Mantel and Partial Mantel Tests in Vegan

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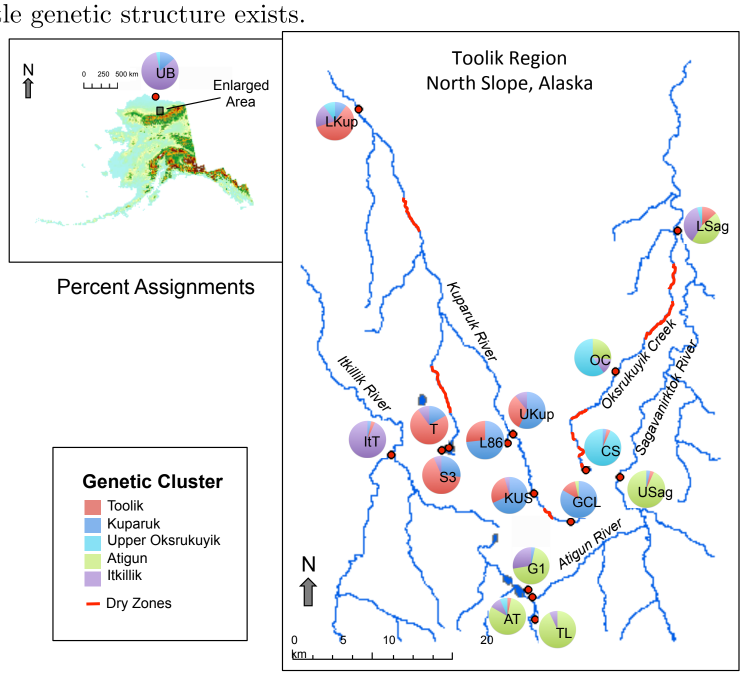
Contents

1 Project Description

For chapter 1 of my dissertation I investigated population structure of a freshwater migratory fish, the Arctic grayling, on the North Slope of Alaska (see figure). Fish fin tissue samples were collected from approximately 30 individuals at each of 16 geographic locations across the aquatic landscape. Using both the Bayesian clustering program, Structure, and discriminant analysis of principal components, DAPC, my data show five genetically distinct clusters. To some extent, genetic differentiation, Fst, appears to be associated with river distance among locations - isolation by distance - but some closely situated locations, such as CS and LSag, show high differentiation at short distances and high assignment probabilities to certain sites, as well. It appears that where river drying is intense, red lines on figure, genetic differentiation tends to be high, even across relatively short river distances. Additionally, where there are no dry zones, i.e. between IT near the headwaters and UB on the coastal plain, little genetic structure exists.



2 Trouble with BIMr

The Bayesian program (BIMr) often used for analysing genetic structure with regard to migration rate and environmental factors, such as distance and dry zones, failed due to assymetry in migration rates among my populations. For example, gene flow was much greater from the Kuparuk River population to Toolik Lake than from Toolik Lake to the Kuparuk River. Thus, model runs would often not converge even after a 50 million iteration burn-in and runs that did show convergence did not agree with one another. The field of Landscape Genetics is currently struggling to address statistical issues with regard to assessing environmental factors associated with population structure.

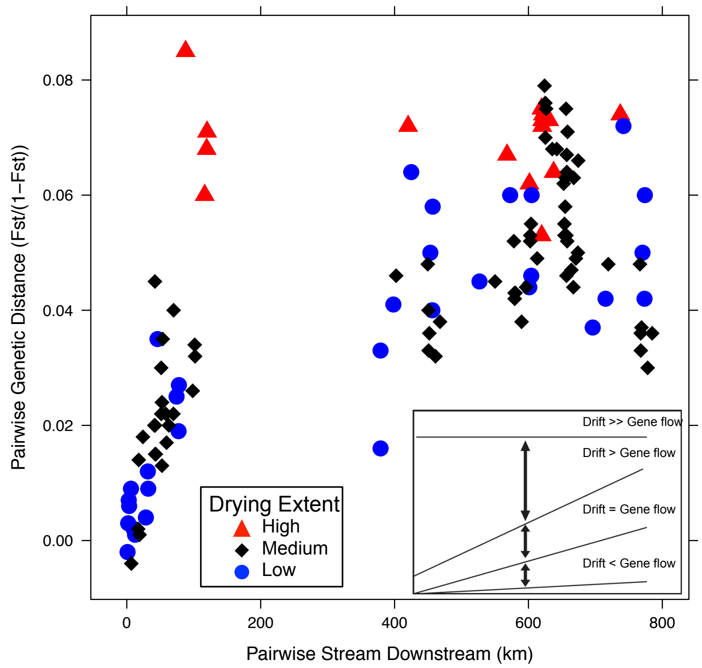
Since proposed in 1967, Mantel and partial Mantel tests have been the most commonly used method to evaluate the relationship between genetic divergence with geographic distance and other environmental factors. Mantel tests, however, have recently come under criticism for having high type 1 error rates, i.e. false positives, and having low power to detect differences when they exist.

