

The Collaborative Cross: A Recombinant Inbred Mouse Population for the Systems Genetic Era

David W. Threadgill, Darla R. Miller, Gary A. Churchill, and Fernando Pardo-Manuel de Villena

Abstract

The mouse is the most extensively used mammalian model for biomedical and aging research, and an extensive catalogue of laboratory resources is available to support research using mice: classical inbred lines, genetically modified mice (knockouts, transgenics, and humanized mice), selectively bred lines, consomics, congenics, recombinant inbred panels, outbred and heterogeneous stocks, and an expanding set of wild-derived strains. However, these resources were not designed or intended to model the heterogeneous human population or for a systematic analysis of phenotypic effects due to random combinations of uniformly distributed natural variants. The Collaborative Cross (CC) is a large panel of recently established multiparental recombinant inbred mouse lines specifically designed to overcome the limitations of existing mouse genetic resources for analysis of phenotypes caused by combinatorial allele effects. The CC models the complexity of the human genome and supports analyses of common human diseases with complex etiologies originating through interactions between allele combinations and the environment. The CC is the only mammalian resource that has high and uniform genomewide genetic variation effectively randomized across a large, heterogeneous, and infinitely reproducible population. The CC supports data integration across environmental and biological perturbations and across space (different labs) and time.

Key Words: aging; Collaborative Cross (CC); mouse model; recombinant inbred mice; systems genetics; genetic reference population

David W. Threadgill, PhD, is a professor in and head of the Department of Genetics at North Carolina State University in Raleigh. Darla R. Miller, BA, is the Project Manager for the CC in the Department of Genetics, Lineberger Comprehensive Cancer Center, Carolina Center for Genome Sciences at the University of North Carolina (UNC) in Chapel Hill. Gary A. Churchill, PhD, is a professor at the Jackson Laboratory in Bar Harbor, Maine. Fernando Pardo-Manuel de Villena, PhD, is an associate professor in the Department of Genetics, Lineberger Comprehensive Cancer Center, Carolina Center for Genome Sciences at UNC.

Address correspondence and reprint requests to Dr. Fernando Pardo-Manuel de Villena, Department of Genetics, University of North Carolina at Chapel Hill, Genetic Medicine Building 5046, 120 Mason Farm Road – CB 7264, Chapel Hill, NC 27599-7264 or email fernando@med.unc.edu.

Recombinant Inbred Lines and Genetic Reference Populations

Recombinant inbred (RI¹) lines have been generated in many organisms including mice and rats (Shisa et al. 1997). Traditional mouse RI lines are derived from two inbred strains through the brother-sister mating of F2 individuals and their descendants until each independent line reaches inbred status (Bailey 1971). A complete RI panel is composed of a defined set of inbred strains, each representing a unique mosaic of the original genomes. RI lines retain all the advantages of inbred strains (reproducibility and integration of data across space and time) while providing a simple and cost-effective approach to genetic mapping (Bailey 1971). RI resources have been widely used in genetic research and have become popular as tools for systems genetics, the integration of data across biological scales (Threadgill 2006).

The usefulness of traditional RI panels as platforms for systems genetics is limited by the presence of only two parental genomes and the number of lines in the RI panels. The first limitation of low genetic diversity is caused by the fact that existing RI panels use two classical inbred strains as founders and a large fraction of the genome of all pairs of classical inbred strains is identical by descent (IBD¹; Yang et al. 2007, Yang et al. submitted). IBD in the founders leads to a nonuniform distribution of genetic variation across the genome in the RI panel (Roberts et al. 2007). More importantly, as a result of the lack of variation, IBD regions are effectively “blind spots” for genetic mapping (Roberts et al. 2007), a critical shortcoming that can be partly remedied by substituting a classical inbred strain with a wild-derived inbred strain in the founder pair. However, it is not possible to completely overcome the presence of blind spots because of the pervasive presence of intersubspecific introgression and contamination in wild-derived laboratory strains (Yang et al. submitted). Others have attempted to overcome the shortcoming of limited genetic diversity by using panels of extant inbred strains (e.g., the Mouse Phenome Project, MPP) that feature variation across most of the mouse genome (Bennett et al. 2010; Paigen and Eppig 2000; Svenson et al. 2007). The intertwined breeding history of extant strains is problematic as

¹Abbreviations used in this article: CC, Collaborative Cross; IBD, identical by descent; ORNL, Oak Ridge National Laboratory; RI, recombinant inbred; SOP, standard operating procedure

multiple variable regions of the genome have similar distribution patterns across strains, making it impossible to uniquely distinguish associations to different areas of the genome (Roberts et al. 2007; Yang et al. 2007).

The second limitation of small numbers of lines in existing RI panels has been overcome by generating larger panels such as the 81 lines of the BXD panel (C57BL/6J × DBA/2J; Peirce et al. 2004) and the 77 lines of the LXS panel (Williams et al. 2004). These panel sizes remain relatively small, but their primary weakness is that they are biparental, so a large fraction of the genomes are IBD and thus invisible for genetic analysis.

The main impetus behind the development of the Collaborative Cross (CC¹) was to overcome the limitations of existing RI panels and to create the first genetic reference population suitable for systems approaches to biomedical research (Threadgill et al. 2002). The CC project involved generation of a large panel of RI strains derived from eight founder inbred strains through randomized breeding (Churchill et al. 2004). The generation of each CC line requires three generations of intercrossing followed by at least 20 generations of brother-sister mating or other selection methods to ensure the lines are inbred (Figure 1). The overall aim of the CC project was to create hundreds of independent RI lines.

The CC: A Community Resource

Since its inception the CC was conceived as a community resource that would complement the traditional strengths of the mouse as a model of human disease and create novel avenues for scientific research (Threadgill et al. 2002). During the initial phase several scientists hosted meetings to discuss the breeding schema, the number and identity of the founder strains, and the logistics of the generation of the cross. The Complex Traits Consortium (CTC, recently renamed the Complex Traits Community) assumed leadership (Churchill et al. 2004) and the CC became its most tangible project along with the annual CTC meeting.²

A critical aspect of the early discussions to establish the CC was the identification of appropriate funding sources. Because generation of the CC was expected to take more than 5 years and its utility would become clear only after most of the lines were completed, securing funds was a challenge. Although long-term planning and support for community resources are common in some scientific fields such as astronomy, they are rare in biomedical research. Funding to initiate the CC was provided by the Department of Energy, the Ellison Medical Foundation, and the Wellcome Trust, with subsequent ongoing support from the National Institutes of Health.

The CC is now a partnership among several national and international universities and research institutions and includes three distinct breeding arms located in the United

States, Israel, and Australia. All three populations are traceable to F1 mice generated by the eight founder strains at the Jackson Laboratory and sent in 2004 to (1) the Oak Ridge National Laboratory (ORNL,¹ since relocated to the University of North Carolina [UNC] at Chapel Hill; Chesler et al. 2008), (2) the International Livestock Research Institute in Kenya (since relocated to Tel Aviv University in Israel; Iraqi et al. 2008), and (3) the Western Australia Institute for Medical Research in Perth (Morahan et al. 2008) (Figure 2). Because we are more actively involved in the generation of the US CC population, the remainder of this article focuses on the US arm, but most of the observations and conclusions also apply to the Israeli and Australian arms.

Production of the CC Population

In contrast to traditional RI lines, the CC was derived from eight genetically diverse inbred strains—A/J, C57BL/6J, 129S1/SvImJ, NOD/ShiLtJ, NZO/HILtJ, CAST/EiJ, PWK/Ph, and WSB/EiJ (Chesler et al. 2008)—to result in high levels of variation distributed uniformly throughout the genome (Roberts et al. 2007; Yang et al. 2007). This was achieved mainly by including wild-derived strains from three mouse subspecies among the eight founder strains (Figure 1C). Unlike most RI panels, no genomic region is IBD in all CC parental strains (Yang et al. 2010). The presence of more than two distinct haplotypes can be easily visualized by determining the subspecific origin of the genome of the eight founders (Figure 1D).

We recently conducted a proof-of-principle experiment using incompletely inbred CC lines that were genotyped using the high-density Mouse Diversity Genotyping Array (MDA; Yang et al. 2009). Genomewide analysis of single nucleotide polymorphism (SNP) data in the incipient strains revealed the three desired characteristics of a genetic reference population: high genetic diversity, balanced allele frequencies, and dense, evenly distributed recombination sites (Aylor et al., in press).

The eight founder inbred strains were bred using a combinatorial funnel design to yield a large number of genetically independent RI lines (Figure 1). Reciprocal matings initially involved all combinations of the eight founder strains: F1 hybrids were intercrossed, a balanced subset of nonoverlapping 4- and 8-way progeny were subsequently mated, and the final 8-way animals were sibling mated to produce incipient RI lines. The aim of this breeding scheme was to generate RI lines with many random allele combinations via recombination and chromosomal assortment.

A CC breeding database (CCDB) was developed to track each animal and to determine, using a random generator, which mice should become breeders (Chesler et al. 2008). Strict criteria were established to meet these randomization goals: breeders were assigned priorities and mating pairs for generation X + 1 were prioritized at weaning. Second- or third-priority mating pairs were used only if their second litter was born before a litter from the first-priority pair. This

²Reports of the initial discussions and of the CTC's annual meetings are available at <http://csbio.unc.edu/CCstatus/> (accessed on December 23, 2010).

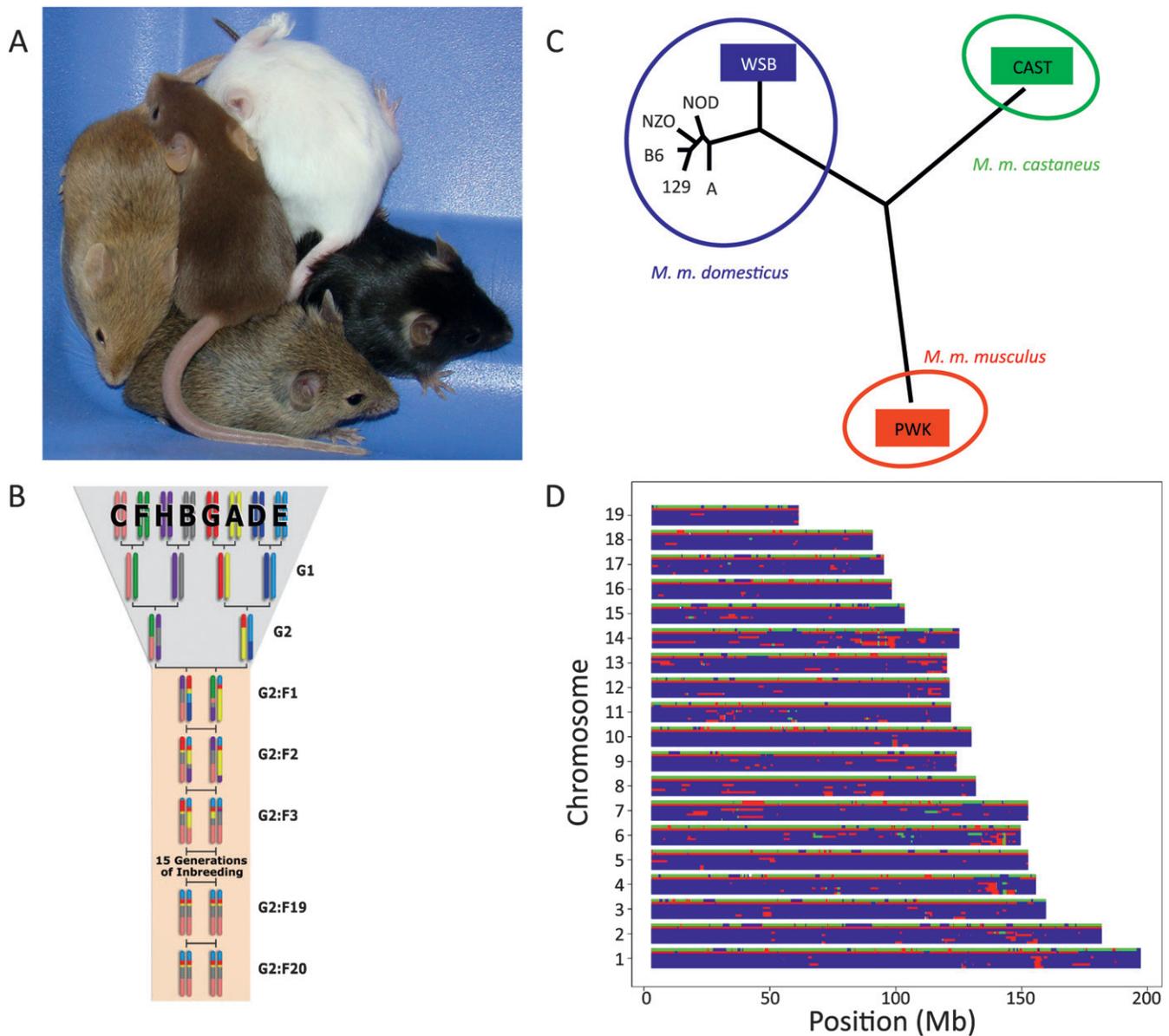


Figure 1 Generation and genetic diversity in the Collaborative Cross (CC). (A) Variable coat color: litter from a CC line at an early generation of inbreeding (G2:F4) in which multiple coat color alleles are still segregating. (B) Breeding scheme. The colors and letters represent the eight founder strains as follows: A/J, A/yellow; C57BL/6J, B/grey; 129S1/SvImJ, C/pink; NOD/LtJ, D/dark blue; NZO/H1LtJ, E/light blue; CAST/EiJ, F/green; PWK/PhJ, G/red; and WSB/EiJ, H/purple (these colors and letters are also used in the website describing the status and availability of CC lines; <http://csbio.unc.edu/CCstatus/>). (C) Phylogenetic tree of the eight CC founder strains based on single nucleotide polymorphism (SNP) data for chromosome 11. Blue denotes *M.m. domesticus* origin; red, *M.m. musculus* origin; and green, *M.m. castaneus* origin. Strains in solid color boxes are wild-derived inbred strains. (D) Subspecific origin of the autosomes of the eight CC founder strains. Colors follow the same conventions as for (C).

schema proved to be overly strict and in hindsight may have caused the loss of more lines than if the first litter from any mating was used (Chesler et al. 2008). Once the preliminary studies demonstrated that all eight strains were represented in lines at intermediate stages of inbreeding, the stringency of randomization was relaxed to allow for the selection of more fecund animals. (For details on recombination, inbreeding rates, and statistical power in the CC, Broman 2005; Valdar et al. 2006.)

Unique Characteristics and Special Considerations

Several of the CC founder strains have particular characteristics that affect their handling, lifespan, and reproduction. Although in this section we use the founder strains as examples, a recent proof-of-principle experiment indicates that all traits studied in the CC lines span—and typically expand—the founders' range of phenotypic variation (Aylor et al., in press).

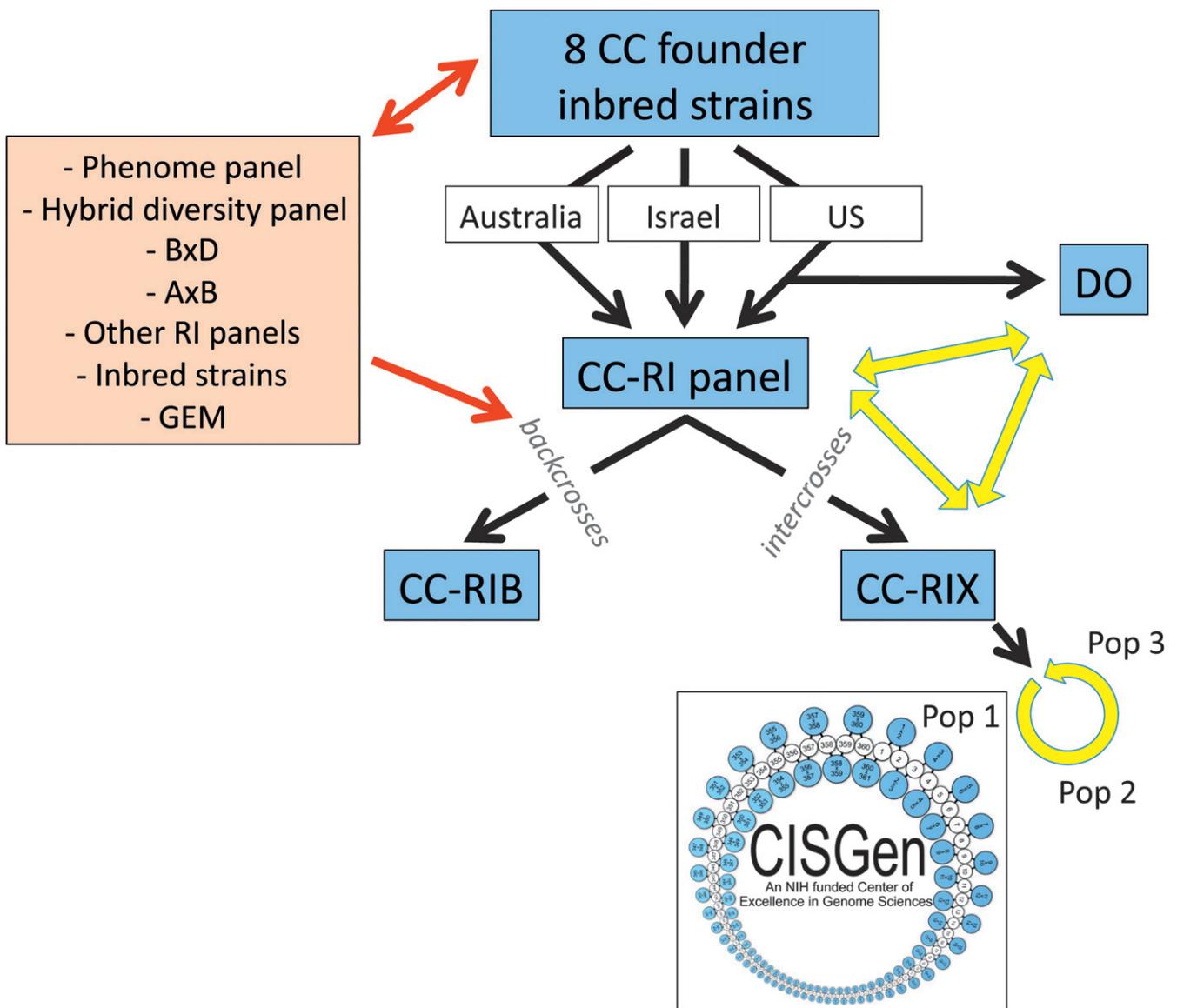


Figure 2 The Collaborative Cross (CC) and related mouse genetic resources. Blue boxes represent the CC, its founder strains, and derived populations; related genetic resources are shown in orange boxes. Black arrows represent the derivation of the CC resources. Yellow arrows indicate the potential combined use of CC-related resources. DO, Diversity Outcross; GEM, genetically engineered model; Pop, population; RI, recombinant inbred; RIB, recombinant backcross; RIX, recombinant intercross.

The inclusion of three wild-derived inbred strains (CAST/EiJ, PWK/PhJ, and WSB/EiJ) ensures high levels and uniform distribution of genetic variation across the genome. In contrast with classical inbred strains (derived from pet mice bred for docility and coat color), wild-derived strains originate from wild-caught mice. Although some form of selection for docility may have occurred, the behavior of these mice is strikingly different from that of most laboratory strains and their handling requires special precautions to reduce the possibility of escape, which can be reduced by handling cages with the least amount of disturbance and by cautiously removing cage tops. Cages should be placed in hoods fitted with shields around three sides so that the only opening is occupied by the handler. Alternatively, it

is useful to place the cage in a large sanitized bin while opening so that any escapees have a third level of containment.

