Landscape geneticis - Hobart

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Contents

Forword

This tutorial consists mainly of four parts.

I. The first part demonstrates howto run an isolation by distance analysis.

II. Then you will learn via an example how you can run a resistance layer approach. Here we explore a relationship between landscape features and their effect expresses as a cost-distance matrix. Very similar to an isolation-by-distance analysis we then use several method to check for a relationship between cost-distance(s) and genetic distances.

III. The third part of the tutorial introduces a simulator, which allows you to "play" with the approach and check its power to detect an effect of the landscape. You learn how to simulate landscapes and populate those landscape with a metapopulation of interest using an individual-based forward simulator.

IV. The final part allows you to explore the approach using your own data. Either you can use your own data if you have a suitable resistance layer, or you can "create" a resistance layer using a simple graphic progam such as Paint to test a "made-up" hypothesis on your data. Obviously you can also skip this part and "play" a bit more with the simulator introduced in the second part.

Hint

You can find an eletronic pdf version of this script at the following github page:
`https://github.com/green-striped-gecko/dartRworkshop`

Before we start, a last word on the required data. To be able to work on your own data (part III) you need a genetic data set (SNPs) in the genlight format. There are several ways to achieve this. The table below (see also [`https://onlinelibrary.wiley.com/doi/abs/10.1111/1755-0998.12745`] (Gruber et al. 2018)) you'll several possible avenues.

In addition you need to have coordinates attached to your samples. Be warned though the tutorial shows you how to project your coordinates into different coordinate systems, you need to be learn and understand some basic GIS skills before you can run an analysis with your own data on your own landscape.

We need two main libraries

```
library(dartR)
```

```
## Warning: package 'dartR' was built under R
## version 3.5.3
```

```
## Loading required package: adegenet
```

```
## Loading required package: ade4
```

```
##
##    /// adegenet 2.1.1 is loaded ////////////
##
##    > overview: '?adegenet'
##    > tutorials/doc/questions: 'adegenetWeb()'
##    > bug reports/feature requests: adegenetIssues()
```

```
library(PopGenReport)
```

```
## Loading required package: raster
```

```
## Loading required package: sp
```

I. Isolation by distance

Calculate a genetic distance

Calculate a "Euclidean Distance"

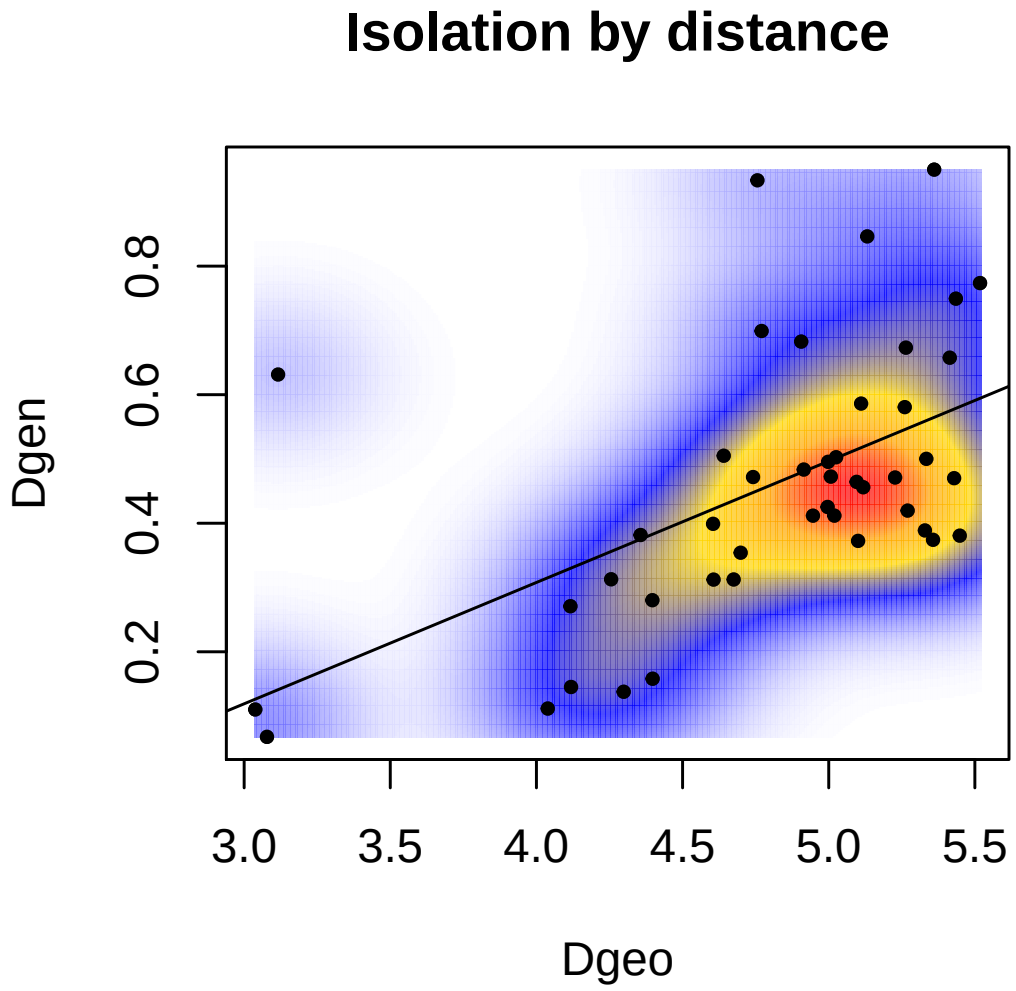
Isolation by distance

The "null model" in landscape genetics is that there is a simple relationship between genetic and euclidean distance. A standard procedure is to study the relationship between log(euclidean distance) and Fst/1-Fst (see ?gl.ibd for details). For a quick check we can use the `gl.ibd`. To be able to use the function the genlight object needs to have the coordinates for each individual in the `@other$latlong` slot. Further we need to provide information if the coordinates are already projected or given in lat/lon.

```
iso <- gl.ibd(possums.gl, projected = TRUE)
```

```
## Standard analysis performed on the genlight object. Mantel test and plot will be Fst/1-Fst versus log(dist
## Coordinates not transformed. Distances calculated on the provided coordinates.
## Mantel statistic based on Pearson's product-moment correlation
##
## Call:
## mantel(xdis = Dgen, ydis = Dgeo, permutations = 999, na.rm = TRUE)
```

```
##
## Mantel statistic r: 0.5513
##       Significance: 0.001
##
## Upper quantiles of permutations (null model):
##   90%   95% 97.5%   99%
## 0.216 0.272 0.323 0.358
## Permutation: free
## Number of permutations: 999
```



The function returns a list of the following components: Dgen (the genetic distance matrix), Dgeo (the Euclidean distance matrix), mantel (the statistics of the mantel test)

A mantel test is basically a simple regression, but the significance takes the non-independence of pairwise distances via a bootstrap approach into account.

