

## **Complex Genetic Architecture Revealed by Analysis of HDL in Chromosome Substitution Strains and F<sub>2</sub> Crosses**

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## ABSTRACT

Intercrosses between inbred lines provide a traditional approach to analysis of polygenic inheritance in model organisms. Chromosome substitution strains (CSS) have been developed as an alternative to accelerate the pace of gene identification in quantitative trait mapping. We compared a classical intercross and three CSS intercrosses to examine the genetic architecture underlying plasma high-density lipoprotein cholesterol (HDL) levels in the C57BL/6J (B) and A/J (A) mouse strains. The BxA intercross revealed significant quantitative trait loci (QTL) for HDL on chromosomes 1, 4, 8, 15, 17, 18 and 19. A CSS survey revealed that many have significantly different HDL levels compared to the background strain B, including chromosomes with no significant QTL in the intercross and, in some cases (CSS-1, CSS-17), effects that are opposite to those observed in the BxA intercross population. Intercrosses between B and three CSSs (CSS-3, CSS-11 and CSS- 8) revealed significant QTL but with some unexpected differences from the BxA intercross. Our inability to predict the results of CSS intercrosses suggests that additional complexity will be revealed by further crosses and that the CSS mapping strategy should be viewed as a complement to, rather than a replacement for, classical intercross mapping.

## INTRODUCTION

The genome wide distribution of allelic variation in an intercross population, as in human populations, provides great potential for epistatic interactions that can mask or enhance the impact of allelic variation at multiple loci. This complexity may hinder our ability to detect the genes and alleles that are most important. One approach to circumvent this possibility is to reduce the number of potential interactions by reducing the amount of segregating variation in a cross. Chromosome substitution strain (CSS, also known as consomic) panels achieve this goal by restricting variation to a single chromosome (BEVOVA *et al.* 2006; COWLEY *et al.* 2004; FERNANDES *et al.* 2004; NADEAU *et al.* 2000). In these panels each strain is derived from a ‘background’ inbred strain except a single chromosome that is derived from a distinct ‘donor’ inbred strain. Consequently any genetic variation seen among CSS is presumably due to allelic variation on the donor chromosome. It is possible that some differences between the CSS may be due to recent mutations (COOK *et al.* 2006). However, it is estimated that only 3% of polymorphisms among strains have arisen within the last 100 years. Thus, most of the differences between inbred strains, and specifically for this report between C57BL/6J (B) and A/J (A), are ancestral and differences among consomic strains due to recent mutations are expected to be quite rare (FRAZER *et al.* 2004).

Ischemic cardiovascular disease (ICD) is the leading cause of morbidity and mortality in developed nations. Major risk factors for ICD are high levels of low-density lipoprotein cholesterol (LDL) and low levels of high-density lipoprotein cholesterol (HDL) in plasma (FRUCHART and DURIEZ 2002). High levels of plasma HDL provide protection against heart disease as shown in both human (GORDON *et al.* 1989; NISSEN *et al.* 2003; WILSON *et al.* 1994) and animal studies (BADIMON *et al.* 1990;

OKAMOTO *et al.* 2000; PLUMP *et al.* 1994; RITTERSHAUS *et al.* 2000; RUBIN *et al.* 1991; SUGANO *et al.* 1998). A successful route towards identifying genes that affect quantitative phenotypes such as HDL levels is through the use of inbred mouse strains and quantitative trait locus (QTL) analysis. A combination of genetic tools and techniques is helping to identify these QTL genes (DIPETRILLO *et al.* 2005; HILLEBRANDT *et al.* 2005; KORSTANJE and PAIGEN 2002; WANG *et al.* 2005). A total of 22 mapping crosses have identified more than 130 loci for HDL and these localize to 37 unique QTL (WANG and PAIGEN 2005). Only a small number of gene-by-gene interactions have been reported for HDL (BROCKMANN *et al.*; ISHIMORI *et al.* 2004), in comparison to other traits such as obesity (CHEVERUD *et al.* 2001; STYLIANOU *et al.* 2006). Thus either HDL interactions are rare or they have been too complex to detect with current methods.

In this study we carried out a standard F<sub>2</sub> intercross between B and A, and detected QTL on seven chromosomes (Chr) including both main effect and interacting QTL. We then surveyed the chromosome substitution strains and carried out intercrosses between B and three CSS. The results showed that CSS do not simply partition the genetic variation as expected. The unique genetic backgrounds present in the CSS and parental line intercrosses alter the genetic architecture of HDL regulation. Some QTL are clearly shared among the different mapping populations but many are not. We demonstrate that epistatic interactions among multiple loci are buffering the potential for phenotypic variation in the parental genomes.

## MATERIALS AND METHODS

**Mice:** Mouse strains were obtained from The Jackson Laboratory (Bar Harbor, ME) and maintained on a 14 hour light/10 hour dark cycle. Mice were housed in individually pressurized cages (Thoren Caging Systems) containing pine shaving bedding, topped with a polyester filter, and allowed *ad libitum* access to acidified water and food (18% protein rodent diet, 6% fat, product #2018; Harlan Teklad, Madison, WI). Animal protocols were reviewed and approved by the Animal Care and Use Committee at The Jackson Laboratory.

