

Genetic characterization of a new set of recombinant inbred lines (LGXSM) formed from the intercross of SM/J and LG/J inbred mouse strains

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Abstract

A new set of LGXSM recombinant inbred (RI) strains is presented. The RI strain panel consists of 18 remaining strains of the original 55 founding strains. Strain characterization is based on 506 polymorphic microsatellites and 4289 single nucleotide polymorphisms (SNPs) distributed across the genome. Average microsatellite intermarker distance is 4.80 ± 4.84 Mb or 2.91 ± 3.21 F₂ cM. SNPs are more densely spaced at 0.57 ± 1.27 Mb. Ninety-five percent of all microsatellite intermarker intervals are separated by less than 15.00 Mb or 8.50 F₂ cM, while 95% of the SNPs are less than 0.95 Mb apart. Strains show expected low levels of nonsynonymous association among loci and complete genomic independence. During inbreeding, the RI strains went through strong natural selection on the *agouti* locus on Chromosome 2, especially when the epistatically interacting tyrosinase locus on Chromosome 7 carried the wild-type allele. The LG/J and SM/J strains differ in a large number of biomedically important traits, and they and their intercross progeny have been used in multiple mapping studies. The LG×SM RI strain panel provides a powerful new resource for mapping the genetic bases of complex traits and should prove to be of great biomedical utility in modeling complex human diseases such as obesity and diabetes.

Introduction

Recombinant inbred (RI) strains have been used to map a wide range of Mendelian loci and quantitative traits (Taylor 1989). They offer compelling advantages for mapping complex genetic traits, particularly those that have low heritabilities. Each recombinant genome is replicated in the form of an entire isogenic line with genotypic variance concentrated among lines and eliminated within lines (Falconer and Mackay 1996). Because of the isogenic nature of the lines, phenotypic and genotypic data can be pooled across different animals and even between generations. The combined effect of concentrating genotypic variance in comparisons among strains and having many replicates of identical genotype within strains greatly improves prospects for mapping quantitative trait loci (QTLs) for traits with lower heritabilities or that require complex, difficult-to-obtain measurements. The phenotypic data collected on RI animals is cumulative in that the results of separate experiments at different times and places can be combined in genetic studies.

A major advantage of using a RI strain mapping panel is that molecular genotypes have to be obtained only once. Since each strain is isogenic, genotypes do not need to be obtained from all individual animals studied but only from a small representative sample. Once the representative sample is genotyped, any researcher can use these genotypes to map any complex trait of interest. This allows research groups that do not have access to genotyping, because of lack of laboratory equipment or funding, to participate fully in complex trait genetic mapping studies. Other mapping panels, such as F₂ intercrosses between isogenic lines, require genotyping

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each individual because individuals are genetically unique in this design.

Another major advantage of RI strains is that genotypes generated by different research groups using a variety of methods can be pooled to generate high-density linkage maps. Although no longer a major issue, this attribute was a significant advantage before the advent of efficient and easy PCR genotyping methods (Weber and Broman 2000) and SNPs (Gabriel et al. 2002; Stickney et al. 2002).

The experimental power of the RI strain design comes at the price of elevated sensitivity to genotyping errors compared to Advanced Inter Cross Line or F₂ designs. Each typing error expands distances between loci and degrades linkage between neighboring markers, inevitably blurring associations between genotypes and phenotypes and making it difficult to map underlying loci. The accumulation of false recombinations has become extreme in some RI strain sets. For example, Williams et al. (2001) found that the map of Chromosome 1 in the BXD RI strain data set (Mouse Genome Informatics release 2.5) is 1305 cM long and made up of 160 linked loci. This map length is just over three times the length expected of an RI strain map. Fortunately, the BXD linkage map expansion has now been largely corrected by Williams et al. (2001). Another disadvantage of the RI strain mapping design is that the number of strains is often rather small, limiting statistical power and resulting in nonsyntenic associations among markers that complicate the interpretation of QTL mapping results.

Strain origin. The strains used in this study are derived from the intercross of the LG/J "Large" and SM/J "Small" inbred lines maintained by The Jackson Laboratory (Bar Harbor, ME). The parental strains used here have a long history. The LG/J strain originated from a population selected for large body size at 60 days (Goodale 1938). The original population subjected to selection was composed of 8 male and 28 female albino mice "of no particular distinction" obtained over a period of nine months from a commercial breeder. The SM/J strain was derived from a separate experiment in which selection was for small body size at 60 days (MacArthur 1944). The source population for MacArthur's (1944) experiment was produced from four crosses of seven different inbred strains, including "dilute brown" (*dba*), "silver chocolate" (*sv ba*), "black and tan" (*a^t*), "pink-eyed, short-eared dilute brown" (*ps^edba*), and the locally derived strains "albino" (*c*), cinnamon spotted (*bs*), and "agouti." These strains were chosen because they were all unexceptional with regard to body size. Only *dba* seems to survive today as two

quite distinct substrains, DBA/1 and DBA/2. Twenty-six F₁ animals were produced from four crosses (*a^t* by *bs*, *c* by *dba*, *sv ba* by *ps^edba*, and *a^t* by "agouti"). The F₁ animals were allowed to interbreed freely in a common cage producing several hundred F₂ mice. Unlike Goodale (1938), MacArthur's (1944) selection experiment included both up and down selection lines. By the end of the experiment these two selection lines were an incredible 28 standard deviation units apart for 60-day body weight (MacArthur 1944). The SM/J strain is derived from MacArthur's (1944) down selected line. The up selected line is no longer extant, thus we used Goodale's LG and MacArthur's SM lines to generate our RI strain panel. Using strains derived from different experiments and progenitors has also the advantage of introducing more genetic diversity into the experimental population.