II. Landscape genetics using a landsacpe restistance approach

A simulated example

Often ecologists want to know if a particular landscape feature is affecting population structure on top of Euclidean distance. The idea is that a particular feature is causing some cost for individuals when moving through it, hence modifying the actual euclidean distance between individuals/populations. Commonly used approaches to calculate so-called cost-distances are the "least-cost" and "circuitscape" approach. Both approaches require a landscape that represents landscape features in terms of their "resistance" values.

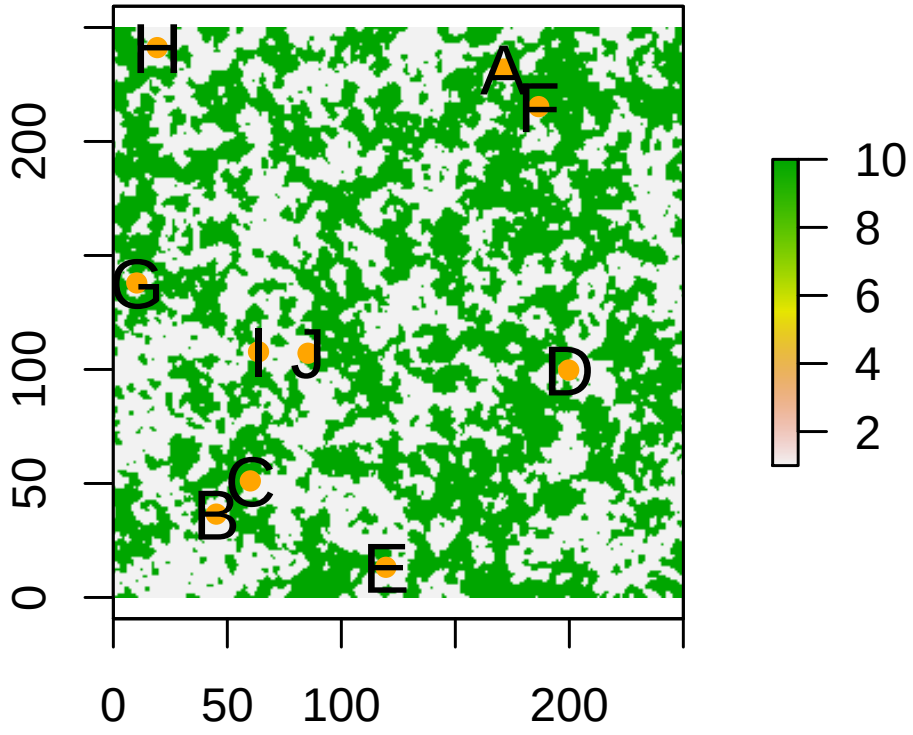
Resistance layer

In this example we use populations as the entity of interest. Hence we need to calculate three population-based pairwise distance matrices, namely a Euclidean distance matrix, a cost distance matrix and finally a genetic distance matrix. The two distance matrices can then be used (very similar to the partial mantel test above) to "compete"" against each other - how well they explain genetic distances. As mentioned we base our analysis on populations, therefore we first need to calculate the coordinates of our population centers. But first we load our (resistance) landscape to be able to plot our data.

```
landscape.sim <- readRDS(system.file("extdata",
    "landscape.sim.rdata", package = "dartR"))
```

We calculate the population centers via:

```
xs <- tapply(possums.gl@other$latlong[, "lon"],
    pop(possums.gl), mean)
ys <- tapply(possums.gl@other$latlong[, "lat"],
    pop(possums.gl), mean)

plot(landscape.sim)
points(xs, ys, pch = 16, col = "orange")
text(xs, ys, popNames(possums.gl), col = "black",
    cex = 1.5)
```




```
coords <- cbind(xs, ys)
```

Euclidean distance matrix

```
eucl <- as.matrix(dist(coords))
```

Task

1. Explore your Euclidean distance matrix.
2. Note the Euclidean distance between A-F, and I-J.

Cost distance matrix

```
cost <- gl.costdistances(landscape = landscape.sim,
    locs = coords, method = "leastcost", NN = 8)
```

Task

1. Explore your Euclidean distance matrix.
2. Note the cost distance between A-F, and I-J and compare it to the Euclidean distances noted above. Can you see the effect of the landscape between A-F compared to I-J?

Genetic distance matrix

We will use pairwise Fsts between population, but in principle any pairwise genetic distance index can be used.

```
gd <- as.matrix(as.dist(gl.fst.pop(possums.gl,
    nboots = 1)))
```

And finally run a partial mantel test

```
library(PopGenReport)
wassermann(gen.mat = gd, eucl.mat = eucl, cost.mats = list(cost = cost),
    plot = F)
```

```
## $mantel.tab
##                   model       r     p
## 1 Gen ~cost | Euclidean  0.4945 0.028
## 2 Gen ~Euclidean | cost -0.2502 0.836
```