**Crosses:** F<sub>2</sub> progeny were obtained by intercrossing (B6 x A/J) F<sub>1</sub> mice to produce 343 F<sub>2</sub> males and 271 female. These two crosses were carried out at different times for different purposes, however the conditions under our control were consistent for both crosses. In particular both crosses were carried out using a standard chow (4% fat) diet. HDL was measured in both crosses and the data were combined for this report.

The C57BL/6J with A/J chromosome substitution strains whose nomenclature is B-Chr#A are simplified to CSS-# throughout this manuscript. The BxCSS intercrosses were generated using F<sub>1</sub> mice from B6 females mated to CSS-3, CSS-8 or CSS-11 males. These F<sub>1</sub> mice were intercrossed to generate F<sub>2</sub> animals. The CSS-3 intercross population consisted of 146 mice (65 females, 65 males); the CSS-8 intercross consisted of 150 F<sub>2</sub> mice (77 females, 71 males); and the CSS-11 intercross consisted of 179 F<sub>2</sub> mice (84 females and 70 males).

**Genotyping:** Genotyping was performed for each CSS intercross using evenly spaced MIT microsatellite markers as previously described (STYLIANOU *et al.* 2004). SNP

genotyping was performed for the larger BxA intercross. For SNP genotyping genomic DNA was isolated from the tail of each mouse and genotyped with markers initially evenly spaced throughout the genome with additional markers added in regions of interest. A total of 97 markers for males and 174 for females were used. SNPs were chosen from a panel designed to facilitate genotyping in inbred mouse strains (PETKOV *et al.* 2004) and was performed by the Allele Typing Service at The Jackson Laboratory in conjunction with KBiosciences (Herts, UK).

**Phenotyping:** Blood samples from mice fasted for 4 hours in the morning were collected in tubes containing EDTA, and centrifuged at 9,000 rpm for 5 min. Plasma was placed into a fresh tube and frozen at  $-20^{\circ}\text{C}$  until analyzed. Plasma samples were thawed, vortexed and analyzed within a week of being collected. Plasma HDL concentrations from each blood sample were measured directly using an enzymatic Reagent Kit (#650207, Beckman Coulter, Inc) used according to manufacturer's recommendations on the Synchron CX Delta System (Beckman Coulter, Inc.).

**CSS Strain Survey:** For the strain survey, 8 to 10 mice of each sex from each strain were collected and phenotyped over a six month period. Control animals were tested concurrently with the CSS. Each strain and sex was represented by animals from at least three independent litters. Statistical significance of HDL between strain B and each CSS was determined using student's t-test. Two-way analysis of variance was used to test for sex specific QTL. Multiple testing was accounted for using a Bonferroni correction within sex. Homogeneity of variances was assessed using Bartlett's and Levene's tests.

**QTL and statistical analyses:** QTL analysis was performed using *Pseudomarker* 2.03 (<http://www.jax.org/staff/churchill/labsite/software/pseudomarker>). The two parental intercrosses were combined and marker locations were exact as base pair positions of the markers were known. Approximate centimorgan coordinates for QTL analysis were obtained by dividing Mb base pair positions by a factor of 2 (Mouse genome build 33). The validity of this approximation was confirmed by comparison to estimated map positions in *Pseudomarker*. For consistency positions of microsatellite markers used to genotype the BxCSS intercrosses were obtained as the base pair position of the amplified fragments by doing a BLAST with the primer sequences. Again Mb base pair positions were converted into centimorgans by dividing by 2. Genetic maps, recombination frequencies and segregation analysis were performed within *Pseudomarker* for quality control. Approximate confidence intervals were computed using the maximum posterior density method (SEN and CHURCHILL 2001). QTL names were assigned by the Mouse Genome Nomenclature Committee and are listed in Table 1.

HDL values were natural log transformed. The log transform reduced the right skew in the HDL distribution but, of greater importance, it also resulted in constant variance among the crosses. When cross populations are combined for analysis purposes, if one population has a greater phenotypic variance, it will tend to dominate the results. All crosses were analyzed with sex as an additive covariate, in addition to performing separate analyses that included a sex-by-QTL interaction to examine sex specific effects. Genome-wide significance was determined using 1000 permutations with stratification by sex (CHURCHILL and DOERGE 1994) . Other statistical analyses were performed using JMP version 6.0 (SAS Institute, NC).