Two of the founder lines, NOD/ShiLtJ and NZO/HILtJ, become diabetic by 4 to 6 months of age and need special attention as both strains exhibit excessive water consumption and urination due to the diabetes. The extra water consumption, combined with the large litter sizes typical of NOD/ShiLtJ mice, results in the need for much more frequent cage changes than for nondiabetic mice. In fact, changes as frequent as every 2 to 3 days are not unusual compared with the normal changing schedule of 14 days for ventilated cages. Moreover, lifespan is usually shorter in diabetic animals—NOD/ShiLtJ mice may live only 6 to 8 months under normal food and water conditions. NOD/ShiLtJ females have even

shorter lifespans and are retired from breeding at a much higher rate than any other CC founder strain. These observations may apply to CC lines susceptible to diabetes.

NZO/HILtJ mice become obese as young adults and have notoriously poor reproductive performance, so they need to be mated immediately upon weaning. Once a male has sired a litter, he is usually able to father 3 or 4 more litters; but if he does not sire his first litter before 10 weeks of age, it is extremely unlikely he will father any offspring. The NZO/HILtJ breeding colony typically requires twice as many cages as any other inbred strain.

Characteristics of the founder strains have been noted in the CC lines and will likely persist, and even increase in severity, in some lines.

Extinction

The extinction rate in the US CC population is greater than 50% (Chesler et al. 2008), possibly because of the presence of incompatible combinations of alleles originating in different subspecies (Figure 1C).

The US population was started in two phases separated by 2 years. The extinction rate in the first phase was significantly higher than in the second; for example, the cumulative extinction rates at the G2:F2, G2:F3, and G2:F4 generations of inbreeding (when it is possible to make fair comparisons between phases 1 and 2) are statistically different ($p < 0.01$). The improvement in extinction rates reflects breeding changes implemented in 2007 to increase the likelihood of survival of phase 1 lines and to minimize the loss of phase 2 lines (Chesler et al. 2008). The changes in breeding involved avoiding combinations of nonproductive F1s, increasing the number of breeding pairs per generation in later generations, and decreasing the time before using lower-priority mating pairs when the first-priority pair was nonproductive. These changes had minimal impact on the randomization inherent to the CC breeding scheme. However, the extinction rate and the relatively small average litter size of the most advanced CC lines highlight the importance of maintaining large colony sizes and cryopreservation of stock to avoid reduction in the CC population.

Housing

Breeding to generate G2 mice started in 2004 at the ORNL William L. and Liane B. Russell Vivarium, a state-of-the-art barrier facility completed just before the initiation of the CC project and ideally suited to support the large-scale breeding program required. Mice were housed in Thoren ventilated racks with duplex cages, which allowed for higher density of breeding pairs without compromising the space needed per animal (each side of a duplex cage can accommodate three adults or a breeding pair and a litter). Each CC line was allotted four breeding pens in the early generations: one from generation X and three from generation X + 1. Keeping

one box from generation X as a known fertile pen and backup helped to reduce the loss of lines. The ORNL vivarium standard operating procedure (SOP) called for the use of autoclaved cages, irradiated food, ALPHA-dri bedding, and automated hyperchlorinated water. Each cage also had a nestlet for nesting material. The number of animal care staff was limited to reduce disturbance and staff entered the facility through an air shower after donning full-body personal protection equipment.

In late 2008, the Department of Energy determined that the recently opened ORNL vivarium should be closed and in March 2009 the CC was transferred to UNC. Investigators at UNC had been involved in the development of the CC resource since its inception and had secured NIH support to complete the breeding of the US CC population, making UNC a logical place for relocation. The relocation schedule was a complex undertaking. Animals were transported once a week for 8 weeks by ORNL staff in a dedicated mouse transport van that carried 250 to 350 cages per trip (breeding boxes with pups less than 7 days old were not transported). As a precaution, at least one breeding cage from each CC line remained at ORNL during colony transportation. Only about 10 animals out of the thousands moved died due to the move.

The CC population was housed in a temporary location in Research Triangle Park (RTP) until completion of a new vivarium in the UNC Genetics Medicine building. The RTP SOP called for the mice to be housed in ventilated, hot-washed Techniplast cages (the equipment available). Food delivery followed manufacturer recommendations and tap water was used to fill water bottles. The bedding used was bed-o-cobs[®] and a nestlet was placed in each breeding cage. We originally included a PVC pipe as well, but quickly discontinued its use after the deaths of some pups when the pipe rolled onto them during routine checks. The animals otherwise adapted quickly to the UNC conditions and exhibited no adverse effects on breeding or health status in the year they resided at the RTP location.

A second move, from RTP to the UNC Genetic Medicine vivarium, took place in February 2010 in five large shipments, two on each of 2 days in one week and the final shipment a week later to move cages housing pups that were less than 7 days old at the time of the first shipment. Care was taken while packing and there was no evidence of loss of animals during or after transport. However, after both moves it took about 4 weeks before litter production resumed normal levels.

The UNC housing conditions are the same as at RTP and, as per SOPs at all three facilities, the CC mice are housed in ventilated racks and the cages changed every 2 weeks. Based on our discussions with the UNC Institutional Animal Care and Use Committee (IACUC) and Division of Laboratory Animal Medicine, a revision to the UNC SOP has increased litter survival by calling for a 24-hour delay in cage changes after the birth of a litter.

Animals are identified by ear notches and/or cage card notations (eventually all weanlings will be identified by ear notching). Numbering is usually consecutive, in order of weaning starting with #1 in a line. The entire litter (including

weanlings that are not needed for breeding or saved for experiments) is recorded on the mother's breeding card. Weanlings are recorded both in a separate ledger for each CC line and in the CCDB.

Breeding Considerations

Weaning

Litters are typically weaned at 3 weeks of age, which is a frequency sufficient to prevent the birth of multiple litters in breeding pens. But in some CC lines the pups are not sufficiently mature to be weaned by that age. At UNC the SOP is to wean at 10 grams. But NZO/HILtJ mice reach 10 g as early as 12 to 14 days of age, whereas the wild-derived strains (CAST/EiJ, PWK/PhJ, and WSB/EiJ) do not weigh 10 g until 24 to 28 days of age. Thus weaning by weight is not feasible because NZO/HILtJ pups would be weaned as early as 12 days of age, when few pups would survive, and wild-derived strains might not be weaned until 28 days of age despite their ability to survive independently at 21 days. Technicians working with the CC are trained to determine when animals can survive and the IACUC has approved exceptions for weaning by age and robustness rather than weight.

A second IACUC-approved exception allows for two litters from one dam to be in the same pen concurrently. Cages are checked daily by the animal care staff for overcrowding. A newborn litter in a mating pair cage with a weaning-age litter is not unusual in highly productive colonies and is promptly corrected when noted. With the use of a log of cages with higher than acceptable densities for the CC breeding program at UNC, the rate of deaths due to trampling or neglect by the dam is determined to be less than 5%.

Mating

The practice since the start of the CC project has been to set up mating pairs at weaning in order to increase the speed of inbreeding and to accurately track time to fertility in the different lines. Animals are usually pair mated, but, depending on availability, some are harem mated (one male mated to one to four females in a single pen). Pregnant harem-mated females are removed to separate pens to litter, then returned to the mating pen after the pups are weaned.

A third IACUC exception allows the sire to stay in the cage with the dam even in the presence of large litters (>11 pups), enabling the pair to continue mating. This exception applies mainly to early generations of the CC in which hybrid vigor prevails. As inbreeding progresses the litter sizes drop and usually by the G2:F4 generation the average litter sizes are four to eight. There are three main justifications for this exception:

- As discussed, three of the CC founder strains are wild-derived inbred lines and the behaviors of many CC lines resemble those of these wild stocks. Each time a cage is opened there is a high risk that the dam, sire, and/or pups

will jump from the cage and be lost. Separating the male from the female before the litter is born would increase the number of times the cage needs to be opened, thus increasing the chance of losing the sire during a cage opening, and would decrease the likelihood of postpartum matings. These outcomes would be in direct conflict with two of the goals of the project: rapid turnover of generations and production of a high number of animals.

- Because breeding is a major goal at this stage, there are about 2,500 breeding cages. If the males are removed from each of the approximately 1,000 breeding cages at any given time, the cage census would dramatically increase. Many of the CC lines show signs of fertility loss, so every breeder could mean the difference of survival of the line.
- The average litter size in the founder inbred lines and in the CC lines themselves ranges from 2 to 12 and litters as large as 14 to 16 are not unusual. The UNC SOP calls for a maximum cage density of a breeding pair plus up to six pups or one adult plus 11 pups after the pups reach 14 days of age. Larger litters are allowed with a breeding pair until 14 days of age. In other projects, these goals are achievable by genotyping pups early or culling one sex if specific classes of animals are not needed. For the CC every animal is important and needs to be kept alive.

Marker-Assisted Inbreeding

To accelerate the completion of the CC project and to minimize the loss of lines, the number of breeding cages has been expanded for later generations of inbreeding. In addition, genotype-based approaches are being used to characterize the genetic makeup and ensure the genetic integrity of each line, and to accelerate inbreeding. To accomplish this, the breeding pairs that are obligate ancestors of all extant mice in a CC line are genotyped using the MDA (Yang et al. 2009). Typically these breeding pairs then have to be euthanized to enable preparation of the high-molecular-weight DNA from their spleens that is necessary for the high-density SNP array. Areas of residual heterozygosity are determined and a second, much smaller panel of SNPs is used to track these regions. For the lower-density array DNA is prepared using tail or ear clips from pups just prior to or at weaning. From animals genotyped using the low-density arrays breeding pairs are selected to be most conducive to rapid inbreeding rather than randomized mating.

Simulations indicate that it is possible to significantly reduce the number of generations to full inbred status with minimal impact on the genetic architecture of the CC population (Catherine Welsh, Fernando Pardo-Manuel de Villena, and Leonard McMillan, unpublished results). High-density genotypes and haplotype reconstructions for the lines undergoing marker-assisted inbreeding (MAI) are available (<http://csbio.unc.edu/CCstatus/>).

A set of CC lines at the most advanced stages of inbreeding was recently imported from the Israeli CC population and most were producing litters within 6 weeks of their arrival at

UNC. These lines will also undergo MAI similar to the UNC population.

Once a line is determined to be inbred, it will be re-derived into a barrier facility at UNC's Mutant Mouse Regional Resource Center so that it is available to any facility in the world. As of November 2010, we have one inbred line and expect to complete 50 lines by mid-2011 and then 50 more every 6 months to a total of at least 200. Each confirmed inbred line will undergo rederivation, which should make the lines available 6 to 12 months after they are confirmed inbred.

The CC and Other Mouse Resources

Notwithstanding its unique characteristics, the CC is intimately connected with other major mouse resources (Figure 2). It is derived from extant inbred strains that are widely used by the mouse genetics community and represented in other genetic reference populations such as traditional RI panels, the MPP, the Hybrid Mouse Diversity Panel (an updated and improved phenome panel; Bennett et al. 2010), and strains commonly used to produce genetically engineered models (GEM; Guan et al. 2010). These relationships can be exploited to integrate and leverage existing data to inform new experiments and to interpret previous experiments.

In addition, CC lines were the starting material for the Diversity Outcross, a heterogeneous stock designed to achieve very high mapping resolution that was seeded with mice from intermediate generations of the US CC population and is being bred at the Jackson Laboratory (GAC, unpublished results) (Figure 2). Finally, CC lines can be intercrossed among themselves or backcrossed to any other line or GEM to generate recombinant intercross (RIX) and recombinant backcross (RIB) panels. A RIX population with a unique loop design will be generated as part of an NIH-funded Center of Excellence in Genome Sciences (Figure 2).

Conclusion

The Collaborative Cross is a community resource that will eventually integrate data from a wide variety of biomedical disciplines. Its benefits to both individual users and the whole biomedical research community will be proportional to its integration of the many disparate datasets generated by individual researchers into a single public database. It is thus essential to develop and implement bioinformatic tools that will allow both geneticists and nongeneticists to take full advantage of this unique resource. Ideally these tools will be complemented with training programs that maximize access and enhance the efficient use and maintenance of the CC population.

Acknowledgments

This work was supported by grants from the National Institutes of Health (U01CA105417, U01CA134240, and

P50MH090338), Ellison Medical Foundation (AG-IA-0202-05), and US Department of Energy (DE-AC05-00OR22725 with UT-Battelle, LLC). Essential support was also provided by the Dean of the UNC School of Medicine, the Lineberger Comprehensive Cancer Center at UNC, and the University Cancer Research Fund from the state of North Carolina. We thank Leonard McMillan for unpublished results and Figure 1B, and Jennifer Shockley and Stephanie Hansen for Figure 1A.

References

- Aylor DL, Valdar W, Foulds-Mathes W, Buus RJ, Verdugo RA, Baric RS, Ferris MT, Frelinger JA, Heise M, Frieman MB, Gralinski LA, Bell TA, Didion JP, Hua K, Nehrenberg DL, Powell CL, Steigerwalt J, Xie Y, Kelada SNP, Collins FS, Yang IV, Schwartz DA, Branstetter LA, Chesler EJ, Miller DR, Spence J, Liu EY, McMillan L, Sarkar A, Wang J, Wang W, Zhang Q, Broman KW, Korstanje R, Durrant C, Mott R, Iraqi FA, Pomp D, Threadgill D, Pardo-Manuel de Villena F, Churchill GA. Genetic analysis of complex traits in the emerging Collaborative Cross. In press.
- Bailey DW. 1971. Recombinant-inbred strains: An aid to finding identity, linkage, and function of histocompatibility and other genes. *Transplantation* 11:325-327.
- Bennett BJ, Farber CR, Orozco L, Kang HM, Ghazalpour A, Siemers N, Neubauer M, Neuhaus I, Yordanova R, Guan B, Truong A, Yang WP, He A, Kayne P, Gargalovic P, Kirchgessner T, Pan C, Castellani LW, Kostem E, Furlotte N, Drake TA, Eskin E, Lusis AJ. 2010. A high-resolution association mapping panel for the dissection of complex traits in mice. *Genome Res* 20:281-290.
- Broman KW. 2005. The genomes of recombinant inbred lines. *Genetics* 169:1133-1146.
- Chesler EJ, Miller DR, Branstetter LR, Galloway LD, Jackson BL, Philip VM, Voy BH, Culiati CT, Threadgill DW, Williams RW, Churchill GA, Johnson DK, Manly KF. 2008. The Collaborative Cross at Oak Ridge National Laboratory: Developing a powerful resource for systems genetics. *Mamm Genome* 19:382-389.
- Churchill GA, Airey DC, Allayee H, Angel JM, Attie AD, Beatty J, Beavis WD, Belknap JK, Bennett B, Berretini W, Bleich A, Bogue M, Broman KW, Buck KJ, Buckler E, Burmeister M, Chesler EJ, Cheverud JM, Clapcote S, Cook MN, Cox RD, Crabbe JC, Crusio WE, Darvasi A, Deschepper CF, Doerge RW, Farber CR, Forejt J, Gaile D, Garlow SJ, Geiger H, Gershenfeld H, Gordon T, Gu J, Gu W, de Haan G, Hayes NL, Heller C, Himmelbauer H, Hitzemann R, Hunter K, Hsu HC, Iraqi FA, Ivandic B, Jacob HJ, Jansen RC, Jepsen KJ, Johnson DK, Johnson TE, Kempermann G, Kendzioriski C, Kotb M, Kooy RF, Llamas B, Lammert F, Lassalle JM, Lowenstein PR, Lu L, Lusis A, Manly KF, Marcucio R, Matthews D, Medrano JF, Miller DR, Mittleman G, Mock BA, Mogil JS, Montagutelli X, Morahan G, Morris DG, Mott R, Nadeau JH, Nagase H, Nowakowski RS, O'Hara BF, Osadchuk AV, Page GP, Paigen B, Paigen K, Palmer AA, Pan HJ, Peltonen-Palotie L, Peirce J, Pomp D, Pravenec M, Prows DR, Qi Z, Reeves RH, Roder J, Rosen GD, Schadt EE, Schalkwyk LC, Seltzer Z, Shimomura K, Shou S, Sillanpaa MJ, Siracusa LD, Snoeck HW, Spearow JL, Svenson K, Tarantino LM, Threadgill D, Toth LA, Valdar W, de Villena FP, Warden C, Whately S, Williams RW, Wiltshire T, Yi N, Zhang D, Zhang M, Zou F. 2004. The Collaborative Cross, a community resource for the genetic analysis of complex traits. *Nat Genet* 36:1133-1137.
- Guan C, Ye C, Yang X, Gao J. 2010. A review of current large-scale mouse knockout efforts. *Genesis* 48:73-85.
- Iraqi FA, Churchill G, Mott R. 2008. The Collaborative Cross, developing a resource for mammalian systems genetics: A status report of the Wellcome Trust cohort. *Mamm Genome* 19:379-381.
- Morahan G, Balmer L, Monley D. 2008. Establishment of "The Gene Mine": A resource for rapid identification of complex trait genes. *Mamm Genome* 19:390-393.

- Paigen K, Eppig JT. 2000. A mouse phenome project. *Mamm Genome* 11: 715-771.
- Peirce JL, Lu L, Gu J, Silver LM, Williams RW. 2004. A new set of BXD recombinant inbred lines from advanced intercross populations in mice. *BMC Genet* 29:5-7.
- Roberts A, Pardo-Manuel de Villena F, Wang W, McMillan L, Threadgill DW. 2007. The polymorphism architecture of mouse genetic resources elucidated using genome-wide resequencing data: Implications for QTL discovery and systems genetics. *Mamm Genome* 18:473-481.
- Shisa H, Lu L, Katoh H, Kawarai A, Tanuma J, Matsushima Y, Hiai H. 1997. The LEXF: A new set of rat recombinant inbred strains between LE/Stm and F344. *Mamm Genome* 8:324-327.
- Svenson KL, Von Smith R, Magnani PA, Suetin HR, Paigen B, Naggert JK, Li R, Churchill GA, Peters LL. 2007. Multiple trait measurements in 43 inbred mouse strains capture the phenotypic diversity characteristic of human populations. *J Appl Physiol* 102:2369-2378.
- Threadgill DW. 2006. Meeting report for the 4th annual Complex Trait Consortium meeting: From QTLs to systems genetics. *Mamm Genome* 17:2-4.
- Threadgill DW, Hunter KW, Williams RW. 2002. Genetic dissection of complex and quantitative traits: From fantasy to reality via a community effort. *Mamm Genome* 13:175-178.
- Valdar W, Flint J, Mott R. 2006. Simulating the Collaborative Cross: Power of quantitative trait loci detection and mapping resolution in large sets of recombinant inbred strains of mice. *Genetics* 172:1783-1797.
- Williams RW, Bennett B, Lu L, Gu J, DeFries JC, Carosone-Link PJ, Rikke BA, Belknap JK, Johnson TE. 2004. Genetic structure of the LXS panel of recombinant inbred mouse strains: A powerful resource for complex trait analysis. *Mamm Genome* 15:637-647.
- Yang H, Bell TA, Churchill GA, Pardo-Manuel de Villena F. 2007. On the subspecific origin of the laboratory mouse. *Nat Genet* 39:1100-1107.
- Yang H, Ding Y, Hutchins LN, Szatkiewicz J, Bell TA, Paigen BJ, Graber JH, Pardo-Manuel de Villena F, Churchill GA. 2009. A customized and versatile high-density genotyping array for the mouse. *Nat Methods* 6:663-666.
- Yang H, Wang JR, Didion JP, Buus RJ, Bell TA, Welsh CE, Bonhomme F, Hon-Tsen Yu A, Nachman MW, Pialek J, Tucker P, Boursot P, McMillan L, Churchill GA, Pardo-Manuel de Villena F. Subspecific origin and haplotype diversity in the laboratory mouse. Submitted.

