**SNAPP (Beast) analysis for phylogenetic reconstruction and divergence time estimates using RAD data**

In order to use Beauti you need to open a putty session with the following configuration as it needs X forwarding to open the GUI:

Host Name: maccolllab.life.nottingham.ac.uk

Port: 22

Under the SSH tab select X11 then check enable X11 forwarding and set the x display location to be localhost:0

***On the maccolllab.life.nottingham.ac.uk server:***

***To convert the file to the correct format to be read into Beauti:***

cp /home/mbzlld/lauras\_files/Post-doc\_work\_2018/RAD\_analysis/my\_RAD\_analysis/Populations\_analysis/batch\_1.vcf /home/mbzlld/lauras\_files/Post-doc\_work\_2018/RAD\_analysis/my\_RAD\_analysis/PGDspider\_SNAPP\_file\_conversion

cd /home/mbzlld/lauras\_files/Post-doc\_work\_2018/RAD\_analysis/my\_RAD\_analysis/PGDspider\_SNAPP\_file\_conversion

java -Xmx1024m -Xms512m -jar PGDSpider2-cli.jar \

-inputfile batch\_1.vcf \

-inputformat VCF \

-outputfile batch\_1\_binary.nex \

-outputformat NEXUS \

-spid VCF\_to\_NEXUS\_binary.spid

***On the maccolllab.life.nottingham.ac.uk server:***

***To make the xml file in beauti:***

cp /home/mbzlld/lauras\_files/Post-doc\_work\_2018/RAD\_analysis/my\_RAD\_analysis/PGDspider\_SNAPP\_file\_conversion/batch\_1\_binary.nex /home/mbzlld/lauras\_files/Post-doc\_work\_2018/RAD\_analysis/my\_RAD\_analysis/SNAPP\_analysis

cd /home/mbzlld/lauras\_files/Post-doc\_work\_2018/RAD\_analysis/my\_RAD\_analysis/SNAPP\_analysis/beast/bin

./beauti

* This opens the Beauti GUI as a new window. In the Beauti GUI go to file, Template, SNAPP then file,import alignment and select the batch\_1.nex file that you edited in the text editer and copied back into the SNAPP\_analysis folder.
* Replace the species/population entries with the correct populations.
* Under the model parameters tab untick the include non-polymorphic sites box and click calculate mutation rates. It calculated:
  + - U = 2.442525248920452
    - V = 0.6286984558573621
    - Coalescence rate = 10.0
* In the priors tab let everything be standard
* Set the MCMC settings to:
  + - Chain length = 10,000,000
    - Store every = 1000
    - Pre Burnin = 1,000,000
    - Nu mini Att = 10
    - For tracelog Filename = snap\_1.log
    - Log every 1000
    - For treelog Filename = snap\_1.trees
    - Log every 1000
* Click save as and save as and save in the SNAPP\_analysis folder.

***To perform the SNAPP analysis:***

screen –S snapp

screen -r 54526.snapp

cd /home/mbzlld/lauras\_files/Post-doc\_work\_2018/RAD\_analysis/my\_RAD\_analysis/SNAPP\_analysis

java -Dbeast.load.jars=true -jar ./beast/lib/beast.jar Beauti\_output\_file\_1.xml

cntrl a d

screen -r 54526.snapp

cntrl a :quit

***On the maccolllab.life.nottingham.ac.uk server:***

***To test the model of nucleotide substitution using jModelTest:***

cp /home/mbzlld/lauras\_files/Post-doc\_work\_2018/RAD\_analysis/my\_RAD\_analysis/PGDspider\_SNAPP\_file\_conversion/batch\_1.nex /home/mbzlld/lauras\_files/Post-doc\_work\_2018/RAD\_analysis/my\_RAD\_analysis/jmodeltest/jmodeltest2-master/dist

cd /home/mbzlld/lauras\_files/Post-doc\_work\_2018/RAD\_analysis/my\_RAD\_analysis/jmodeltest/jmodeltest2-master/dist

java -jar jModelTest.jar \

-d /home/mbzlld/lauras\_files/Post-doc\_work\_2018/RAD\_analysis/my\_RAD\_analysis/Populations\_analysis/batch\_1.phylip \

-g 4 \

-i \

-f \

-AIC \

-BIC \

-a

***On the maccolllab.life.nottingham.ac.uk server:***

***To test the model of nucleotide substitution using PAUP and MrModelTest (never completed troubleshooting of this method as the batch\_1\_model\_out file indicated errors that I coundn’t seem to fix and sparse info on the MrModelTest program on the web):***

WITH THE BINARY FILE (doesn’t work):

* Open the nexus file in a text editor and delete the lines about datatype and missing data but make sure there is symbols = "012" followed by a semi colon after the word format.
* replace the line FORMAT with FORMAT symbols = "012"; then transfer it back to the same place using filezilla.
* Open the nexus file in a text editor and replace the line format with FORMAT datatype=standard missing=? symbols = "012"; and remove the lines datatype=SNP and missing=? Below. Name this file batch\_1\_paup.nex and put it in the PAUP folder
* Put the MrModelBlock file that comes with MrModelTest in the paup folder. I had to edit this file to get it to work with non-nucleotide data. I had to replace every ‘base’ word in each of the 24 models with ‘genFreq’ I called the new file MrModelBlock2 and this file executed.

cp /home/mbzlld/lauras\_files/Post-doc\_work\_2018/RAD\_analysis/my\_RAD\_analysis/PGDspider\_SNAPP\_file\_conversion/batch\_1\_binary.nex /home/mbzlld/lauras\_files/Post-doc\_work\_2018/RAD\_analysis/my\_RAD\_analysis/PAUP

screen -RD

cd /home/mbzlld/lauras\_files/Post-doc\_work\_2018/RAD\_analysis/my\_RAD\_analysis/PAUP

./paup4a164\_ubuntu64

exe batch\_1\_binary.nex

exe MrModelblock2

CNTRL A D

* Open a new shell. In the new shell:

cd /home/mbzlld/lauras\_files/Post-doc\_work\_2018/RAD\_analysis/my\_RAD\_analysis/PAUP

mrmodeltest2 <mrmodel.scores> batch\_1\_model\_out

less batch\_1\_model\_out