## RESULTS

**B x A F<sub>2</sub> intercross:** A total of 578 BxA F<sub>2</sub> were measured for HDL cholesterol. A genome-wide scan with an adjustment for sex detected significant QTL on Chrs 1, 4 and 8 and several suggestive QTL (Figure 1A). A pairwise genome scan indicated several potential QTL interactions (Figure 2). Joint modeling of all main effects and interactions was performed by merging all significant and suggestive results and then removing one QTL or interaction at a time until all terms remaining in the multiple QTL model were significant ( $p < 0.01$ ). The final model (Table 1) includes QTL on Chr 1, 4, 8, 15, 17, 18, 19 and two interaction terms Chr1@76cM by Chr8@30cM (Figure 2A and 2C) and Chr1@2cM by Chr1@48cM (Figure 2B and 2C). Sex explained 48% and the Chr 8 QTL explained 5% of the total variance. In a cross of this size we can detect QTL that explain as little as 1% of the total variation after accounting for sex. Note that it is not possible to distinguish sex effects from cross effects in this study but it is important to account for this source of variation by using an additive covariate in the genome scans. In the supplementary data (supplementary figure 1) we show that fitting ‘sex-by-QTL’ interaction reveals that loci on Chr 11 and 17 are further influenced by sex.

[Insert Table 1, Figure 1 & 2]

**CSS strain survey:** Males and females for each of the 21 strains of the CSS panel and both parental strains A and B were analyzed for HDL levels (Figure 3). When compared to parental strain B, the CSS show evidence for a QTL in at least one sex for all chromosomes except Chr 7, 13 ( $p < 0.05$ ), after accounting for multiple testing using a Bonferroni correction ( $P < 0.002$  [ $P < 0.05/N=21$ ]), approximately half the strains within each sex are still significantly different from the B strain. With sample sizes of 10 per

group, it should be possible to detect differences between CSS-# and B with 80% power of 1.5 standard deviation units at  $\alpha=0.01$  significance level and 1.25 standard deviation units at  $\alpha=0.05$  significance. Despite some differences in QTL detection between male and female CSS, tests for sex specific QTL did not exceed the multiple test adjusted  $\alpha=0.05$  significance threshold, however there is suggestive evidence for sex specific QTL on CSS-1, 14, 15, 16 and 17. The variance heterogeneity apparent in Figure 3 is statistically significant ( $p<0.001$ ) for both males and females, suggesting that genetic background may influence individual variability in HDL levels.

[Insert Figure 3]

**B x CSS F<sub>2</sub> intercrosses:** Intercrosses between B and three CSS were performed. We chose CSS-11 because it showed a suggestive QTL in the BxA intercross that would presumably become significant in the BxCSS-11 intercross. We chose CSS-8 because it had the strongest QTL in the parental intercross, and we chose CSS-3 because Chr 3 had no QTL in the parental F<sub>2</sub> intercross but the CSS-3 was significantly different from B (Figure 3).

The HDL values were natural log transformed to obtain stable variances (Figure 4). Prior to transformation, the standard deviation in HDL of male mice is roughly twice that of females. Following the natural log transformation, male and female standard deviations are approximately equal. The trait variances in each of the intercross populations, reflects an environmental component plus contributions due to segregating genetic variance (Table 2). As expected, the variance in each of the intercross populations is greater than the within strain variance in the survey population. The trait variance in the BxA intercross is largest, reflecting the genetic complexity of this population. The

BxCSS-8 cross is intermediate in variability due to segregation of a QTL with large effect. The BxCSS-3 and BxCSS-11 intercrosses are segregating small QTL and have correspondingly smaller standard deviations.

[Insert Figure 4]

All crosses were analyzed to detect sex-specific effects by fitting sex\*QTL interaction in the genome scans. We also fit models with 1, 2 and 3 linked QTL on each chromosome. Power to detect linked QTLs when linkage is tight (with 10 to 20 cM) can be limited and it is always possible that an apparently singular QTL is the result of two or more linked polymorphisms. There was no evidence to support either sex specific effects or multiple linked QTL in any of the BxCSS intercrosses.

Thresholds for significant (0.05) and suggestive (0.63) QTL were calculated using 1000 permutations as described in the methods. The smaller target genome size of the BxCSS intercrosses substantially reduced the significance thresholds from 3.2 in the BxA cross to about 2.2 in the BxCSS intercrosses as summarized in Table 2. We note that the chromosome specific thresholds for the BxA cross are the same as those for the BxCSS thresholds (0.8 and 2.1 for Chr 8 and Chr 11, 0.8 and 2.2 for Chr 3), indicating that the reduced target genome size is the most important factor for determining significance thresholds.

*B x CSS-3*: The main effect genome scan (Figure 1C) shows one significant QTL with a peak at 16cM (LOD 4.2) and there is a second peak at 40cM; however, a two-QTL analysis does not find support for a second QTL. The B allele is associated with higher HDL in both the CSS-3 strain (Figure 3) and in the BxCSS-3 intercross. The BxA

intercross data did not indicate any QTL on Chr 3 (Figure 1A, 1B). This surprising difference could be explained by a complex epistatic interaction requiring B alleles at multiple locations. This genotype would occur in only a few animals in a BxA intercross population.

