 2-generation outcross-backcross breeding protocol for the purpose of identifying alcohol preference QTLs. With this breeding strategy, a large number of N2 mice (335 in the 1st series, and 160 in the 2nd series) were generated. All of the N2 animals were phenotyped for alcohol preference using a 2-bottle choice test adopted from that of McClearn and Rodgers (1959). The extreme alcohol-preferring animals from the 1st series of mice were selected for genotyping at markers spaced evenly over all 20 chromosomes. Regions emerging as suggestive from this genome scan were then genotyped in the preferring and avoiding N2 animals of both series. Our results have led to the mapping of 2 B6 alcohol-preference (ALCP) QTLs (Melo et al. 1996) as well as the preliminary identification of 2 others.

MATERIALS AND METHODS

Animals

C57BL/6 (B6), DBA/2 (DBA), and B6xDBA (BXD) animals were purchased from the Jackson Laboratory or bred at Princeton University. N2 (BXDxB6 and B6xBXD) animals were bred at Princeton University. N2 animals in the 1st series (used for the initial genome scan and in locating *Alcp1* and *Alcp2*) were from 2-9 mo old at the time of testing. Animals in the 2nd series were from 3-5 mo old at the time of testing.

Alcohol preference phenotyping

Alcohol preference was measured for singly housed animals over the course of several 3-d trials. Animals in the 1st series were tested for 9-d, and animals in the 2nd series were tested for 12-d.

Animals were presented with 2 identical bottles, 1 containing tap water, and the other containing a 10% ethanol/water (w/v) solution in addition to unlimited food. The bottles were removed, weighed, and replaced with different, freshly prepared bottles every 3-d. The position of the ethanol solution-containing bottle was reversed after each trial to control for possible position effects. Preference was defined as the fraction of total liquid intake derived from the 10% ethanol solution.

The 'bottle' for the 1st series consisted of a 30-ml Corex tube topped by a #3 one-hole stopper implanted with a 2-inch ball-less sipper tube. The 2nd series bottle was identical except that the Corex tube was replaced by a similar plastic tube. In the 2nd series, preference ratios were adjusted by subtracting the average evaporation from identically prepared tubes placed in empty cages before calculating the ratio.

Tests for consistency

A consistency test was applied to each set of

3 consumption values (for the 1st series) or 4 values (for the 2nd series) obtained for each N2 animal. When all values were within 2 sex-specific B6 standard deviations of each other, they were averaged together to generate a consumption value for the animal. In the 1st series, when all values were not within 2 standard deviations of each other, the 2 values closest together were retained to calculate an animal average. In the 2nd series, the method was similar, except that the 3 values closest together were retained and the fourth was removed. If no 2 (for the 1st series) or 3 (for the 2nd series) of the values were within 2 standard deviations of each other, the animal was removed from consideration. This was the case for 3 of 338 total N2 animals in the 1st series and for 2 of 160 total N2 animals in the 2nd series. This test was implemented before knowledge of genotypes was correlated.

Genotypic analysis

Genomic DNA was prepared from animal tissues according to standard protocols in both series of animals. For the 1st series, microsatellite markers polymorphic between B6 and DBA were chosen approximately every 30 cM across the genome (Figure 2). Microsatellite markers are highly polymorphic dinucleotide "CA" repeats flanked by regions of constant sequence. Primers flanking the dinucleotide repeats were purchased from Research Genetics and PCR was performed as indicated by the manufacturer (Dietrich et al. 1994). When possible, markers with large polymorphisms were typed by electrophoresis in 6% acrylamide gels followed by staining with ethidium bromide. When necessary, markers with smaller polymorphisms were amplified with ^{32}P-labeled primers and separated by electrophoresis on denaturing gels. For the 2nd series, markers showing suggestive or significant linkage when genotyped across the whole population of the 1st series were genotyped for the top and bottom quarters of the population.

Linkage analysis

All data input and analyses were performed using the Microsoft Excel software package (Microsoft) on a Macintosh computer (Apple). Chi-squared tests were performed using a macro from within Microsoft Excel or with the use of a hand calculator. *T*-tests were performed from within Microsoft Excel, and 2-tail values are reported for the 1st series. Since the 2nd series incorporates a directional hypothesis about the effect of the locus, a 1-tail *t*-test was used in analysis of the known or suspected loci in this series.

