

Gene expression profiles in HS-CC founder strains

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Background and Context

Heterogenous Stock-Collaborative Cross (HS-CC)

- Derived from 8 founder strains: 3 wild and 5 laboratory
- Spans 89% of genetic diversity available in *Mus musculus*
- "Source of extreme phenotypic variability and associated candidate loci"
- **Useful for studying complex, polygenetic quantitative traits**

Portland Alcohol Research Center

- Uses HS-CC mice to study risk for high drinking via selection for low vs. high ethanol preference
- CC Founders have wide variation in preference
- **Two high drinking founders: PWK and B6**

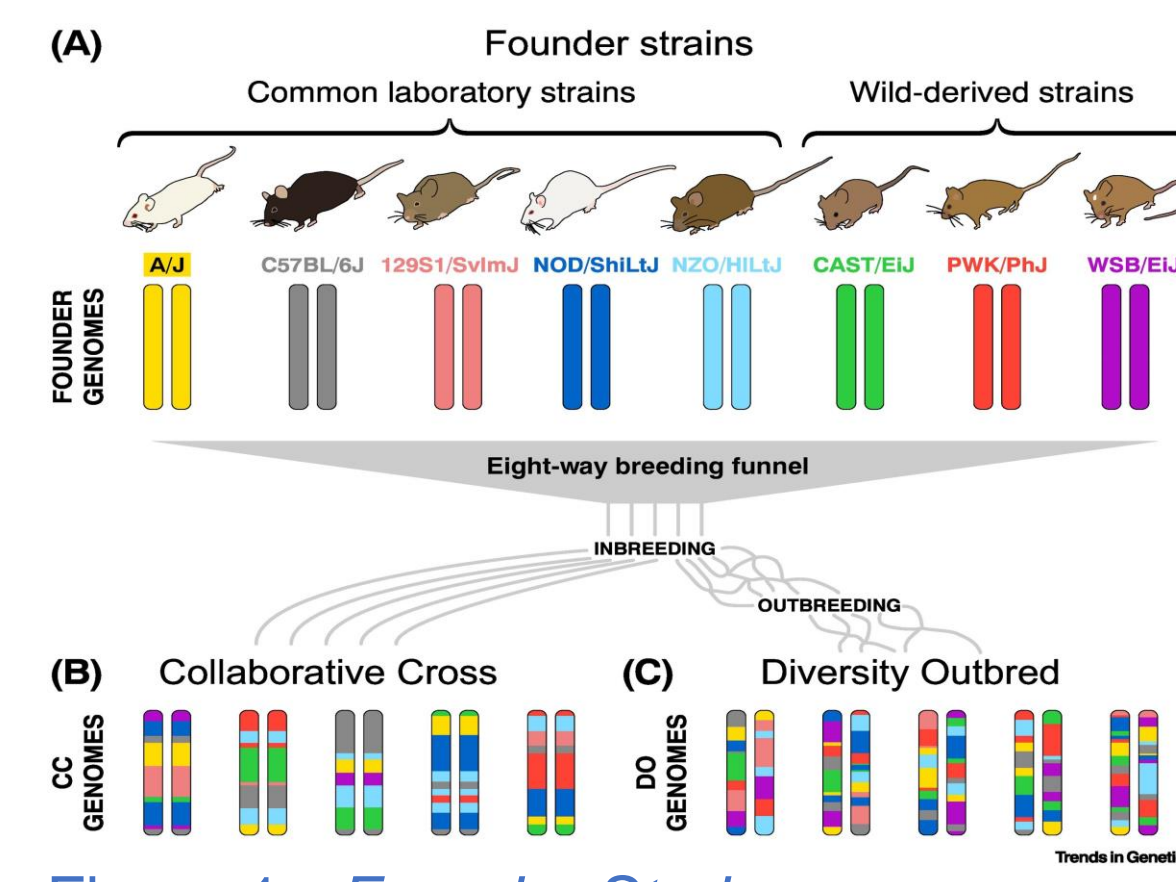


Figure 1 - Founder Strains
Schematic of the 8 founder strains and the breeding schemes used to generate the: (1) collaborative cross and (2) diversity outbred. 5 common laboratory strains and 3 wild derived strains were used.
doi: 10.1016/j.tig.2019.04.003