*B x CSS-8*: This cross confirms both the data from the CSS survey, where the CSS-8 strain is significantly different from B6 for both sexes, and the BxA intercross where the Chr 8 QTL was highly significant. The Chr 8 QTL from the BxA intercross has a peak at 30cM (95% CI 20cM-40cM) however the LOD plot is rather broad suggesting the influence of multiple linked QTL. The QTL peak from the CSS-8 intercross population is at 50cM and its 95% CI is outside that of the BxA intercross Chr 8 QTL. As mentioned above, within the BxA intercross there is a significant interaction between the HDL QTL on Chr1@76cM with Chr8@30cM (Figure 2A, and 2C). When homozygous A/J alleles are fixed within the BxA population at Chr1@76cM, the Chr8@30cM QTL is absent seemingly being fully dependent on at least one B allele being present at the Chr 1 location (Figure 5). This demonstrates that two QTL exist on Chr 8: one seen in the BxA intercross and one seen in the BxCSS-8 intercross. Both QTL are large, highly significant and dependent on the background genotype. The BxA Chr8@30cM QTL is dependent on B alleles on Chr1@76cM, and the CSS-8 Chr8@50cM QTL is dependent on B6 alleles at locations that were not elucidated from these crosses.

[Insert Figure 5]

*B x CSS-11*: The main effect genome scan (Figure 1C) looks almost identical to that of the BxA intercross (Fig 1B) with some minor differences in the effect plots. Whereas the

Chr 11 QTL in the BxA intercross is only suggestive, the QTL in the BxCSS-11 intercross is significant. The LOD threshold for the significance is 2.1 in the CSS intercross compared to the genome-wide threshold of 3.2 in the BxA intercross.

## DISCUSSION

Chromosome substitution strains have been developed as tools for genetic analysis. They complement recombinant inbred, recombinant congenic and other types of strain resources. A benefit of using CSS is that fine mapping crosses can be accelerated due to the fixed genetic background. Typically 5-10 backcross generations are required to isolate a QTL on a fixed background and generate a congenic model, breeding that is already completed for CSS. The B.A CSS panel with C57BL/6J background and A/J donor chromosomes are available to the research community through The Jackson Laboratory; a panel of C57BL/6J with PWD donor chromosomes will soon become available; and a third panel of DBA/2 with donor chromosomes from an obese strain Du6i are being generated by BEVOVA *et al.* (2006). These three panels of mouse CSS will enhance the potential for mapping QTL since each panel will harbor different allelic variation.

In this report we demonstrate that using the CSS lines to dissect genetic architecture may not be as straightforward as was originally proposed (BELKNAP 2003; SINGER *et al.* 2005). Combining a QTL analysis of a large intercross between the donor strains with a CSS survey and intercrosses among three of the BxCSS indicates that a variety of outcomes are possible. The most straightforward outcome was the Chr 11 QTL in the BxCSS-11 intercross. This QTL illustrated that, consistent with predictions

made by BELKNAP (2003), SINGER *et al.* (2005) and others, CSS intercrosses can provide greater power to detect QTL of small effect. It is worth noting that the Chr 11 QTL has a similar LOD profile in both crosses and that it exceeds the chromosome-wide significance criteria in the BxA intercross. Thus, the difference in background genetic variation in the BxCSS-11 intercross had little impact on our ability to detect this particular QTL, and the difference between the BxCSS-11 intercross and the BxA intercross is largely due to the reduced target genome which provides a lower significance threshold. Confidence intervals for the QTL location are similar in the two crosses.

The assumption underlying theoretical analyses of the CSS strategy is that QTL with the same effect size will be present in both the parental and CSS intercross population and that the only difference will be a reduction of the background genetic variation in the latter (BELKNAP 2003; SINGER *et al.* 2005). This was observed for the BxCSS-11 intercross but is not consistent with our findings on Chr 3, where a QTL appears in the BxCSS-3 intercross that was not present in the BxA intercross. It is not the case that we simply fail to detect it in the BxA intercross. The estimated effect size of the QTL in the BxCSS-3 intercross (-0.12 log HDL additive effect) is sufficiently large to detect if it were present in the BxA intercross. It is likely that the Chr 3 QTL is masked by epistatic loci segregating in the mixed background of the BxA intercross.

On Chr 8, we observe QTLs in both the BxA and CSS intercrosses, but the position is changed and the pattern of epistatic interactions is at odds with our expectations. The QTL in the BxA intercross is broad which suggests that multiple QTL may be present. Examination of the effect plots (Figure 1B and 1C) suggests that the QTL with a peak at 30cM identified in the BxA intercross may be present in the BxCSS-8 intercross. However the peak of the QTL in BxCSS-8 intercross at 50cM indicates a

new QTL with a larger dominance and additive effect than the QTL at 30cM. Epistatic effects exist between Chr 1 and Chr 8 in the BxA intercross, this interaction requires at least one B allele on Chr 1 for the QTL on Chr8@30cM to yield increased HDL. In the BxCSS-8 intercross homozygous B alleles are fixed on Chr 1 and thus we would expect to see a large peak on Chr8@30cM. This indicates that alleles on other chromosomes that we did not detect in the BxA intercross may be interacting with one or more loci on Chr 8.