In each case, the original selected lines were systematically inbred and maintained by brother-sister mating to the present (Chai 1956a, 1956b, 1957, 1961, 1968). This involved over 125 generations of brother-sister mating for the LG/J and SM/J strains (Festing 1996). Chai's studies (1956a, 1956b) showed a 24-g difference in body weight at 60 days between the two strains. He found that strain differences were due to many genes—at least 11 effective loci—each of relatively small effect. In our own recent experiment, after correcting for gender differences, we found an approximately 20-g body weight difference between these two strains at 10 weeks (Kramer et al. 1998). Cheverud et al. (1996) and Routman and Cheverud (1997) also found a multigenic basis of body size differences between the LG/J and SM/J strains. Eighteen potential QTLs of small but varying effects and their epistatic interactions for weight at week 10 were identified in an earlier intercross experiment (Cheverud et al. 1996; Routman and Cheverud 1997), a number consistent with Chai's (1956a) quantitative genetic results. LG/J animals in our laboratory weigh the same as they did in Chai's studies 50 years ago. The SM/J strain is now 6 g heavier than it was in the 1950s, probably a result of unintentional selection for fertility during strain maintenance (Kramer et al. 1998).

The SM/J strain is maintained in a heterozygous condition at the agouti locus. This is accomplished by breeding black mice (*a* allele at agouti) with white-bellied agouti mice (*A^w* at agouti). The *A^w* allele is dominant to the *a* allele for coat color, so white-bellied heterozygotes are mated with black homozygotes to continue the line. Investigation into the reasons for this mating scheme found it to be historical. This mating scheme was started by Chai and has been continued by The Jackson Laboratory

Table 1. Traits with known genetic variation in populations from the intercross of the LG/J and SM/J strains

<i>Traits</i>	<i>References</i>
Brain morphology	Williams, personal communication
Cranial morphology	Leamy et al. 1999; Wolf et al. 2005
Cranial nonmetric traits	Leamy et al. 1998
ENU-induced cancer	Graubert, personal communication
Growth	Cheverud et al. 1996; Routman and Cheverud 1997; Vaughn et al. 1999; Wu et al. 2004
<i>Helicobacter pylori</i> colonization	Akada et al. 2003
Litter size	Peripato et al. 2004
Liver triglycerides	Schoenfeld, personal communication
Long bone lengths (femur, tibia, humerus, ulna)	Kenney-Hunt et al. 2006
Mandibular asymmetry	Leamy et al. 1997, 2002
Mandibular morphology	Cheverud et al. 1997, 2004c; Mezey et al. 2000; Klingenberg et al. 2001, 2004; Ehrich et al. 2003b
Maternal care	Peripato and Cheverud 2002; Peripato et al. 2002
Maternal effects on neonatal growth	Wolf et al. 2002
Molar morphology	Workman et al. 2002; Leamy et al. 2005
Obesity	Cheverud et al. 2001, 2004a, 2004b; Ehrich et al. 2003a, Ehrich et al. 2005
Organ weights (heart, liver, spleen, kidneys)	Kramer et al. 1998; Cheverud et al. 2004a, 2004b; Kenney-Hunt et al. 2006
Plasma levels (glucose, leptin, insulin, free fatty acids, cholesterol, and triglyceride)	Ehrich et al. 2003a, 2005; Cheverud et al. 2004a, 2004b
Response to dietary fat	Cheverud et al. 1999a, 2004a, 2004b; Ehrich et al. 2003a
Sexual dimorphism in growth	Zhao et al. 2004
Tissue regeneration	Heber-Katz, personal communication

ever since the acquisition of these strains (Chai, personal communication). Although not explicitly stated, it is likely that attempts to fix this strain for either allele were unsuccessful. To date, Chai's studies (1956a, 1956b) have held up in replication 50 years later, and the SM/J and LG/J strains and intercrosses have contributed greatly to our understating of the genetic basis of complex phenotypes. The creation and characterization of RI lines derived from the LG/J × SM/J intercross will further our knowledge of the genetic basis of complex phenotypes and their interactions with the environment by providing us with replicated experimental mapping populations and allowing us to implement powerful experimental breeding designs.

Our studies of the LG/J × SM/J intercross have mapped multiple QTLs for a wide variety of traits including studies of growth (Cheverud et al. 1996), obesity (Kramer et al. 1998; Cheverud et al. 2001), response of obesity and diabetes-related traits to high levels of dietary fat (Cheverud et al. 1999b; Ehrich et al. 2003a), skeletal morphology (Cheverud et al. 1997; Leamy et al. 1999; Ehrich et al. 2003b; Kenney-Hunt et al. 2006), litter size (Peripato et al. 2004), maternal effects on neonatal growth (Wolf et al. 2002), and maternal behavior (Peripato and Cheverud 2002; Peripato et al. 2002). Because of the wide range of phenotypes for which the LG/J × SM/J cross has shown genetic variation (for complete list

see Table 1) and considering the many advantages offered by the RI strain design, we developed our own set of RI strains derived from the LG/J × SM/J cross.

In this report we describe the production of the LGXSM RI strains from the original F₂ intercross to the present. We also describe the molecular genetic composition of the strains and their utility for gene mapping studies. In addition, we tested for mutation and natural selection associated with production of the RI lines. New mutations were reported when a RI strain carried an allele distinct from both parental alleles. In testing selection, we specifically focused on the agouti locus which has been maintained in a heterozygous state in the otherwise inbred SM/J line.

Materials and methods

Mouse population. The mouse population used in this study is represented by 18 lines of fully inbred recombinant inbred (RI) strains (Taylor 1989) derived from the LG/J and SM/J inbred lines. A F₂ intercross of the LG/J and SM/J strains was performed and then served as the source of animals for the RI strain set. Five hundred ten F₂ animals were generated from 54 F₁ hybrids of the parental strains. The F₂ animals were randomly mated to produce 200 full-sib F₃ families ($n = 1600$). In an experiment

measuring the effects of epistasis on the evolution of additive genetic variation through a population bottleneck (Cheverud et al. 1999b), 55 lines were started with two males and two females per generation to produce inbreeding from the F₃ to the F₇ generations. We began brother-sister mating in the F₇ generation instead of the standard F₃ generation. There are two minor consequences for gene mapping in the RI lines arising from our modified design. First, we have had to complete 22 generations of inbreeding (F₂₅) to reach the level of inbreeding obtained after 20 generations of standard brother-sister mating. Reduced inbreeding in the first few generations actually improves our ability to use these strains for genetic mapping because delayed brother-sister mating increases the amount of recombination between linked markers in our RI strain set relative to sets created purely by brother-sister mating (Haldane and Waddington 1931; Hanson 1959; Liu et al. 1996). Eighteen strains are currently fully inbred ($F > 0.9863$) and range from the F₂₈ to the F₃₂ generation; the remaining 37 lines have been lost.