The Genome Architecture of the Collaborative Cross Mouse Genetic Reference Population

Collaborative Cross Consortium¹

ABSTRACT The Collaborative Cross Consortium reports here on the development of a unique genetic resource population. The Collaborative Cross (CC) is a multiparental recombinant inbred panel derived from eight laboratory mouse inbred strains. Breeding of the CC lines was initiated at multiple international sites using mice from The Jackson Laboratory. Currently, this innovative project is breeding independent CC lines at the University of North Carolina (UNC), at Tel Aviv University (TAU), and at Geniad in Western Australia (GND). These institutions aim to make publicly available the completed CC lines and their genotypes and sequence information. We genotyped, and report here, results from 458 extant lines from UNC, TAU, and GND using a custom genotyping array with 7500 SNPs designed to be maximally informative in the CC and used a novel algorithm to infer inherited haplotypes directly from hybridization intensity patterns. We identified lines with breeding errors and cousin lines generated by splitting incipient lines into two or more cousin lines at early generations of inbreeding. We then characterized the genome architecture of 350 genetically independent CC lines. Results showed that founder haplotypes are inherited at the expected frequency, although we also consistently observed highly significant transmission ratio distortion at specific loci across all three populations. On chromosome 2, there is significant overrepresentation of WSB/EiJ alleles, and on chromosome X, there is a large deficit of CC lines with CAST/EiJ alleles. Linkage disequilibrium decays as expected and we saw no evidence of gametic disequilibrium in the CC population as a whole or in random subsets of the population. Gametic equilibrium in the CC population is in marked contrast to the gametic disequilibrium present in a large panel of classical inbred strains. Finally, we discuss access to the CC population and to the associated raw data describing the genetic structure of individual lines. Integration of rich phenotypic and genomic data over time and across a wide variety of fields will be vital to delivering on one of the key attributes of the CC, a common genetic reference platform for identifying causative variants and genetic networks determining traits in mammals.

Copyright © 2012 by the Genetics Society of America

doi: 10.1534/genetics.111.132639

Manuscript received July 11, 2011; accepted for publication October 3, 2011

Available freely online through the author-supported open access option.

Supporting information is available online at <http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.111.132639/-DC1>.

¹Tel Aviv University, Ramat Aviv, Tel Aviv 69978, Israel: Fuad A. Iraqi, Mustafa Mahajne, Yasser Salaymah, Hani Sandovski, Hanna Tayem, and Karin Vered; Geniad, Ltd., University of Western Australia, and Animal Resources Centre, Australia: Lois Balmer, Michael Hall, Glynn Manship, Grant Morahan, Ken Pettit, Jeremy Scholten, Kathryn Tweedie, Andrew Wallace, and Lakshini Weerasekera; Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford OX3 7BN, United Kingdom: James Cleak, Caroline Durrant, Leo Goodstadt, Richard Mott and Binnaz Yalcin; University of North Carolina, Chapel Hill, NC 27599: David L. Aylor, Ralph S. Baric, Timothy A. Bell, Katharine M. Bendt, Jennifer Brennan, Jackie D. Brooks, Ryan J. Buus, James J. Crowley, John D. Calaway, Mark E. Calaway, Agnieszka Cholka, David B. Darr, John P. Didion, Amy Dorman, Eric T. Everett, Martin T. Ferris, Wendy Foulds Mathes, Chen-Ping Fu, Terry J. Gooch, Summer G. Goodson, Lisa E. Gralinski, Stephanie D. Hansen, Mark T. Heise, Jane Hoel, Kunjie Hua, Mayanga C. Kapita, Seunggeun Lee, Alan B. Lenarcic, Eric Yi Liu, Hedi Liu, Leonard McMillan, Terry R. Magnuson, Kenneth F. Manly, Darla R. Miller, Deborah A. O'Brien, Fanny Odet, Isa Kemal Pakatci, Wenqi Pan, Fernando Pardo-Manuel de Villena², Charles M. Perou, Daniel Pomp, Corey R. Quackenbush, Nashiya N. Robinson, Norman E. Sharpless, Ginger D. Shaw, Jason S. Spence, Patrick F. Sullivan, Wei Sun, Lisa M. Tarantino, William Valdar, Jeremy Wang, Wei Wang, Catherine E. Welsh, Alan Whitmore, Tim Wiltshire, Fred A. Wright, Yuying Xie, Zaining Yun, Vasyl Zhabotynsky, Zhaojun Zhang, and Fei Zou; North Carolina State University, Raleigh, NC 27695: Christine Powell, Jill Steigerwalt, and David W. Threadgill; The Jackson Laboratory, Bar Harbor, ME 04607: Elissa J. Chesler, Gary A.

Churchill, Daniel M. Gatti, Ron Korstanje, and Karen L. Svenson; National Institutes of Health, Bethesda, MD 20892: Francis S. Collins, Nigel Crawford, Kent Hunter, Samir N. P. Kelada, Bailey C. E. Peck, Karlyne Reilly, and Urraca Tavares; Oregon Health and Science University, Portland, OR 97239: Daniel Bottomly, Robert Hitzeman, and Shannon K. McWeeney; University of Arizona, Tucson, AZ 85719: Jeffrey Frelinger, Harsha Krovi, and Jason Phillippi; University of Colorado Denver, Denver, CO: Richard A. Spritz; University of Washington, Seattle, WA 98195: Lauri Aicher, Michael Katze, and Elizabeth Rosenzweig; Faculty of Dental Medicine, Hadassah Medical Centers and The Hebrew University, Jerusalem, Israel: Ariel Shusterman, Aysar Nashef, Ervin I. Weiss, and Yael Hour-Haddad; Hebrew University, Jerusalem, Israel: Morris Soller; University of Tennessee Health Science Center, Memphis, TN 38163: Robert W. Williams; Helmholtz Centre for Infection Research & University of Veterinary Medicine Hannover, Braunschweig, Germany: Klaus Schughart; Duke University, Durham, NC 27710: Hyuna Yang; National Institute of Environmental Health Sciences, National Toxicology Program, Research Triangle Park, NC 27709: John E. French; University of Nebraska-Lincoln, Lincoln, NE 68583: Andrew K. Benson, Jaehyoung Kim, Ryan Legge, Soo Jen Low, Fangrui Ma, Ines Martinez, and Jens Walter; University of Wisconsin-Madison, Madison, WI 53706: Karl W. Broman; The Alberta Children's Hospital Research Institute, University of Calgary, 3330 Hospital Dr. NW, Calgary, Alberta T2N 4N1, Canada: Benedikt Hallgrímsson; University of California San Francisco, San Francisco, CA 94143: Ophir Klein; The Genome Institute at Washington University, St. Louis, MO 63108: George Weinstock and Wesley C. Warren; University of Colorado School of Medicine, Denver, CO 80206: Yvana V. Yang and David Schwartz.

²Corresponding author: Department of Genetics, 5046 Genetics Medicine Bldg., University of North Carolina, Campus Box 7264, Chapel Hill, NC, 27599. E-mail: fernando@med.unc.edu

GENETIC reference populations (GRPs) are defined as sets of individuals with fixed and known genomes that can be replicated indefinitely. Typically they consist of dozens to hundreds of inbred lines related by descent from a set of common ancestors (*i.e.*, the founders). GRPs have been developed for many organisms, including yeast, plants, flies, and mammals (Bailey 1971; Crow 2007; Buckler *et al.* 2009; Ayroles *et al.* 2009; Kover *et al.* 2009; Cubillos *et al.* 2011). GRPs are popular for the study of complex traits and biological systems in both medical and life science applications because genotyping is required only once (described as the “genotype once, phenotype many times” paradigm); replicate individuals can be produced with the same genotype allowing for optimal case/control and gene-by-environment designs, and custom analysis tools can be developed to pave the way for the use of these resources by nonexperts (Wang *et al.* 2003; Chesler *et al.* 2004; Kang *et al.* 2008). GRPs are also attractive because over time the phenotypic, genetic, and genomic data associated with each line becomes richer, making possible the integration of data from distinct biological fields that support a more holistic view of biological processes.

Most mouse GRPs are collections of inbred lines derived from pairs of inbred strains. In mice, these include panels of chromosome substitutions strains (*i.e.*, consomics), recombinant inbred lines (RIL), and subcongenics (Bailey 1971; Taylor *et al.* 1971; Hudgins *et al.* 1985; Demant and Hart 1986; Nadeau *et al.* 2000). Alternative GRPs include panels of extant inbred lines with complex population structures and nonuniform genetic relationships among the lines, such as the Laboratory Strain Diversity Panel derived from the Mouse Phenome Project (Paigen and Eppig 2000) and com-

binations of diversity panels and pairwise panels (Bennett *et al.* 2010). Key parameters that determine the usefulness of GRPs for the analysis of complex traits are the number of lines; the density, distribution, and functional significance of the genetic variation present in the GRP; the number and distribution of unique recombination sites; the presence of population structure; and the level of inbreeding and genetic drift.

The Collaborative Cross (CC) concept of a multiparental RIL panel was proposed in 2002, as a project aimed at generating a common platform for mammalian complex traits genetics that overcomes the limitations of existing resources (Threadgill *et al.* 2002) and that can advance the field beyond complex trait analyses toward systems genetics (Threadgill 2006). The final eight-way RIL design of the CC was community driven (Churchill *et al.* 2004) and included founders from five classical inbred strains (A/J, C57BL/6J, 129S1/SvImJ, NOD/ShiLtJ, and NZO/HILtJ) and three wild-derived strains that were selected to represent three *Mus musculus* subspecies (CAST/EiJ, PWK/PhJ, and WSB/EiJ). The CC lines were generated via a funnel breeding scheme that combined the eight founder genomes in three outbreeding generations prior to repeated generations of inbreeding through sibling mating (Figure 1). The eight founder strains capture a much greater level of genetic diversity than existing RIL panels or other extant mouse GRPs, and the genetic variants are more uniformly distributed across the genome than in other GRPs (Roberts *et al.* 2007; Keane *et al.* 2011; Yalcin *et al.* 2011; Yang *et al.* 2011). In the absence of selection and errors, the breeding design predicts that the captured genetic variation will be randomly distributed among the CC lines with each line being

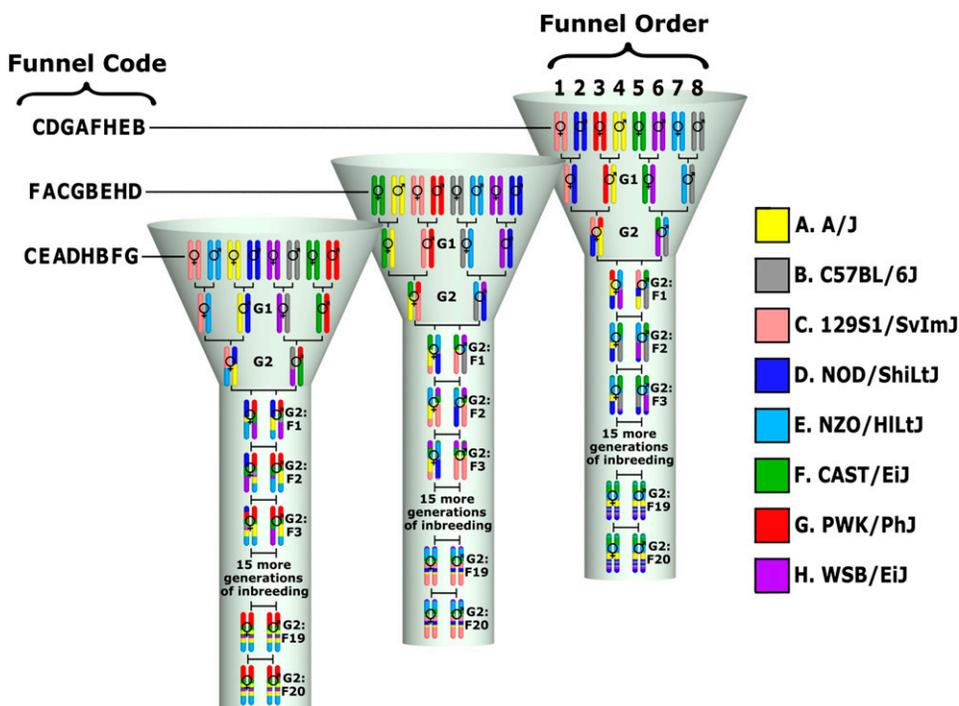


Figure 1 Breeding scheme of CC lines. The figure shows the breeding scheme for three independent CC lines. Each line has a funnel section followed by an inbreeding section. The eight founder strains are arranged in different positions (1–8) in each line, and this order determines the funnel code on the basis of a single letter code for each line. Founder order is randomized and not repeated across lines. The colors used for founder strains are seen throughout this article. Each mouse is represented by a pair of homologous autosomes and a symbol denoting its sex.

independent (*i.e.*, CC lines do not share recombination events and local founder contributions). Therefore, the use of the CC should not result in spurious associations in mapping studies that frequently occur in other GRPs (Manenti *et al.* 2009).

Because of practical and budgetary constraints, breeding of CC lines started simultaneously in 2004 at different locations from common founder lines. The US lines were started at Oak Ridge National Laboratory (ORNL) in Tennessee and were subsequently relocated to the University of North Carolina in 2009 (hereafter referred to as the CC-UNC). A second set of CC lines was started at the International Livestock Research Institute (ILRI) in Kenya and relocated to Tel Aviv University (Israel) in 2006 (hereafter referred to as CC-TAU). A third set of CC lines was started in Western Australia by Geniad Ltd. (hereafter referred to as CC-GND). The combined CC-UNC, CC-TAU, and CC-GND populations are the focus of this study. Initial status reports for each of these populations were published in 2008 (Chesler *et al.* 2008; Iraqi *et al.* 2008; Morahan *et al.* 2008) with subsequent publications detailing breeding, simulation, and statistical modeling (Broman 2005, 2007, 2012a, 2012b; Valdar *et al.* 2006; Teuscher and Threadgill *et al.* 2011; Gong and Zou 2012; Lenarcic *et al.* 2012; Zhang *et al.* 2012). Phenotypic and mapping results for a variety of traits using incompletely inbred CC lines are available (pre-CC) (Mathes *et al.* 2010; Aylor *et al.* 2011; Durrant *et al.* 2011; Philip *et al.* 2011; Kelada *et al.* 2012). These pivotal proof-of-concept studies used various subsets of pre-CC lines from either the CC-UNC or CC-TAU populations, some of which have since become extinct. The previous analyses of subsets of lines from each of the three populations provided only a limited view into the combined genome architecture of the “final” CC population as a whole due to use of different genotyping platforms, haplotype reconstruction methods, and analytical pipelines. Furthermore, most of these studies did not incorporate recent results on the subspecific origin and haplotype diversity present in the founder strains (Yang *et al.* 2011), nor the whole genome sequence of the eight founder inbred strains recently completed by the Mouse Genome Project from the Wellcome Trust/Sanger Institute (Keane *et al.* 2011; Yalcin *et al.* 2011). This project reported the presence of at least 36,155,524 SNPs in the founder strains of the CC. Initial analyses in CC founders and incompletely inbred lines indicate that the high level of genetic diversity is responsible for the vast number and strength of differences in gene expression in the CC (Aylor *et al.* 2011; Sun *et al.* 2012). Finally, The Jackson Laboratory is leading an ongoing effort to create a complementary resource, the Diversity Outcross (DO), derived from partially inbred CC lines originating from the CC-UNC population (Svenson *et al.* 2012).

Here, we report the joint genetic analysis of all three populations. This study was conducted by the Collaborative Cross Consortium and in what we expect will be an ongoing community effort to popularize this resource. We focused only on extant lines that will be part of the final CC population and conducted the analysis to provide the

research community with a more complete picture of the genome architecture expected to appear in the set of CC lines that are publicly available. All genotypes are available (Supporting information, Table S1) and use of these data should cite this publication as a reference. Genotypes will also be available at a dedicated website (<http://csbio.unc.edu/CCstatus/>). We have created a novel genome browser inspired by the Mouse Phylogeny Viewer (MPV; Yang *et al.* 2011; Wang *et al.* 2011) to facilitate visualization and interaction with the genomes of any given CC line (<http://csbio.unc.edu/CCstatus/?run=CCV>). Finally, we provide details of a Material Transfer Agreement that ensures availability of the CC population for use by the research community.

Materials and Methods

Mice and DNA

CC-TAU lines are bred and maintained in the small animal facility at The Sackler Faculty of Medicine, TAU. Mice are housed on hardwood chip bedding in open-top cages and are given tap water and rodent chow *ad libitum*. CC-UNC lines are bred and maintained under specific pathogen-free conditions in the Genetics Medicine Vivarium at UNC, where rodent chow and tap water are provided *ad libitum* and mice are maintained on bed-o’cobs with a nestlet placed in each breeding cage. CC-GND lines are bred and maintained at the Animal Resources Centre (ARC) in Western Australia and are housed under specific pathogen-free conditions with tap water and chow *ad libitum*. The Institutional Animal Care and Use Committees of TAU, UNC, and ARC have approved all experimental protocols at their respective institutions. During the generation of the CC population, CC-UNC lines are named with the prefix OR (that stands for the two first letters of the Oak Ridge National Laboratory) followed by a number with two to four digits. CC-TAU lines are named IL (which represents the first two letters of the International Livestock Research Institute) followed by a number with two to four digits. CC-GND lines have unique names followed by a two-letter code reflecting the strain located in positions 1 and 8 of the funnel (Figure 1 and also see Chesler *et al.* 2008; Aylor *et al.* 2011; Threadgill *et al.* 2011). Once the CC lines are deemed complete (>97% inbred), they will be renamed in accordance with the rules of the International Nomenclature Committee (see *Discussion*). Specifically, each line will be named CC#/@, where # are four digits from a consecutive sequence across all three CC populations and @ is the location from whence the line originated (Unc, US lines; Tau, Israeli lines; and Geni, Geniad lines). For example, the first completed line, OR867, is now CC0001/Unc and the second line, IL6211, is CC0002/Tau.

DNA isolation and genotyping

Tail clips were used to isolate DNA using Qiagen Genra Puregene blood kits from 458 lines (199 from CC-UNC, 214

from CC-TAU, and 45 from the CC-GND lines at the most advanced generations of inbreeding that were available at the time of analysis from approximately 230 extant lines). DNA was resuspended in water and 15- μ l aliquots at concentrations ranging from 50 to 200 ng/ μ l were sent in 96-well plates to Neogen's GeneSeek division for genotyping. Genotyping was conducted using our custom designed Mouse Universal Genotyping Array (MUGA). MUGA is a 7851-SNP marker genotyping array built on the Illumina Infinium platform. SNP markers are distributed throughout the mouse genome with an average spacing of 325 kb (SD 191 kb). The markers were chosen to be maximally informative and maximally independent for the eight founder strains of the CC. This combination was achieved by selecting SNPs with high minor-allele frequencies (maximizing entropy) and low local pairwise linkage disequilibrium (minimizing mutual information). The design criteria make the platform optimal for detecting heterozygous regions, while in homozygous regions they allow for optimal discrimination between haplotypes. These optimization criteria are population dependent. All genotypes are available in [Table S1](#). (If you use these genotypes or the updated genotypes available on the Collaborative Cross Consortium website, <http://www.csbio.unc.edu/CCstatus/index.py/>, we request that you also cite this article.)