The Chr1 x Chr8 interaction seems critical to the Chr 8 QTL since the presence of homozygous A alleles at Chr1@76cM neutralizes the Chr 8 QTL (Figure 5). Two previous studies have reported HDL QTL interactions, Ishimori *et al.* (2004) used a C57BL/6J x 129S1/SvImJ F<sub>2</sub> cross and observed one interaction between Chr1@104cM by Chr2@90cM, and Brockmann *et al.* (2006) who used a DU6i x DBA/2 F<sub>2</sub> cross observed four interactions, one of which was a Chr1@72cM by Chr10@20cM. It is clear therefore that Chr 1 is a key player in the epistatic genetics of HDL QTL.

The genetic architecture of HDL in CSS lines is altered in comparison to the parental intercross presumably due to multiple epistatic interactions. We can pinpoint one such interaction (Chr1 x Chr8) in the intercross and we can detect how these changes in genetic architecture affect other QTL. For example, in the BxA intercross the QTL located at Chr1@48cM, Chr1@76cM and Chr17@26cM are caused by high A/J alleles. The CSS survey indicates however that there must also be alleles for reducing HDL on chromosomes 1 and 17, or at least the absence of interactions, since CSS-1 and CSS-17 females actually have significantly lower HDL than B6 ( $P < 0.0001$ ) (Figure 3) not higher as would have been expected from the BxA intercross results.

**Is the CSS strategy effective?** It has been proposed that the CSS strains can be used to map QTL using fewer mice. Sample size is an important parameter in determining statistical power and confidence interval size in any statistical application. The sample size required to achieve a desired power or precision scales in proportion to the variance of the sample data. Phenotypic variances are smaller in the CSS intercrosses than in the BxA intercross population (Table 2) and we can obtain an empirical estimate of the relative efficiencies of the cross designs by computing the ratio of variances (square of SDs). Comparing CSS-3, -8 and -11 to the BxA cross, the estimated relative efficiencies are 0.35, 0.58 and 0.28, respectively. This means that 35 mice in the CSS-3 population will provide the same power and precision as 100 mice in the BxA intercross. This efficiency of the CSS cross is a consequence of reduced genetic background variation (BELKNAP 2003). Multiple locus mapping procedures (ZENG 1994) could improve the relative efficiency of the BxA cross but would not close the gap completely. Another factor to consider is the genomic coverage provided by the different types of crosses. A CSS cross provides information only for a single chromosome, and the number of CSS crosses required to cover the whole genome will vary depending on the outcome of the strain survey. In the extreme case where crosses are made for each of 20 chromosomes, as many as 700 animals would be required to obtain the same precision and coverage as 100 BxA intercross animals.

Using ~600 mice in the BxA intercross we detect nine QTL on seven chromosomes and two interactions (Table 1). The CSS strategy employs two stages. First the strain survey, with a total of 371 mice, indicated that almost all chromosomes carried HDL QTL. We confirmed three of these QTL with BxCSS intercrosses using 130-154 mice for each cross. The total number of mice in the CSS study was ~900. Given the different objectives and outcomes of the two cross designs, it is difficult to formulate a



fair basis for the numbers of mice needed to obtain the same information. However, when we compare the results from both cross types we find significant complementary information about the genetics of the HDL trait. Genetic background can have unpredictable effects and this comparison provides specific insights into the nature of genetic architecture. We conclude that the combination of different cross designs provides novel information that is not available from either approach considered alone.

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BxCSS-8	Sex	1	3.09	52.14										
	Chr8@50cM	2	0.86	14.5	$1.78 \times 10^{-11}$	<i>Hdlq50</i>	8	50	44-54	10.8	100	B6	dominant	
	error	144	2.17											
BxCSS-11	Sex	1	2.85	64.85										
	Chr11@22cM	2	0.10	2.34	0.0056	<i>Hdlq51</i>	11	22	6-32	2.3	44	B6	dominant	
	error	150	1.45											

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\* Confidence intervals cannot be accurately estimated due to multiple linked QTL. † Mb positions based on mapped position of peak marker to NCBI build33. 1, includes contributions of the interactions in main effects; 2, based on regression F test for this term; 3, obtained from the single QTL genomescans.



**Table 2: Summary statistics and significant thresholds**

Cross	Sex	N	<u>Log HDL</u>		<u>Threshold</u>	
			Mean	SD	Suggestive ( $p < 0.63$ )	Significance ( $p < 0.05$ )
BxCSS-3	F	65	3.98	0.11	0.8	2.2
	M	65	4.31	0.11		
BxCSS-8	F	77	3.96	0.13	0.8	2.1
	M	71	4.24	0.16		
BxCSS-11	F	84	4.1	0.11	0.8	2.1
	M	70	4.37	0.09		
BxA	F	270	3.87	0.19	2.0	3.2
	M	341	4.24	0.19		

N is the number of mice in each cross.