General husbandry procedures consisted of animals being weaned at 3 weeks of age, at which time each family was evenly segregated by sex, with no more than 5 animals per cage. Animals were fed PicoLab Rodent Chow 20 (No. 5053) *ad libitum*. The animal facility was maintained at a constant temperature of 21°C with 12-h light and dark cycles. brother-sister pairs were mated at 7 weeks of age, allowing production of 4–5 generations a year. Animals not used to maintain the strains or not used in experiments were sacrificed after weaning. All animals used in genetic characterization have an inbreeding coefficient greater than 0.986.

PCR procedures. Microsatellite loci distributed across all autosomes and the X Chromosome were typed using a modified version of a polymerase chain reaction (PCR) protocol optimized in our laboratory (Routman and Cheverud 1994, 1995). A total of 506 primer pairs that selectively amplify microsatellite loci polymorphic for LG/J and SM/J strains were purchased from Research Genetics (www.res-gen.com), or from Integrated DNA Technologies (www.idtdna.com). Almost all primer sets were originally designed by Whitehead Institute/MIT Center for Genome Research, based on their screens of polymorphic microsatellite loci in mice (Dietrich et al. 1992). A few primers were from other sources such as the Washington University School of Medicine (Wsm) or the Nuffield Department of Surgery (NDs). A complete list of markers and their physical position is available in Appendix 1.

Each 10- μ l PCR reaction mixture contained 1× PCR buffer, 1.92 mM MgCl₂, 0.25 unit of Promega® Taq DNA polymerase, 0.2 mM of each deoxynucleotide, 132 nM of the primers, and 50 ng of genomic DNA. Reactions were set up using a 96-channel pipetting station. A loading dye (60% sucrose, 1.0 mM cresol red) was added to the reaction before PCR (Routman and Cheverud 1994). PCR reactions were carried out in 96-well PCR plates and cycled in MJ Research PTC-200 or Eppendorf MasterCycler thermocyclers. To assure that we were amplifying the correct genomic region, we used a high-stringency touchdown PCR protocol. The temperature profile consisted of (1) preheating at 68°C for 60 sec, (2) denaturation at 93°C for 10 sec, (3) annealing at 55–50°C for 35 sec, (4) extension at 68°C for 60 sec, and (5) a final extension at 68°C for 10 min. Steps 2–4 were repeated 25 times; in the first 9 cycles the annealing temperature was lowered by 0.5°C until an annealing temperature of 50°C was reached. After 35 PCR cycles, PCR products were run on 5% Fisher brand Agarose Low EEO, stained with ethidium bromide, and visualized under UV in a BioRad UV chamber. Photos were recorded as hard and electronic copies, scored, and then directly entered into a Microsoft Excel database file.

Microsatellite markers and error checking. Appendix 1 identifies the microsatellite markers that we scored on each chromosome in the RI strains. To minimize genotyping errors, we retyped and rescored all markers. The order of microsatellite loci on each chromosome was determined by actual physical position given in the Ensembl (www.ensembl.org) build m32 of the mouse strain C57BL/6J genome. The positions of microsatellite markers that could not be physically mapped on to the C57BL/6J genome were determined from the Mouse Genome Database (MGD) recombination map. Marker strings were checked for double recombination. Markers that introduced double recombinations within short genomic regions were regenotyped and rechecked to minimize errors associated with future QTL analyses. With few exceptions, marker order determined from the physical map based on the Ensembl m32 build of the mouse strain C57BL/6J genome and the MDG recombination map is congruent.

SNP genotyping. The 13,377 SNP loci distributed at 0.19 ± 0.16-Mb intervals across all 19 autosomes and the X Chromosome were typed in collaboration with the Wellcome Trust Centre for

Human Genetics (<http://www.well.ox.ac.uk/mouse/INBREDS>). Typing used Illumina DNA chip technology (www.illumina.com).

Coat color of RI lines. In our LG/J × SM/J cross, coat color is determined by the agouti locus on Chromosome 2 and the tyrosinase (*Tyr*) locus on Chromosome 7. LG/J carries the non-agouti allele (*a*) on Chromosome 2 and the albino (*c*) allele on Chromosome 7. SM/J is either homozygous for the non-agouti allele (*a*) or heterozygous for the non-agouti and white-bellied agouti (A^w) alleles on Chromosome 2 and wild-type (+) at the tyrosinase locus. All F₁ intercross animals were either black (*a/a*;*+/c*) or white-bellied agouti (A^w/a ;*+/c*). In the F₂ and F₃ generations, the ratio of black and agouti (*a/a* or A^w/a ; *+/+* or *+/c*) to albino was 0.73:0.27 in 1995 animals. This represents a very slight but statistically significant ($p = 0.014$) deviation favoring the *cc* homozygote at the tyrosinase locus.

Selection at coat color loci will be examined by comparing the two locus genotypes of the various surviving RI strains to random expectations. Genotypes at the agouti and tyrosinase loci are measured using microsatellite markers closely linked to their genomic locations, *D2Mit286* and *D7Mit62*, respectively. We will also examine the relative survivorship of strains having been fixed for the black or white-bellied agouti versus albino coat color using the Cox regression.