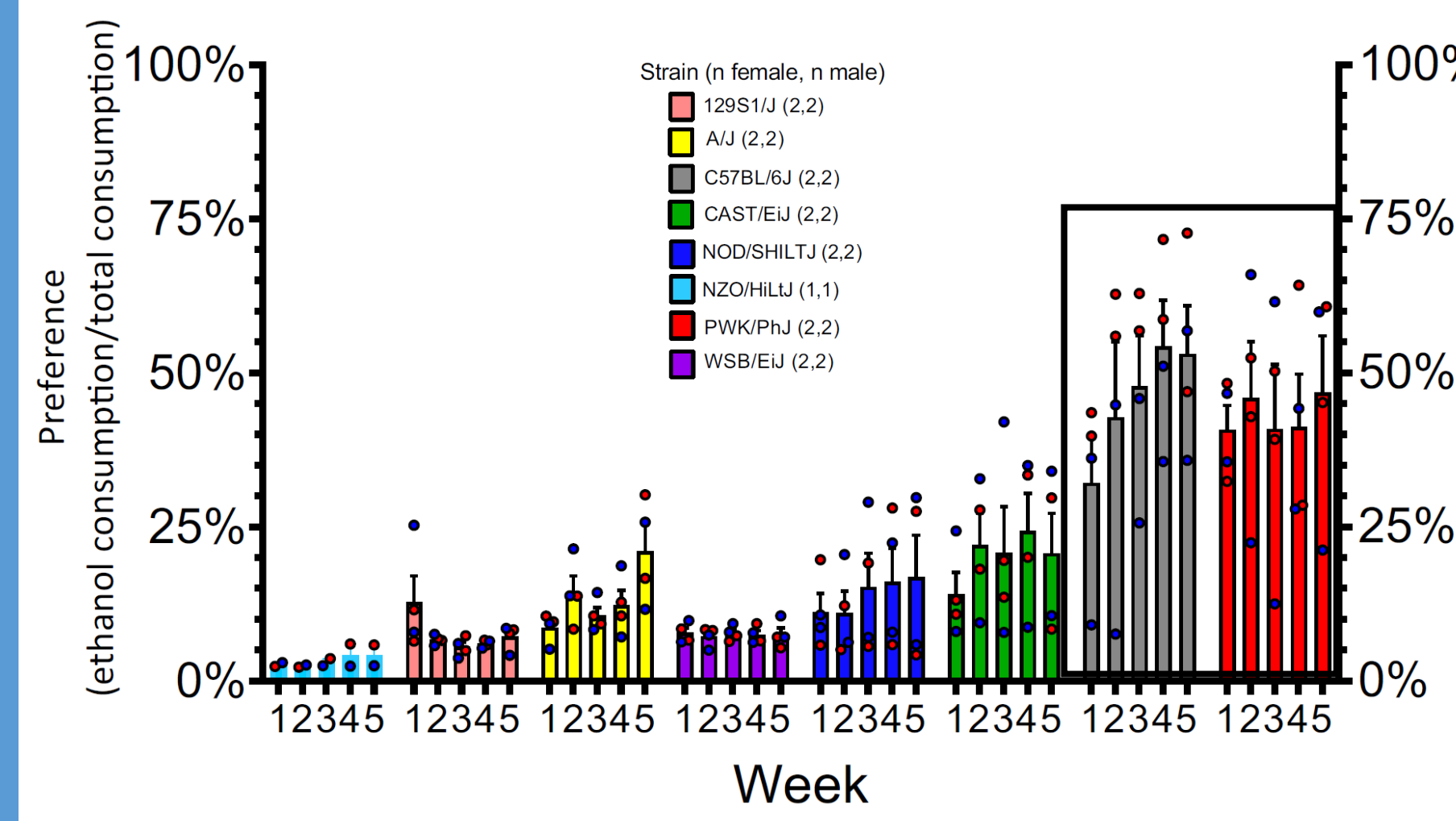


Figure 2 - Founder Strain Ethanol Preference
Preference for consuming alcohol for the 8 HS-CC founding strains. PWK and B6, shown inside the highlight box, are identified as high drinking relative to other founders. Their preference for voluntary ethanol consumption is consistently 200% higher than other strains.
doi: 10.1111/acer.14582

Summary

Explained variance in expression among founder strains:

- 26% by brain region (color), 17% by strain (shape)
- PWK/CAST most unique

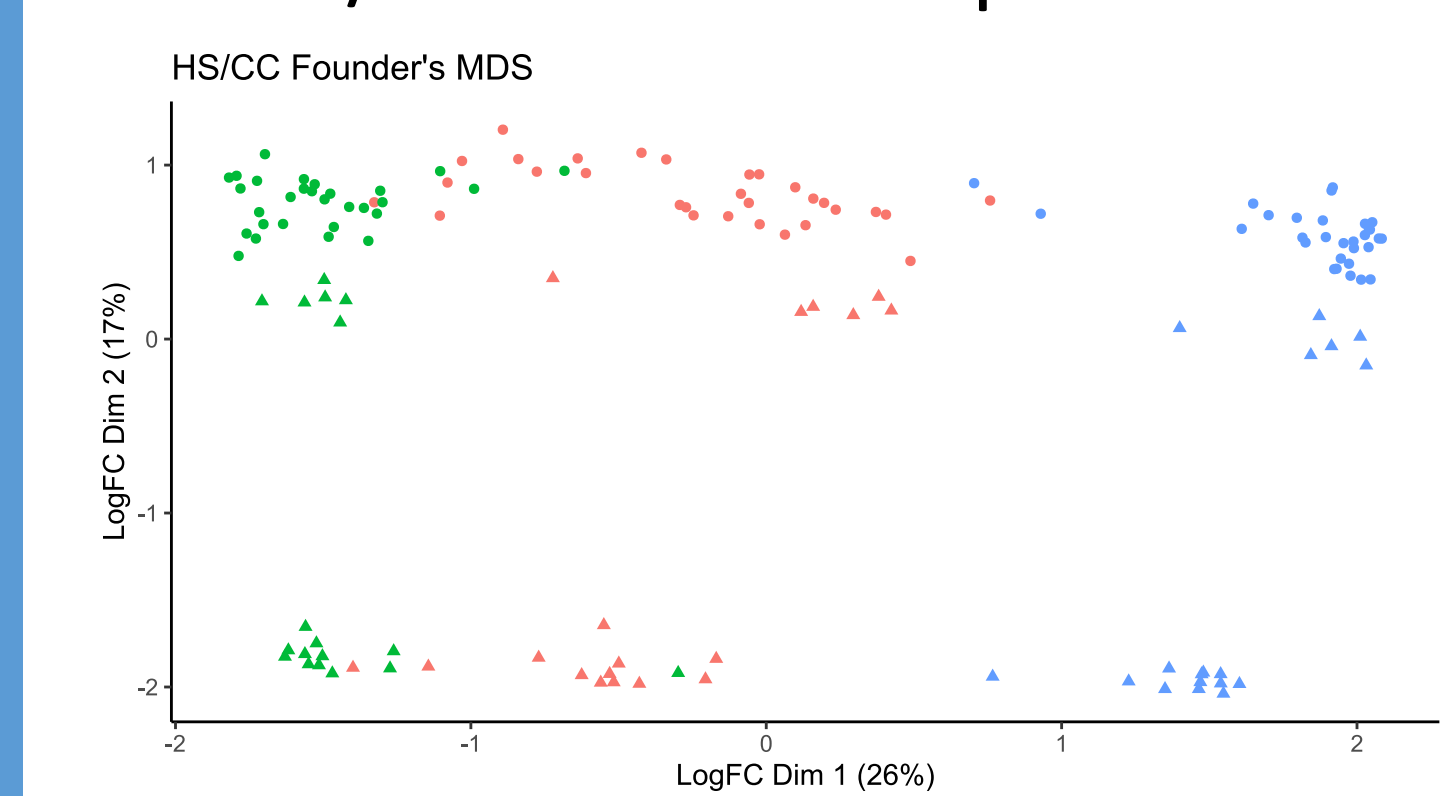


Figure 3 - Founders Expression
Multidimensional scaling of the 8 founder strain's RNA expression. Differences among strains (shape) are lower than differences across region (color). Strains group by subspecies (see figure 4), with wild-derived strains PWK and CAST being the most different than 6 *M. m. domesticus* strains.

Future and ongoing work

- Differential expression, slicing and variance, co-expression networks and eQTL for ethanol preference across replicate selections
- 400 samples, powered to explore sex differences

Pairwise Differential Expression: How different are the founders?