**Figure 1: Interval mapping.** [A] Genome-wide scan of the BxA Intercross for HDL with sex as an additive covariate. [B] Chromosome specific plots of Chr 3, Chr 8 and Chr 11 from BxA Intercross with genomewide thresholds (upper horizontal lines indicated significance ( $P < 0.01$ ) and the lower lines indicate suggestive thresholds ( $P < 0.63$ )). [C] Mainscan plots for each of the  $F_2$  consomic crosses, CSS-3, CSS-8 and CSS-11 along with chromosome-wide thresholds (upper horizontal lines indicate significant ( $P < 0.01$ ) and lower lines indicate suggestive thresholds ( $P < 0.63$ )) (LANDER and KRUGLYAK 1995). Effect size estimates are shown at each putative QTL location. Additive effect is half of the difference between the AA and BB genotype classes. Positive values indicate that A is the high allele. Dominance effect is the difference between the mean of the AB genotype class and the midpoint of the AA and BB genotype class means. Positive values indicate dominance of the B allele. All analysis are performed on natural log transformed HDL. Positions are given in cM.

**Figure 2: Interaction analysis.** Effect sizes of the two interactions between [A] Chr1@2cM-by-Chr1@48cM and [B] Chr1@76cM-by-Ch8@30cM. Heat map from part of the genome-wide pairwise scan focusing on Chrs 1 and 8 [C]. *Int LOD* score refers to the interaction LOD score and applies to the area above the diagonal. *Full LOD* score is the LOD score for the full model and applies to the area below the diagonal.

**Figure 3: B.A chromosome substitution strain survey.** HDL natural log transformed for [A] male and [B] female CSS and parental B and A mice. The mean of strain B is given by the horizontal broken line and the grey area represents the  $\pm 1$  SEM boundaries, an \* indicates significance between the CSS-# and B6 after correction for multiple testing.  $N = 8-10$  for each group. Panel [C] summarizes the presence (+) or absence (-) of

QTL as detected in the CSS survey compared and BxA intercross. For BxA intercross animals, presence (+) indicates at least a suggestive QTL as discussed in the text, for the CSS animals presence (+) indicates a significant difference from parental strain B after a Bonferroni correction for multiple testing ( $P < 0.002$  [ $P < 0.05/N=21$ ]).

**Figure 4: Box plots HDL variation in intercross populations.** Natural log transformed HDL for each of the four intercrosses BxCSS-3, BxCSS-8, BxCSS-11 and BxA.

**Figure 5: Chromosome 8 QTL analysis.** Mainscan plots of Chr 8 intercrosses for [A] BxCSS-8; [B] BxA; [C] the BxA where alleles at the interacting locus on Chr1@76cM are homozygous for A/J; [D]; heterozygous; and [E] homozygous for B alleles. Grey horizontal bars indicate the 95% confidence intervals and dashed lines indicate the significance threshold.

**Supplementary Figure 1**

Genome scans of the BxA intercross for HDL [A], includes sex as an additive covariate [B], sex and sex\*QTL interaction as covariates, and [C] the difference between the two models indicating strong sex effects if a LOD of 2 is exceeded. The upper horizontal line indicates the significance threshold ( $P < 0.01$ ) and the lower lines indicate the suggestive thresholds ( $P < 0.63$ ).