2.1 Partial Mantel tests and correlation among factors

Type 1 errors occur most often with partial Mantel tests when factor matrices show correlation with each other. For example, if the true underlying causal factor were elevation and elevation was correlated with temperature, partial Mantel test might incorrectly identify temperature as a significant factor associated with genetic structure. Thus, partial Mantel tests are best used for testing specific hypotheses, using non-correlated environmental factors and not for model selection. See Diniz-Filho et al. 2013. Mantel test in population genetics. Genetics and Molecular Biology. 36 4: 475-485.

3 Hypotheses

For this chapter of my dissertation, I hypothesize that Arctic grayling genetic differentiation (Fst) is influenced by river distance among locations, isolation by distance. Other factors, however, likely contribute to higher than expected differentiation at relatively short river distances, such as number of dry zones or the extent of river drying, which might restrict gene flow among sites. Similarly, the presence of estuarine water between sample locations might influence genetic structure as fish might be less likely to enter saline environments. And, elevation differences among locations might add resistance to the aquatic landscape, such that upstream movement might be restricted compared to downstream movement. Thus, I want to test the following factors as contributors to Arctic grayling genetic differentiation: stream distance (km), number of dry zones, dry extent (km), watershed boundaries, elevation (m), estuaries, and estuary extent (km).



3.1 Drift versus gene flow

In the figure above, sites separated by low to medium river drying (0 to 15 km) exhibit a pattern associated with isolation by distance, but sites with high river drying (>15 km) appear to be dominated by drift. The inset graph was taken from Koizumi et al.(2006).

4 Mantel Test for Isolation by Distance

The Mantel test tests for isolation by distance. Here the standardized version of Mantel's test gives the Pearson correlation r between the standardized elements of the matrices. Relationships range from -1 to 1, where zero is no relationship, one is a positive relationship and -1 is a negative relationship.

4.1 Load pairwise Fst matrix

My Fst matrix was created using the Genodive program. One issue I have with using this matrix is that it does not capitalize upon the individual level genetic data, such that 437 individuals genotyped at 10 microsatellite loci are reduced to a 16 x 16 matrix of Fst values.

```
> setwd("/Users/heidigolden/Dropbox/02_UConn/Data/Genetics/Adult_DNA/MantelTests/")
> library(vegan)
> fst <- as.matrix(read.csv("PairwiseFst_GenoDiveMatrix_11_11_2015.csv"))
> fst <- fst[ , -1]
> fst
      AT      G1      TL      US      LS      OC      CS      UB      IT
 [1,] "0.000" "0.007" "0.006" "0.004" "0.024" "0.025" "0.057" "0.048" "0.048"
 [2,] "0.007" "0.000" "0.009" "0.012" "0.019" "0.033" "0.064" "0.038" "0.040"
 [3,] "0.006" "0.009" "0.000" "0.009" "0.026" "0.031" "0.066" "0.055" "0.057"
 [4,] "0.004" "0.012" "0.009" "0.000" "0.034" "0.038" "0.078" "0.060" "0.067"
 [5,] "0.024" "0.019" "0.026" "0.034" "0.000" "0.018" "0.043" "0.032" "0.036"
 [6,] "0.025" "0.033" "0.031" "0.038" "0.018" "0.000" "0.014" "0.044" "0.046"
 [7,] "0.057" "0.064" "0.066" "0.078" "0.043" "0.014" "0.000" "0.067" "0.069"
 [8,] "0.048" "0.038" "0.055" "0.060" "0.032" "0.044" "0.067" "0.000" "0.016"
 [9,] "0.048" "0.040" "0.057" "0.067" "0.036" "0.046" "0.069" "0.016" "0.000"
[10,] "0.047" "0.048" "0.062" "0.064" "0.042" "0.050" "0.060" "0.037" "0.035"
[11,] "0.045" "0.042" "0.059" "0.064" "0.037" "0.047" "0.068" "0.031" "0.029"
[12,] "0.050" "0.044" "0.060" "0.065" "0.040" "0.050" "0.067" "0.032" "0.032"
[13,] "0.055" "0.049" "0.066" "0.070" "0.041" "0.052" "0.069" "0.035" "0.036"
[14,] "0.042" "0.044" "0.057" "0.057" "0.043" "0.043" "0.063" "0.039" "0.040"
[15,] "0.052" "0.050" "0.063" "0.071" "0.041" "0.049" "0.068" "0.038" "0.035"
[16,] "0.058" "0.059" "0.070" "0.073" "0.049" "0.058" "0.070" "0.046" "0.046"
      GCL      KUS      K       L86     LK      S3       T
 [1,] " 0.047" " 0.045" "0.050" "0.055" "0.042" " 0.052" " 0.058"
 [2,] " 0.048" " 0.042" "0.044" "0.049" "0.044" " 0.050" " 0.059"
 [3,] " 0.062" " 0.059" "0.060" "0.066" "0.057" " 0.063" " 0.070"
 [4,] " 0.064" " 0.064" "0.065" "0.070" "0.057" " 0.071" " 0.073"
 [5,] " 0.042" " 0.037" "0.040" "0.041" "0.043" " 0.041" " 0.049"
 [6,] " 0.050" " 0.047" "0.050" "0.052" "0.043" " 0.049" " 0.058"
 [7,] " 0.060" " 0.068" "0.067" "0.069" "0.063" " 0.068" " 0.070"
 [8,] " 0.037" " 0.031" "0.032" "0.035" "0.039" " 0.038" " 0.046"
 [9,] " 0.035" " 0.029" "0.032" "0.036" "0.040" " 0.035" " 0.046"
[10,] " 0.000" "-0.004" "0.002" "0.001" "0.022" " 0.017" " 0.022"
[11,] "-0.004" " 0.000" "0.002" "0.001" "0.020" " 0.013" " 0.022"
[12,] " 0.002" " 0.002" "0.000" "0.003" "0.023" " 0.015" " 0.020"
[13,] " 0.001" " 0.001" "0.003" "0.000" "0.034" " 0.015" " 0.020"
[14,] " 0.022" " 0.020" "0.023" "0.034" "0.000" " 0.023" " 0.029"
[15,] " 0.017" " 0.013" "0.015" "0.015" "0.023" " 0.000" "-0.002"
[16,] " 0.022" " 0.022" "0.020" "0.020" "0.029" "-0.002" " 0.000"
```