Linkage analysis was conducted in 3 stages. First, the top 40 most phenotypically extreme animals in the N2 population were chosen to be genotyped at each of the above-mentioned markers. Markers found to have greater than a 0.6 ratio of the B/B genotype to the B/D genotype (a departure from the 1:1 ratio expected for an unlinked locus) were then used to genotype additional animals at the high and low preference ends of the N2 population. Using such a liberal cut-off ratio reduces the likelihood that QTLs of detectable effect size were missed, even though a relatively small number of extremes were examined. Markers which remained promising were genotyped across the entire N2 population and analyzed using a 2-tail *t*-test.

The initial group of 40 high-preferring animals was also analyzed by sex due to strong a priori evidence of sex specificity. The results for these sex-specific analyses were treated in a similar manner as described above.

The 2nd set of animals were genotyped directly at markers near the putative loci they were intended to examine. A *t*-test between the animals B/B and B/D at the locus was also used here to determine significance of the locus in that set of animals.

RESULTS

The 1st step in our analysis of alcohol preference in C57BL/6 mice was determining the consumption values for the parental and F1 animals (B6, DBA, and B6xDBA). All animals were tested for consumption as described for the 1st series under Methods. Alcohol consumption in the B6 mice was much higher than consumption in the DBA strain, as expected from previous studies (McClearn and Rodgers 1959). Also, as expected, there was a sex-specific difference in consumption between B6 males and B6 females (Rodgers and McClearn 1962) (p = 0.02). Table 1 shows the mean alcohol consumption values and standard deviations for the B6, DBA, and F1 males and females.

The F1 animals show an alcohol preference which falls between the B6 and DBA values and is approximately equivalent to the strains which show moderate consumption (McClearn and Rodgers 1959). This suggests that there are separate, mostly recessive loci for both the preference exhib-

ited by B6 and the avoidance exhibited by DBA. Because of this fact, alcohol preference loci were mapped using an outcross-backcross model (Fig. 1).

After an N2 population was generated, animals were tested and genotyped as described above. Linkage analysis was likewise performed on the 1st set of animals as described and significant loci were reported. These investigations resulted in the identification of 2 sex-specific loci, *Alcp1* and *Alcp2*. *Alcp1* is a male-specific locus on chromosome 2, and *Alcp2* is a female-specific locus on chromosome 11. *Alcp2* in the entire population of females was not significant, but when the female animals with a B6 father were separated from the rest of the population of females, the significance of the locus increased dramatically. Division of the data in a post hoc manner, such as by cross, requires that the significance criterion be adjusted downward 2-fold, which we have done.

Figs. 3 and 4 show the log plots of *p*-values for these 2 loci. *Alcp1* has a minimum *p*-value of 4.3 $\times$ 10^{-7} and maps into a 15 cM 90% confidence interval. *Alcp2* has a minimum *p*-value of 3.2 $\times$ 10^{-5} and has a 90% confidence interval 17 cM long. The *p*-values for both of these loci, particularly that

