Examining correlates of female-limited polymorphism using MCMCglmm (incl. diagnostics)

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May 2020

Set your working directory to the parent directory containing all of your MCMC analyses

```
knitr::opts_knit$set(root.dir = '..' )
```

1) Read in distribution data and sample of trees from StarBEAST analysis

Call the "ape" package for reading trees

```
require(ape)
```

Read in tree samples file

```
tres <- read.nexus("MCMCglmm/sample.2100.trees")
length(tres)
```

```
## [1] 2100
```

Read in data from literature

```
isch_dat <- read.csv("MCMCglmm/ischnura_distribution.csv")
```

Get list of countries by ISO code

```
ischISO <- unique(unlist(c(strsplit(as.character(isch_dat$Countries), split = ","))))
```

Get area for all countries with Ischnura species

```
require(raster)
```

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

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

```
##
## Attaching package: 'raster'
## The following objects are masked from 'package:ape':
##
##     rotate, zoom
```

```
x <- shapefile('MCMCglmm/maps/countries.shp')
area <- vector(length = length(ischISO))

for (i in 1:length(ischISO)){
  area[i] <- raster::area(x[which(x$ISO == paste0(ischISO[i])),])/1000000
}
```

Get area for all states/provinces/terriories in Australia, Brazil, Canada, China, India, Russia, and US

```
AUS <- shapefile('MCMCglmm/maps/AUS.shp')
```

```
## Warning in rgdal::readOGR(dirname(x), fn, stringsAsFactors = stringsAsFactors, :
## Dropping null geometries: 3
```

```
for (i in 1:length(AUS$NAME_1)){
  area <- append(area, raster::area(AUS[which(AUS$NAME_1 == paste0(AUS$NAME_1[i])),])/1000000)
}

BRA <- shapefile('MCMCglmm/maps/BRA.shp')
for (i in 1:length(BRA$NAME_1)){
  area <- append(area, raster::area(BRA[which(BRA$NAME_1 == paste0(BRA$NAME_1[i])),])/1000000)
}

CAN <- shapefile('MCMCglmm/maps/CAN.shp')
for (i in 1:length(CAN$NAME_1)){
  area <- append(area, raster::area(CAN[which(CAN$NAME_1 == paste0(CAN$NAME_1[i])),])/1000000)
}

CHN <- shapefile('MCMCglmm/maps/CHN.shp')
for (i in 1:length(CHN$NAME_1)){
  area <- append(area, raster::area(CHN[which(CHN$NAME_1 == paste0(CHN$NAME_1[i])),])/1000000)
}

IND <- shapefile('MCMCglmm/maps/IND.shp')
for (i in 1:length(IND$NAME_1)){
  area <- append(area, raster::area(IND[which(IND$NAME_1 == paste0(IND$NAME_1[i])),])/1000000)
}

IDN <- shapefile('MCMCglmm/maps/IDN.shp')
for (i in 1:length(IDN$NAME_1)){
  area <- append(area, raster::area(IDN[which(IDN$NAME_1 == paste0(IDN$NAME_1[i])),])/1000000)
}

RUS <- shapefile('MCMCglmm/maps/RUS.shp')
for (i in 1:length(RUS$NAME_1)){
  area <- append(area, raster::area(RUS[which(RUS$NAME_1 == paste0(RUS$NAME_1[i])),])/1000000)
}

TUR <- shapefile('MCMCglmm/maps/TUR.shp')
```

```
for (i in 1:length(TUR$NAME_1)){
  area <- append(area, raster::area(TUR[which(TUR$NAME_1 == paste0(TUR$NAME_1[i])),])/1000000)
}

US <- shapefile('MCMCglmm/maps/USA.shp')
for (i in 1:length(US$NAME_1)){
  area <- append(area, raster::area(US[which(US$NAME_1 == paste0(US$NAME_1[i])),])/1000000)
}

area.dat <- data.frame("division" = c(ischISO, AUS$NAME_1, BRA$NAME_1, CAN$NAME_1, CHN$NAME_1, IND$NAME_
```

Add areas for each species

```
Range <- vector()
for (j in 1:length(isch_dat$Taxon)){
  divs <- unlist(c(strsplit(as.character(isch_dat$Countries[j]), split = ","),
             strsplit(paste0(isch_dat$Other_division[j]), split = ",")))
  ar <- 0
  for (i in divs){
    temp <- area.dat$area[which(area.dat$division == paste0(i))]
    ar <- ar + temp
  }
  Range[j] <- ar
}

isch_dat <- cbind(isch_dat[2:5],Range)
```