4.2 Load pairwise stream distance matrix

Pairwise stream distances were obtained using the STARS package in ArcGIS, where I created a stream network and extracted the pairwise distances among each of my sampling locations along that network.

```
> StrmDist1 <- as.matrix(read.csv("Edge_LengPairwiseMatrix_11_19_2015.csv", header = FALSE))
> strm <- StrmDist1[-1 , -1]
> strm
```

```
      V2    V3    V4    V5    V6    V7    V8    V9    V10   V11   V12   V13
 [1,] "0"   "3"   "4"   "29"  "74"  "98"  "116" "453" "770" "671" "664" "654"
 [2,] "3"   "0"   "6"   "31"  "77"  "101" "119" "456" "773" "674" "667" "657"
 [3,] "4"   "6"   "0"   "32"  "78"  "102" "120" "457" "774" "674" "668" "657"
 [4,] "29"  "31"  "32"  "0"   "46"  "70"  "88"  "425" "742" "642" "636" "625"
 [5,] "74"  "77"  "78"  "46"  "0"   "24"  "42"  "379" "696" "597" "590" "579"
 [6,] "98"  "101" "102" "70"  "24"  "0"   "18"  "402" "719" "620" "613" "603"
 [7,] "116" "119" "120" "88"  "42"  "18"  "0"   "420" "737" "638" "631" "621"
 [8,] "453" "456" "457" "425" "379" "402" "420" "0"   "379" "468" "461" "451"
 [9,] "770" "773" "774" "742" "696" "719" "737" "379" "0"   "785" "778" "768"
[10,] "671" "674" "674" "642" "597" "620" "638" "468" "785" "0"   "7"   "17"
[11,] "664" "667" "668" "636" "590" "613" "631" "461" "778" "7"   "0"   "10"
[12,] "654" "657" "657" "625" "579" "603" "621" "451" "768" "17"  "10"  "0"
[13,] "655" "658" "658" "626" "581" "604" "622" "452" "769" "19"  "12"  "2"
[14,] "601" "604" "605" "573" "527" "550" "568" "398" "715" "70"  "63"  "53"
[15,] "654" "657" "657" "625" "579" "603" "621" "451" "768" "59"  "53"  "42"
[16,] "653" "656" "656" "624" "578" "601" "619" "450" "767" "58"  "51"  "41"
      V14   V15   V16   V17
 [1,] "655" "601" "654" "653"
 [2,] "658" "604" "657" "656"
 [3,] "658" "605" "657" "656"
 [4,] "626" "573" "625" "624"
 [5,] "581" "527" "579" "578"
 [6,] "604" "550" "603" "601"
 [7,] "622" "568" "621" "619"
 [8,] "452" "398" "451" "450"
 [9,] "769" "715" "768" "767"
[10,] "19"  "70"  "59"  "58"
[11,] "12"  "63"  "53"  "51"
[12,] "2"   "53"  "42"  "41"
[13,] "0"   "54"  "43"  "42"
[14,] "54"  "0"   "53"  "51"
[15,] "43"  "53"  "0"   "1"
[16,] "42"  "51"  "1"   "0"
```

4.3 Conduct Mantel test for IBD

```
> mantel(fst, strm, permutations = 10000, method="pearson")
```

```
Mantel statistic based on Pearson's product-moment correlation

Call:
```

```
mantel(xdis = fst, ydis = strm, method = "pearson", permutations = 10000)

Mantel statistic r: 0.7038
      Significance: 9.999e-05

Upper quantiles of permutations (null model):
  90%   95% 97.5%   99%
0.132 0.179 0.228 0.292
Permutation: free
Number of permutations: 10000
```

Mantel test indicates significant isolation by distance, with stream distance among locations accounting for 70 percent of the variance in pairwise Fst values.