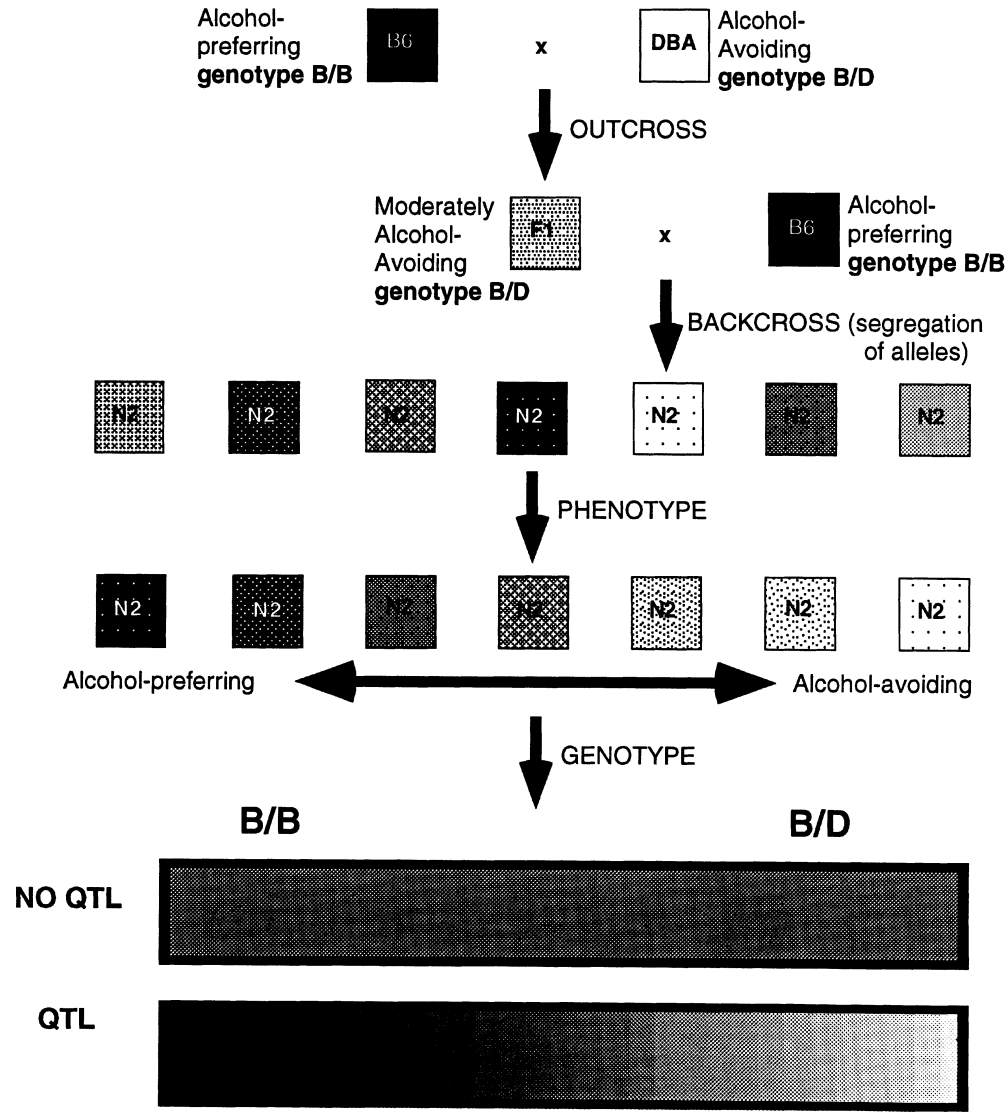

Fig. 1. Simplified illustration of how the outcross-backcross strategy can be used to detect quantitative trait loci controlling B6 alcohol preference.

Table 1. Alcohol consumption in B6, DBA, and B6 x DBA F1 animals by sex

Strain	Aggregate	Male	Female	Sex difference
B6	0.77 ± 0.14 (68)	0.72 ± 0.18 (32)	0.81 ± 0.10 (36)	$p = 0.02$
DBA	0.07 ± 0.03 (40)	0.06 ± 0.02 (16)	0.07 ± 0.03 (24)	not significant
F1	0.21 ± 0.15 (122)	0.20 ± 0.14 (59)	0.22 ± 0.15 (63)	not significant