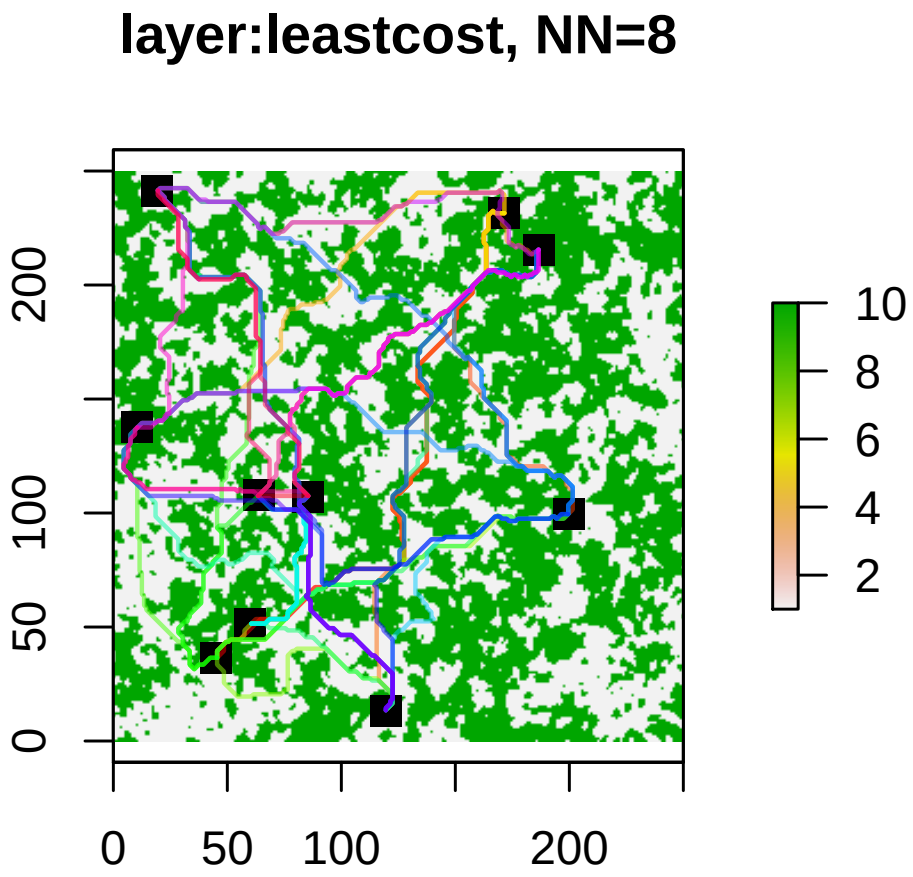
```
lgrMMRR(gen.mat = gd, cost.mats = list(cost = cost),
    eucl.mat = eucl)
```

```
## $mmrr.tab
##       layer   coefficient tstatistic tpvalue
## 2      cost  0.0014649146   3.686690   0.054
## 3 Euclidean -0.0009384741  -1.674577   0.350
## 1 Intercept  0.1228057756   4.419399   1.000
##    Fstat Fpvalue        r2
## 2 24.532   0.001 0.5387859
## 3     NA      NA        NA
## 1     NA      NA        NA
```

II. A landscape genetics analysis in a single command

dartR has a convinience function that does it all in once, but is less flexible. It has the benefit that it shows the actual least cost path in the landscape (but runs slower).

```
glc <- gl.genleastcost(possums.gl, fric.raster = landscape.sim,
    gen.distance = "D", NN = 8, pathtype = "leastcost")
```




```
wassermann(gen.mat = gd, eucl.mat = eucl, cost.mats = list(cost = cost),
    plot = F)
```

```
## $mantel.tab
##                  model       r     p
```

```
## 1 Gen ~cost | Euclidean  0.4945 0.026
## 2 Gen ~Euclidean | cost -0.2502  0.83
```

Task

Possums in New Zealand

Load the provided data set and the provided raster data set.

Run a resistance layer analysis to check if the proportion of forest in the area do have an effect on the population structure of possums.

III. A simulated resistance surface landscape analysis

An excellent way to test an approach and understand its limitation, is to use a simulation appraoch. You will be provided with functions to:

A. simulate a landscape (a resistance layer) B. Populate this landscape with populations of a simulated species C. "Run" the species over the landscape for a number of generations and perform a landscape genetic analysis using a resistance layer.

Then you can go back and explore the parameters and its effect on the performance of the approach (e.g. change the number of loci, change the number of generations etc.)

We need to install another package: `secr`.

```
install.packages("secr")
library(secr)
library(PopGenReport)  #should already be installed
library(raster)  #should already be installed
```

Before we can start we also need to download some additional functions that simplifies the coding and scripts.

```
source("https://raw.github.com/green-striped-gecko/lgfun/master/lgfuns.r")
```

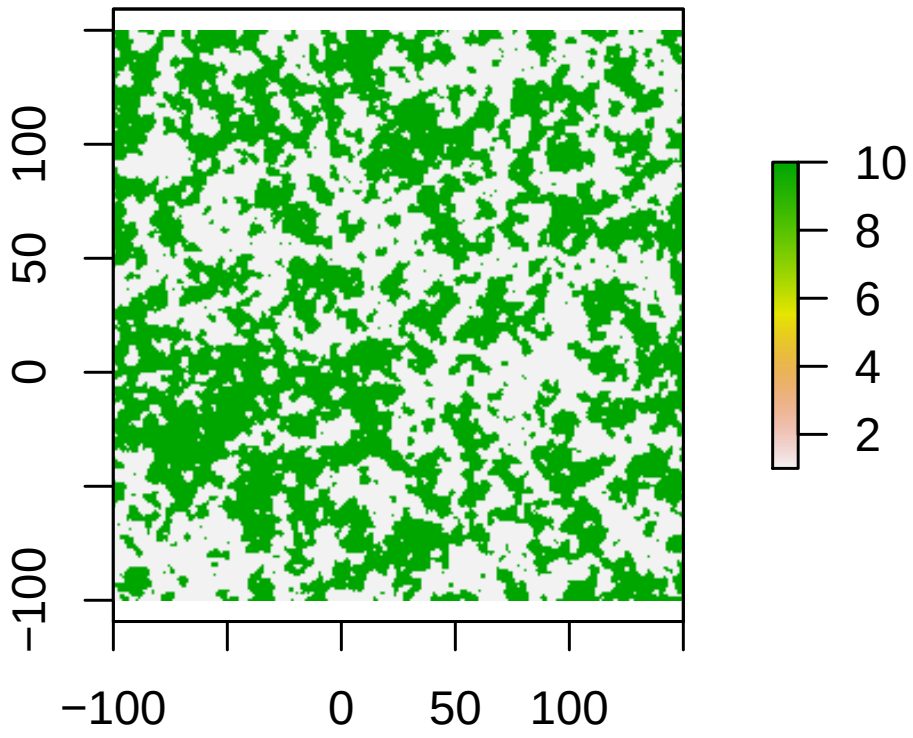
A. Simulate a resistance layer

We will use the 'randomHabitat' function from the 'secr' package to create a resistance layer, but you could simply load a png file or any other image file format with the 'raster' function from package 'raster' (?raster::raster, see the examples in there).