5 Correlations Among Factors

5.1 Load factor matrices

Matrices for pairwise stream distance (km), number of dry zones, dry extent (km), watershed boundaries, elevation (m), estuaries, and estuary extent (km) are loaded and tested for correlations. Any correlated matrices can then be identified and re-evaluated prior to proceeding with hypothesis testing using partial Mantel tests.

```
> # dry extent (km)
> DryExt <- as.matrix(read.csv("DryLengthKm_PairwiseMatrix_11_19_2015.csv", header = FALSE))
> dext <- DryExt[ -1, -1]
> dext. <- as.numeric(dext)
> # number of dry zones
> DryZones2 <- as.matrix(read.csv("Adult_NumberDryZonesMatirx_11_11_2015.csv"))
> dz <- DryZones2[ , -1]
> dz. <- as.numeric(dz)
> # Estuary Extent Km
> EstExt <- as.matrix(read.csv("EstuaryLengthMatrix_11_19_2015.csv"))
> est.ext <- EstExt[ , -1]
> est.ext. <- as.numeric(est.ext)
> # Estuary (yes or no)
> EstPres <- as.matrix((read.csv("EstuaryMatrixFor_vegan_11_11_2015.csv")))
> est.pres <- EstPres[ , -1]
> est.pres. <- as.numeric(est.pres)
> Elev <- as.matrix(read.csv("/Users/heidigolden/Dropbox/02_UConn/Data/Genetics/Adult_DNA/Ma
> elev <- Elev[ , -1]
> elev. <- abs(as.numeric(elev))
> # Major Watersheds
> wshd <- as.matrix(read.csv("/Users/heidigolden/Dropbox/02_UConn/Data/Genetics/Adult_DNA/Ma
```

5.2 Environmental Factor Matrices

Dry Extent Matrix (km)

	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11
[1,]	"0.0"	"0.0"	"0.0"	"0.0"	"0.0"	"19.2"	"20.6"	"0.0"	"0.0"	"3.3"
[2,]	"0.0"	"0.0"	"0.0"	"0.0"	"0.0"	"19.2"	"20.6"	"0.0"	"0.0"	"3.3"
[3,]	"0.0"	"0.0"	"0.0"	"0.0"	"0.0"	"19.2"	"20.6"	"0.0"	"0.0"	"3.3"
[4,]	"0.0"	"0.0"	"0.0"	"0.0"	"0.0"	"19.2"	"20.6"	"0.0"	"0.0"	"3.3"
[5,]	"0.0"	"0.0"	"0.0"	"0.0"	"0.0"	"19.2"	"20.6"	"0.0"	"0.0"	"3.3"
[6,]	"19.2"	"19.2"	"19.2"	"19.2"	"19.2"	"0.0"	"1.4"	"19.2"	"19.2"	"22.4"
[7,]	"20.6"	"20.6"	"20.6"	"20.6"	"20.6"	"1.4"	"0.0"	"20.6"	"20.6"	"23.8"
[8,]	"0.0"	"0.0"	"0.0"	"0.0"	"0.0"	"19.2"	"20.6"	"0.0"	"0.0"	"3.3"
[9,]	"0.0"	"0.0"	"0.0"	"0.0"	"0.0"	"19.2"	"20.6"	"0.0"	"0.0"	"3.3"
[10,]	"3.3"	"3.3"	"3.3"	"3.3"	"3.3"	"22.4"	"23.8"	"3.3"	"3.3"	"0.0"
[11,]	"2.7"	"2.7"	"2.7"	"2.7"	"2.7"	"21.9"	"23.3"	"2.7"	"2.7"	"0.5"
[12,]	"2.7"	"2.7"	"2.7"	"2.7"	"2.7"	"21.9"	"23.3"	"2.7"	"2.7"	"0.5"
[13,]	"2.7"	"2.7"	"2.7"	"2.7"	"2.7"	"21.9"	"23.3"	"2.7"	"2.7"	"0.5"
[14,]	"0.0"	"0.0"	"0.0"	"0.0"	"0.0"	"19.2"	"20.6"	"0.0"	"0.0"	"3.3"
[15,]	"7.2"	"7.2"	"7.2"	"7.2"	"7.2"	"26.4"	"27.8"	"7.2"	"7.2"	"5.0"
[16,]	"7.2"	"7.2"	"7.2"	"7.2"	"7.2"	"26.4"	"27.8"	"7.2"	"7.2"	"5.0"

	V12	V13	V14	V15	V16	V17
[1,]	"2.7"	"2.7"	"2.7"	"0.0"	"7.2"	"7.2"
[2,]	"2.7"	"2.7"	"2.7"	"0.0"	"7.2"	"7.2"
[3,]	"2.7"	"2.7"	"2.7"	"0.0"	"7.2"	"7.2"
[4,]	"2.7"	"2.7"	"2.7"	"0.0"	"7.2"	"7.2"
[5,]	"2.7"	"2.7"	"2.7"	"0.0"	"7.2"	"7.2"
[6,]	"21.9"	"21.9"	"21.9"	"19.2"	"26.4"	"26.4"
[7,]	"23.3"	"23.3"	"23.3"	"20.6"	"27.8"	"27.8"
[8,]	"2.7"	"2.7"	"2.7"	"0.0"	"7.2"	"7.2"
[9,]	"2.7"	"2.7"	"2.7"	"0.0"	"7.2"	"7.2"
[10,]	"0.5"	"0.5"	"0.5"	"3.3"	"5.0"	"5.0"
[11,]	"0.0"	"0.0"	"0.0"	"2.7"	"4.5"	"4.5"
[12,]	"0.0"	"0.0"	"0.0"	"2.7"	"4.5"	"4.5"
[13,]	"0.0"	"0.0"	"0.0"	"2.7"	"4.5"	"4.5"
[14,]	"2.7"	"2.7"	"2.7"	"0.0"	"7.2"	"7.2"
[15,]	"4.5"	"4.5"	"4.5"	"7.2"	"0.0"	"0.0"
[16,]	"4.5"	"4.5"	"4.5"	"7.2"	"0.0"	"0.0"