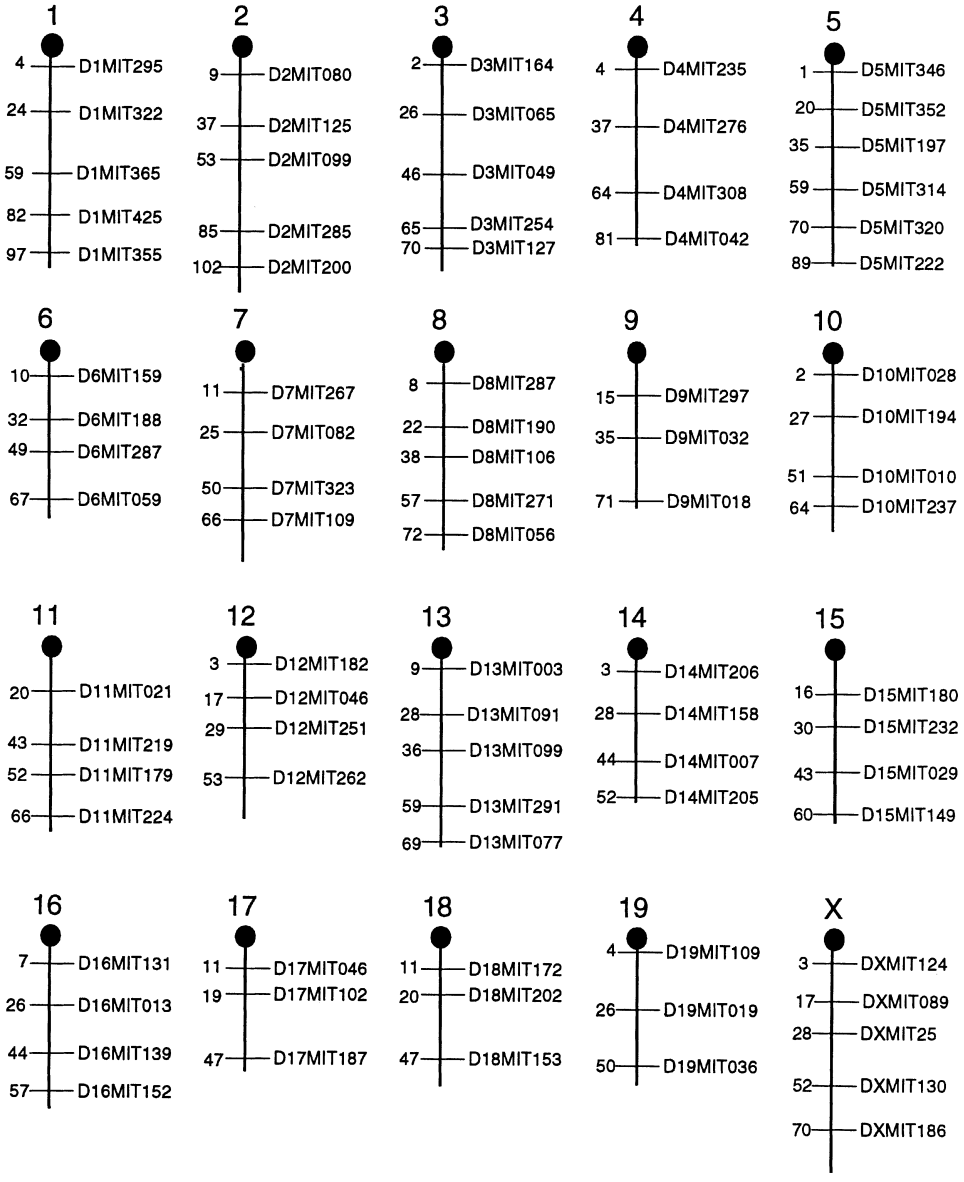

Fig. 2. Microsatellite marker maps used in the Genome Scan for QTLs influencing B6 alcohol preference. The maps are drawn approximately to scale, and the number to the left of the hash mark represents the marker's position in centimorgans. All of the markers chosen show polymorphisms between the B6 and DBA alleles.

for *Alcp1*, fall well below the criterion suggested by Lander and Kruglyak (1995) for significance in a whole genome scan (for a nominal $p = 0.05$ in a whole genome scan in the mouse, this value is approximately $p = 1 \times 10^{-4}$).

In addition to the *Alcp1* and *Alcp2* loci, there were several loci that exceeded the requirement for suggestive linkage when genotyped across the 1st series of animals. To determine the actual status of these loci, we created a 2nd series of 160 N2 animals and phenotyped them as noted in Methods. In this study, a subset of extreme animals of the appropriate sex were genotyped for each locus to be tested. Using this new population of mice, we were able to confirm the existence of 1 new female-specific locus and 1 new male-specific locus, (Peirce et al. in preparation) which are each slightly weaker in effect than the *Alcp1* and *Alcp2* loci described and which account for 22% and 18% of the genetic variance, respectively.