Parameter A is the amount of habitat and p controls the amount of clumping. Nx, ny determine the extend of the landscape and resVal determines the resistance values of non-Habitat features.[1]

[1] Check ?randomHabitat for details

```
r <- create.resistance(nx = 50, ny = 50, p = 0.5,
    A = 0.5, resVal = 10)
```



Task

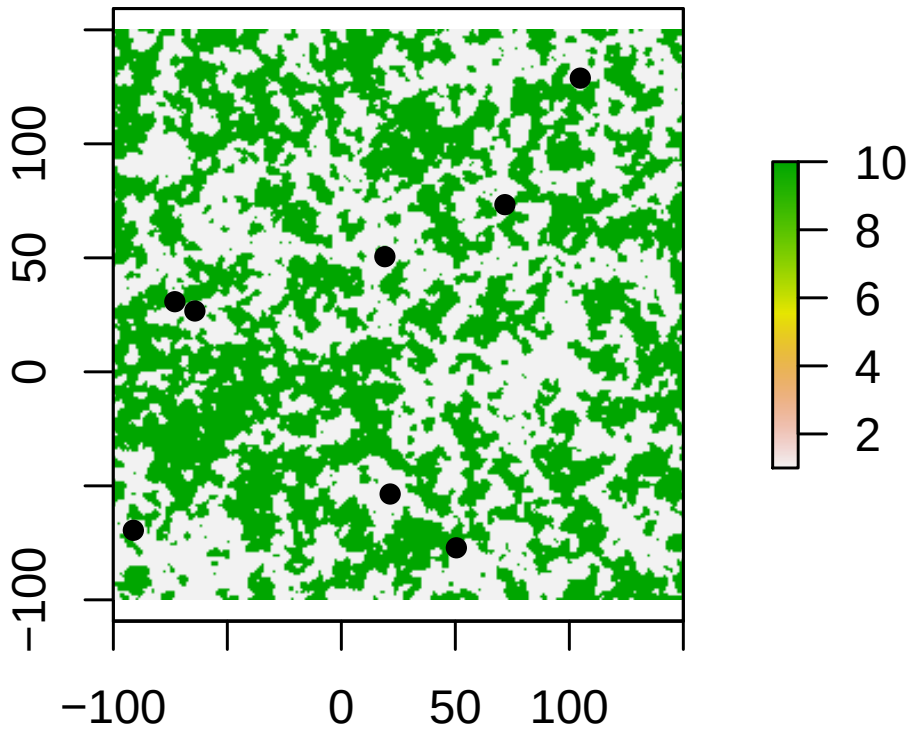
1. Rerun the command above until you "like" the resistance layer. You can change the parameter values (but be aware that p should not be higher than 0.55, otherwise the algorithm does not work reliably).

B. Populate the landscape with population of a species

B.1. Add populations to the landscape (using minimal distance)

`createpops` allows us to set up 'n' subpopulations in the habitat only (non green areas). The subpopulations should be at least 'mindist' units apart.

```
locs <- create.pops(n = 8, mindist = 3, landscape = r,
    plot = TRUE)
```




```
locs
```

```
##          lx       ly
## 1  19.08356  50.51776
## 2  71.75057  73.32838
```

```
## 3 -73.02937  30.76285
## 4  50.44226 -77.18287
## 5 -64.24840  26.69431
## 6 104.81158 128.82332
## 7  21.39996 -53.58770
## 8 -91.21844 -69.49850
```

B.2. Initialise a metapopulation

We use 'init.popgensim' from package PopGenReport to initialise a metapopulation based on the locations we created earlier. To do this we need to initialise a number of parameters (the locations of the subpopulations, the number of individuals per subpopulation, the number of loci and alleles per loci. For a full list check '?init.popgensim').

To store all the parameters we create a list called para where we store all of them

Define metapopulation:

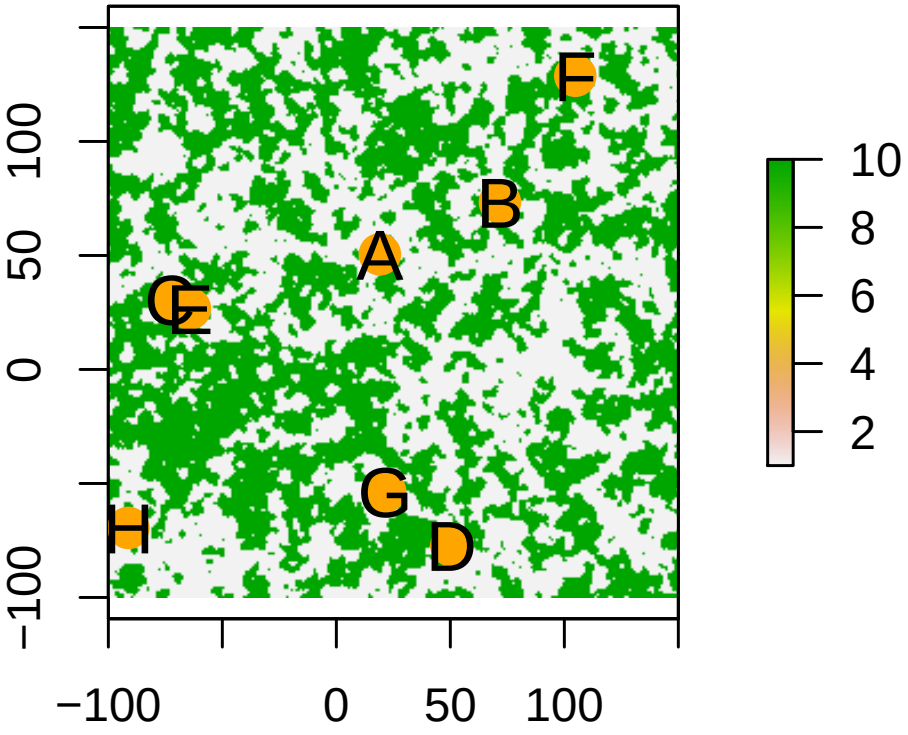
```
para <- list()
# Define populations (dynamics)
para$n.pops = 8
para$n.ind = 50

para$sex.ratio <- 0.5
# age distribution....

para$n.cov <- 3
# number of covariates (before the loci in the
# data.frame, do not change this!!)
```

Define population dynamics:

```
# reproduction
para$n.offspring = 2

# migration
para$mig.rate <- 0.2

# dispersal: exponential dispersal with
# maximal distance in map units
para$disp.max = 100  #average  dispersal of an individual in meters
para$disp.rate = 0.1  #proportion of dispersing individuals

# Define genetics [create 100 SNPs]
para$n.allels <- 2
para$n.loci <- 200
para$mut.rate <- 1e-05
```