144 samples for RNA-seq

- 3 addiction associated brain regions
- Central nucleus of the amygdala (CeA)
- Nucleus accumbens core (NAcc)
- Prelimbic cortex (PrL)
- 3 mice per sex / region / strain

~15,000 genes investigated

28 possible pairwise comparisons per region

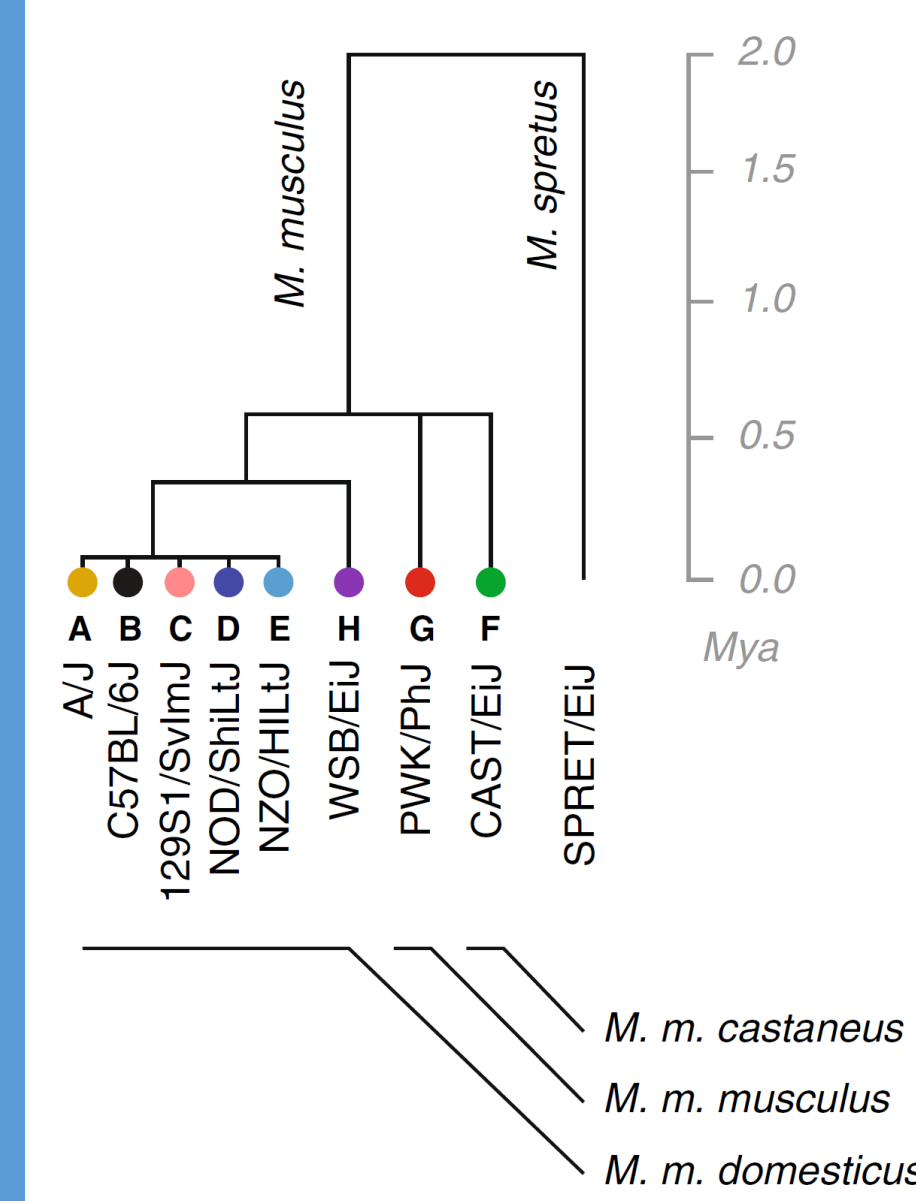


Figure 4 - Founder strain phylogeny
The 8 founder strains represent all three subspecies of *M. musculus*. The 5 common laboratory strains and the wild derived strain WSB are all primarily *M. m. domesticus* in origin and are expected to be the most similar. PWK and CAST, representing the subspecies *musculus* and *castaneus*, are expected to be the most different.
doi: 10.1007/s00335-015-9581-z