Results and discussion

Strains. The RI strains were not started in the usual way because they were also being used in a population bottleneck experiment in which the level of heritable variation is followed as populations pass through a bottleneck of small population size. As described above, this variation in breeding has no effect on the utility of the strains for gene mapping, except allowing for slightly higher resolution in our strains. Data from our mapping experiments suggested that epistasis was common for body size traits (Routman and Cheverud 1997), and theoretical considerations indicated that its effects on heritable variance would be strongest in a population of size four in the F₇ generation ($F = 0.4$). A smaller population size ($n = 2$) does not allow for the maximum effects of epistasis on heritable variance to be observed because double fixation at multiple interacting loci occurs too quickly. We started forming 55 inbred lines using two males and two females chosen randomly from a pair of F₃ sibships. These animals were mated in April 1996. The lines were maintained in this fashion to the F₇ generation and

the expected experimental effects on levels of heritable variance were observed (Cheverud et al. 1999b). Eight strains (15%) were lost by the F₇ generation. Obligate brother-sister mating was begun at the F₇ generation and has been continued to date. At first, four brother-sister pairs were mated for each strain. However, as strains were lost this was increased to six brother-sister pairs per strain to increase the probability of successful breeding and rearing of the young. A few lines were also either bred with control animals or the LG/J strain to boost fertility and strain survival. The negative aspect of this is the addition of several generations of inbreeding to those needed to reach fully inbred status.

Strains could be considered inbred by the F₂₅ generation. The time at which individual strains entered their F₂₅ generation varied somewhat because of the vagaries of breeding, although most had reached this stage by late 2001, a period of more than 5 years from the start of breeding. This is a little longer than would have been required with brother-sister mating starting in the F₃ generation. Eighteen strains (33%) reached the F₂₅ generation. Animals began to be transferred to experimental populations in mid-2002 (Cheverud et al. 2004a, 2004b). The strains are presently in the F₃₂–F₄₁ generations, varying with the rate of production for the strains. Eleven of these strains breed well while the remaining seven breed relatively poorly, resulting in longer generation intervals and smaller populations in these strains.

Genotyping and marker distribution. We have scored 506 polymorphic microsatellite markers in the 18 RI strains (Table 2). This represents approximately 35% of the total number of markers tested initially for between-strain polymorphism. The genotypes of all RI strains are presented in Appendix 1 and have been deposited in both the Mouse Phenome Database (MPD:178; <http://www.jax.org/phenome>) and at the WebQTL site (<http://webqtl.org>). The genotypes are also available on our laboratory web site in Microsoft Excel format (<http://thalamus.wustl.edu/cheverudlab/>). We also typed 15 of these RI strains using 13,377 SNP markers, 4289 of which were polymorphic between the LG/J and SM/J strains. Strains 5, 10, 15, and 18 were not included because we had insufficient DNA for accurate SNP typing.

Despite our best attempt, the spacing of genetic markers is not completely uniform. The average distance between microsatellite markers is 4.80 Mb [standard deviation (SD) = 4.84 Mb] or 2.91 F₂ cM (SD = 3.21 F₂ cM). The average distance between polymorphic SNP markers is 0.59 Mb (SD = 1.27

Table 2. Largest gap size characterization for microsatellite markers

Chr	Length		Marker No.	Recomb No.	Largest gap	
	MGD (cM)	Physical (Mb)			MGD (cM)	Physical (Mb)
1	112	195.2	55	88	8.0	15.1
2	114	181.7	31	54	22.3	25.2
3	95	160.6	27	60	6.8	18.7
4	84	154.1	37	55	9.5	16.2
5	92	149.2	21	49	13.0	20.5
6	75	149.7	31	68	10.4	15.9
7	74	133.1	31	46	11.0	16.0
8	82	128.7	16	35	15.0	23.4
9	74	124.2	31	43	8.0	20.4
10	77	130.6	26	51	10.0	22.5
11	80	121.6	32	53	15.0	22.3
12	61	115.1	24	50	6.0	13.9
13	80	116.5	34	41	9.0	16.6
14	69	117.1	16	23	20.0	18.1
15	75	104.1	19	44	14.7	8.7
16	72	98.8	13	26	7.4	9.5
17	58	93.6	17	38	8.7	13.1
18	59	91.1	17	40	6.0	8.2
19	57	60.7	16	28	17.0	6.2
X	77	160.6	12	27	25.0	38.6
Total	1567	2932.4 ^a	506	919		

95% intervals < 15 Mb.

^aTotal base pairs. Golden Path Length = 2615.2 Mb.

Mb). During the process of inbreeding of the RI strains, additional recombination occurs, resulting in the expansion of the F₂ cM map. In a study of 100 independent RI strains, Williams et al. (2001) observed an average 3.7 map expansion in the RI strains compared with the F₂ generation. Using this expansion factor, the intermarker distance in our RI strain set is 10.77 ± 11.88 RI cM. Although variances around means are large, 95% of all markers are less than 15 Mb apart at microsatellite loci (Fig. 1) and only 1.93 Mb apart at SNP markers. The outlier microsatellite interval, at 38.5 Mb, occurs at the central portion of Chromosome X. No markers between *DXMit163* (at position 11,927,923) and *DXMit64* (89,040,048) were variable. This region spans 25 F₂ cM, the equivalent of over 90 RI cM. There are also three large invariant regions of SNP markers on Chromosome X spanning 16 Mb (28,890,781–44,656,596), 7 Mb (45,612,522–52,389,338), and 22 Mb (61,660,457–83,872,209). The largest invariant region in the SNP data spans 27 Mb in the proximal end of Chromosome 8 from position 26,454,785 to 53,532,210. This region also lacked microsatellite markers.