Define a cost distance method:

```
para$method <- "leastcost"  #rSPDdistance, commute
para$NN <- 8  #number of neighbours for the cost distance method

# Initialize simulation of populations from
# scratch

landscape <- r  #<-raster(system.file('external/rlogo.grd', package='raster'))

# Define x and y locations
para$locs <- locs
# give the population some names
rownames(para$locs) <- LETTERS[1:para$n.pops]



# Create a costdistance matrix

cost.mat <- gl.costdistances(landscape, para$locs,
    para$method, para$NN)
# needed for the simulation
eucl.mat <- as.matrix(dist(para$locs))  #needed for the analysis later

# Plot your landscape with the populations....

plot(landscape)
points(para$locs[, 1], para$locs[, 2], pch = 16,
    cex = 2, col = "orange")
text(para$locs[, 1], para$locs[, 2], row.names(para$locs),
    cex = 1.5)
```




```
# Check the parameter list

para
```

```
## $n.pops
## [1] 8
##
## $n.ind
## [1] 50
##
## $sex.ratio
## [1] 0.5
##
```

```
## $n.cov
## [1] 3
##
## $n.offspring
## [1] 2
##
## $mig.rate
## [1] 0.2
##
## $disp.max
## [1] 100
##
## $disp.rate
## [1] 0.1
##
## $n.allels
## [1] 2
##
## $n.loci
## [1] 200
##
## $mut.rate
## [1] 1e-05
##
## $method
## [1] "leastcost"
##
## $NN
## [1] 8
##
## $locs
##          lx        ly
## A  19.08356  50.51776
## B  71.75057  73.32838
## C -73.02937  30.76285
## D  50.44226 -77.18287
## E -64.24840  26.69431
## F 104.81158 128.82332
## G  21.39996 -53.58770
## H -91.21844 -69.49850
```

B.3 Initialise your population on the landscape

Now finally we can initialise our population using the init function

```
simpops <- init.popgensim(para$n.pops, para$n.ind,
    para$sex.ratio, para$n.loci, para$n.allels,
    para$locs, para$n.cov)
```

You may want to check the simpops object, which is simply a list of our subpopulation and each individual is coded in a single run in one of the subpopulations.

```
names(simpops)  #the names of the subpopulations
```

```
## [1] "A" "B" "C" "D" "E" "F" "G" "H"
```

We can also analyse our simpop object. (e.g. calculate the pairwise Fst value between all the populations).

To be able to do that we first need to convert it into a genlight object, then we can use our usual functions (e.g `gl.fst.pop`).

```
glsp <- pops2gl(simpops, locs = para$locs)
```

```
## Loading required package: parallel
```

```
glsp  #check the genlight object
```

```
##  /// GENLIGHT OBJECT /////////
##
##  // 400 genotypes,  200 binary SNPs, size: 648.3 Kb
##  0 (0 %) missing data
##
##  // Basic content
##    @gen: list of 400 SNPbin
##    @ploidy: ploidy of each individual  (range: 2-2)
##
##  // Optional content
##    @ind.names:  400 individual labels
##    @loc.names:  200 locus labels
##    @pop: population of each individual (group size range: 50-50)
##    @other: a list containing: xy
```

```
summary(glsp)
```

```
##   Length    Class     Mode
##        1 genlight       S4
```

```
gen.mat <- as.matrix(as.dist(gl.fst.pop(glsp,
    nboots = 1)))
# overall pairwise fst
mean(as.dist(gen.mat))
```

```
## [1] -1.442734e-05
```

Task

1. Check the pariwise Fsts between populations. Why are they so low? Is there an effect of the landscape on the population structure, yet?
2. Run a partial mantel test and check the result []

Now we run our simulation by simply passing our simpops, with some additional parameters that are needed for the simulation. The number of generations the simulation should run is in the steps parameter. (check ?run.popgensim for a description of all parameters).

Important to understand is the idea of the cost.mat (which is the cost matrix that is used for the distance between subpopulation).

*C. Run simulations and analyze results***

C.1. Run your population several generations (steps) on the landscape

```
simpops <- run.popgensim(simpops, steps = 3, cost.mat,
    n.offspring = para$n.offspring, n.ind = para$n.ind,
    para$mig.rate, para$disp.max, para$disp.rate,
    para$n.allels, para$mut.rate, n.cov = para$n.cov,
    rec = "none")
```

In essence we were running a metapopulation with 100 individuals per subpopulation on our resistance landscape for 3 generations. The question is now, was that enough time to create an effect on the population structure?

We can check now the pairwise Fst values and then do a landscape genetic analysis using partial mantel tests.

Task

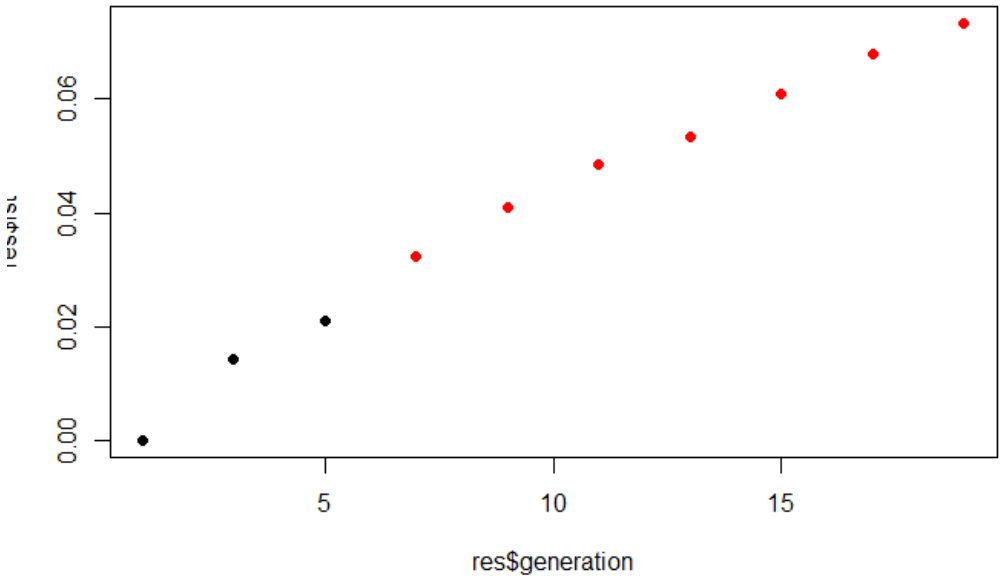
1. Check the pairwise Fsts between populations after 3 generation have passed. Is there now an effect of the landscape on the population structure, yet?
2. Run a partial mantel test and check the result
3. Run now your population another 50 generation.

simpops <- run.popgensim(simpops, steps=50,....)
How did the overall mean pairwise Fst change?
Is there a significant effect of the landscape on the population structure after 53 generations?

