**ADVANCED ANALYSES**

**RRHO**

Rank Rank Hypergeometric Overlap (RRHO) allows for comparison of gene expression signatures in a p-value independent way, in that it compares sets of genes based on their relative ranks. Once you have log fold changes for expression values (e.g. from differential expression analysis) this analysis is easy to do. There is an R program to do it, but there is also an online interface where all you need is an appropriately formatted text document.

<http://systems.crump.ucla.edu/rankrank/rankranksimple.php>

To save us time I’ve provided you a text document that is ready to go that is a continuation of the guppy dataset we’ve been working with. Remember, yesterday you compared high-predation and low-predation populations. These were from the same drainage, but now what we would like to know is to what extent the expression differences we see in one population pair are mirrored by expression differences in other population pairs. This question is particularly interesting in guppies, because we know that fish from different drainages represent independent evolutionary lineages that have undergone convergent phenotypic evolution.

The file has the following columns (order is important!):

Gene ID

Gene name (same as gene ID in our case)

Rank 1 (rank of LFC in drainage A)

Rank 2 (rank of LFC in drainage B)

Metric 1 (LFC between HP and LP in drainage A)

Metric 2 (LFC between HP and LP in drainage B)

Go to the website and follow the instructions to generate some graphs!

**WGCNA**

Weighted Gene Correlation Network Analysis (WGCNA) constructs gene coexpression modules based on weighted correlations among transcripts. Module construction is unbiased and unsupervised and requires no previous knowledge of the biological relationships among genes. Each modules is assigned a color to differentiate it graphically and verbally and module expression can be mathematically summarized using the first principal component of the module (the module ‘eigengene’) to then relate module expression to trait differences.

Open the “WGCNAcode” R script in the folder of files I gave you. This should automatically open in R and then we can go from there.