Although there is a highly significant association between recombinational distance (cM) and physical distance (Mb; $p < 0.001$), the correlation between these two distance measures is relatively low ($r = 0.546$). The low correlation is best explained by

recombinational hotspots and coldspots. Several outstanding examples include regions on Chromosomes 6, 10, and 14. Chromosome 6 marker pair *D6Mit268/D6Mit223* spans 10.1 F₂ cM and 9.8 Mb, while the adjacent pair *D6Mit223/D6Mit183* spans

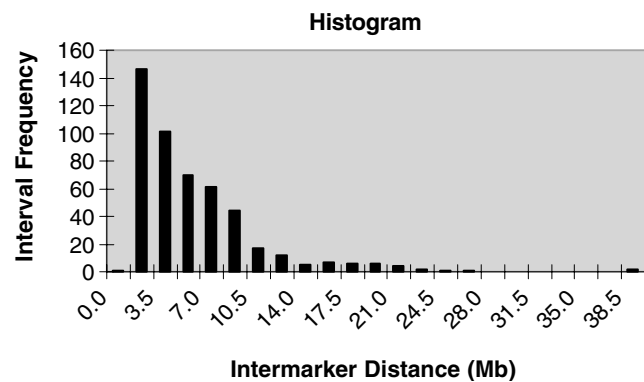


Fig. 1. Histogram of microsatellite intermarker distances. Ninety-five percent of all markers are less than 15 Mb apart. Average intermarker distance is 4.80 Mb with a mode of 3.47 Mb. This corresponds to a mean of 2.91 F₂ cM (10.76 RI cM) and a mode of 1.00 F₂ cM (3.7 RI cM). Outlier at 38.5 Mb represents the central portion of Chromosome X for which no markers between *DXMit163* (at position 11,927,923) and *DXMit64* (89,040,048) were variable. This area spans 25 F₂ cM which is equivalent to over 90 RI cM. Conversion from F₂ to RI cM is based on an average 3.7 map expansion from F₂ to RI strains observed for 100 independent RI strains by William et al. (2001).

only 3.4 F₂ cM over a 10.7-Mb physical distance. Chromosome 10 marker pair *D10Mit170/D10Mit130* spans 10.0 F₂ cM and 22.5 Mb, while the adjacent pair *D10Mit130/D10Mit15* spans only 2.4 F₂ cM and over an 18.3-Mb interval. Chromosome 14 marker pair *D14Mit5/D14Mit225* spans 14.1 F₂ cM and 15.1 Mb while the adjacent pair *D14Mit225/D14Mit7* spans only 2.0 F₂ cM over 15.8 Mb.

Some regions may simply not be polymorphic as a result of shared ancestry in the mouse strains used to generate the common inbred strains (Wade et al. 2002). Because of the mosaic nature of the mouse laboratory strains, it is expected that only approximately one third of the genome will be highly polymorphic between any two strains, with the remaining two thirds showing medium to very low rates of polymorphism. This is confirmed by the empirical observation that 4289 of the 13,377 typed SNPs are polymorphic, which represents a polymorphism rate of 32%. Furthermore, the lack of polymorphism is not randomly distributed through the genome and will exist in patches of completely conserved blocks between any two strains, and variable blocks.

The 38.5-Mb (25 F₂ cM) central portion of Chromosome X typed in our RI strain panel may represent such a case of highly conserved block as a result of shared ancestry of the LG/J and SM/J strains. We typed this region spanned by *DXMit163* at position 11,927,923 and *DXMit64* at position 89,040,048 with all published microsatellite markers at intervening positions, but found no markers that could differentiate between the parental LG/J and SM/J genomes. SNP data show similar results. From position 28,890,781 to 52,389,338 56 SNP markers were typed, but only marker *rs13483756* at position 45,475,262 was polymorphic between the LG/J and SM/J strains. No polymorphic markers are found from 61,660,457 to 83,872,209 on Chromosome X, a region spanning 68 SNPs. The intervening 9.5-Mb region between 52,389,338 and 61,660,457 contains 25 variable SNPs; however, this region contains no polymorphic microsatellite markers. Large sections, defined as spans of 4 Mb or greater, of Chromosomes 5, 8, 9, 10, 14, 16, and 17 are also not variable in SNP markers between the LG/J and SM/J strains and can represent a significant portion of the total chromosome length. For example, the large conserved blocks on Chromosome 17 comprise over 25% of the total chromosomal length. Overall, the SNP genotyping indicates that approximately 20% of the genome is potentially identical by descent between LG/J and SM/J. These regions are quite unlikely to contain QTLs unless they are due to new mutations.

RI strain genetic composition. All RI strains animals were genotyped in the F₂₂–F₂₇ generations. At the time of initial genotyping, strains 4 and 35 were not yet fully inbred because of crossing with an F₁ LG/J × SM/J animal to reinvigorate these strains during the inbreeding process. Strain 33 was mistakenly crossed with a strain 38 animal. These three strains were approximately 25% heterozygous during initial rounds of genotyping. These three strains were re-genotyped and analyzed for SNP variation after additional 13 generations of brother-sister mating after the initial round of genotyping, after which their heterozygosity levels approached those of other strains. The residual heterozygosity of all strains is only 1.51%. Approximately 30% of this residual heterozygosity is contributed by strains 4 and 35. Residual heterozygosity in the remaining inbred strains is 1.00%. Distribution and size of heterozygous regions in all RI strains across all chromosomes is presented in Appendix 2.

Two strains, 6 and 31, were breeding very poorly and were backcrossed to the LG/J strain in the F₈ generation. While the heterozygosity is low in these strains, 2.40% and 0.59%, respectively, they carry the expected 3:1 LG/J to SM/J allele ratio instead of the usual 1:1 ratio expected for RI strains. In this respect the strains 6 and 31 resemble recombinant congenic strains. Considering only the remaining 16 strains, 49.21% of the 8096 alleles are from the SM/J parent and 49.28% from the LG/J parent, with the remaining 1.51% of the loci not yet fixed. There is no significant difference between the frequency of parental alleles ($p = 0.937$) despite the incredibly large sample of alleles. The frequency of both LG/J and SM/J alleles ranges from 12.50% to 81.25% across the loci. The distribution of allele frequencies across loci in all RI strains is given in Fig. 2.