Example how to use the simulator: We are interested how many generation it takes until the average Fst values becomes so "different", that the effect of the resistance approach can be detected. We create a loop and calculate mean pairwise Fst values every second generation (up to 20). We plot generations against average pairwise Fst and colour the dots by the significance of the Mantel test.

```
# initialise
simpops <- init.popgensim(para$n.pops, para$n.ind,
    para$sex.ratio, para$n.loci, para$n.allels,
    para$locs, para$n.cov)
# Calculate overall Fsts
glsp <- pops2gl(simpops, locs = para$locs)
gen.mat <- as.matrix(as.dist(gl.fst.pop(glsp,
    nboots = 1)))
# overall pairwise fst
tempfst <- mean(as.dist(gen.mat))

glc <- gl.genleastcost(glsp, fric.raster = landscape,
    gen.distance = "D", NN = 8, pathtype = "leastcost")
temp.mantel <- wassermann(gen.mat = glc$gen.mat,
    eucl.mat = glc$eucl.mat, cost.mats = glc$cost.mats,
    plot = F)

# find the p value in temp
mantelp <- as.numeric(temp.mantel$mantel.tab["1",
    "p"])

res <- data.frame(generation = 0, fst = tempfst,
    mantelp)
```

```
for (i in 1:10) {
    simpops <- run.popgensim(simpops, steps = 2,
        cost.mat, n.offspring = para$n.offspring,
        n.ind = para$n.ind, para$mig.rate, para$disp.max,
        para$disp.rate, para$n.allels, para$mut.rate,
        n.cov = para$n.cov, rec = "none")

    glsp <- pops2gl(simpops)
    gensim.mat <- as.matrix(as.dist(gl.fst.pop(glsp,
        nboots = 1)))
    # overall pairwise fst
    tempfst <- mean(as.dist(gensim.mat))
    temp.mantel <- wassermann(gen.mat = gensim.mat,
        eucl.mat = glc$eucl.mat, cost.mats = glc$cost.mats,
        plot = F)

    mantelp <- as.numeric(temp.mantel$mantel.tab["1",
        "p"])


    res[i, ] <- c(i * 2, tempfst, mantelp)

    cat(paste("Generation:", i * 2, "\n"))

}

plot(res$generation, res$fst, col = (res$mantelp <
    0.05) + 1, pch = 16)

res
```



Task

You can now "play" with the simulator using different landscapes, number of subpopulations, different locations, number of loci etc. For example rerun your analysis for only 4 subpopulations. How does this affect your ability to detect an effect of the landscape? Or you can try to use your own data and create a resistance layer. Be aware that the coordinate system between your landscape and genlight object needs to be the same preferably projected coordinate system.

IV. Create a resistance layer for your own data (a bit experimental!!!)

Admittedly this is a bit of a stretch, as originally you would like to have a gis data set with landscape features to create a resistance layer. Here will will use a "quick and dirty" approach and create a resistance landscape from open source maps, which may be not too useful for your data set.

The idea of this tutorial is to demonstrate the approach for an example of our fox data

Make sure you have installed two additional libraries:

```
# install via: library(devtools)
# install_github('dkahle/ggmap')
library(ggmap)
library(dartR)
library(osmdata)
# also load some helper functions

source("https://raw.github.com/green-striped-gecko/dartRworkshop/master/code/lgfuns.r")



## define your genlight data set here we use
## the bandicoots in NSW
glNSW <- bandicoot.gl[pop(bandicoot.gl) == "NSW",
    1:100]

## bounding box in lat/lon
ll <- glNSW@other$latlong
bb <- matrix(c(min(ll$lon), min(ll$lat), max(ll$lon),
    max(ll$lat)), nrow = 2, ncol = 2)

# or you can use geocode functions bb <-
# getbb('South Australia')

# get a map you may need to play with zoom to
# get a suitable map also you could try
# terrain-lines to get roads if your study is
# close to a city
map <- get_stamenmap(bb, zoom = 6, maptype = "terrain-background")
plot(map)
```

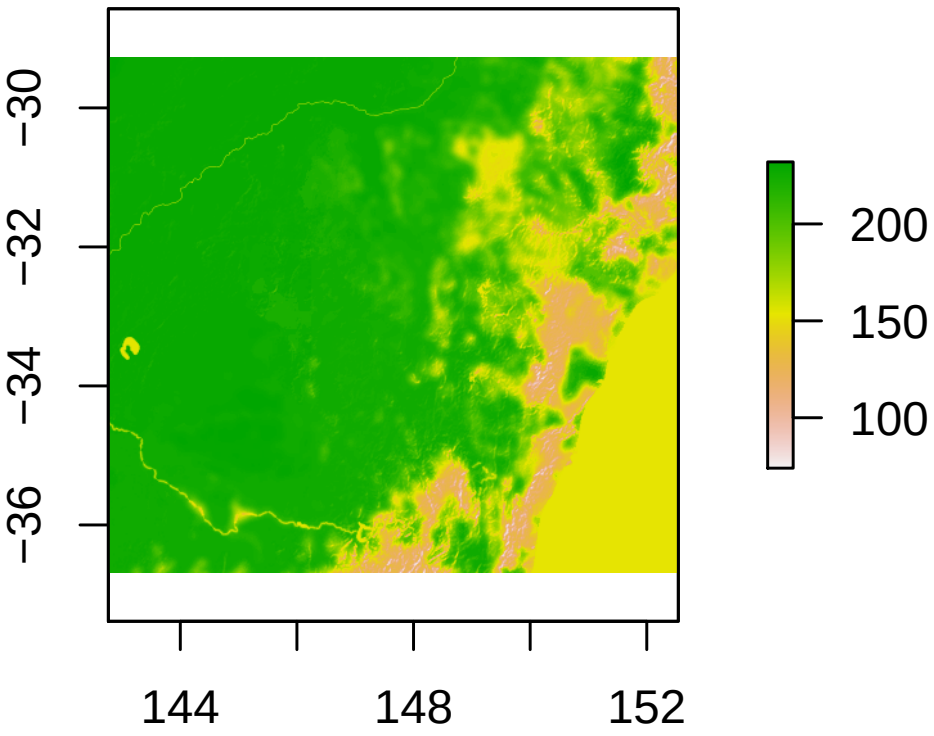
We then need to convert the image to a raster

```
## converts the image to a raster (only the red
## values are returned)
map.raster <- ggmap_to_raster(map)[[1]]
map.raster
```

```
## class       : RasterLayer
## dimensions  : 402, 444, 178488  (nrow, ncol, ncell)
## resolution  : 0.02201689, 0.01840868  (x, y)
## extent      : 142.7657, 152.5412, -36.6801, -29.27981  (xmin, xmax, ymin, ymax)
## coord. ref. : NA
## data source : in memory
## names       : layer.1
```

```
## values      : 74, 232  (min, max)
```

```
plot(map.raster)
```