Figure 1

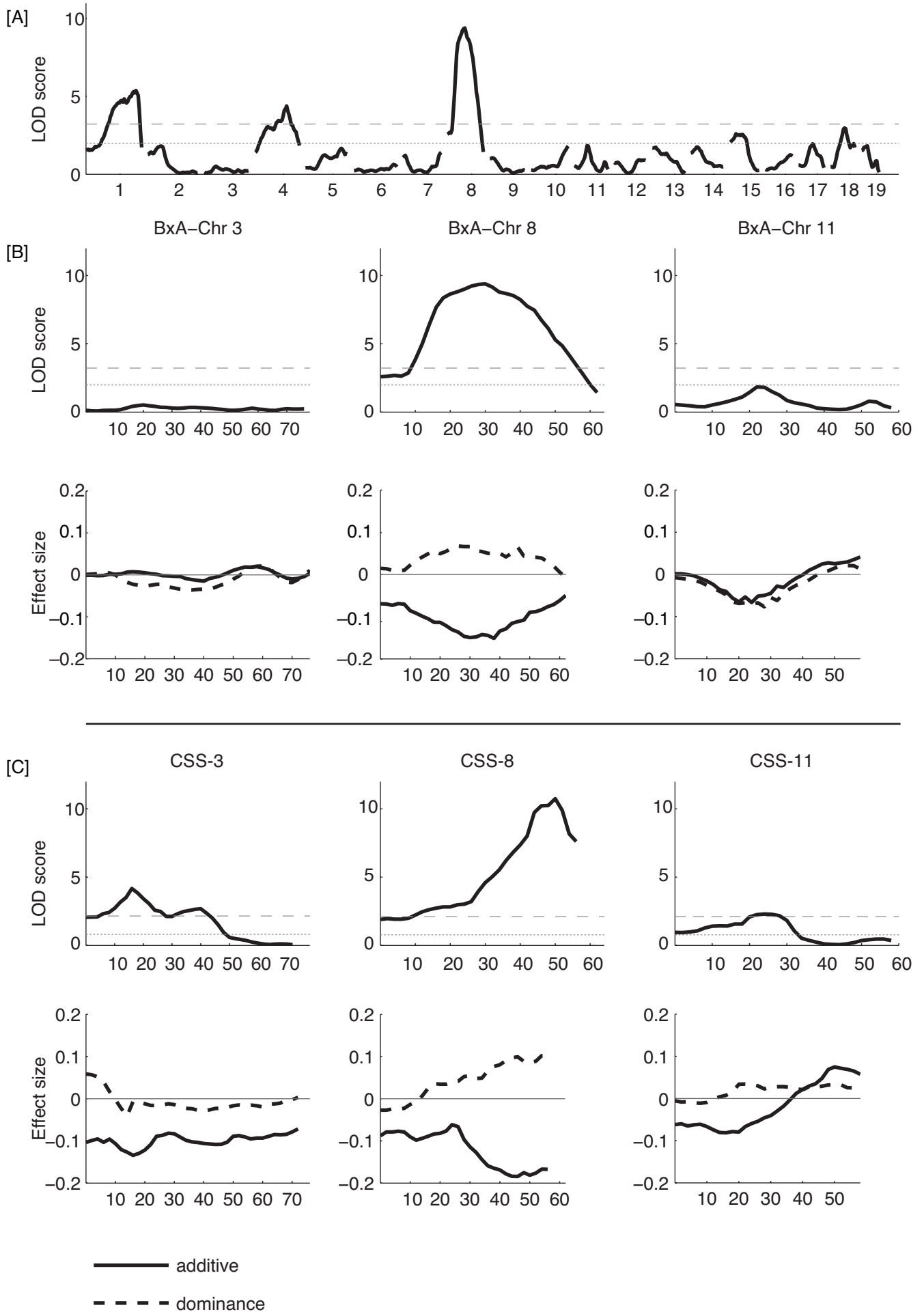
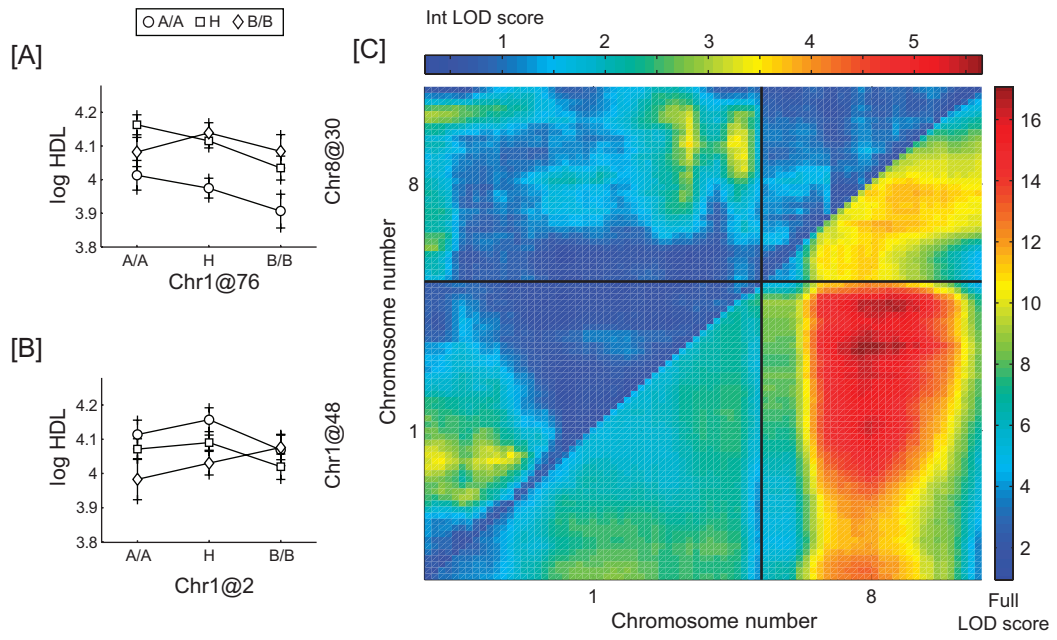
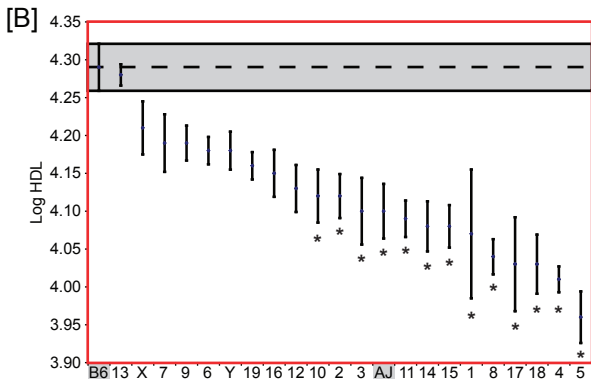
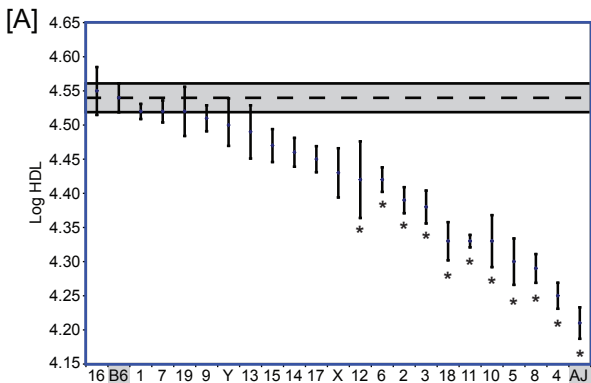