CC founder haplotype inference

Existing techniques for minimizing recombination breakpoints (Zhang *et al.* 2009), and for haplotype inference such as in GAIN (Liu *et al.* 2010) and HAPPY (Mott *et al.* 2000), use four discrete genotype calls as input (homozygous allele 1, homozygous allele 2, heterozygous, or no-call). Rather than using discrete genotype calls, our haplotype reconstructions directly use Illumina's normalized intensity values. This is based on our observation that the allele clusters seen in a genotyping probe set can often be further subclustered according to the intensity values of the eight founders and the 28 possible F1's ([Figure S1](#)). This subclustering within genotype clusters can be attributed to subtle differences in the genomic sequence, such as unreported genetic variants within or nearby probes. Our use of subclusters from intensity values transforms the standard 4-state genotyping classification problem to one with 36 possible states for the CC population. The most likely founder at each position is assigned using a hidden Markov model (HMM) similar to the one used in GAIN, a genotype call-based method designed for pedigrees with inbreeding such as the CC (Liu *et al.* 2010).

The founder states are based on 2D distributions of intensity clusters of biological and technical replicates of CC founders and F1's at each marker (163 replicates in total: 8 replicates for each founder except C57BL/6J, which has 9, and 3.5 replicates on average for each of the 28 F1's). These distributions are then used as reference models for each founder and F1 combination. We estimate the likelihood that a test sample fits a particular model as a function of

the test sample's probe intensities Euclidean 2D distance from the model's mean. These distance-derived probabilities are combined with a transition probability between adjacent markers using an HMM. The transition probability parameters were selected so that evidence of sufficient distance from approximately three sequential markers is necessary to change founder state. Moreover, the transition penalty varies depending on the number of shared founders between adjacent states, with the highest penalty assigned to adjacent states with no shared founders. A dynamic programming algorithm was then used to calculate the maximum-likelihood founder assignment for each genomic position.

Identification of related lines and lines with breeding errors

Related IDs (for example, IL1912 and IL3912 or IL51 and IL551) were purposely used to identify cousin lines in the CC-TAU population, as well as mice from CC-TAU lines that were shipped from TAU to UNC for accelerated completion through marker-assisted inbreeding (MAI; Welsh and McMillan 2012). Note that samples from CC-TAU lines used for MAI at UNC are renamed with the OR prefix for colony-management purposes ([Table S2](#)). The cousin lines were segregated from the original lines between 6 and 11 generations of the inbreeding process ([Figure 1](#)). We used shared recombination events to confirm the identity of related lines. Shared recombination events are defined as those involving the same two strains in the same proximal-to-distal orientation at the same chromosome position. We determined the number of shared recombination events in the autosomes between all pairwise combinations of the 458 genotyped CC samples. Events that are fixed in a strain were counted only once. As expected, most pairs of lines do not share any recombination events (mean 0.0653 ± 0.7552) but a subset of pairs had a significantly higher rate of shared events ([Figure S2](#)). All known related lines have at least three shared events, while not a single pair of independent lines with three shared recombination events exists, and only 5% of 47,278 pairwise combinations between independent lines have one or two shared events ([Figure S2](#)). We identified 99 related CC samples that define 46 sets of related lines ([Table S3](#)). For each set we retained the sample with the lowest heterozygosity for further analyses.

Among the 405 independent lines, only 330 have alleles from each of the eight founder strains present in the autosomes ([Table S2](#) and [>Table S3](#)). Based on the simulation of 7 million CC lines, we estimate that 0.05% will have <1% of any given founder. The rate of CC lines missing one or more founders was significantly higher than the results of the simulation, and we eliminated any line with more than one founder missing. Finally, eight CC-UNC lines were eliminated because they represent four pairs of lines, with each pair missing one founder strain caused by the incorrect use of one of four G1 males ([Figure 1](#)) that were likely not hybrids between the expected two CC founder

lines. Twenty CC lines with one missing founder were retained in the analyses (Table S2). The 350 independent lines passing these quality metrics were used to analyze genome architecture in the CC populations.

Transmission ratio distortion (TRD)

In the autosomes, the frequency of the haplotypes inherited from each CC founder strain should be ~12.5% (one out of eight equally likely founders) in the final CC population (as well as in the individual populations). To determine the significance of local distortion in founder frequency we simulated the inbreeding of mouse genomes (19 autosomes + 2 sex chromosomes) using the same breeding scheme as the CC with a Haldane recombination model including interference (Welsh and McMillan 2012). We simulated 20,000 independent sets of 350 lines and tabulated the founder contribution over all haplotype segments within each set (a haplotype segment is the region from one recombination breakpoint to the next in any of the 350 lines). Each simulation used the same funnel code (Figure 1) as the actual CC-UNC, CC-TAU, and CC-GND populations when available. A random funnel code was used for lines where that line's funnel code was unknown or inconsistent with the genotypes. The funnel code reflects the position of the founder strains in the funnel (Figure 1). This position has consequences for the inheritance of mitochondrial genome (inherited from the strain in position 1), chromosome (chr) Y (inherited from the strain in position 8), and chr X. For chr X, the expected contribution of each CC founder depends on the funnel order. Founders in positions 4, 7, and 8 cannot contribute a chr X to the line while the founder in position 3 has double the opportunity to contribute compared with the rest of the positions. Finally, after the G1 generation (Figure 1) no CC mouse can be heterozygous for alleles from founder strains located in positions 1 and 2, 3 and 4, 5 and 6, and 7 and 8 in that line. We found that reported funnel codes did not match the expectations in many CC-TAU lines.

Expectations for the founder contribution for chr X (and estimation of the TRD significance) would be best achieved by simulations based on the actual funnel codes of the 350 independent CC lines. However, given the issues with the funnel codes of the CC-TAU population, the significance of local distortion in founder allele frequency was modeled on the basis of equal contribution from each founder in the CC-TAU population. Actual contributions for the CC-UNC and CC-GND populations are provided in Table S4.

Finally, we assigned the subspecific origin of each CC line using the subspecific assignments of each CC founder (Yang *et al.* 2011) overlaid on the inferred CC haplotype mosaics.

Linkage disequilibrium

We partitioned the genome into 5295 nonoverlapping 500-kb windows and binned all of the previously reported mouse diversity array (MDA; Yang *et al.* 2011) SNPs into these windows. We then computed the maximum linkage disequilibrium (LD) value, on the basis of the r^2 metric (Pearson

correlation squared), among all SNP pairs within each pair of windows.

The genotypes of CC lines were imputed at MDA resolution by assembling MDA founder genotypes according to the haplotype mosaics inferred from the founder assignment algorithm described previously. For each recombination we defined a recombination interval flanked by the most distal SNP assigned to the proximal haplotype and the proximal SNP assigned to the distal haplotype. We used the midpoint of these recombination intervals as the dividing point between the founder haplotypes. Each chromosome was imputed separately, giving two haplotype sequences per sample. We modeled a final predicted genome of each inbred CC line by randomly choosing one of the two haplotypes associated with a given line in each chromosome.

The comparative analyses with a panel of 88 inbred strains required matching population sizes. Therefore, we randomly chose an equal number ($n = 88$) of CC lines to compute the LD for the panel using the same metric. We repeated the random selection of 88 haplotypes 100 times and then found the average maximum LD value for each window pair. We considered all SNPs with fewer than 5% H or N calls across all samples, and of the SNPs considered, we calculated LD for only those SNPs with a minor allele frequency of 5% or higher.

The panel of classical inbred strains includes the following 88 inbred strains: 129P1/ReJ, 129P3/J, 129S1SvlmJ, 129S6, 129T2/SvEmsJ, 129X1/SvJ, A/J, AEJ/GnLeJ, AEJ/GnRka^{e/a^e}, AKR/J, ALR/LtJ, ALS/LtJ, BALB/cByJ, BDP/J, BPH/2J, BPL/1J, BPN/3J, BTBR T⁺tf/J, BUB/BnJ, BXSB/MpJ, C3H/HeJ, C3HeB/FeJ, C57BL/10J, C57BL/6J, C57BLKS/J, C57BR/cdJ, C57L/J, C58/J, CBA/CaJ, CBA/J, CE/J, CHMU/LeJ, DBA/1J, DBA/1LacJ, DBA/2HaSmnJ, DBA/2J, DDK/Pas, DDY/JclSidSeyFrkJ, DLS/LeJ, EL/SuzSeyFrkJ, FVB/NJ, HPG/BmJ, I/LnJ, IBWSR2, ICOLD2, IHOT1, IHOT2, ILS, ISS, JE/LeJ, KK/HlJ, LG/J, LP/J, LT/SvEiJ, MRL/MpJ, NOD/ShiLtJ, NON/ShiLtJ, NONcNZO10/LtJ, NONcNZO5/LtJ, NOR/LtJ, NU/J, NZB/BINJ, NZM2410/J, NZO/HlLtJ, NZW/LacJ, P/J, PL/J, PN/nBSwUmabJ, RF/J, RHJ/LeJ, RIIS/J, RSV/LeJ, SB/LeJ, SEA/GnJ, SEC/1GnLeJ, SEC/1ReJ, SH1/LeJ, SI/Col Tyrp1, Dnahc11/J, SJL/J, SM/J, ST/bJ, STX/Le, SWR/J, TALLYHO/JngJ, TKDU/DnJ, TSJ/LeJ, YBR/EiJ, ZRDCT Rax⁺ChUmd. This set of strains represents the largest panel of classical inbred strains genotyped with the MDA after excluding substrains that are identical by descent (IBD) genome wide (Yang *et al.* 2011; Wang *et al.* 2012). The panel overlaps significantly with the strains of the Mouse Phenome Project (Paigen and Eppig 2000) and the Hybrid Mouse Diversity Panel (Bennett *et al.* 2010). All genotypes have been reported previously (Yang *et al.* 2011).

Ancestral haplotype diversity in the CC founders

We generated compatible intervals on the basis of the four-gamete rule (Hudson and Kaplan 1985) for the five classical founder inbred strains of the CC using MDA genotypes

(Wang *et al.* 2010; Yang *et al.* 2011). We then generated the intersection between these intervals and the transitions between subspecific origin in one or more of the eight CC founder strains (Yang *et al.* 2011). Among strains with the same subspecific origin we estimated the number of haplotypes on the basis of MDA genotype similarity, using a threshold of 97% to identify regions that are IBD among CC founders. The rationale for this threshold has been described in a recent study of haplotype diversity in a large panel of laboratory strains (Yang *et al.* 2011), and it is supported by validation of large-scale SNP genotype imputation in mouse inbred strains (Wang *et al.* 2012) and the mouse genome sequencing project (Keane *et al.* 2011).

CC viewer

We have developed a web-based genome browser for visualizing genomic data over multiple CC lines to aid in comparative analysis. This tool is freely available online at <http://csbio.unc.edu/CCstatus/?run=CCV>. Available data includes 458 incipient CC lines. We visualize subspecific origin, founder haplotype, and haplotype identity mosaics as stacked horizontal tracks to align coincident features. Our tool includes dynamic panning and zooming, which allows for intuitive navigation about the genome. It also has dynamic interaction features that are applied to the various data sets, including sample sorting based upon similar features as a selected locus. The tool also automatically generates stacked histograms that show the distribution of subspecific origin and founder contribution for a user-selected subset of lines.

Results

Breeding, extinction, and reproductive performance in the CC

Although this report focuses on extant lines, data on all initiated lines in the CC-UNC population are provided to frame our results within the larger context of the CC project. Importantly, the value of our characterization of the genome landscape of the CC resource depends on whether a given CC line that is extant today eventually survives the inbreeding process and becomes available to the research community.

In the CC-UNC population, we included only CC lines that bore a litter within 6 months of this study's starting date (December 2010). The extinction rate in the UNC arm of the CC project was 73.04% (199 extant lines out of 738 lines started at ORNL). The high rate of extinction is consistent with previous reports (Chesler *et al.* 2008; Philip *et al.* 2011). Since the last status report, we have attempted to reduce loss of lines due to colony management, and we started MAI of the most advanced lines (Welsh and McMillan 2012). We also relocated the project to the University of North Carolina upon closure of the Mouse Genetics Program at ORNL (Threadgill *et al.* 2011). We determined the re-

productive performance of the extant lines on the basis of average litter size per generation and time between generations (Figure S3). As expected, reproductive performance decreases significantly during inbreeding but stabilizes after generation G2:F7. On the basis of data available for the most advanced generations of inbreeding (>12), the "final" CC lines will have reproductive performances within the range observed in the founder CC strains. The CC lines and corresponding reproductive performance data will be available at the Collaborative Cross Consortium website (<http://www.csbio.unc.edu/CCstatus/index.py>). (Please cite this article when using this information.)

Genotyping and haplotype reconstruction

We selected a single male from 458 CC lines for genotyping, 199 from the CC-UNC population, 214 from the CC-TAU population, and 45 from the CC-GND population (Table S3). The genotyped male either belonged to the most advanced generation of each line at the time of sample collection or was the most inbred male in the case of lines with multiple males genotyped (*i.e.*, lines actively undergoing MAI). All samples passed the initial QC step on the basis of the fraction of SNP genotypes called (Table S1). We then performed founder assignment (see *Materials and Methods*) and determined the contribution of each founder strain to each CC line (Table S2). Unexpectedly, we found that numerous CC lines had fewer than eight CC founders' alleles in their genome. This result could be explained by breeding errors (the missing founder was never present in the line), selection against a given CC founder genome, or chance. Given that one of our main goals seeks to compare the genome composition of the final CC population to what may be expected based on the genome of the CC founders, we established a set of criteria to identify CC lines with breeding errors and to identify related lines in the CC-TAU population ("cousin" lines and sister lines of CC-TAU lines sent to UNC for MAI). These criteria include the frequency of shared recombination events between pairs of samples and the number of missing founders (*Materials and Methods*). We identified 55 samples with more than one CC founder missing. Eight of these lines belong to the CC-UNC population, 44 to the CC-TAU population, and 3 to the CC-GND population (Table S2 and Table S3). Among the remaining 403 samples, 99 are related and represent 46 independent lines. Related lines are denoted as rCC while incomplete lines are denoted as iCC in Table S2. After these quality-control steps, our final sample set for analysis consists of 350 independent CC lines, 191 CC-UNC lines, 117 CC-TAU lines, and 42 CC-GND lines.

For each line we estimated the residual heterozygosity as the fraction of the genome for which a line has contributions from two different CC founders (Table S2). Average heterozygosity was 25.38% in the CC population genotyped for this study, but the range varied between 0.21% and 66.96% (Figure S4). Note that most of the CC lines have progressed between one and three generations since the mice were

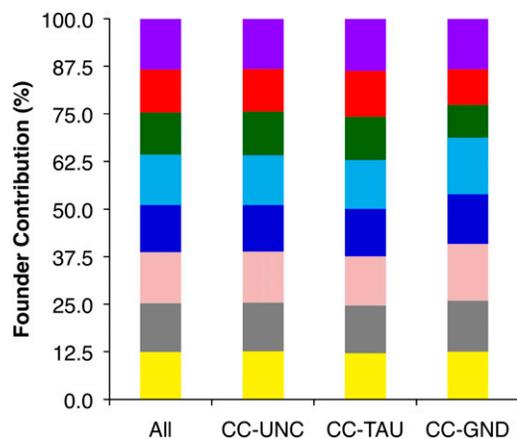


Figure 2 Overall contribution of the eight CC founder strains to the autosomes of the CC lines. The stacked columns show the founder contribution to the overall CC, CC-UNC, CC-TAU, and CC-GND populations.

genotyped. The distribution of residual heterozygosity was as expected for the number of generations of inbreeding (Broman, 2012a; Welsh and McMillan 2012) and both the CC-UNC and CC-TAU populations having two waves of production that started 3–4 years apart.

Founder contribution

Overall the eight founder strains' alleles were similarly represented when averaged across the autosomes of the CC lines (Figure 2), and their contribution varied between 11.06% for CAST/EiJ and 13.40% for 129S1/SvImJ (Table S2). The lower contribution of CAST/EiJ holds true for all three populations, CC-TAU, CC-GND, and CC-UNC (Figure 2), and becomes more pronounced when chr X is included (see below). On the other hand, founder contribution varied significantly along the autosomes (Figure 3A). In general, deviation from an expected 12.5% contribution resulted from an overrepresentation of a single founder strain, while a similar level of underrepresentation of a founder was less frequent.

Notably, there is a significant ($P < 0.05$, corrected for genome-wide significance) excess of WSB/EiJ alleles spanning a 51.6-Mb genomic region (73.25–124.85 Mb) on chr 2 in the overall set. Similar levels of distortion were observed in the independent CC-UNC, CC-TAU, and CC-GND populations (Figure 3, B–D). This region overlaps with a putative region of TRD in favor of WSB/EiJ reported previously in the pre-CC experiments (Aylor *et al.* 2011; Durrant *et al.* 2011). There are 66 CC-UNC lines in common between one of the

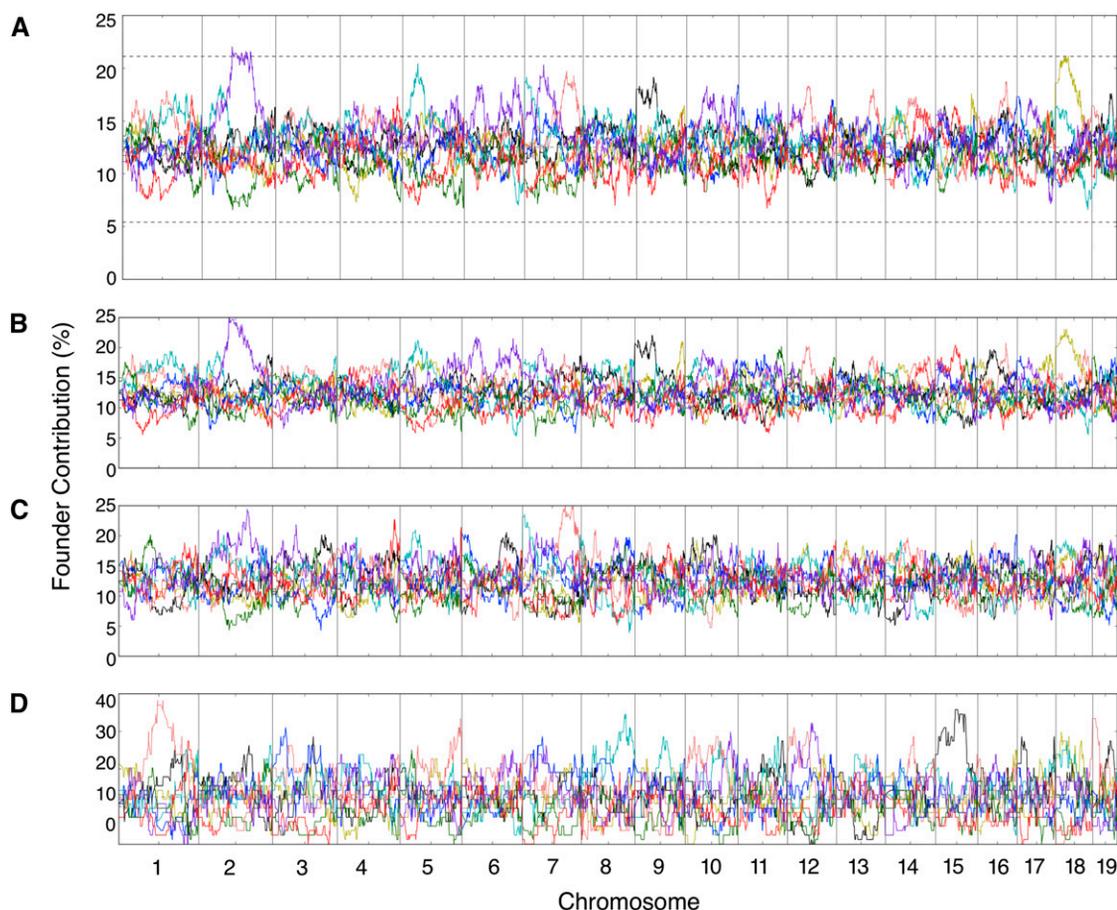


Figure 3 Local founder strain contribution along the autosomes. (A) The CC population, (B) CC-UNC population, (C) CC-TAU population, and (D) CC-GND population. The percentage contribution from each founder is represented as a continuous line using the color schema shown in Figure 1. The dotted lines represent the threshold for TRD at $P = 0.05$ adjusted for genome-wide significance.