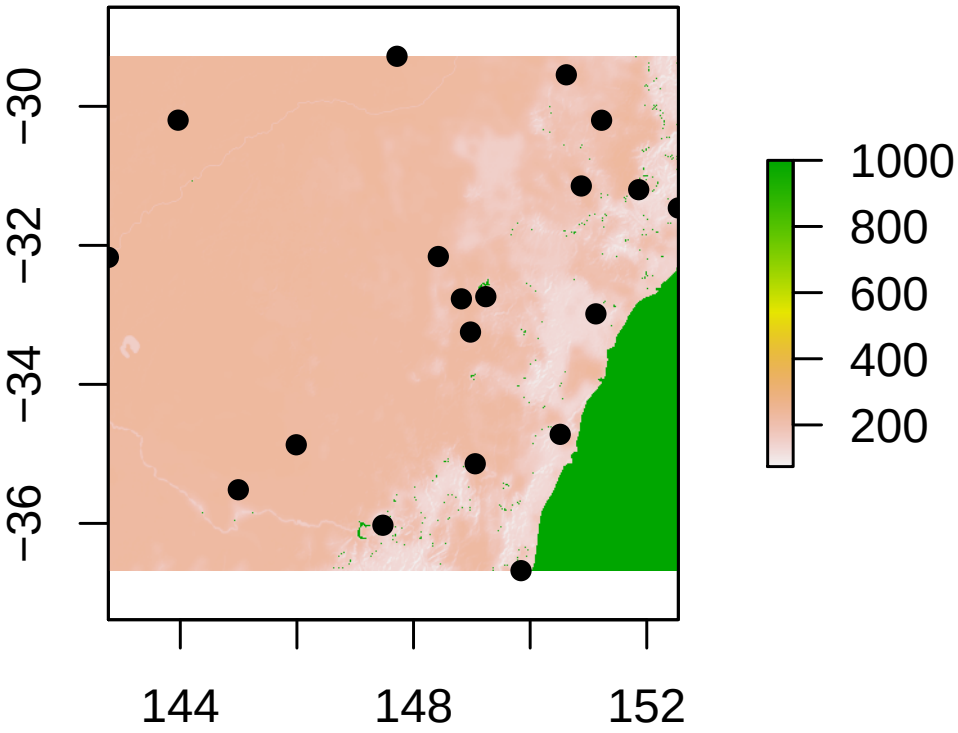


Obiously this is not a very suitable map, basically colors (the amount of red of each pixel is converted into resistance), nevertheless for demonstration purposes we will use it.

To make it a bit more realistic we want to convert the ocean to NAs. As we can see the ocean has a "greeness" that refers to a value around 150. If you table the values of the raster you see, that values of 153 are very promiment. To make them "unavailable"" we set those to very high resistance values=1000 (to make sure paths are not runnnig through the ocean), we use:

```
reslay <- map.raster  #copy to keep the original map
reslay[values(reslay) == 153] <- 1000
plot(reslay)
```

```
points(glNSW@other$latlong, pch = 16)
```



Now we can run a resistance layer analysis. The difference this time is that we run it on individuals, hence we need to specify an individual distance meassure (the fastest in terms of computer time is the proportion of Shared alleles [=propShared]).

Two more issues:

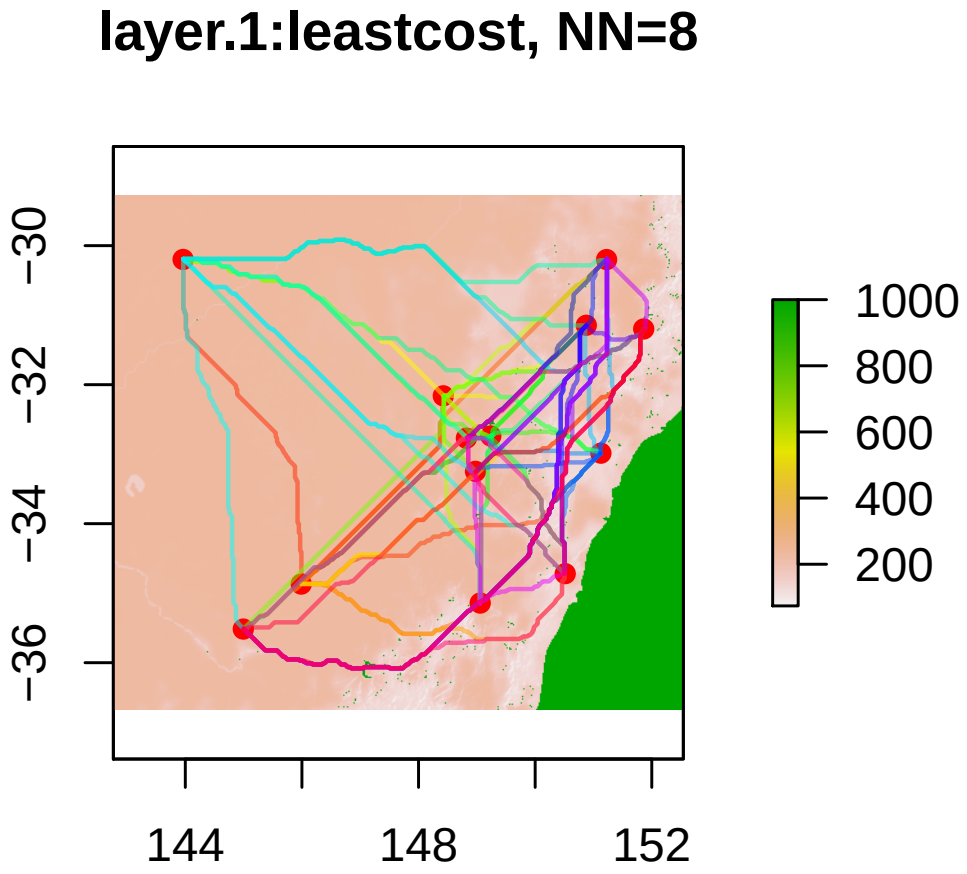
- If you inspect the points in the map above closely you see that four individuals are outside/at the boundary of the actual resistance layer. We would like to delete them.

```
glNSW2 <- glNSW[glNSW$other$latlong$lon < 152 &
    glNSW$other$latlong$lon > 143 & glNSW$other$latlong$lat <
    -30 & glNSW$other$latlong$lat > -36, ]
```