Number of Dry Zones Matrix

	AT	G1	TL	US	LS	OC	CS	UB	IT	GCL	KUS	K	L86	LK	S3	T
[1,]	"0"	"0"	"0"	"0"	"0"	"1"	"3"	"0"	"0"	"2"	"1"	"1"	"1"	"0"	"2"	"2"
[2,]	"0"	"0"	"0"	"0"	"0"	"1"	"3"	"0"	"0"	"2"	"1"	"1"	"1"	"0"	"2"	"2"
[3,]	"0"	"0"	"0"	"0"	"0"	"2"	"3"	"0"	"0"	"2"	"1"	"1"	"1"	"0"	"2"	"2"
[4,]	"0"	"0"	"0"	"0"	"0"	"1"	"3"	"0"	"0"	"2"	"1"	"1"	"1"	"0"	"2"	"2"
[5,]	"0"	"0"	"0"	"0"	"0"	"1"	"3"	"0"	"0"	"2"	"1"	"1"	"1"	"0"	"2"	"2"

```
 [6,] "1" "1" "1" "1" "1" "0" "2" "1" "1" "3" "2" "2" "2" "1" "3" "3"
 [7,] "3" "3" "3" "3" "3" "2" "0" "3" "3" "5" "4" "4" "4" "3" "5" "5"
 [8,] "0" "0" "0" "0" "0" "1" "3" "0" "0" "2" "1" "1" "1" "0" "2" "2"
 [9,] "0" "0" "0" "0" "0" "1" "3" "0" "0" "2" "1" "1" "1" "0" "2" "2"
[10,] "2" "2" "2" "2" "2" "3" "5" "2" "2" "0" "1" "1" "1" "2" "2" "2"
[11,] "1" "1" "1" "1" "1" "2" "4" "1" "1" "1" "0" "0" "0" "1" "1" "1"
[12,] "1" "1" "1" "1" "1" "2" "4" "1" "1" "1" "0" "0" "0" "1" "1" "1"
[13,] "1" "1" "1" "1" "1" "2" "4" "1" "1" "1" "0" "0" "0" "1" "1" "1"
[14,] "0" "0" "0" "0" "0" "1" "3" "0" "0" "2" "1" "1" "1" "0" "2" "2"
[15,] "2" "2" "2" "2" "2" "3" "5" "2" "2" "2" "1" "1" "1" "2" "0" "0"
[16,] "2" "2" "2" "2" "2" "3" "5" "2" "2" "2" "1" "1" "1" "2" "0" "0"
```

Estuary Extent Matrix (km)

```
      AT    G1    TL    US    LS    OK    CS    UB    IT    GCL   KUS   UK
 [1,] "  0" "  0" "  0" "  0" "  0" "  0" "  0" "184" "183" " 47" " 47" " 47"
 [2,] "  0" "  0" "  0" "  0" "  0" "  0" "  0" "184" "183" " 47" " 47" " 47"
 [3,] "  0" "  0" "  0" "  0" "  0" "  0" "  0" "184" "183" " 47" " 47" " 47"
 [4,] "  0" "  0" "  0" "  0" "  0" "  0" "  0" "184" "183" " 47" " 47" " 47"
 [5,] "  0" "  0" "  0" "  0" "  0" "  0" "  0" "184" "183" " 47" " 47" " 47"
 [6,] "  0" "  0" "  0" "  0" "  0" "  0" "  0" "184" "183" " 47" " 47" " 47"
 [7,] "  0" "  0" "  0" "  0" "  0" "  0" "  0" "184" "183" " 47" " 47" " 47"
 [8,] "184" "184" "184" "184" "184" "184" "184" "  0" " 33" "161" "161" "161"
 [9,] "183" "183" "183" "183" "183" "183" "183" " 33" "  0" "160" "160" "160"
[10,] " 47" " 47" " 47" " 47" " 47" " 47" " 47" "161" "160" "  0" "  0" "  0"
[11,] " 47" " 47" " 47" " 47" " 47" " 47" " 47" "161" "160" "  0" "  0" "  0"
[12,] " 47" " 47" " 47" " 47" " 47" " 47" " 47" "161" "160" "  0" "  0" "  0"
[13,] " 47" " 47" " 47" " 47" " 47" " 47" " 47" "161" "160" "  0" "  0" "  0"
[14,] " 47" " 47" " 47" " 47" " 47" " 47" " 47" "161" "160" "  0" "  0" "  0"
[15,] " 47" " 47" " 47" " 47" " 47" " 47" " 47" "161" "160" "  0" "  0" "  0"
[16,] " 47" " 47" " 47" " 47" " 47" " 47" " 47" "161" "160" "  0" "  0" "  0"
      L86   LK    S3    T
 [1,] " 47" " 47" " 47" " 47"
 [2,] " 47" " 47" " 47" " 47"
 [3,] " 47" " 47" " 47" " 47"
 [4,] " 47" " 47" " 47" " 47"
 [5,] " 47" " 47" " 47" " 47"
 [6,] " 47" " 47" " 47" " 47"
 [7,] " 47" " 47" " 47" " 47"
 [8,] "161" "161" "161" "161"
 [9,] "160" "160" "160" "160"
[10,] "  0" "  0" "  0" "  0"
[11,] "  0" "  0" "  0" "  0"
[12,] "  0" "  0" "  0" "  0"
[13,] "  0" "  0" "  0" "  0"
[14,] "  0" "  0" "  0" "  0"
[15,] "  0" "  0" "  0" "  0"
```

```
[16,] "  0" "  0" "  0" "  0"
```