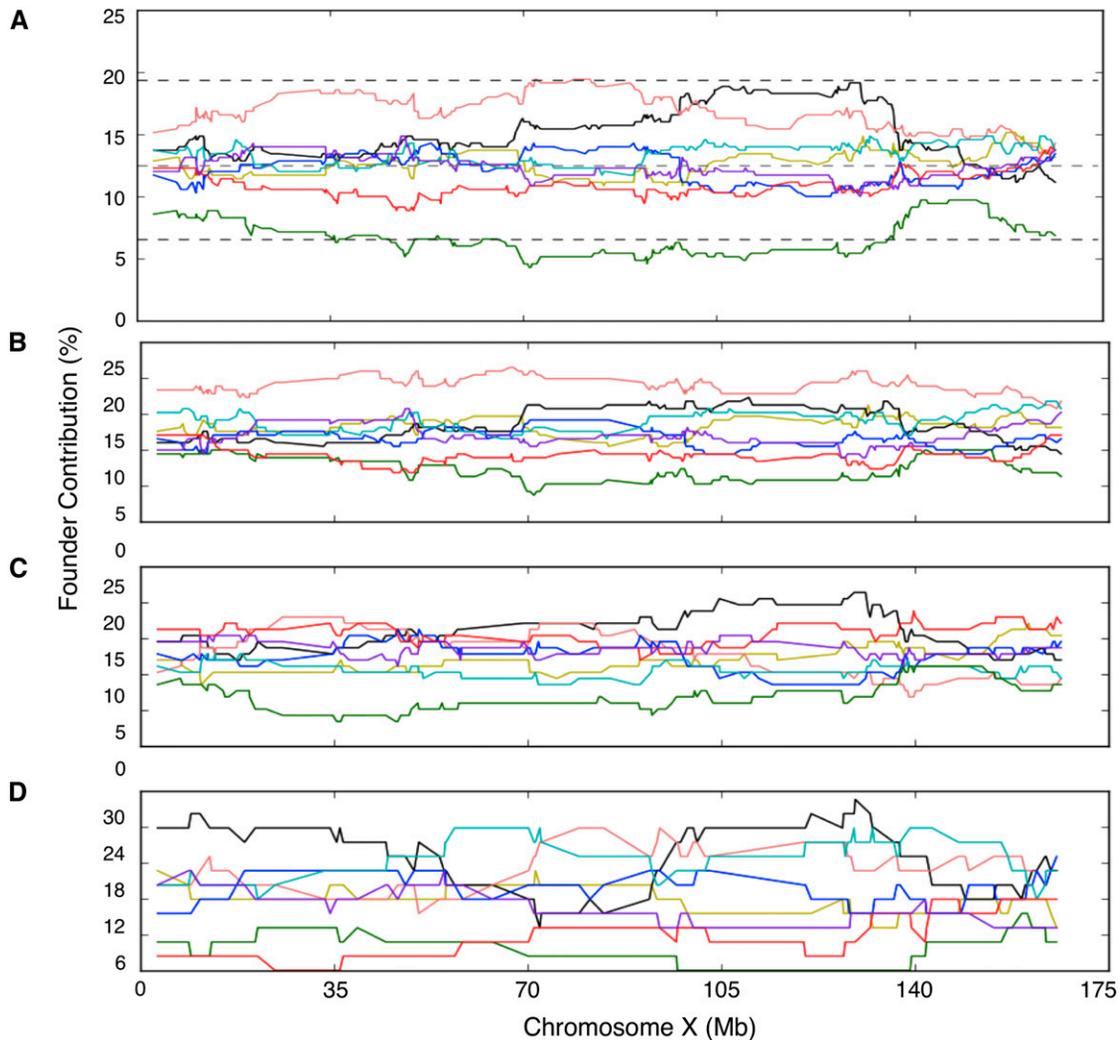


Figure 4 Local founder strain contribution on chromosome X. (A) Final CC population, (B) CC-UNC population, (C) CC-TAU, and (D) CC-GND population. The percentage contribution from each founder is represented as a continuous line using the color schema shown in Figure 1. The dotted lines represent the threshold for TRD at $P = 0.05$ adjusted for genome-wide significance.

pre-CC experiments (Aylor *et al.* 2011) and the 191 CC-UNC lines in this study; the level of distortion among the animals used in the pre-CC experiments is not significantly different than the final CC set (23.5%, 31 WSB/EiJ chromosomes out of 132 total: two chromosomes \times 66 samples). Therefore, we conclude that the distortion in favor of WSB/EiJ at this locus is a general feature of the CC rather than simply a chance event. The large size of the region and the shape of the TRD peak on the overall population (Figure 3A) strongly suggest the involvement of multiple loci.

Three additional regions of distortion are consistent between CC-UNC and CC-TAU populations (the CC-GND population was not considered in this analysis because its smaller size leads to highly variable allele frequencies; Figure 2D): overrepresentation of NZO/HILtJ on chr 5 and overrepresentation of WSB/EiJ and 129S1/SvImJ on chr 7 (Figure 3). Multiple examples of strong deviation from expectations are population specific. For example, an excess of WSB/EiJ, C57BL/6J, and A/J is found on chrs 6, 9, and 18,

respectively, in the CC-UNC population. There is an excess of WSB/EiJ, CAST/EiJ, and NOD/ShiLtJ on chrs 3, 4, and 6, respectively, in the CC-TAU population. Whether these findings are due to differential selection based on differences in husbandry between the two sites or due to chance is not known.

In contrast with the situation in the autosomes, we observed consistent underrepresentation of founder strains' alleles on chr X (Figure 4). The most striking observation is a significant ($P < 0.05$, corrected for genome-wide significance) underrepresentation of the CAST/EiJ contribution for much of chr X in all populations. TRD spans at least a 100-Mb region (35–135 Mb) that includes the center of chr X (Figure 4). Estimation of TRD significance was based on assuming equal contribution of each founder rather than the actual contribution dictated by the frequency at which each founder was at each position in the funnel (funnel order, see *Materials and Methods* and Figure 1). However, the actual contribution for 233 known CC lines (Table S4) indicates that underrepresentation of chr X from CAST/EiJ

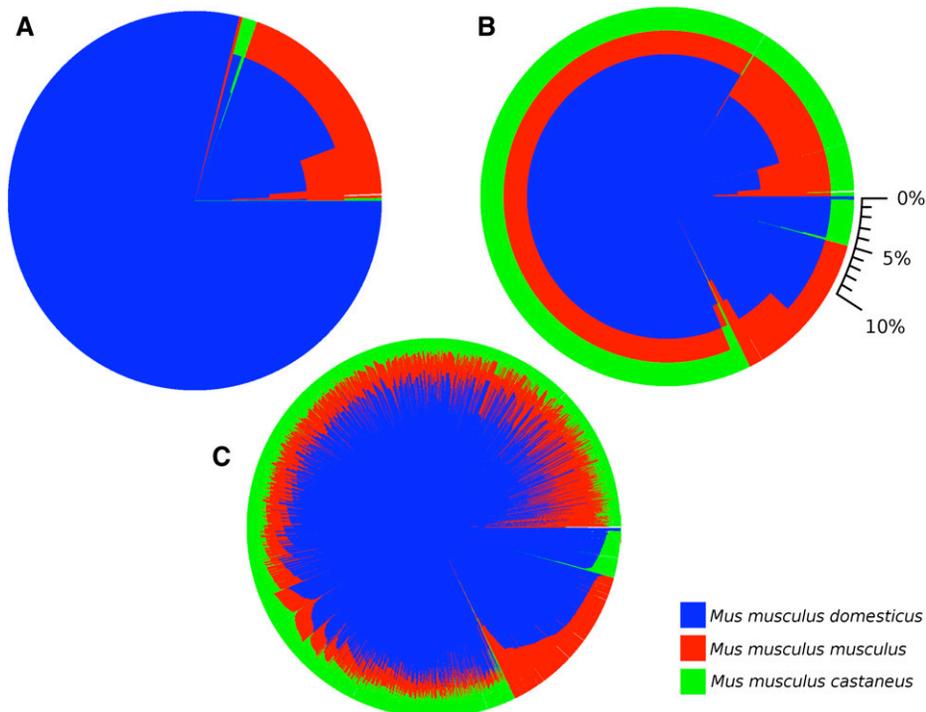


Figure 5 Subspecific contribution to the genome of the CC lines. Each pie chart depicts the fraction of the genome that has a given pattern of subspecific contribution in each set of lines. (A) Subspecific contribution in the five CC founder strains that are classified as classical (AJ, 129S1SvMj, C57BL/6J, NOD/ShiLtJ, and NZO/HILtJ). (B) Subspecific contribution in the eight CC founders. (C) Subspecific contribution in the 308 lines that represent the combined CC-UNC and CC-TAU populations. Blue represents *M. m. domesticus*, red represents *M. m. musculus*, and green represents *M. m. castaneus*. A scale in percentage is provided in B.

at the initial generations of the CC can not be responsible for the observed TRD.

We have recently assigned each region of the genome of the eight CC founders to one of three *M. musculus* subspecies (Yang *et al.* 2011). On the basis of this assignment we determined the subspecific origin of each CC line (Figure S5). When the CC founder strains were selected, an important consideration was the inclusion of three wild-derived strains thought to be pure representatives of three major *M. musculus* subspecies (Chesler *et al.* 2008). We now know, however, that in two of the wild-derived strains, CAST/EiJ (assumed to be *M. m. castaneus*) and PWK/PhJ (assumed to be *M. m. musculus*), a significant amount of their genome originates from *M. m. domesticus* due to intersubspecific introgression (Yang *et al.* 2007, Yang *et al.* 2011). Furthermore, classical inbred strains have little contribution of subspecies other than *M. m. domesticus* and that contribution is not randomly distributed across the genome. The impact of inclusion of wild-derived strains and the overall representation of the three subspecies is shown in Figure 5; the representation of each subspecies in the individual CC lines varies dramatically (Figure S5). Although the overall subspecies representation is not dramatically distorted, a small excess of *M. m. domesticus* exists compared to simulations. This conclusion is based on comparing the subspecies distribution observed in the extant CC lines with the anticipated subspecies distribution of founder strains in simulations of the generation of similar number of independent CC lines.

Linkage and gametic disequilibrium

We determined the extent and strength of LD and gametic disequilibrium (GD), which is also known as long-range LD,

in the CC. LD decays rapidly in the final population (Figure S6 and Figure S7).

More interestingly for users of mouse GRPs, we compared LD and GD between the CC population and a large panel of 88 classical inbred strains (see *Materials and Methods*). To facilitate comparisons between these two GRPs, we subsampled the CC to ensure the same population size ($n = 88$). We further selected only one representative among recently derived substrains (Yang *et al.* 2011). Figure 6 shows the striking differences in genome-wide LD/GD between these two populations. The genome-wide LD/GD in the entire set of 350 CC lines is shown in Figure S8.

In the CC, high LD is observed only between SNP loci that are in close physical proximity, and we see no evidence of significant GD among any unlinked markers. In contrast, the panel of classical inbred strains shows limited local LD but high GD is pervasive throughout the genome. The LD decay is considerably different in these two populations (Figure S6). In the panel of classical inbred strains LD decays very rapidly, but at distances over 20 Mb it stabilizes at 0.17. In the CC, LD decay is initially slower but it continues to decrease over longer distances. At distances over 55 Mb (~27 cM) LD is substantially lower in the CC than in the classical inbred panel (Figure S6). For example, at 80 Mb the mean LD in 88 CC lines is approximately two-thirds that of the LD observed in the classical inbred panel (and less than one-third in the complete set of 350 CC lines compared to the panel of classical inbred strains).

We estimated the mean and the maximum GD between unlinked markers (>100 Mb that represents 50 cM on average in the mouse) (Figure S6). The mean GD in both populations has a unimodal distribution but with very

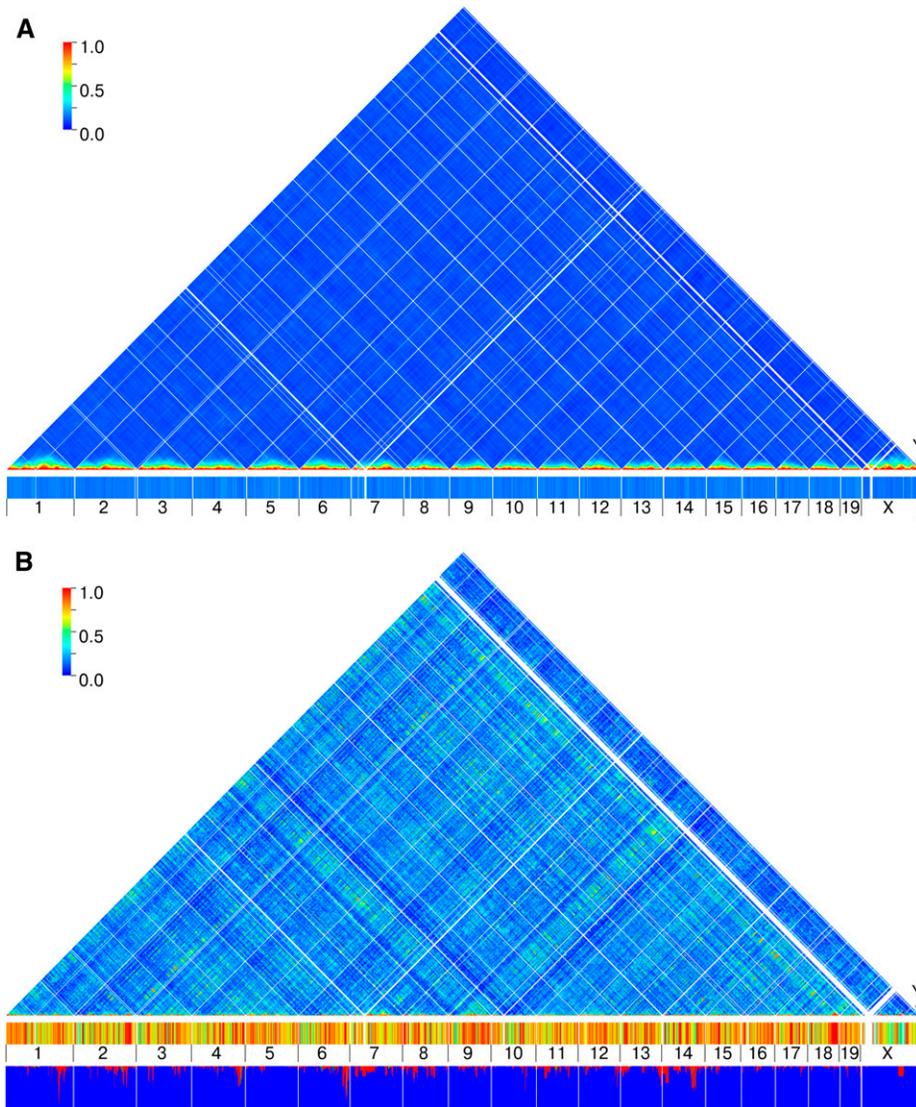


Figure 6 Linkage and gametic disequilibrium in mouse GRPs. Chromosomes are arranged in sequential order in the horizontal axis and the color of each pixel represents the maximum level of LD at that pair. The tick boxes denote the maximum level of gametic disequilibrium found genome-wide for each 500-kb window. (A) Mean level of maximum LD in 100 random sets of 88 CC lines. (B) A panel of 88 mouse inbred strains. The additional box at the bottom of the panel represents the cumulative contribution of the subspecies to the panel 88 of inbred strains. Blue represents *M. m. domesticus*, red represents *M. m. musculus*, and green represents *M. m. castaneus*.

different means and variance. In the panel of classical inbred strains, the mean is 0.1733 but we observe wide variance. In the CC the mean is 0.0968, and the variance is low. The distribution of maximum GD shows similar but more extreme features (Figure S6). The most striking result is the number of 500-kb windows that have at least one SNP locus very high LD (>0.75) with unlinked SNP loci in the panel of classical inbred strains (Figure 6 and Figure S7).

Discussion

We provide the first comprehensive view of the genetic architecture of the extant CC breeding populations, a framework for future use of this resource, and the ways it complements ongoing research and related resources such as the DO (Svenson *et al.* 2012). This study has the advantage of combining the three populations (CC-UNC, CC-TAU, and CC-GND) that will be publicly available. We also focus on lines that are most likely to survive inbreeding and, therefore, will be used in future research.

Our analysis also benefits from consistency in genotyping and analyses; the MUGA genotyping platform was primarily designed as a tool to help accelerate inbreeding and detect breeding errors during the generation of the CC population. However, MUGA was not designed to provide a definitive resolution description of the genome of CC lines. During MUGA development, containing costs, a reasonable turn-around time, and operational simplicity were the main considerations. The number of SNP loci was dictated by the price and real estate of the Illumina Infinium platform. The average number of SNPs required to infer founder-strain origin dictates that we will not have resolution under 1 Mb. This is confirmed by the fact that the number of recombination events and segments per CC line (Figure S9) is lower than predicted by simulations (Broman 2005; Teuscher and Broman 2007; Welsh and McMillan 2012) and observed in the pre-CC (Aylor *et al.* 2011; Durrant *et al.* 2011), which used the much denser MDA (Yang *et al.* 2009). The average number of segments in our analysis (92.1 ± 12.8) is 30–50% lower than these estimates.

This is explained in part by a marked reduction in the number of small segments under 2 Mb in CC founder haplotype reconstructions (Figure S9). However, LD and TRD distortion analyses should be largely unaffected by resolution of founder haplotype assignments.

Founder–strain contribution varies widely among the 350 CC lines included in this study (Figure S10). TRD is common in mouse crosses (Eversley *et al.* 2010) and can be due to multiple causes (Pardo-Manuel de Villena and Sapienza 2001). Our results suggest the operation of both positive and negative selection during the generation of the CC. Positive selection for the WSB/EiJ haplotype on chr 2 occurred at the expense of all other founder strains (Figure 3) and is observed uniformly over a wide range of generations of inbreeding, suggesting that it operated in the outcross generations and/or the earliest generations of inbreeding. TRD in favor of WSB/EiJ alleles is also observed in the early generations of the DO (Svenson *et al.* 2012).