- The other issue is that we would need to reproject the data set so coordinates are in suitable non-geographic units. For simplicity we skip this step here and will receive a warning.

```
glc <- gl.genleastcost(glNSW2, fric.raster = reslay,
    gen.distance = "propShared", NN = 8, pathtype = "leastcost")
```

```
## No projected coordinates in @other$xy found. Hence will use latlongs (if provided), which are not projecte
```



You can see the warning about the projection and yes I would encourage to project your data set to a suitable coordinate system.

The final step is now to run a partial mantel test on the matrices. Same code as before:

```
wassermann(gen.mat = glc$gen.mat, eucl.mat = glc$eucl.mat,
    cost.mats = glc$cost.mats, plot = F)
```

```
## $mantel.tab
##                       model       r     p
## 2 Gen ~Euclidean | layer.1  0.0126 0.453
## 1 Gen ~layer.1 | Euclidean -0.0123 0.548
```

Not surprisingly there is not effect of the resistance layer on the genetic structure (nor an isolation by distance effect).

Task

Now you should have all the ingredients to create your own resistance layer and to run your own data set.

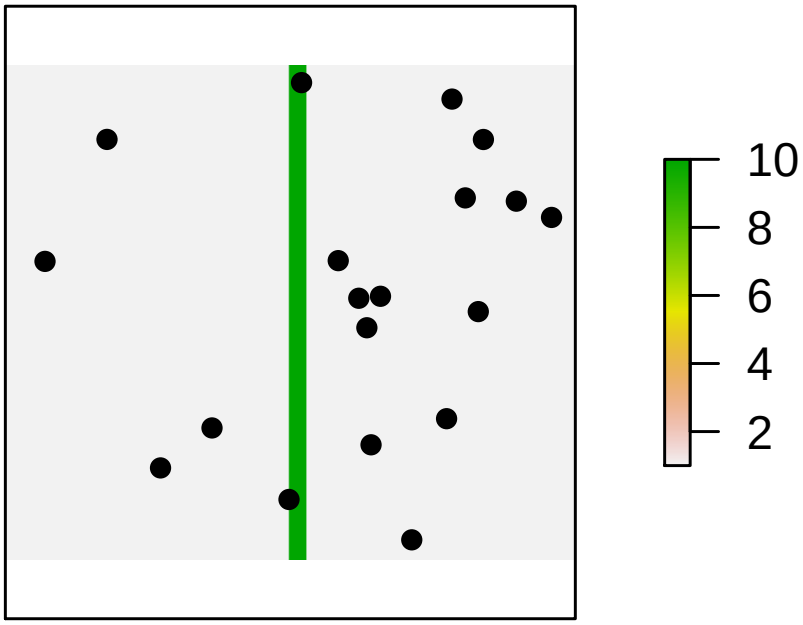
Hint

Another, way to create a resistance layer is to "draw" it in R. Here you basically create a raster with the correct extend and then draw lines on top of it.
Or you simply draw it in your favourite image editor and load it into R with the `raster` function. Be aware here you would need to set the extent manually to match your coordinates.

```
r <- raster(xmn = 142, xmx = 153, ymn = -37, ymx = -29)
r[] <- 1
r
```

```
## class       : RasterLayer
## dimensions  : 180, 360, 64800  (nrow, ncol, ncell)
## resolution  : 0.03055556, 0.04444444  (x, y)
## extent      : 142, 153, -37, -29  (xmin, xmax, ymin, ymax)
## coord. ref. : +proj=longlat +datum=WGS84 +ellps=WGS84 +towgs84=0,0,0
## data source : in memory
## names       : layer
## values      : 1, 1  (min, max)
```

```
rbarrier <- r
rbarrier[, 180:190] <- 10
plot(rbarrier, axes = FALSE)
points(glNSW@other$latlong, pch = 16)
```


10
8
6
4
2

Reproject your coordinates

From geographical projections (lat-lon) to UTM

You need to know two projections, basically which one your coordinates are in. If you have lat-lon you most likely have coordinates in the WGS84 system. Though be aware that GDA94 (the most common system in Australia) is using a different Datum GRS80, which is slightly different and results in "errors" in the order of 1-2 meters if used unknowingly.

In addition you need to know which your new projection is, for example UTM Zone 55.

We use the project function from rgdal

```
library(rgdal)
canberra <- c(149.13, -35.2809)
hobart <- c(147.3272, -42.8821)

ll <- rbind(canberra, hobart)
ll.sp <- SpatialPoints(ll, CRS("+proj=longlat +ellps=WGS84"))

xy <- spTransform(ll.sp, CRS("+proj=utm +zone=55 +south +ellps=WGS84 +datum=WGS84"))
coordinates(xy)
```

```
##          coords.x1 coords.x2
## canberra  693714.4   6093725
## hobart    526720.5   5252226
```

To backtransform is a bit more difficult as we need to define in which projection we are in.

From UTM to lat-lon

```
utms <- SpatialPoints(xy, CRS("+proj=utm +zone=55 +south +ellps=WGS84 +datum=WGS84"))
ll.sp2 <- spTransform(utms, CRS("+proj=longlat +ellps=WGS84"))
coordinates(ll.sp2)
```

```
##          coords.x1 coords.x2
## canberra  149.1300  -35.2809
## hobart    147.3272  -42.8821
```

```
coordinates(ll.sp)
```

```
##          coords.x1 coords.x2
## canberra  149.1300  -35.2809
## hobart    147.3272  -42.8821
```


Well done!!

That is the end. Well done you finished. Feel free to have another go or have a well deserved rest.
Cheers, Bernd