DISCUSSION

We have discovered 4 loci that significantly contribute to the alcohol-preference behavior of B6 mice. All of these loci are sex specific; 2 affect only female preference and 2 affect only male preference. The identification of sex-specific loci suggests that non-identical QTLs influence alcohol preference in males and females. Observations of sex-specific differences for mean fraction intakes, phenotype distributions, and heritability estimates also support this hypothesis (Melo et al. 1996).

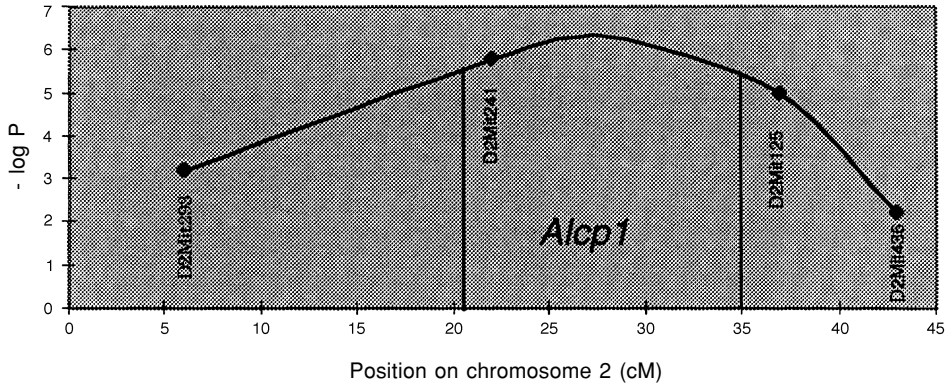

Fig. 3. *Alcp1* localization to chromosome 2. *P*-values associated with individual markers were determined by *t*-tests. Values between typed markers were ascertained by regression. Since *Alcp1* has a male-specific effect, only male animals were used to calculate the *p*-value.

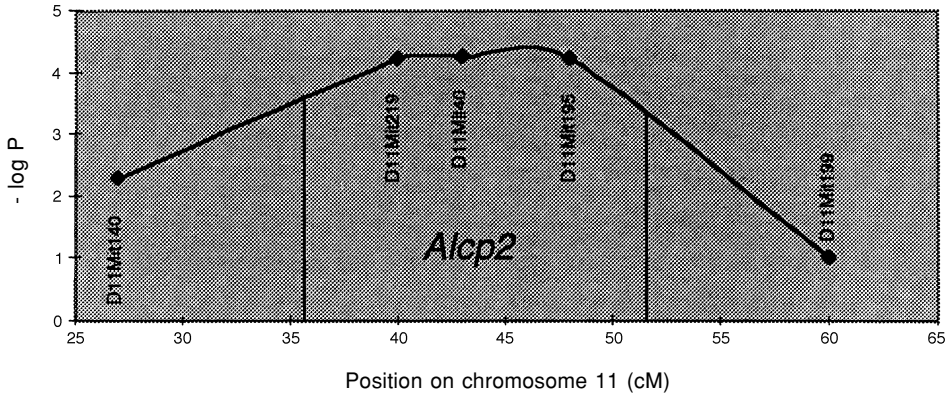

Fig. 4. *Alcp2* localization to chromosome 11. *P*-values associated with individual markers were determined by *t*-tests. Values between typed markers were ascertained by regression. Since *Alcp2* has a female-specific and cross-specific effect, only female animals with B6 fathers were used to calculate the *p*-value.

Alcp2 is not only female specific, but also cross-specific; the locus seems to exert an effect only on those N2 females derived from a B6 father and an F1 mother. When comparing alcohol consumption values for females with B/B and B/D genotypes from each cross, it is clear that animals with both the B/D genotype and a B6 father show lower alcohol fraction intakes than the 3 other subgroups. Surprisingly, the females with the B/D genotype at *Alcp2* and a B6 mother drink alcohol in the same high quantities as the B/B genotype animals. The existence of an X-linked *Alcp* locus with complementary activity to *Alcp2* could explain this finding; however, we were able to reject this explanation based on the results of a marker scan of the X chromosome (data not shown). Another explanation that has yet to be confirmed or refuted involves genomic imprinting at the *Alcp2* locus (Cattanach and Kirk 1985). In this scenario, the paternal copy of *Alcp2* is imprinted, leaving it nonfunctional. Thus, because the important copy of Alcp2 is the 1 inherited from the mother, alcohol preference is lowered by a B/D genotype at *Alcp2* only when the D allele is maternally inherited.