Conversely, our results suggest that negative selection against the CAST/EiJ haplotype is responsible for the distortion on chr X. The involvement of the sex chromosomes in TRD in populations derived from multiple mouse subspecies is not unexpected (Payseur *et al.* 2005; Mihola *et al.* 2009; White *et al.* 2011) and may provide an elegant model for speciation. However, we believe that the selection against the *M. m. castaneus* X chromosome in a population that is mostly *M. m. domesticus* is novel. Furthermore, we expect that most TRD in the CC will involve epistatic interactions between multiple loci. Because of the wide range of heterozygosity in the current CC population (Figure S4), we did not attempt to perform analyses involving more than one locus. When the CC population is fully inbred, such analyses should be conducted.

Among the most important characteristics of the CC as a GRP is the presence of multiple haplotypes and the high minor allele frequencies for every SNP. We have shown previously that the use of eight allele models (representing the eight founder strains) can improve mapping (Valdar *et al.* 2006; Aylor *et al.* 2011) compared to standard biallelic SNP models (Zhang *et al.* 2012). However, the founder strains of the CC have their own population history and structure (Yang *et al.* 2011). Therefore, it is important to determine the number of founder haplotypes on a local scale. For more discussion of the eight alleles model, see Svenson *et al.* (2012). Almost half of the genome has six distinct haplotypes represented in the eight founder strains (Table S5). Most of the remaining genome has four to eight haplotypes while almost none have fewer than four haplotypes. The regions of consistent haplotypes are dictated by the historical recombinations in the founder strains (Yang *et al.* 2011) and on average are 371 kb long but vary widely across the genome. Comparison with the distribution of haplotypes in the five classical founder strains clearly demonstrates the value of including the three wild-derived strains. The spatial variation in ancestral haplotype diversity is reflected in the CC genome browser. Ultimately, we plan

to determine haplotype diversity on the basis of whole-genome sequence of the founders and the new recombination intervals created during the generation of the CC.

One major finding in our analysis of the CC compared to extant classical inbred strain panels is the difference in long range LD (GD), particularly across chromosomes. Existing classical inbred strains have high levels of long-range LD, likely due to their complicated breeding histories and limited founder populations. High GD in essence creates a situation in which association mapping has high type I error rates (false positives). This has been previously noted (Burgess-Herbert *et al.* 2009), although the mechanism responsible for the high false-positive rate was unknown. Here we show that this is due to extensive long-range LD in extant inbred strain panels that, while partially overcome by taking population structures into account (Kang *et al.* 2008), will still lead to extraordinarily high rates of false positives. In contrast, because of the independent inheritance of all genomic intervals, the independent breeding lines of the CC are devoid of long-range LD and present an ideal population for association studies.

The pattern of long-range LD observed in our panel of classical inbred strains is very similar to the one reported previously in laboratory strains (Petkov *et al.* 2005) despite the differences in strain composition, marker density, and ascertainment bias. These results combined with our more complete understanding of the origin of the genome of the laboratory mouse strongly suggest that history rather than selection was the major driving force in setting these patterns. In fact, there is no evidence that long-range LD in the panel of classical inbred strains is driven by combination of alleles from different subspecies (Figure 6b). However, the high extinction rates observed during the derivation of the CC (Chesler *et al.* 2008; Iraqi *et al.* 2008; Morahan *et al.* 2008; Threadgill *et al.* 2011), the presence of replicable and significant TRD, and the reduction in breeding performance (Figure S3) indicates that the role of biological selection in shaping the CC resource needs to be explored in the future.

The long-range LD structure in extant classical inbred lines negatively affects other QTL mapping studies. Biological systems analyses based on correlation structures are predicted to contain multiple erroneous correlations when using extant classical inbred lines because of the preexisting genomic correlations. Because the CC lacks long-range LD and thus preexisting correlation structures, the CC is also optimally suited for systems-level analyses.

To ensure unfettered community access to the CC, a Material Transfer Agreement (MTA) was executed between all parties who developed this new resource. This MTA will promote efficient distribution and use of the CC. The five institutions involved in developing the CC include The Jackson Laboratory, the University of North Carolina, Tel Aviv University, Oxford University, and Geniad Ltd. and are parties to an MTA that establishes policies for distribution of CC mice. CC mice, regardless of where they were originally developed, as well as services for their use, are available from any of the MTA parties. Conditions of use

(COU) for the mice are based on community standards and are identical to the COU currently covering mice from The Jackson Laboratory. To promote use of the CC population, genotypes of the sampled CC lines will be made publicly available (<http://www.csbio.unc.edu/CCstatus>). Because the MTA also aims to preserve the genetic integrity of the CC lines, distribution centers will repopulate from a common source of mice or embryos. UNC and TAU will act as distribution centers for CC mice in the United States and Europe. Furthermore, the U.S. center at UNC has established an external advisory board to provide guidance and advice on completion, archiving, and distribution of CC mice (Table S6). As CC lines are deemed inbred, they will be cryopreserved and rederived by the UNC Mutant Mouse Regional Repository Center and the Wellcome Trust into a vendor-quality health status. Finally, The Genome Institute at Washington University is carrying out an ongoing effort to sequence the genomes of each CC line as the line is completed. Full genome sequence information for each line will also be publicly available.

Acknowledgments

This work is published under a consortium authorship (listed on opening page) that includes mouse breeders, tool developers, and users of the resources. We acknowledge the following members of the consortium who have made special significant contributions to generation of mice, genotyping, and analysis reported in this manuscript: Fuad A. Iraqi, Mustafa Mahajne, Yasser Salaymah, Hanna Tayem, Karin Vered, Hani Sandovski, Richard Mott, Caroline Durrant, David L. Aylor, Ryan J. Buus, John P. Didion, Chen-Ping Fu, Terry J. Gooch, Stephanie D. Hansen, Leonard McMillan, Kenneth F. Manly, Darla R. Miller, Fernando Pardo-Manuel de Villena, Ginger D. Shaw, Jason S. Spence, David W. Threadgill, Jeremy Wang, Catherine E. Welsh, Grant Morahan, Lois Balmer, Ken Pettit, and Michael Hall. This work was supported by grants from the National Institutes of Health U01CA134240, P50MH090338, P50HG006582, and U54AI081680; Ellison Medical Foundation grant AG-IA-0202-05, National Science Foundation grants IIS0448392, IIS0812464, the Australian Research Council grant DP-110102067, and the Wellcome Trust grants 085906/Z/08/Z, 083573/Z/07/Z, and 090532/Z/09/Z. Essential support was provided by the Dean of the University of North Carolina (UNC) School of Medicine, the Lineberger Comprehensive Cancer Center at UNC, and the University Cancer Research Fund from the state of North Carolina. We also thank Tel-Aviv University for their core funding and technical support.

Literature Cited

Aylor, D. L., W. Valdar, W. Foulds-Mathes, R. J. Buus, R. A. Verdugo *et al.*, 2011 Genetic analysis of complex traits in the emerging Collaborative Cross. *Genome Res.* 21: 1213–1222.

Ayroles, J. F., M. A. Carbone, E. A. Stone, K. W. Jordan, R. F. Lyman *et al.*, 2009 Systems genetics of complex traits in *Drosophila melanogaster*. *Nat. Genet.* 41: 299–307.

Bailey, D. W., 1971 Recombinant-inbred strains: an aid to finding identity, linkage, and function of histocompatibility and other genes. *Transplantation* 11: 325–327.

Bennett, B. J., C. R. Farber, L. Orozco, H. M. Kang, A. Ghazalpour *et al.*, 2010 A high-resolution association mapping panel for the dissection of complex traits in mice. *Genome Res.* 20: 281–290.

Broman, K. W., 2005 The genomes of recombinant inbred lines. *Genetics* 169: 1133–1146.

Broman, K. W., 2012a Genotype probabilities at intermediate generations in the construction of recombinant inbred lines. *Genetics* 190: 403–412.

Broman, K. W., 2012b Haplotype probabilities in advanced intercross populations. *G3: Genes, Genomes, Genetics* 2: 199–202.

Buckler, E. S., J. B. Holland, P. J. Bradbury, C. B. Acharya, P. J. Brown *et al.*, 2009 The genetic architecture of maize flowering time. *Science* 325: 714–718.

Burgess-Herbert, S. L., S. W. Tsaih, I. M. Stylianou, K. Walsh, A. J. Co *et al.*, 2009 An experimental assessment of in silico haplotype association mapping in laboratory mice. *BMC Genet.* 10: 81.

Chesler, E. J., L. Lu, J. Wang, R. W. Williams, and K. F. Manly, 2004 WebQTL: rapid exploratory analysis of gene expression and genetic networks for brain and behavior. *Nat. Neurosci.* 7: 485–486.

Chesler, E. J., D. R. Miller, L. R. Branstetter, L. D. Galloway, B. L. Jackson *et al.*, 2008 The Collaborative Cross at Oak Ridge National Laboratory: developing a powerful resource for systems genetics. *Mamm. Genome* 19: 382–389.

Churchill, G. A., D. C. Airey, H. Allayee, J. M. Angel, A. D. Attie *et al.*, 2004 The Collaborative Cross, a community resource for the genetic analysis of complex traits. *Nat. Genet.* 36: 1133–1137.

Crow, J. F., 2007 Haldane, Bailey, Taylor and recombinant-inbred lines. *Genetics* 176: 729–732.

Cubillos, F. A., E. Billi, E. Zörgö, L. Parts, P. Fargier *et al.*, 2011 Assessing the complex architecture of polygenic traits in diverged yeast populations. *Mol. Ecol.* 20: 1401–1413.

Demant, P., and A. A. Hart, 1986 Recombinant congenic strains: a new tool for analyzing genetic traits determined by more than one gene. *Immunogenetics* 24: 416–422.

Durrant, C., H. Tayem, B. Yalcin, J. Cleak, L. Goodstadt *et al.*, 2011 Collaborative Cross mice and their power to map host susceptibility to *Aspergillus fumigatus* infection. *Genome Res.* 21: 1239–1248.

Eversley, C. D., T. Clark, Y. Xie, J. Steigerwalt, T. A. Bell *et al.*, 2010 Genetic mapping and developmental timing of transmission ratio distortion in a mouse interspecific backcross. *BMC Genet.* 11: 98.

Gong, Y., and F. Zou, 2012 Varying coefficient models for mapping quantitative trait loci using recombinant inbred intercrosses. *Genetics* 190: 475–486.

Hudgins, C. C., R. T. Steinberg, D. M. Klinman, M. J. Reeves, and A. D. Steinberg, 1985 Studies of consomic mice bearing the Y chromosome of the BXS mouse. *J. Immunol.* 134: 3849–3854.

Hudson, R., and N. Kaplan, 1985 Statistical properties of the number of recombination events in the history of a sample of DNA sequences. *Genetics* 111: 147–164.

Iraqi, F. A., G. Churchill, and R. Mott, 2008 The Collaborative Cross, developing a resource for mammalian systems genetics: a status report of the Wellcome Trust cohort. *Mamm. Genome* 19: 379–381.

Kang, H. M., N. A. Zaitlen, C. M. Wade, A. Kirby, D. Heckerman *et al.*, 2008 Efficient control of population structure in model organism association mapping. *Genetics* 178: 1709–1723.

Keane, T. M., L. Goodstadt, P. Danecek, M. A. White, K. Wong *et al.*, 2011 Mouse genomic variation and its effect on phenotypes and gene regulation. *Nature* 477: 289–294.

- Kelada, S. N. P., D. L. Aylor, B. C. E. Peck, J. F. Ryan, U. Tavarez *et al.*, 2012 Genetic analysis of hematological parameters in incipient lines of the Collaborative Cross. *G3: Genes, Genomes, Genetics* 2: 157–165.
- Kover, P. X., W. Valdar, J. Trakalo, N. Scarcelli, I. M. Ehrenreich *et al.*, 2009 A multiparent advanced generation inter-cross to fine-map quantitative traits in *Arabidopsis thaliana*. *PLoS Genet.* 5: e1000551.
- Lenarcic, A. B., K. L. Svenson, G. A. Churchill, and W. Valdar, 2012 A general Bayesian approach to analyzing diallel crosses of inbred strains. *Genetics* 190: 413–435.
- Liu, E. Y., Q. Zhan, L. McMillan, F. P. de Villena, and W. Wang, 2010 Efficient genome ancestry inference in complex pedigrees with inbreeding. *Bioinformatics* 26: 199–207.
- Manenti, G., A. Galvan, A. Pettinicchio, G. Trincucci, E. Spada *et al.*, 2009 Mouse genome-wide association mapping needs linkage analysis to avoid false-positive Loci. *PLoS Genet.* 5: e1000331.
- Mathes, W., D. Aylor, D. Miller, G. Churchill, E. Chesler *et al.*, 2010 Architecture of energy balance traits in emerging lines of the Collaborative Cross. *Am. J. Physiol.* 300: E1124–E1134.
- Mihola, O., Z. Trachtulec, C. Vlcek, J. C. Schimenti, and J. Forejt, 2009 A mouse speciation gene encodes a meiotic histone H3 methyltransferase. *Science* 323: 373–375.
- Morahan, G., L. Balmer, and D. Monley, 2008 Establishment of “The Gene Mine”: a resource for rapid identification of complex trait genes. *Mamm. Genome* 19: 390–393.
- Mott, R., C. J. Talbot, M. G. Turri, A. C. Collins, and J. Flint, 2000 A method for fine-mapping quantitative trait loci in outbred animal stocks. *Proc. Natl. Acad. Sci. USA* 97: 12649–12654.
- Nadeau, J. H., J. B. Singer, A. Matin, and E. S. Lander, 2000 Analysing complex genetic traits with chromosome substitution strains. *Nat. Genet.* 24: 221–225.
- Paigen, K., and J. T. Eppig, 2000 A mouse phenome project. *Mamm. Genome* 11: 715–717.
- Pardo-Manuel de Villena, F., and C. Sapienza, 2001 Nonrandom segregation during meiosis: the unfairness of females. *Mamm. Genome* 12: 331–339 Review.
- Payseur, B. A., J. G. Krenz, and M. W. Nachman, 2005 Differential patterns of introgression across the X chromosome in a hybrid zone between two species of house mice. *Evolution* 58: 2064–2078.
- Petkov, P. M., J. H. Graber, G. A. Churchill, K. DiPetrillo, B. L. King *et al.*, 2005 Evidence of a large-scale functional organization of mammalian chromosomes. *PLoS Genet.* 1: e33.
- Philip, V. M., G. Sokoloff, C. L. Ackert-Bicknell, M. Striz, L. Branstetter *et al.*, 2011 Genetic analysis in the Collaborative Cross breeding population. *Genome Res.* 21: 1223–1238.
- Roberts, A., F. Pardo-Manuel de Villena, W. Wang, L. McMillan, and D. Threadgill, 2007 The polymorphism architecture of mouse genetic resources elucidated using genome-wide resequencing data. *Mamm. Genome* 18: 473–481.
- Sun, W., S. Lee, V. Zhabotynsky, F. Zou, F. A. Wright, 2012 Transcriptome atlases of mouse brain reveals differential expression across brain regions and genetic backgrounds. *G3: Genes, Genomes, Genetics* 2: 203–211.
- Svenson, K. L., D. M. Gatti, W. Valdar, C. E. Welsh, R. Cheng *et al.*, 2012 High resolution genetic mapping using the mouse Diversity Outbred population. *Genetics* 190: 437–447.
- Taylor, B. A., H. Meier, and D. D. Myers, 1971 Host-gene control of C-type RNA tumor virus: inheritance of the group-specific antigen of murine leukemia virus. *Proc. Natl. Acad. Sci. USA* 68: 3190–3194.
- Teuscher, F., and K. W. Broman, 2007 Haplotype probabilities for multiple-strain recombinant inbred lines. *Genetics* 175: 1267–1274.
- Threadgill, D. W., 2006 Meeting report for the 4th annual Complex Trait Consortium meeting: from QTLs to systems genetics. *Mamm. Genome* 17: 2–4.
- Threadgill, D. W., K. W. Hunter, and R. W. Williams, 2002 Genetic dissection of complex and quantitative traits: from fantasy to reality via a community effort. *Mamm. Genome* 13: 175–178.
- Threadgill, D. W., D. R. Miller, G. A. Churchill, and F. Pardo-Manuel de Villena, 2011 The Collaborative Cross: recombinant inbred panels in the systems genetics era. *ILAR J.* 52: 24–31.
- Valdar, W., J. Flint, and R. Mott, 2006 Simulating the collaborative cross: power of quantitative trait loci detection and mapping resolution in large sets of recombinant inbred strains of mice. *Genetics* 172: 1783–1797.
- Wang, J., R. W. Williams, and K. F. Manly, 2003 WebQTL: web-based complex trait analysis. *Neuroinformatics* 1: 299–308.
- Wang, J., F. Pardo-Manuel de Villena, K. J. Moore, W. Wang, Q. Zhang *et al.*, 2010 Genome-wide compatible SNP intervals and their properties. *Proceedings of ACM International Conference on Bioinformatics and Computational Biology, Niagara Falls, NY.*
- Wang, J., F. Pardo-Manuel Villena, and L. McMillan, 2011 Dynamic visualization and comparative analysis of multiple collinear genomic data. *Proceedings of ACM International Conference on Bioinformatics and Computational Biology, Chicago, IL.*
- Wang, J. R., F. Pardo-Manuel de Villena, H. A. Lawson, J. M. Cheverud, G. A. Churchill *et al.*, 2012 Imputation of single-nucleotide polymorphisms in inbred mice using local phylogeny. *Genetics* 190: 449–458.
- Welsh, C. E., and L. McMillan, 2012 Accelerating the inbreeding of multi-parental recombinant inbred lines generated by sibling matings. *G3: Genes, Genomes, Genetics* 2: 191–198.
- White, M. A., B. Steffy, T. Wiltshire, and B. A. Payseur, 2011 Genetic dissection of a key reproductive barrier between nascent species of house mice. *Genetics* 189: 289–304.
- Yalcin, B., K. Wong, A. Agam, M. Goodson, T. M. Keane *et al.*, 2011 Sequence-based characterization of structural variation in the mouse genome. *Nature* 477: 326–329.
- Yang, H., T. A. Bell, G. A. Churchill, and F. Pardo-Manuel de Villena, 2007 On the origin of the laboratory mouse. *Nat. Genet.* 39: 1100–1107.
- Yang, H., Y. Ding, L. N. Hutchins, J. Szatkiewicz, T. A. Bell *et al.*, 2009 A customized and versatile high-density genotyping array for the mouse. *Nat. Methods* 6: 663–666.
- Yang, H., J. R. Wang, J. P. Didion, R. J. Buus, T. A. Bell *et al.*, 2011 Genome-wide maps of subspecific origin and identity by descent in the laboratory mouse. *Nat. Genet.* 43: 648–655.
- Zhang, Q., W. Wang, L. McMillan, F. Pardo-Manuel de Villena, and D. Threadgill, 2009 Inferring genome-wide mosaic structure. *Proc. PSB* 14: 150–161.
- Zhang, Z., X. Zhang, and W. Wang, 2012 HTreeQA: using semi-perfect phylogeny trees in quantitative trait loci study on genotype data. *G3: Genes, Genomes, Genetics* 2: 175–189.

