**Genetic Reproductive Incompatibility in the Mouse Collaborative Cross**

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Widespread Extinction of Collaborative Cross lines

The Collaborative Cross is a panel of mouse recombinant inbred lines derived from eight diverse founder strains. The CC panel was conceived to be genetically diverse, absent of population structure, and infinitely reproducible. Eight inbred strains were chosen to represent important classical inbred strains (BALB/c, DBA/2J, 129/SvJ, NOD/LtJ, NZB/ SGd) and well-derived strains from each major species subspecies (CAST/EiJ, PWK/PhJ, WSB/EiJ). A phylogenetic tree of the founder strains (below) shows that the wild-derived strains contribute most of the genetic diversity (60%) and that there is a high degree of convergence between major branches. The CC 'funnel' breeding scheme (right) crosses those strains such that the fourth generation (G3F1) of any particular funnel will further alleles from all eight founders, and subsequent generations will be bred with each other until they are completely interbred. In this way, each line is designed to be a randomized mosaic of the eight founder strains. Breeding of CC lines began at three international sites in 2005.

**Reproductive Phenotyping Reveals Multiple Causes**

We took male mice from 340 independent lines in order to study the genetic causes of the observed reproductive incompatibilities. First, we identified males from lines with a high rate of reproductive outcrosses. These male mice were tested with unrelated females to determine male infertility or offspring-specific incompatibility. About half of the males tested were infertile. The other lines went extinct due to female infertility or other causes. This confirmed multiple causes of extinction in the CC. We collected phenotypes including reproductive organs weight, sperm counts, sperm motility, abnormal sperm morphology, and testis histological parameters. Mice infertile mice had sperm present in the epididymis. These sperm may have been unable to fertilize eggs or may have produced inviable embryos. Other mice had no sperm at all, confirming multiple mechanisms of male infertility.

**Support for a Recessive Dobzhansky-Muller Model**

The Dobzhansky-Muller (DM) model predicts genetic incompatibility between hybrids and new alleles that arise in allopatric populations. In the figure below (adapted from Nature Reviews Genetics 5, 118), A and B are new alleles that arise in the context of the ancestral genotype AA/BB. When A and B recombine in a hybrid, epistasis results in sterility or inviable offspring.

During the course of breeding, some lines ceased to produce offspring and were therefore declared extinct. Lines can go extinct for several reasons including infertility, inviability of offspring or husbandry. Very early on we found that two of the 56 possible mating between inbred founder strains were unproductive (129S1/PWk, 129S6/Cast), and one cross produced sterile F1 hybrid males (PWKx129S6). The first-generation pairings combinations were removed from future CC lines. CC lines continued to go extinct as breeding progressed beyond the initial generations. The vast majority of lines in the U.S. population were ultimately unproductive. Out of 798 lines started as part of the U.S. CC population, 564 went extinct. The Histogram below shows the number of inbreeding generations until extinction. The median number of six, which suggests a recessive epistatic genetic architecture.

85% of CC lines are ultimately unproductive.

**Genetic Architecture of Male Infertility in the CC**

We used a haplotype-based mapping method to associate variation in reproductive traits with the eight founder haplotypes segregating in the CC lines. A peak on Chr X exceeds a 10% error threshold in a genome-wide scan (maroon curve below) and represents the marginal effect of an epistatic interaction. Allele effect estimates suggest that the PWK mouse allele is associated with male infertility. This is a gene-rich region of Chr X and proximal to a separate region recently associated with hybrid sterility. The orange curve shows a modifier within the 44 Mb region containing the PWK allele at the Chr X QTL. Several suggestive peaks are present. The peak on Chr 2 displays long-range linkage disequilibrium within the implicated Chr 2 region, a possible signature of epistatic selection.

**Transmission Ratio Distortion on Chr X**

Chr X is also implicated by transmission ratio distortion (TRD) on the chromosomal level. The pie chart on right shows the contribution of each founder strain to the Chr X. PWK is overrepresented in the infertile lines. While some of this TRD is represented by the QTL above, we found that infertile mice without the PWK QTL allele still had an overrepresentation of PWK on Chr X overall. This may suggest multiple Chr X loci with a role in reproductive incompatibility. This TRD is exceptionally pronounced among lines that went extinct in the first five generations of inbreeding (F5), opening the possibility of further subcharacterizing causes of extinction. X-linked incompatibility is consistent with existing literature and evolutionary theory as described by Haldane’s rule.

**Genetic Control of Sperm Production**

Ten percent of mice in our study had no sperm or nearly no sperm in the cauda epididymis at necropsy (50% of infertile mice). We found an increased association with loci on Chr 1 and Chr 8. Both of these QTL co-localized with QTLs for testes weight and size, which is an expected feature of spermatogenic arrest.