The *Alcp1* locus maps to a region on chromosome 2 in mice that shows conserved synteny with the human chromosomal region 9q32-q34. The *Alcp2* locus maps to mouse chromosome 11 in a region syntenic with the human chromosomal region 17q21. Although no probable candidate genes map within the *Alcp1* 90% confidence interval, a particularly interesting candidate gene, the serotonin transporter (Htt) maps to the center of the *Alcp2* confidence interval (Gregor 1993). Since the serotonin transporter is involved in the re-uptake of serotonin into the presynaptic terminals, alternative allelic forms of 5-Htt could create the different synaptic serotonin levels which have been seen to affect alcohol preference in animal models and in humans. Specifically, research points to a negative correlation between alcohol preference and synaptic serotonin levels. One particularly interesting study, which found a significantly higher rate for platelet serotonin uptake in men with family histories of alcoholism, suggests that kinetics involving the serotonin transporter can contribute to low synaptic serotonin levels, and consequently to alcoholism (Rausch et al. 1991). Another fascinating finding concerning the serotonin transporter is the discovery of a polymorphism in the 5-Htt gene regulatory region in humans that seems to be associated with anxiety-related traits (Lesch et al. 1996). In order to ascertain whether 5-Htt is also affecting alcohol-preference traits, direct functional studies and, ultimately, knock-in strategies are required.

Our search for candidate genes for the *Alcp* QTLs can be facilitated by methods that narrow the centimorgan length of the alcohol-preference loci's confidence intervals, and consequently reduce the number of genes they contain. We are currently pursuing this goal of finer mapping in 2 ways. First, we are constructing advanced intercross lines (AILs), which will give us animals with genomes reflecting a very large number of recombinational events, that should increase mapping resolution by approximately 5-fold (Darvasi and Soller 1995). Second, sets of interval specific-congenic strains are being built for each locus to allow for break-point mapping. Break-point mapping, which utilizes a pool of animals recombinant across the region of interest, may also be able to localize the QTL to a 1-2 cM interval (Darvasi 1997).

The isolation of genes that predispose individuals to alcoholism could allow for the early identification of individuals at risk, help elucidate the environmental factors critical in the progression of alcoholism, and ultimately improve pharmacotherapy and lead to better treatments for alcoholism (NIAAA 1997). Because candidate genes for complex traits like alcoholism are usually not obvious, the identification of candidate genes often requires a QTL approach which proceeds from phenotype to genotype. Our findings reporting significant genetic loci influencing the extreme alcohol preference behavior of B6 mice demonstrate that such an approach can successfully map, to relatively defined intervals, loci that contribute to multifactorial behaviors. Because the B6 mouse is a potential model for the human alcoholic, our results represent a potentially important advance toward understanding the genetics of alcoholism.

Acknowledgments: This research is supported by a grant from the NIH to LMS. We thank Irina Agulnik and the Departmental Animal Facility for excellent technical support.

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小家鼠之酒精偏好遺傳

Rachel L. Derr[1] Jeremy L. Peirce[1] Lee M. Silver[1]

好幾個小家鼠的近親交配品系顯示品系內穩定但品系間差異很大的酒精攝取量。大部份品系的酒精攝取量屬中等，但 C57BL/6 品系的老鼠攝取大量的酒精，而 DBA/2 品系的老鼠攝取微量的酒精。和這個表型有關的基因座，一個位於第二對染色體上，另有一個位於第十一對染色體上。本文闡述這個研究的進展以及我們對於發現的基因座進行高解析度基因定位和分析的策略。

關鍵詞：乙醇，C57BL/6，DBA/2，小家鼠，雙瓶選擇。

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