*Edited by Lauren M. McIntyre, Dirk-Jan de Koning,
and 4 dedicated Associate Editors*

GENETICS

Supporting Information

<http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.111.132639/-/DC1>

The Genome Architecture of the Collaborative Cross Mouse Genetic Reference Population

Collaborative Cross Consortium

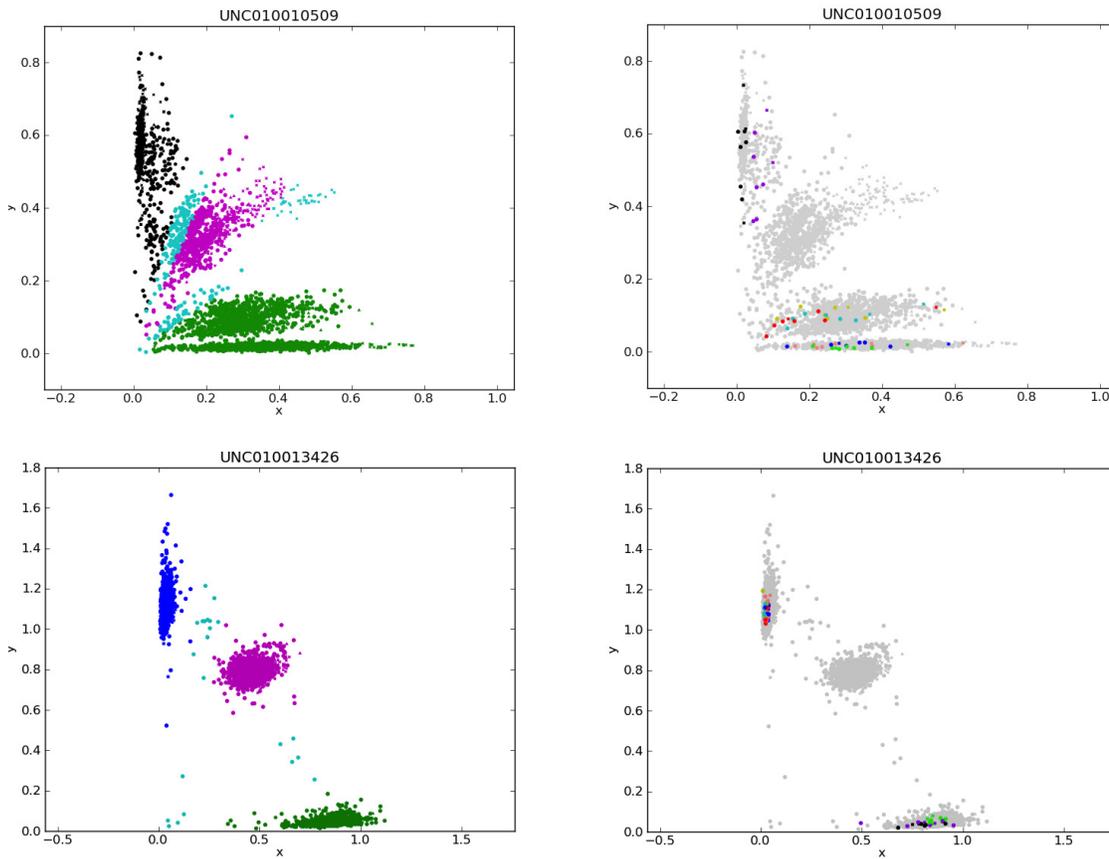


Figure S1 Intensity based haplotype assignment. The figures show the intensity plots for two SNPs (IDs shown above each panel) on Chromosome 1 (8,267,657bp and 8,833,220bp, top and bottom respectively). The top left panel is colored according to Illumina's calls (GG, black; AA, green; H, purple and N, light blue). In the top right panel, the same samples are shown as grey dots with the exception of colored glyphs that represent biological replicates of CC founders. This example exhibits four different alleles distributed as follows in the CC founders G1: C57BL/6J; G2: WSB/EiJ; A1: A/J, NZO/HILtJ and PWK/PhJ, and A2: 129S1/SvImJ, NOD/ShiLtJ and CAST/EiJ. A nearby SNP on is shown on the bottom panels. Calls from Illumina are shown on the left (CC, blue; AA, green; H, purple and N, light blue), and the CC founders are highlighted as colored glyphs on the right. Here, the CC founders exhibit two different alleles as expected from Illumina's calls. The combination of these two SNPs discriminates between six groups of founders B,H,(AE,G),(CD,F) which is two more than possible with bi-allelic genotype calls (the parentheses enclose the two clusters from the first SNP that are broken up by the second).

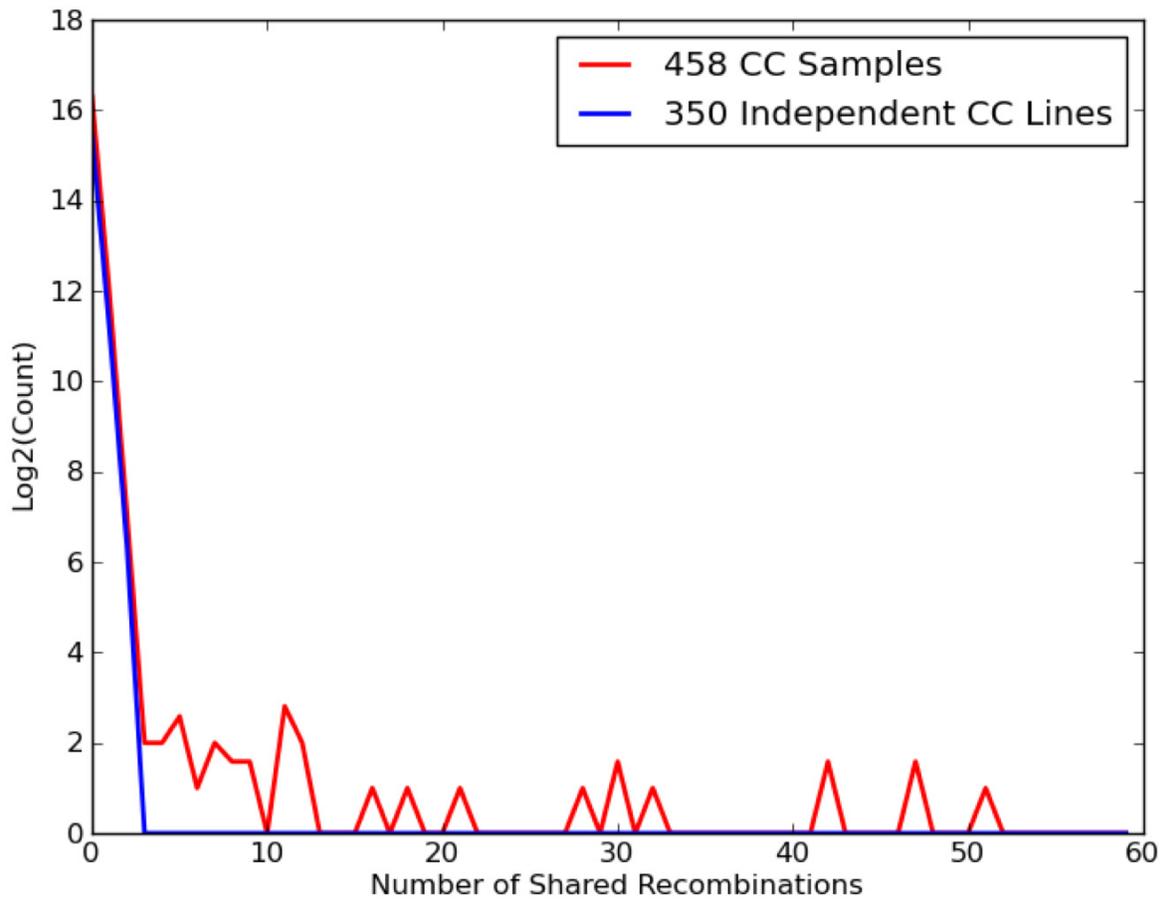


Figure S2 Distribution of shared recombination events before and after identifying related samples.

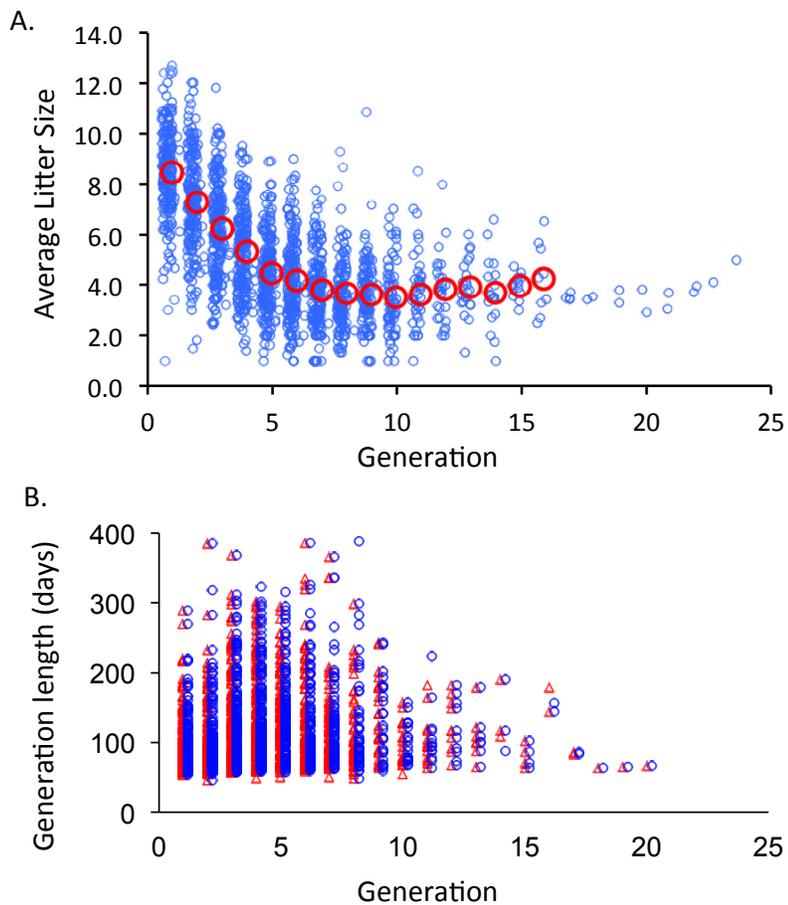


Figure S3 Breeding Performance in the extant CC-UNC population. A) Average litter size per generation. The figure shows average litter size by generation for 191 extant lines. Average litter size by funnel and generation was calculated by query of the CCDB database (Chesler *et al.* 2008). Generation indicates generation of inbreeding (Figure 1). Points are offset along the horizontal axis by a constant amount according to their group and by a smaller random amount to help reveal multiple averages of the same value. Red circles represent the grand average litter size in each generation. B) Generation length in days as a function of the generation number of the productive litter. Female (red triangles): age of female parent at birth of a productive litter; Male (blue circles): age of male parent at birth of a productive litter. Pedigrees include some father-daughter matings.

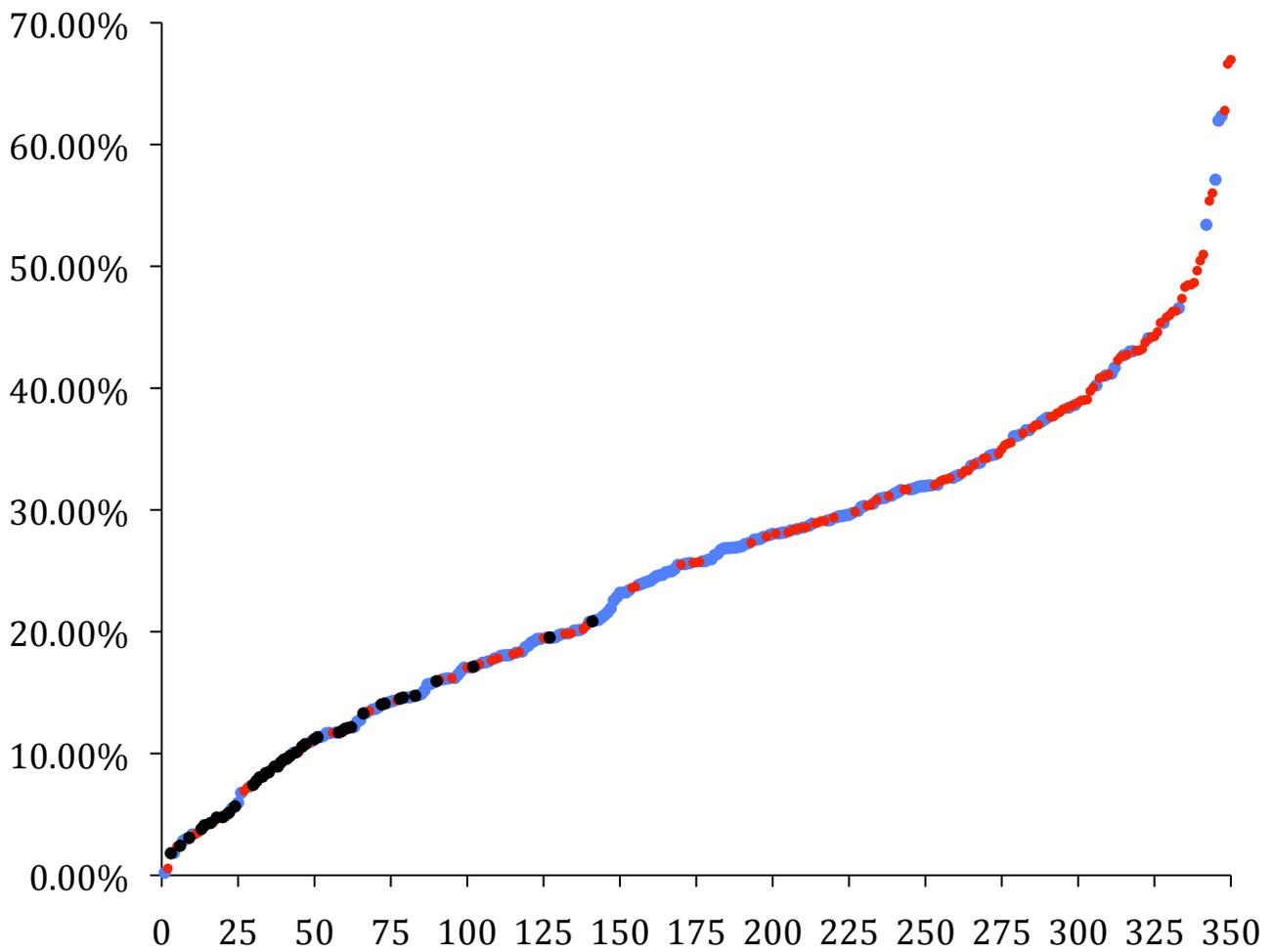


Figure S4 Heterozygosity in the autosomes CC lines. The vertical axis represents the percent heterozygosity in a single male per line. The 350 CC lines are shown ordered by increasing levels of heterozygosity. Red circles represent CC-TAU lines, blue circles represent CC-UNC lines and black circles represent CC-GND lines.

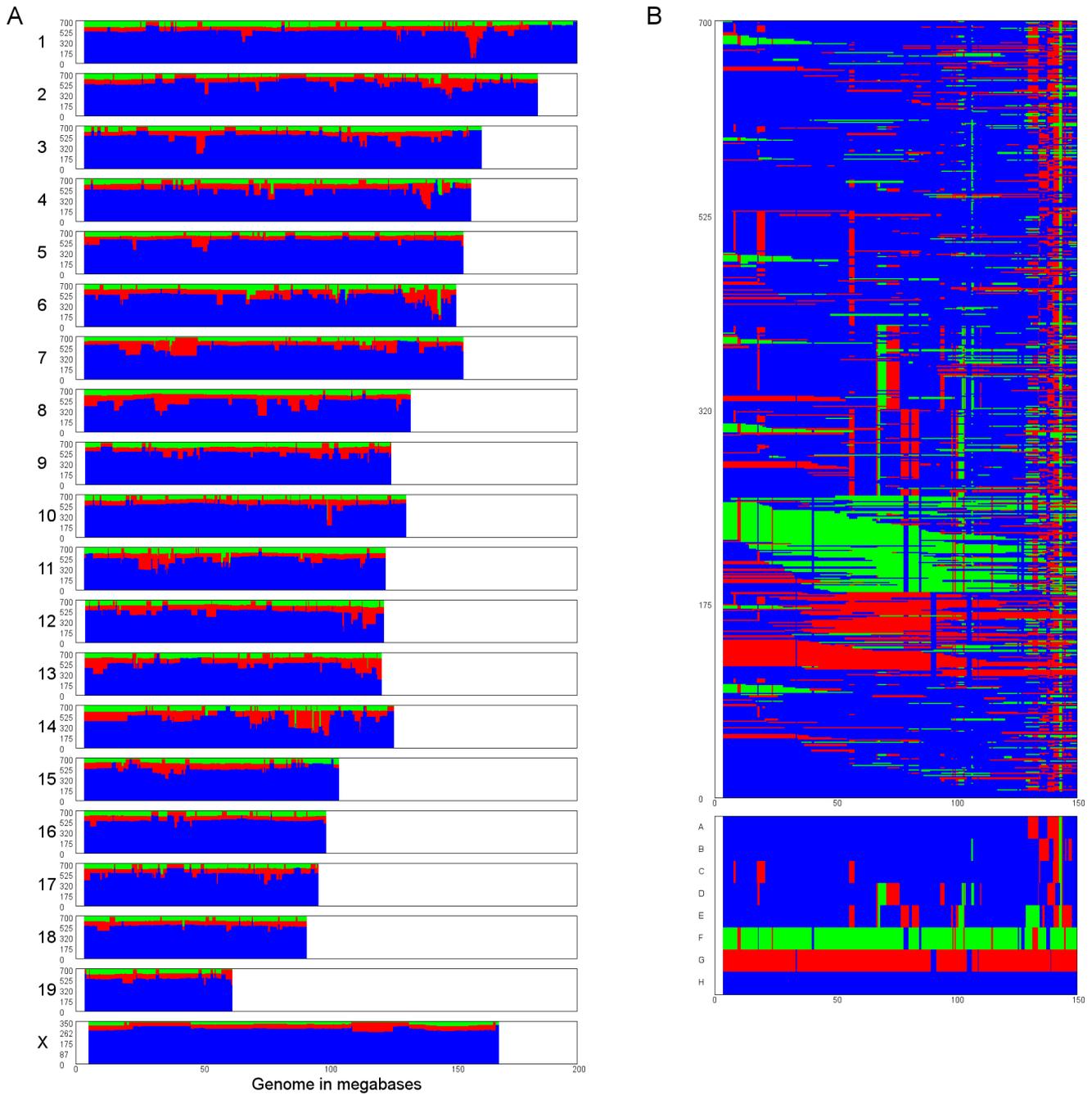


Figure S5 Subspecific origin in the CC population. A) The figure shows the cumulative number of chromosomes (n=700 for the autosomes, n=350 for the X chromosome) that inherit haplotypes from each of the three major *Mus musculus* subspecies in the 350 independent CC lines. Blue represents *M. m. domesticus*, red represents *M. m. musculus* and green represents *M. m. castaneus*. B) The subspecific mosaic of the 350 CC lines and the CC founders on chr 6.