Pairwise in NAcc

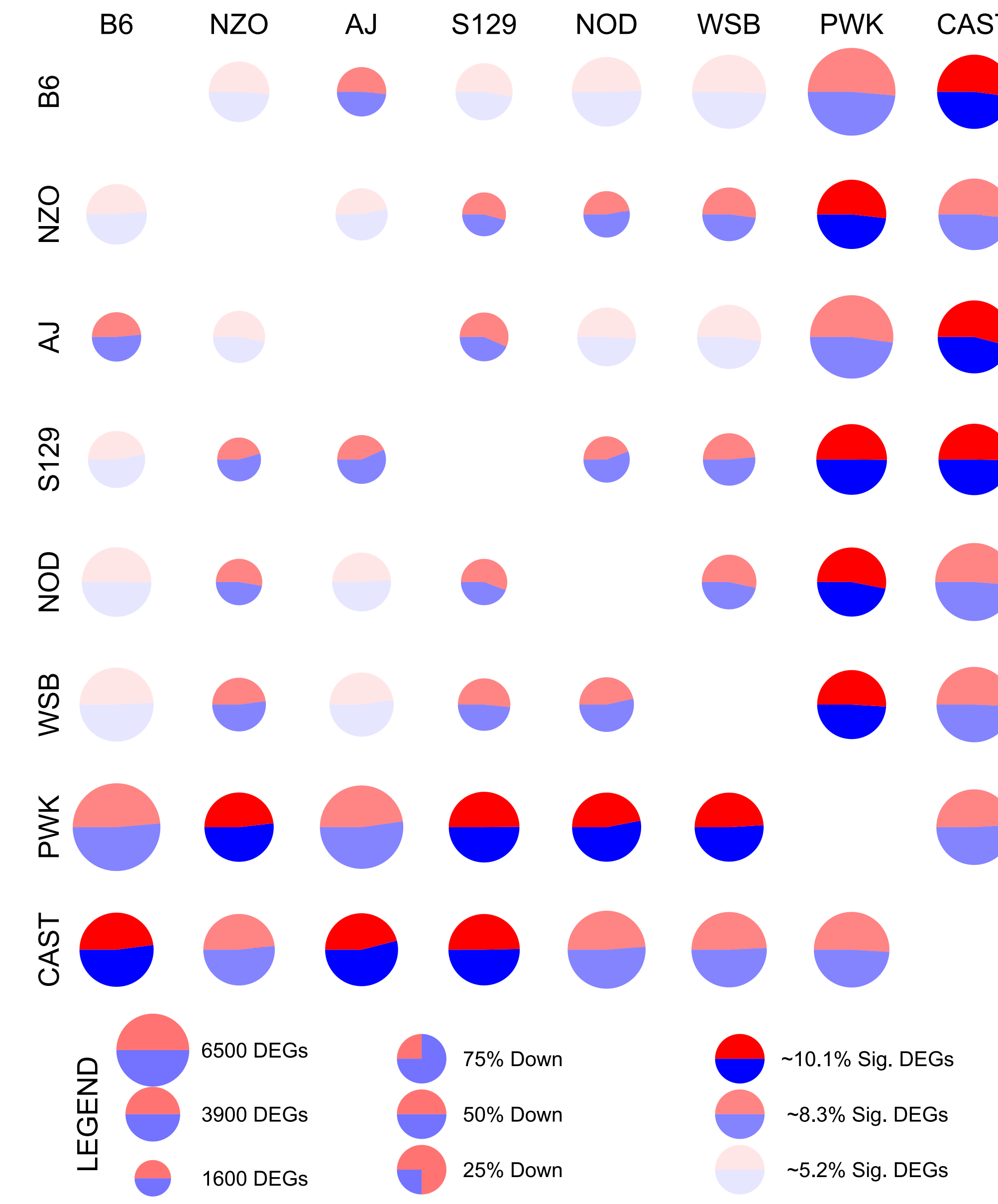


Figure 5 - Pairwise differential expression of the 8 founder strains

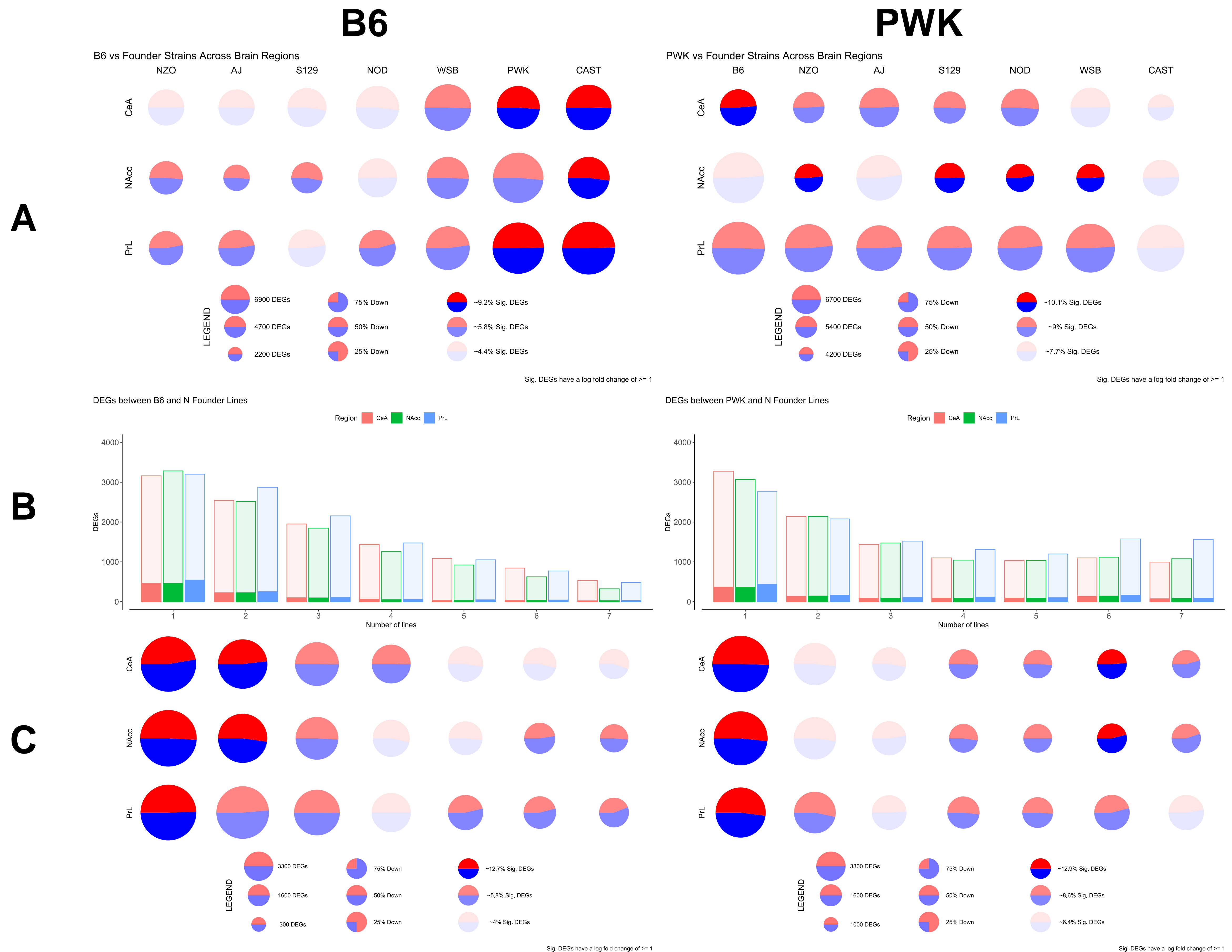
Differentially expressed genes for all pairwise comparisons of the 8 founder strains in the nucleus accumbens core. Comparisons are across rows. For each comparison the chart shows:

1. The number of differentially expressed genes (**Size of the circle**).
2. The number of significantly differentially expressed genes (log fold change greater than 1) (**Color intensity**). The brightest circles are the upper quartile, the dimmest are the lower quartile.
3. The ratio of up (**red**) versus down (**blue**) differentially expressed genes

As expected the wild-derived strains PWK and CAST exhibit the highest number of both differentially expressed genes and significantly differentially expressed genes relative to other strains.

Sig. DEGs have a log fold change of >= 1

High Drinkers Differential Expression: How different are B6 and PWK from the other founders?



Row A - Pairwise differential expression of B6 (left) and PWK (right) versus remaining founder strains in each brain area.

- B6**
- Highest differential expression against wild-derived strains.
 - Significant numbers of highly differentially expressed genes against other laboratory strains in the NAcc and PrL

- PWK:**
- Highest differential expression in the PrL
 - Most significant differential expression in NAcc

Row B - Genes differentially expressed between B6 (left) or PWK (right) and N other founder strains

- B6**
- Numbers of differentially expressed genes drops as expected with higher N
- PWK:**
- Numbers of differentially expressed genes levels off at N=4

Row C - Genes differentially expressed between B6 (left) or PWK (right) and N other founder strains

- B6**
- High number of significantly expressed genes different between B6 and at least 5 other founder strains in the PrL and NAcc
- PWK:**
- Significantly expressed genes between PWK and at least 4 other founder strains across all brain regions.