Independence of strain genotypes. The sharing of large regions of identical or similar genotypes across a number of chromosomes between RI strain pairs is generally caused by breeding errors. These breeding mistakes and the resulting nonindependence of the strain genotypes reduce the information content of the RI strain set. To test for nonindependence, we computed all pairwise correlations of microsatellite genotypes of the LGXSM RI strain panel. Figure 3 shows the genetic similarity based on 506 markers of this 18-strain RI panel. The near-normal distribution of genotypic similarity indicates that the percent identities are randomly distributed as expected, and thus all 18 strains are informative for genetic mapping. Mean identity is 50.10% with a mode of 51.38%. The outlier at 72.13% genomic similarity represents a comparison between RI

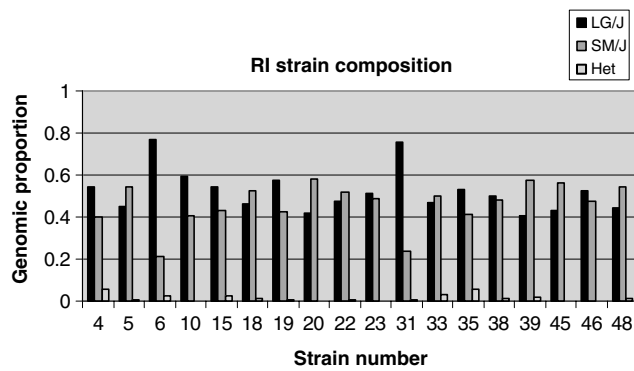


Fig. 2. LGXSM RI strain genomic compositions. Strains 6 and 31 are 76.6% and 76.0% LG/J, respectively, resulting from the hybridization of the original strains with a LG/J animal. All remaining strains have approximately 50% LG/J genomic background. Largest residual heterozygosity (6.0%) remains in strain 35 since this strain together with strain 33 was reinvigorated by crossing it with F₁ LG/J × SM/J animals around generation 20.

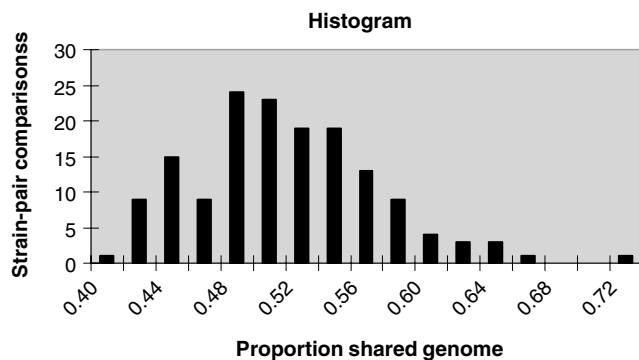


Fig. 3. All possible pairwise strain genome comparisons determined for all 18 RI strains. The near-normal distribution of genotypic similarity indicates that the percent identities are randomly distributed as expected, and thus all 18 strains are informative for genetic mapping. The percent mean identity is 50.10% with a mode of 51.38%. The outlier at 72.13% genomic similarity represents a comparison between RI strains 33 and 38. The approximate normal distribution of percentage of shared genotypes between LGXSM RI strains is comparable to the distribution of BXN RI strains (Williams et al. 2001) and LXS RI strains (Williams et al. 2004).

strains 22 and 33. The approximate normal distribution of percentage of shared genotypes between LGXSM RI strains is comparable to the distribution of BXN RI strains (Williams et al. 2001) and LXS RI strains (Williams et al. 2004).

Correlations between pairs of strains are typically low, averaging 0.043 with a standard deviation of 0.27. This is as expected for a set of independent strains (Sokal and Rohlf 1995). The exception is the relationship between strains 33 and 38 noted above. As strain 33 approaches fixation, the correlation

between the two strains will approach 0.46. While not independent, these strains share only 25% of their variance in common and so do provide much independent information about phenotype and genotype.

Genotype correlations. The relatively small number of strains included in this set will lead to instances of nonsyntenic correlations and potential “shadow” QTLs (Cheverud et al. 2004a). The average correlation between nonsyntenic markers is 0.02, close to the expected value of 0.00. The standard deviation of correlations among nonsyntenic markers is 0.261, again very close to the expected value of 0.267. This means that we expect each marker, on average, to have one nonsyntenic association greater than 0.77. The distribution of intermarker correlations is shown in Fig. 4.

Recombination. The LGXSM RI strains carry an average of 51.1 recombinations per strain (SD = 16.3) for a total of 919 recombinations across the whole set. This number of recombinations is just slightly higher than the number found by Williams et al. (2001) in RIs involving C57BL/6J (45 recombinations) and expected under standard strain formation by brother-sister mating after the F₂ generation. This increased value was expected because of the delay in brother-sister mating until the F₇ generation. Further contribution to the increase and variance in recombination number comes from the strains 4, 33, and 35, which were crossed with control animals, or strain 38 during the inbreeding process. This resulted in continued accumulation of recombinations in these strains. Removing these strains from our sample results in an average of 45.1 ± 9.8 recombinations per remaining strain. These recombination rates are qualitatively the same as those found by Williams et al. (2001).

Generation of new alleles. In a study of our size, we would expect a certain percentage of new microsatellite alleles to arise in the population by mutational processes. Pedigree analysis of human data suggests that microsatellite mutations arise at an average rate of 2.5×10^{-3} per observation (Brinkmann et al. 1998; Sajantila et al. 1999; Kayser and Sajantila 2001), although older estimates (Weber and Wong 1993) reported a slightly lower mutation rate of 5.6×10^{-4} . During the course of our analysis, we scored 18,261 genotypes—506 markers, 18 strains, 2 individuals per strain. At the two observed mutation rates, we would expect between 45.5 and 10.2 new alleles, respectively, in our data set. We observe 11 new alleles at seven loci. *D1Mit463*, *D2Mit286*,

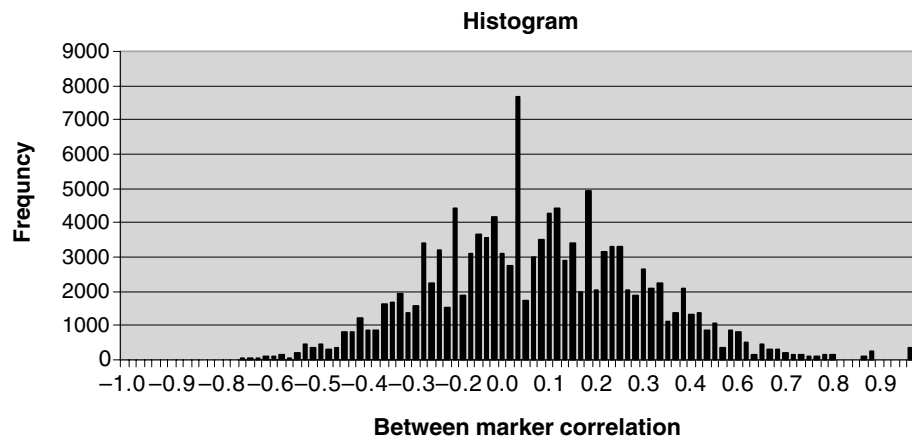


Fig. 4. Histogram of marker correlations; correlation = 0.043 ± 0.27 . This is as expected for a set of independent strains. Because of the small number of strains, we also expect each marker, on average, to have one nonsynthetic association greater than 0.77. This is manifested by outliers on the right-hand side of the figure.