Figure 2





**[C]**

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	X	Y	
CSS Male <sup>-</sup>	-	+	+	+	+	+	-	+	-	+	+	+	-	-	-	-	-	+	-	-	-	
BXA F <sub>2</sub> Male <sup>+</sup>	+	-	-	+	-	-	-	+	-	-	+	-	-	-	-	+	-	+	+	+	NA	NA
CSS Female <sup>-</sup>	+	+	+	+	+	-	-	+	+	+	+	-	-	+	+	-	+	+	-	-	-	
BXA F <sub>2</sub> Female <sup>+</sup>	+	-	-	+	-	-	-	+	-	-	-	-	-	-	-	+	-	+	-	+	NA	NA
BXA F <sub>2</sub> <sup>+</sup>	+	-	-	+	-	-	-	+	-	-	-	-	-	-	-	+	-	+	+	+	NA	NA

Figure 4

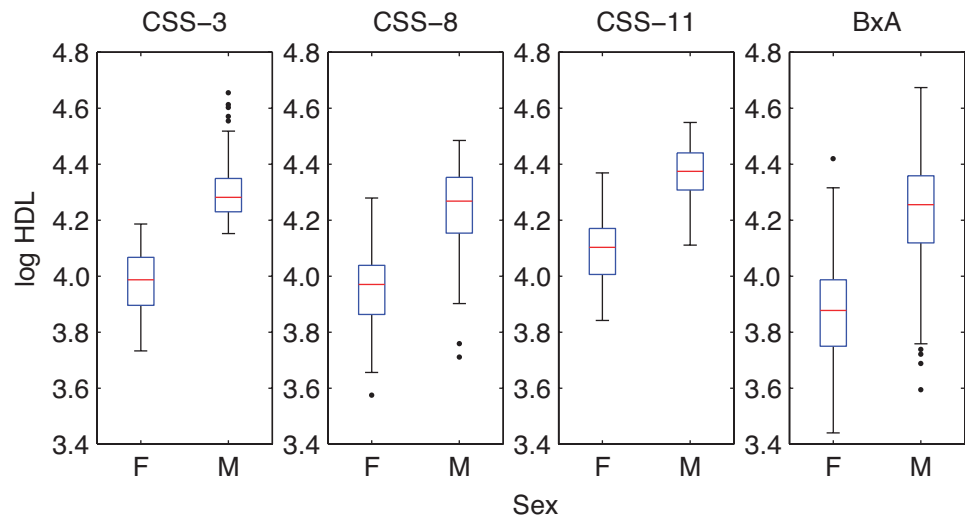
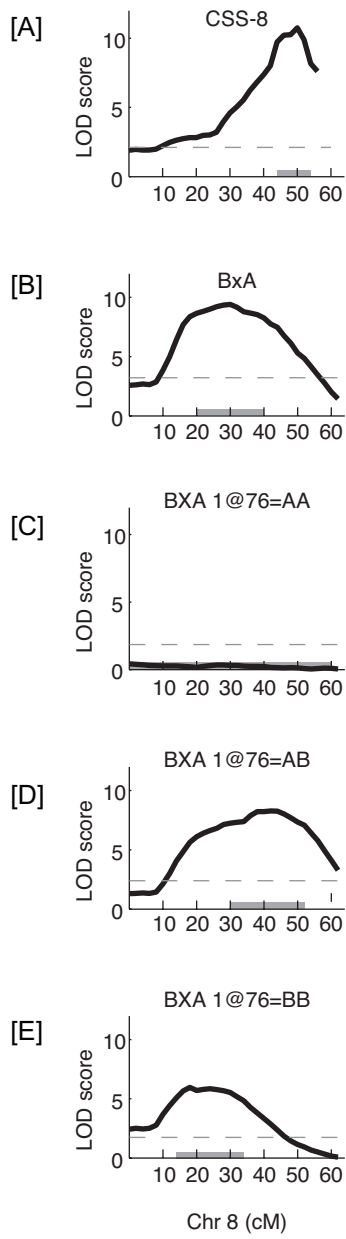




Figure 5



Supplementary Figure 1

LogHDL  
BXA intercross