Estuary Present Matrix (0 or 1)

```
      AT  G1  TL  US  LS  OK  CS  UB  IT  GCL KUS K   L86 LK  S3  T  
 [1,] "0" "0" "0" "0" "0" "0" "0" "1" "1" "1" "1" "1" "1" "1" "1" "1"
 [2,] "0" "0" "0" "0" "0" "0" "0" "1" "1" "1" "1" "1" "1" "1" "1" "1"
 [3,] "0" "0" "0" "0" "0" "0" "0" "1" "1" "1" "1" "1" "1" "1" "1" "1"
 [4,] "0" "0" "0" "0" "0" "0" "0" "1" "1" "1" "1" "1" "1" "1" "1" "1"
 [5,] "0" "0" "0" "0" "0" "0" "0" "1" "1" "1" "1" "1" "1" "1" "1" "1"
 [6,] "0" "0" "0" "0" "0" "0" "0" "1" "1" "1" "1" "1" "1" "1" "1" "1"
 [7,] "0" "0" "0" "0" "0" "0" "0" "1" "1" "1" "1" "1" "1" "1" "1" "1"
 [8,] "1" "1" "1" "1" "1" "1" "1" "0" "0" "1" "1" "1" "1" "1" "1" "1"
 [9,] "1" "1" "1" "1" "1" "1" "1" "0" "0" "1" "1" "1" "1" "1" "1" "1"
[10,] "1" "1" "1" "1" "1" "1" "1" "1" "1" "0" "0" "0" "0" "0" "0" "0"
[11,] "1" "1" "1" "1" "1" "1" "1" "1" "1" "0" "0" "0" "0" "0" "0" "0"
[12,] "1" "1" "1" "1" "1" "1" "1" "1" "1" "0" "0" "0" "0" "0" "0" "0"
[13,] "1" "1" "1" "1" "1" "1" "1" "1" "1" "0" "0" "0" "0" "0" "0" "0"
[14,] "1" "1" "1" "1" "1" "1" "1" "1" "1" "0" "0" "0" "0" "0" "0" "0"
[15,] "1" "1" "1" "1" "1" "1" "1" "1" "1" "0" "0" "0" "0" "0" "0" "0"
[16,] "1" "1" "1" "1" "1" "1" "1" "1" "1" "0" "0" "0" "0" "0" "0" "0"
```

Elevation Matrix (m)

```
        V1   V2   V3   V4   V5   V6   V7   V8   V9  V10  V11  V12  V13 V14  V15
 [1,]    0  -68   -6  -84  177  -21   -3  454  391   65   74   77  -23 794   58
 [2,]   68    0   62  -16  245   47   65  522  459  133  142  145   45 862  126
 [3,]    6  -62    0  -78  183  -15    3  460  397   71   80   83  -17 800   64
 [4,]   84   16   78    0  261   63   81  538  475  149  158  161   61 878  142
 [5,] -177 -245 -183 -261    0 -198 -180  277  214 -112 -103 -100 -200 617 -119
 [6,]   21  -47   15  -63  198    0   18  475  412   86   95   98   -2 815   79
 [7,]    3  -65   -3  -81  180  -18    0  457  394   68   77   80  -20 797   61
 [8,] -454 -522 -460 -538 -277 -475 -457    0  -63 -389 -380 -377 -477 340 -396
 [9,] -391 -459 -397 -475 -214 -412 -394   63    0 -326 -317 -314 -414 403 -333
[10,]  -65 -133  -71 -149  112  -86  -68  389  326    0    9   12  -88 729   -7
[11,]  -74 -142  -80 -158  103  -95  -77  380  317   -9    0    3  -97 720  -16
[12,]  -77 -145  -83 -161  100  -98  -80  377  314  -12   -3    0 -100 717  -19
[13,]   23  -45   17  -61  200    2   20  477  414   88   97  100    0 817   81
[14,] -794 -862 -800 -878 -617 -815 -797 -340 -403 -729 -720 -717 -817   0 -736
[15,]  -58 -126  -64 -142  119  -79  -61  396  333    7   16   19  -81 736    0
[16,] -201 -269 -207 -285  -24 -222 -204  253  190 -136 -127 -124 -224 593 -143
       V16
 [1,]  201
 [2,]  269
 [3,]  207
 [4,]  285
 [5,]   24
```

```
 [6,]  222
 [7,]  204
 [8,] -253
 [9,] -190
[10,]  136
[11,]  127
[12,]  124
[13,]  224
[14,] -593
[15,]  143
[16,]    0
```