D5Mit352, and *D7Mit117* are fixed for the new allelic state in RI strains 39, 18, and 5, respectively, and thus represent only one mutational event. At *D1Mit452*, *D2Mit354*, and *D7Mit332* only one of the genotyped individuals per strain possessed a new allelic state; these new allelic states occurred in strains 39, 15, and 18, respectively. These 11 new alleles represent seven new mutations. The new allelic states are not equally distributed, with five new alleles (three mutations) occurring in strain 39 and three new alleles (two mutations) in strain 18. Our results are on par with previous estimates of microsatellite mutation rates, but they are closer to the lower mutation rate of 5.6×10^{-4} estimated by Weber and Wong (1993). However, because we genotyped our RI panel on agarose gels (Routman and Cheverud 1994) and because our genotype estimates were conservative, it is also possible that not all newly arisen allelic states were detected.

Coat color of RI lines. The survivorship curves for strains fixed for the black or agouti versus albino coat color are given in Fig. 5. Nine of the 55 strains went extinct still carrying both alleles (+/c) at the tyrosinase locus. Survival to 30 generations was 50% among albino strains (c) but only about 10% for black strains (+). The Cox regression indicated that this difference was significant at the 0.01 level. However, the shapes of the survivorship curves are also different. The black and agouti strains show a nearly linear decline in survivorship over the generations, with a loss of 3% of the strains per generation (0.75 strains per generation). The albino strains suffer a similar rate of loss (0.70 strains per generation) to the F₁₂ generation but then the rate of loss slows to only 1.2% (0.30 strains per generation). Most of these remaining albino strains are very good breeders.

New breeders in each strain were and are selected randomly with respect to coat color by the

primary investigator who knew only the animal ID number, i.e., no consideration was given to coat color in choosing breeders. The black strains are thus clearly being lost due to natural selection. The rate of survival for 30 generations is significantly different between coat colors ($p = 0.0025$). However, it is not clear from these data alone how the loci involved

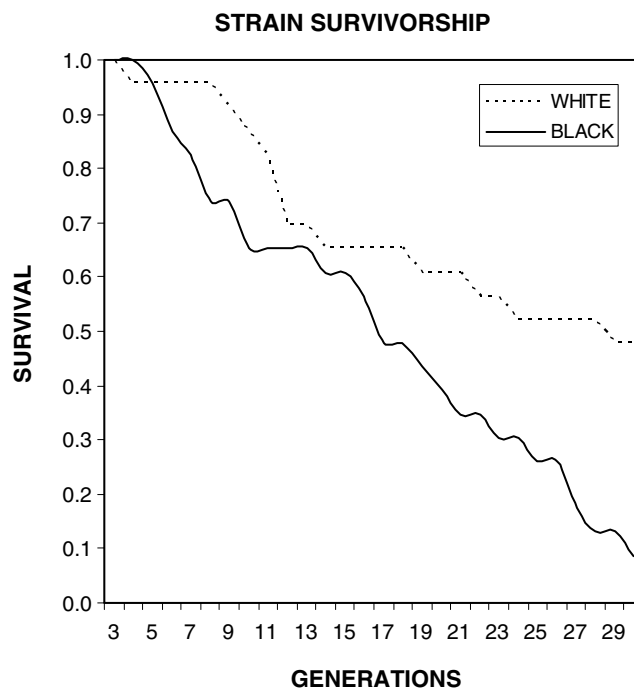


Fig. 5. Cox strain survivorship curve. Of the original 55 RI founding strains, 26 were black carrying the + allele and 29 were white carrying the c allele at the tyrosinase locus on Chromosome 7. At generation 30, 12 strains have the + locus and 6 have the c locus and are nonrandomly associated with genomic background at the Chromosome 2 agouti locus. These differences represent a significant difference in survival rate among the black and white mouse strains.

Table 3. Distribution of genotypes among RI strains surviving to the 30th generation

		Chromosome 2		
		LG/J(<i>a</i>)	SM/J(<i>a</i>)	
Chromosome 7	LG/J(<i>c</i>)	8	4	12
	SM/J(+)	6	0	6
		14	4	18

experience this selection. Genotypes at microsatellite loci closely linked to the agouti and tyrosinase loci were used to identify parental alleles. There were no recombinations separating these markers from the coat color loci in the RI strains. Table 3 provides the distribution of genotypes across strains. Strains carrying the tyrosinase *c* allele are albino, while those with the + allele are either black or white-bellied agouti. Overall, there appears to be selection against the SM/J(*a*, A^w) allele at the agouti locus because only 4 of 18 surviving strains carry it ($p = 0.018$). There is no selection against the LG/J(*a*) allele ($p = 0.16$).

When both the agouti and the tyrosinase loci are considered jointly, they are in strong linkage disequilibrium ($\phi = 0.66$; $p = 0.005$) indicating the possibility of epistatic interactions between them affecting strain survival. In fact, if the LG/J(*c*) allele resides on Chromosome 7, there is no significant selection against the SM/J(*a*) allele on Chromosome 2 ($p = 0.25$). It is only in the presence of the SM/J(+) allele on Chromosome 7 that the SM/J(*a*) allele on Chromosome 2 is exposed to selection ($p = 0.014$), indicating an epistatic interaction for fitness between these loci. Likewise, when the LG/J(*a*) allele is present on Chromosome 2, there is no selection on the tyrosinase locus, but when the SM/J(*a*) allele is at the agouti locus, there is selection against the SM/J(+) allele at tyrosinase. Strains survive best if they carry the LG/J alleles (LG/J(*a*);LG/J(*c*)) at both loci. These results suggest a molecular difference between the LG/J and SM/J *a* alleles at agouti or at very closely linked loci. Likewise, there is an epistatic interaction affecting strain survival between the agouti and the tyrosinase loci or between a pair of loci closely linked to them.