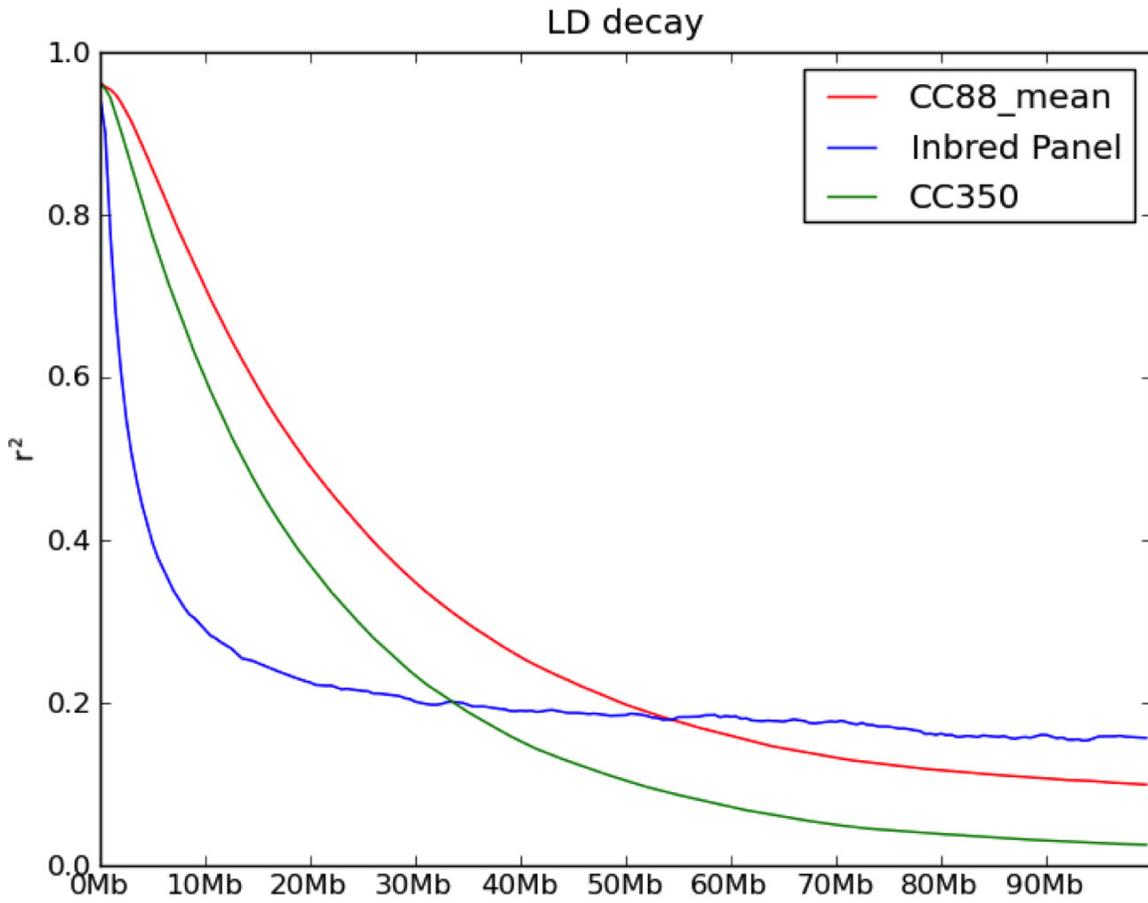


Figure S6 LD decay in the CC. The green line represents the LD decay in the final CC population. The red line represents the mean LD decay in random samples of 88 CC lines and the blue line represents the LD decay in the panel of 88 classical inbred strains. The vertical axis represents r^2 .

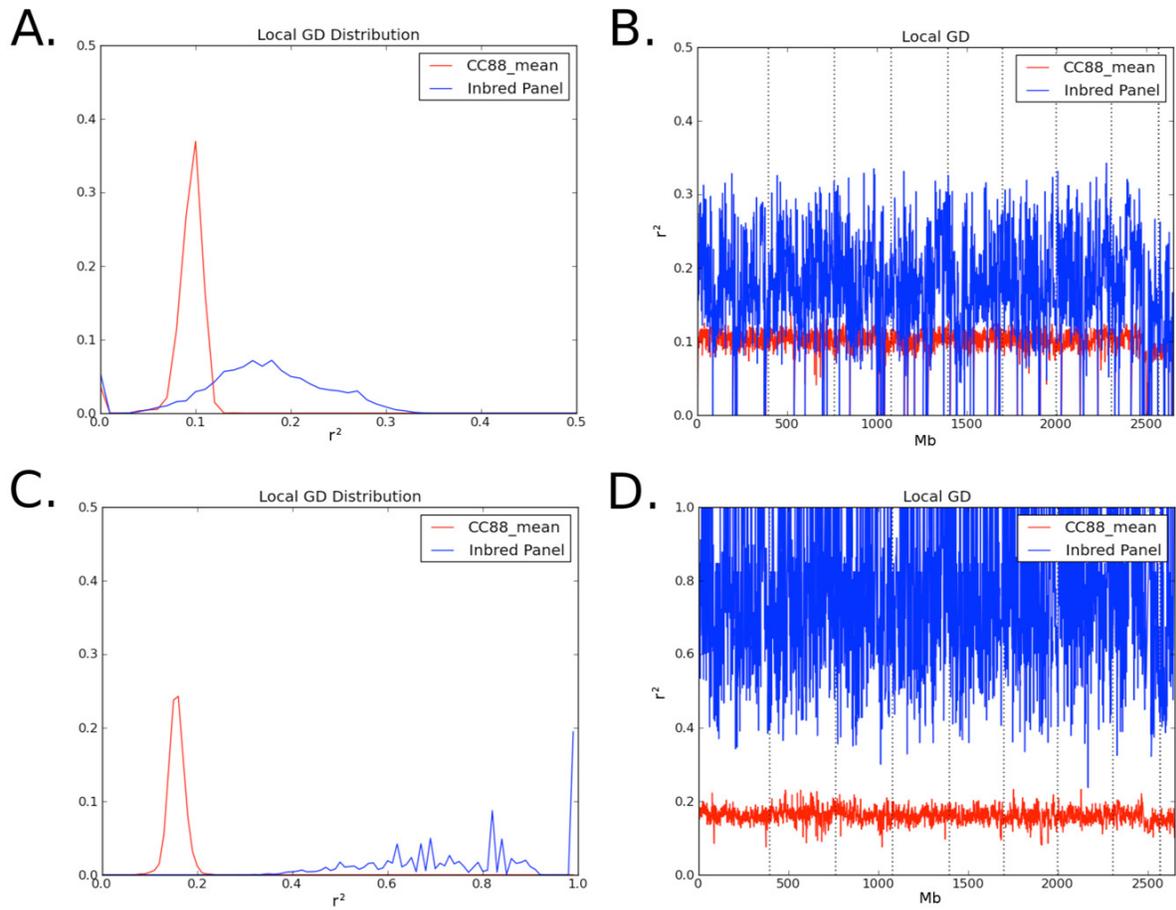


Figure S7 Gametic disequilibrium in the CC and in the panel of 88 inbred strains. a) Distribution of the average GD for each 500 kb interval of the genome. b) Average GD along the genome. c) Distribution of the maximum GD for each 500 kb interval of the genome. d) Maximum GD along the genome.

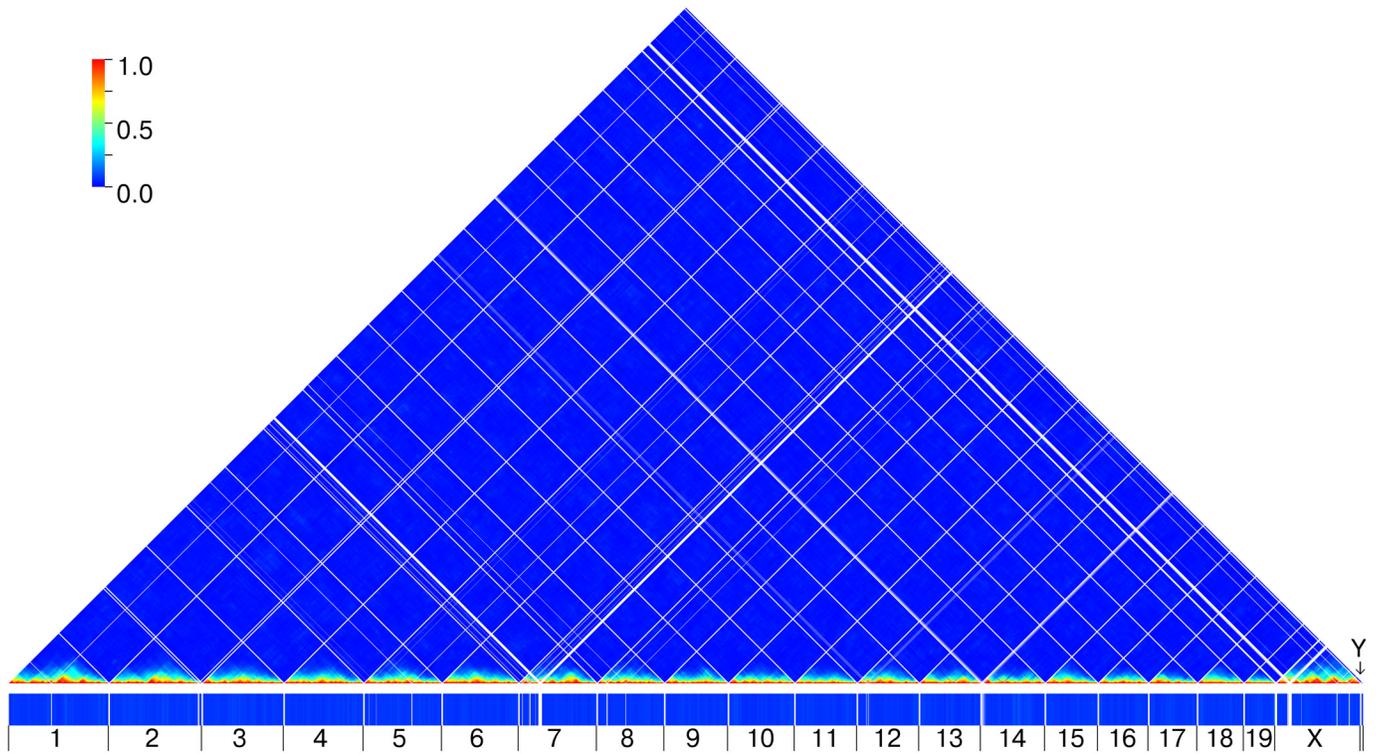


Figure S8 Linkage and gametic disequilibrium in the 350 independent CC lines. Chromosomes are arranged in sequential order in the horizontal axis and the color of each pixel represents the maximum level of LD at that pair. Under each panel the tick box denotes the maximum level of gametic disequilibrium found genome-wide for each 500 kb window.

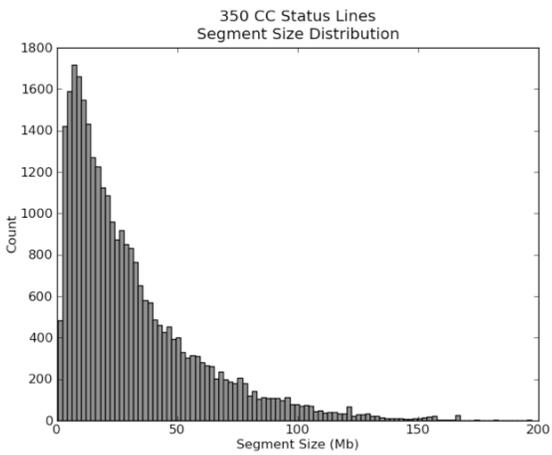
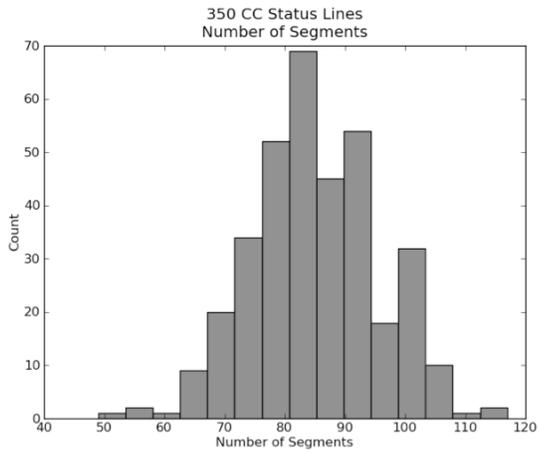


Figure S9 Recombination in the CC lines. The top histogram represents the distribution of segments assigned to each founder strain in each of the 350 CC lines. The bottom figure shows the distribution of segments sizes among the entire CC population.

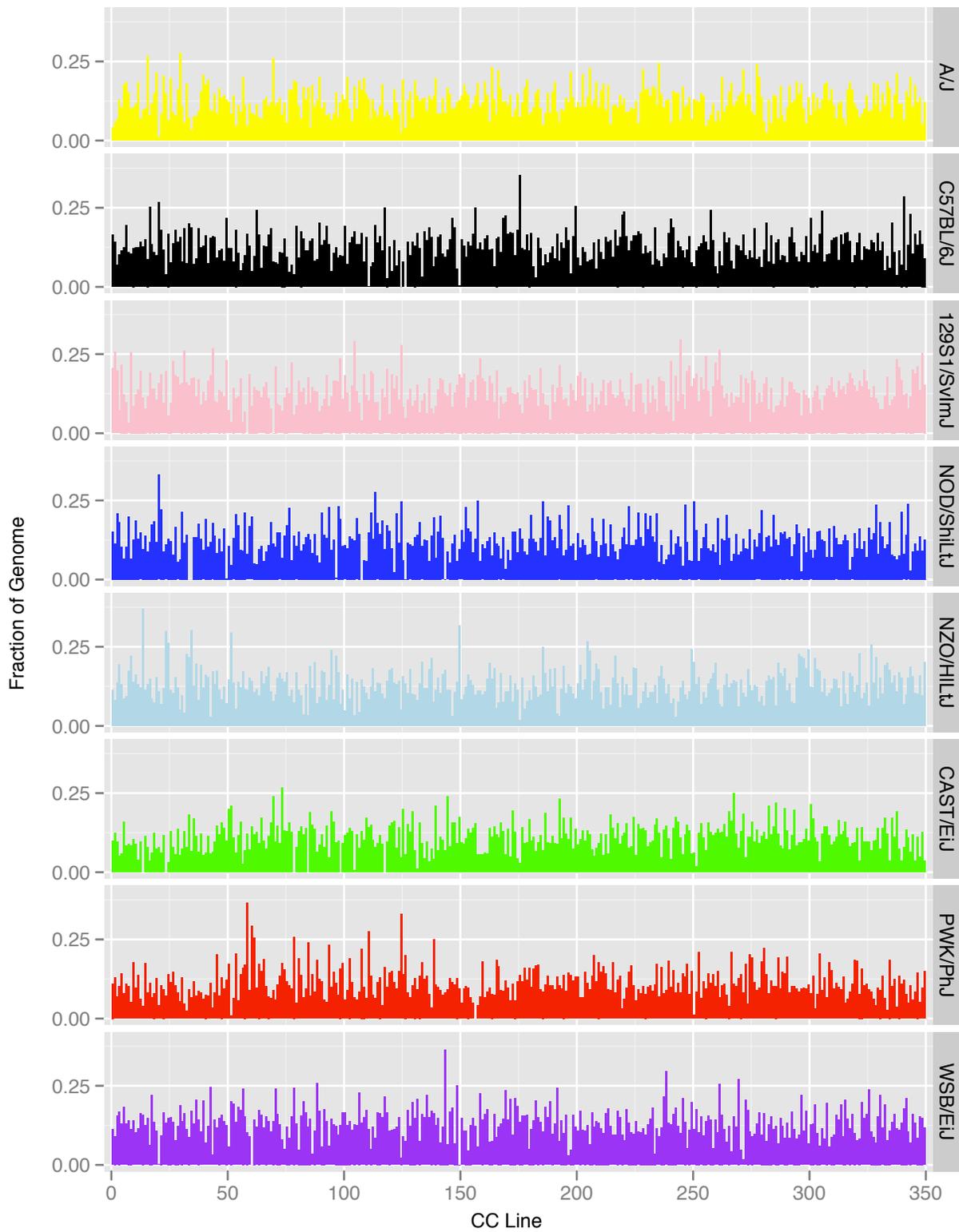


Figure S10 Founder contribution in each of the 350 CC lines. Each vertical bar represents a CC line. Colors follow the same conventions as in Figure 1.

Tables S1 and S2 are available for download at <http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.111.132639/-/DC1> as compressed files.

Table S1 The table provides the MUGA genotypes for 458 CC lines and the eight founder strains, including SNP ID, chromosome and position on mouse build 37. For each sample we provide the Illumina genotype calls and the x and y intensities. Use of these data should cite this publication as a reference

Table S2 The table provides the following information for 458 CC samples used in this study: sample name, population, funnel code, percent contribution from each founder (using the single letter code, see Figure 1), heterozygosity, identity of missing founders, identity of overrepresented founders and the number of recombinations.

Table S3 Number of lines genotyped in each CC population

	TAU	UNC	GND	UNC+TAU+GND
Total # lines genotyped	214	199	45	458
Lines with <7 founders (iCC)	44	8	3	55
Lines with ≥ 7 founders	170	191	42	403
Related Samples	99	0	0	99
# of independent lines in the related samples set	46	0	0	46
# independent lines	117	191	42	350

Table S4 Expected representation of founder strains on Chr X based on the funnel code (Figure 1) in the CC-UNC and CC-GND populations.

Founder	CC-UNC	CC-GND	UNC + GND
A/J	12.40	10.71	12.10
C57BL/6J	12.10	15.87	12.78
129S1/SvImJ	14.00	13.49	13.91
NOD/ShiLtJ	10.70	13.10	11.13
NZO/HILtJ	12.10	15.87	12.78
CAST/EiJ	12.40	9.52	11.88
PWK/PhJ	12.80	11.90	12.64
WSB/EiJ	13.40	9.52	12.70

Table S5 Ancestral haplotype diversity in the CC. The table provides the fraction of the genome with different number of haplotypes and whether these haplotypes are from one, two or three subspecies for the five classical founder strains (5) and all eight CC founders (8).

# of haplotypes	<u># of subspecies</u>					
	1 (5)	1 (8)	2 (5)	2 (8)	3 (5)	3 (8)
1	0.018	0.000	0.000	0.000	0.000	0.000
2	0.083	0.000	0.005	0.000	0.000	0.000
3	0.378	0.000	0.055	0.000	0.000	0.000
4	0.284	0.000	0.083	0.009	0.000	0.012
5	0.044	0.001	0.047	0.033	0.002	0.069
6	0.000	0.001	0.000	0.067	0.000	0.377
7	0.000	0.001	0.000	0.051	0.000	0.296
8	0.000	0.000	0.000	0.014	0.000	0.068

Table S6 CC-UNC External Advisory Board.

Miriam H Meisler, University of Michigan, Ann Arbor, MI 48109, USA

William J Pavan, National Institutes of Health, Bethesda, MD 20892, USA

Roger H Reeves, Johns Hopkins School of Medicine, Baltimore, MD 21205, USA

John C Schimenti, Cornell University, Ithaca, NY 14853, USA

Linda D Siracusa, Thomas Jefferson University, Philadelphia, PA 19107, USA