Watershed Matrix (0, 1, 2)

```
      V1 V2 V3 V4 V5 V6 V7 V8 V9 V10 V11 V12 V13 V14 V15 V16
 [1,]  0  0  0  0  0  0  0  1  1   2   2   2   2   2   2   2
 [2,]  0  0  0  0  0  0  0  1  1   2   2   2   2   2   2   2
 [3,]  0  0  0  0  0  0  0  1  1   2   2   2   2   2   2   2
 [4,]  0  0  0  0  0  0  0  1  1   2   2   2   2   2   2   2
 [5,]  0  0  0  0  0  0  0  1  1   2   2   2   2   2   2   2
 [6,]  0  0  0  0  0  0  0  1  1   2   2   2   2   2   2   2
 [7,]  0  0  0  0  0  0  0  1  1   2   2   2   2   2   2   2
 [8,]  1  1  1  1  1  1  1  0  0   1   1   1   1   1   1   1
 [9,]  1  1  1  1  1  1  1  0  0   1   1   1   1   1   1   1
[10,]  2  2  2  2  2  2  2  1  1   0   0   0   0   0   0   0
[11,]  2  2  2  2  2  2  2  1  1   0   0   0   0   0   0   0
[12,]  2  2  2  2  2  2  2  1  1   0   0   0   0   0   0   0
[13,]  2  2  2  2  2  2  2  1  1   0   0   0   0   0   0   0
[14,]  2  2  2  2  2  2  2  1  1   0   0   0   0   0   0   0
[15,]  2  2  2  2  2  2  2  1  1   0   0   0   0   0   0   0
[16,]  2  2  2  2  2  2  2  1  1   0   0   0   0   0   0   0
```

5.3 Test for correlations among factors

```
> # Factors: dext, dz, est.ext, est.pres, elev, wshd
>
> cor.test(c(dext.), c(dz.)) #0.794, p-value < 2.2e-16  *******

        Pearson's product-moment correlation

data:  c(dext.) and c(dz.)
t = 20.849, df = 254, p-value < 2.2e-16
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 0.7443739 0.8356752
sample estimates:
      cor
0.7944711
```

```
> cor.test(c(dext.), c(est.ext.)) #-0.047, p-value = 0.4501

        Pearson's product-moment correlation

data:  c(dext.) and c(est.ext.)
t = -0.75647, df = 254, p-value = 0.4501
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 -0.16903152  0.07562954
sample estimates:
        cor
-0.04741208

> cor.test(c(dext.), c(est.pres.)) #0.145, p-value = 0.02057

        Pearson's product-moment correlation

data:  c(dext.) and c(est.pres.)
t = 2.3305, df = 254, p-value = 0.02057
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 0.02248469 0.26263101
sample estimates:
      cor
0.1446878

> cor.test(c(dext.), c(elev.)) #-0.111, p-value = 0.07562

        Pearson's product-moment correlation

data:  c(dext.) and c(elev.)
t = -1.784, df = 254, p-value = 0.07562
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 -0.23069699  0.01151774
sample estimates:
       cor
-0.1112413

> cor.test(c(dext.), c(wshd)) #0.219, p-value = 0.00041

        Pearson's product-moment correlation

data:  c(dext.) and c(wshd)
t = 3.581, df = 254, p-value = 0.00041
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
```

```
 0.09929339 0.33288154
sample estimates:
      cor
0.2192266

> cor.test(c(dz.), c(est.ext.)) #-0.039, p-value = 0.5304

        Pearson's product-moment correlation

data:  c(dz.) and c(est.ext.)
t = -0.62832, df = 254, p-value = 0.5304
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 -0.16121722  0.08361195
sample estimates:
        cor
-0.03939387

> cor.test(c(dz.), c(est.pres.)) #0.267, p-value = 1.544e-05

        Pearson's product-moment correlation

data:  c(dz.) and c(est.pres.)
t = 4.4074, df = 254, p-value = 1.544e-05
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 0.1488027 0.3768298
sample estimates:
     cor
0.266542

> cor.test(c(dz.), c(elev.)) #0.142, p-value = 0.02343

        Pearson's product-moment correlation

data:  c(dz.) and c(elev.)
t = -2.2801, df = 254, p-value = 0.02343
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 -0.25971698 -0.01935871
sample estimates:
       cor
-0.1416246

> cor.test(c(dz.), c(wshd)) #0.365, p-value = 1.795e-09

        Pearson's product-moment correlation
```

```
data:  c(dz.) and c(wshd)
t = 6.2426, df = 254, p-value = 1.795e-09
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 0.2534458 0.4664593
sample estimates:
     cor
0.364715

> cor.test(c(est.ext.), c(est.pres.)) #0.691, p-value < 2.2e-16  *******

        Pearson's product-moment correlation

data:  c(est.ext.) and c(est.pres.)
t = 15.229, df = 254, p-value < 2.2e-16
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 0.6208523 0.7499473
sample estimates:
      cor
0.6908671

> cor.test(c(est.ext.), c(elev.)) #0.399, p-value = 3.464e-11

        Pearson's product-moment correlation

data:  c(est.ext.) and c(elev.)
t = 6.9295, df = 254, p-value = 3.464e-11
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 0.2903271 0.4970400
sample estimates:
      cor
0.3987362

> cor.test(c(est.ext.), c(wshd)) #0.324, p-value = 1.183e-07

        Pearson's product-moment correlation

data:  c(est.ext.) and c(wshd)
t = 5.4513, df = 254, p-value = 1.183e-07
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 0.2093388 0.4292064
sample estimates:
      cor
0.3236346
```

```
> cor.test(c(est.pres.), c(elev.)) #0.269, p-value = 1.312e-05

        Pearson's product-moment correlation

data:  c(est.pres.) and c(elev.)
t = 4.4454, df = 254, p-value = 1.312e-05
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 0.1510483 0.3787991
sample estimates:
      cor
0.2686748

> cor.test(c(est.pres.), c(wshd)) #0.907, p-value < 2.2e-16  *******

        Pearson's product-moment correlation

data:  c(est.pres.) and c(wshd)
t = 34.221, df = 254, p-value < 2.2e-16
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 0.8819265 0.9261777
sample estimates:
      cor
0.9065112

> cor.test(c(elev.), c(wshd)) #0.121, p-value = 0.05335

        Pearson's product-moment correlation

data:  c(elev.) and c(wshd)
t = 1.9411, df = 254, p-value = 0.05335
alternative hypothesis: true correlation is not equal to 0
95 percent confidence interval:
 -0.001725681  0.239947191
sample estimates:
      cor
0.1209019
```