The period of greatest loss for the albino strains occurred while average inbreeding levels increased from 0.60 to 0.80 (F_9 – F_{14}). This is the inbreeding level at which deleterious recessives are most commonly exposed to selection (Crow and Kimura 1970). Thus, strains that survive this level of inbreeding have enhanced future survival because the deleterious recessive alleles they carried have been purged (Templeton and Read 1984).

Our breeding data indicate that there has been selection against the SM/J(*a*) allele at the agouti locus in the production of these RI lines, especially when the SM/J(+) allele is present at the tyrosinase locus. This is consistent with the historical practice followed at The Jackson Laboratory, indicating that it is not possible to fix the SM/J strain for either the SM/J(*a*) or SM/J(A^w) allele at the agouti locus.

Other recombinant inbred strain sets involving SM/J have had contradictory results. In the NXSM recombinant inbreds, all surviving strains carry the NZB/BLNJ parent alleles on distal Chromosome 2, in the region of the agouti locus (Eicher and Lee 1990; Frankel et al. 1992). All of these strains also carry the wild-type allele at the tyrosinase locus, under which there was strong selection against the SM/J agouti alleles in our strains. There has been natural selection against the SM/J *a* allele and in favor of the NZB *a* allele at the agouti locus in a tyrosinase + allele background during production of these strains, again consistent with the possibility that the SM/J(*a*) allele is different in some way from the *a* allele in other strains.

However, no such selection was apparent during the production of the SMXA recombinant inbred strains (Nishimura et al. 1995). In this case there is a borderline significant excess of black relative to white coat colors (18:8), and there is no selection for or against the SM/J *a* allele in the presence of the tyrosinase + allele. Instead, it is the A/J *a* allele combined with the A/J *c* allele that is relatively rare (Nishimura et al. 1995). Apparently, there are alleles at other loci carried by the A/J strain that obviate the deleterious effects of homozygosis for the SM/J(*a*, A^w) alleles.

Studies of maternal care (Peripato et al. 2002) and litter size (Peripato et al. 2004) in the LG/J × SM/J intercross provide some insights into the mechanisms of strain loss in the production of the RI lines. We noted a behavioral syndrome in our LG/J × SM/J intercross population in which some mothers did not care for their offspring (Peripato et al. 2002), leading to loss of the entire litter soon after birth. This syndrome has been recorded in 27% of our SM/J matings, 60% of our LG/J matings, none of our F_1 matings, and in 13% of the F_2 matings. We used QTL mapping to identify genomic locations associated with this outcome in the F_2 intercross population. We found suggestive evidence for an overdominant QTL in the agouti locus region of Chromosome 2 for this lack of maternal performance. Heterozygous mothers (SM/J(*a*),LG/J(*a*)) in this region performed better than either SM/J or LG/J homozygotes, again indicating some difference between the non-agouti

alleles carried by these strains. This same region was also involved in several epistatic interactions, the strongest of which involved proximal Chromosome 9 (*D9Mit4* + 12 cM) and proximal Chromosome 14 (*D14Nds1* + 12 cM).

QTL mapping of litter size in the LG/J × SM/J intercross also implicates the agouti region of Chromosome 2 in epistatic interactions affecting litter size (Peripato et al. 2004). Interactions of Chromosome 2 with proximal Chromosome 5 (*D5Mit47* + 12 cM) and distal Chromosome 15 (*D15Mit2* + 26 cM) indicate larger litter sizes for the single heterozygotes than for either double or mixed parental homozygotes.

Several independent lines of evidence implicate the agouti region of Chromosome 2 in SM/J as being responsible for loss of RI strains as a result of interactions with other loci, including the region around the tyrosinase locus. These include the historic breeding practices followed for SM/J at The Jackson Laboratory, the results obtained in the production of the LGXSM and NXSM RI lines, and QTL mapping for litter size and maternal performance in the LG/J × SM/J intercross. It remains to be determined whether this is due to polymorphism in the coat color genes themselves (agouti and/or tyrosinase) or in genes tightly linked to them. However, it also indicates that much greater RI line survival could be obtained with the LG/J × SM/J populations if artificial selection for white coat color and the LG/J(*a*) non-agouti allele were practiced during strain formation. It may also be possible to produce a SM/J-related strain carrying many of its unusual phenotypic characteristics without the necessity of forced heterozygosity at the agouti locus by making a congenic strain with the agouti region donated by another strain.

The LGXSM RI strain set has been used successfully to map QTLs affecting obesity- and diabetes-related traits and also gene-by-diet interactions (Cheverud et al. 2004a, 2004b). In addition to the RI strains, we also maintain an advanced intercross (AI) line derived from the LG/J × SM/J intercross. The AI line complements the LG×SM RI strain set by allowing fine mapping of regions previously detected in analyses of the RI mapping panel. By randomly mating the AI line over generations, recombinations accumulate, expanding the genetic map and thereby allowing finer-scale mapping of QTLs. Ehrich et al. (2005) followed up and confirmed gene-by-environment interactions on proximal Chromosome 13 first discovered in the LGXSM RI strains using the F₁₆ generation of the AI line. The combination of the RI strain set and AI line provides a powerful resource for genetic mapping of complex traits.

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Appendix 1

This appendix is a table with all microsatellite and SNP markers and their states for the 18 RI strains. It is available at <http://thalamus.wustl.edu/cheverudlab/>

Appendix 2

This appendix shows the analysis of heterozygosity in all 18 RI strains across all 19 autosomes and the X sex chromosome. It is available at <http://thalamus.wustl.edu/cheverudlab/>

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