5.4 Correlation results

Correlations equal or greater than 0.7 occured between dext and dz; est.ext and est.pres; and est.pres and wshd.

Choosing dry extent (dext) over number of dry zones (dz) and removing estuary presence (est.pres) from consideration should eliminates correlation issues among environmental matrices.

Question: What level of correlation is okay? Here I use 0.7 as an arbitrary cut off.

6 Partial Mantel Tests

Partial Mantel tests evaluate how two matrices are correlated after controlling, or keeping statistically constant, the effects of other matrices. Here I partial out the effects of stream distance and test for significance of the other environmental factors.

6.1 Partial Mantel test with dry zone extent

```
> mantel.partial(fst, dext, strm, permutations = 10000)
```

```
Partial Mantel statistic based on Pearson's product-moment correlation

Call:
mantel.partial(xdis = fst, ydis = dext, zdis = strm, permutations = 10000)

Mantel statistic r: 0.5298
      Significance: 9.999e-05

Upper quantiles of permutations (null model):
  90%   95% 97.5%   99%
0.199 0.262 0.322 0.365
Permutation: free
Number of permutations: 10000
```

```
> #Extent of dry extent is significant: 53%
```

6.2 Partial Mantel test with elevation

```
> mantel.partial(fst, elev, strm, permutations = 10000)
```

```
Partial Mantel statistic based on Pearson's product-moment correlation

Call:
mantel.partial(xdis = fst, ydis = elev, zdis = strm, permutations = 10000)

Mantel statistic r: -0.2429
      Significance: 0.977

Upper quantiles of permutations (null model):
  90%   95% 97.5%   99%
0.128 0.159 0.188 0.225
Permutation: free
Number of permutations: 10000
```

```
> # Elevation is not significant
```

```
> mantel.partial(fst, est.ext, strm, permutations = 10000)
```

```
Partial Mantel statistic based on Pearson's product-moment correlation

Call:
mantel.partial(xdis = fst, ydis = est.ext, zdis = strm, permutations = 10000)

Mantel statistic r: -0.1993
      Significance: 0.9877

Upper quantiles of permutations (null model):
  90%   95% 97.5%   99%
0.192 0.282 0.316 0.364
Permutation: free
Number of permutations: 10000
```

```
> # Estuary extent is not significant
```

```
> mantel.partial(fst, wshd, strm, permutations = 10000)
```

```
Partial Mantel statistic based on Pearson's product-moment correlation

Call:
mantel.partial(xdis = fst, ydis = wshd, zdis = strm, permutations = 10000)

Mantel statistic r: 0.3064
      Significance: 0.0015998

Upper quantiles of permutations (null model):
  90%   95% 97.5%   99%
0.130 0.160 0.185 0.227
Permutation: free
Number of permutations: 10000
```

```
> # Watershed is significant: 30%
```

7 Conclusions

I found significant differences for factors likely contributing to the observed genetic structure among my study locations. These factors included stream distance (70 percent), dry extent (53 percent) and watershed boundaries (30 percent). After removing variance associated with stream distance among locations, extent of river drying accounted for 53 percent of the remaining variance. Likewise, watershed within which sites were located accounted for 30 percent of the remaining variance after removing variance associated with stream distance among locations.

These tests, however, used 16x16 square matrices to test for significance differences amonge locations with regard to Fst and contributing factors. Unfortunately, this techniques looses the power of my individual level genetic data, which includes 437 individuals genotyped at 10 microsatellite loci. Next, I would like to try using Mantel tests with the individual data using the r package adegenet.

We'll see if I can get that accomplished by Tuesday. Cheers, Heidi