I. ezoin is endemic of Ogasawara islands in Japan, according to IUCN the estimated extent of occurrence is 258 km2

```
isch_dat$Range[which(isch_dat$Taxon  == "Ischnura_ezoin")] <- 258
```

Call the "geiger" package for "name.check" function

```
require(geiger)
```

Check that species names from literature data match species names in tree file

```
Taxa <- data.frame(Taxon = unique(isch_dat$Taxon))
rownames(Taxa) <- Taxa$Taxon
name.check(tres[[1]], Taxa)
```

```
## [1] "OK"
```

Call the "MCMCglmm" package for creating MCMC objects

```
require(MCMCglmm)
```

Calculate inverted relatedness matrix

```
inv.phylo <- inverseA(tres[[1]], nodes="TIPS",scale=TRUE)
```

2) Analysis

Run 1

Set prior

```
prior.1 <- list(B = list(mu = c(0,0), V = diag(2) * (1 + pi^2/3)),
                G=list(G1=list(V=1,nu=1000,alpha.mu=rep(0,1),alpha.V=diag(1))),
                R = list(V = 1, fix = 1))
```

Start run

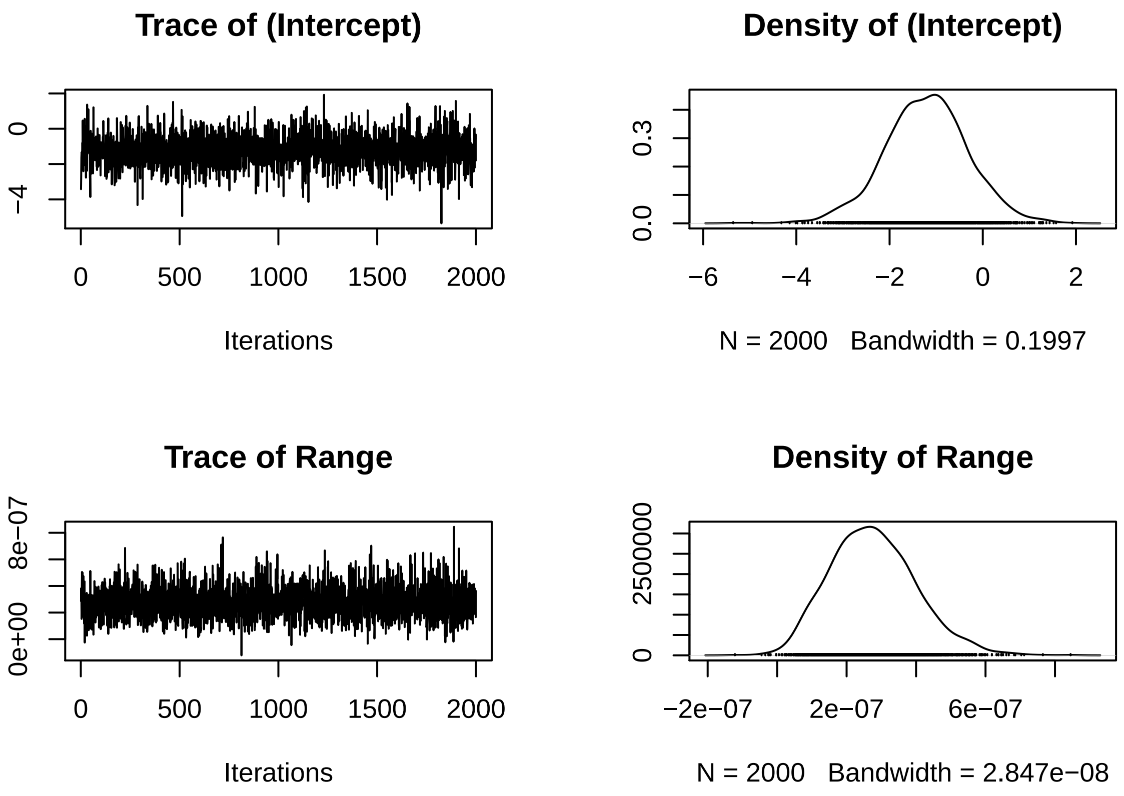
```
m1.bin.start <- MCMCglmm(FemState.bin ~ Range,
                         random = ~ Taxon,
                         rcov = ~ units,
                         ginverse=list(Taxon=inv.phylo$Ainv),
                         family ="categorical", data = isch_dat,
                         prior = prior.1, pl=TRUE,slice=TRUE,
                         nitt=2000, burnin=0, thin=1,verbose = F)
```

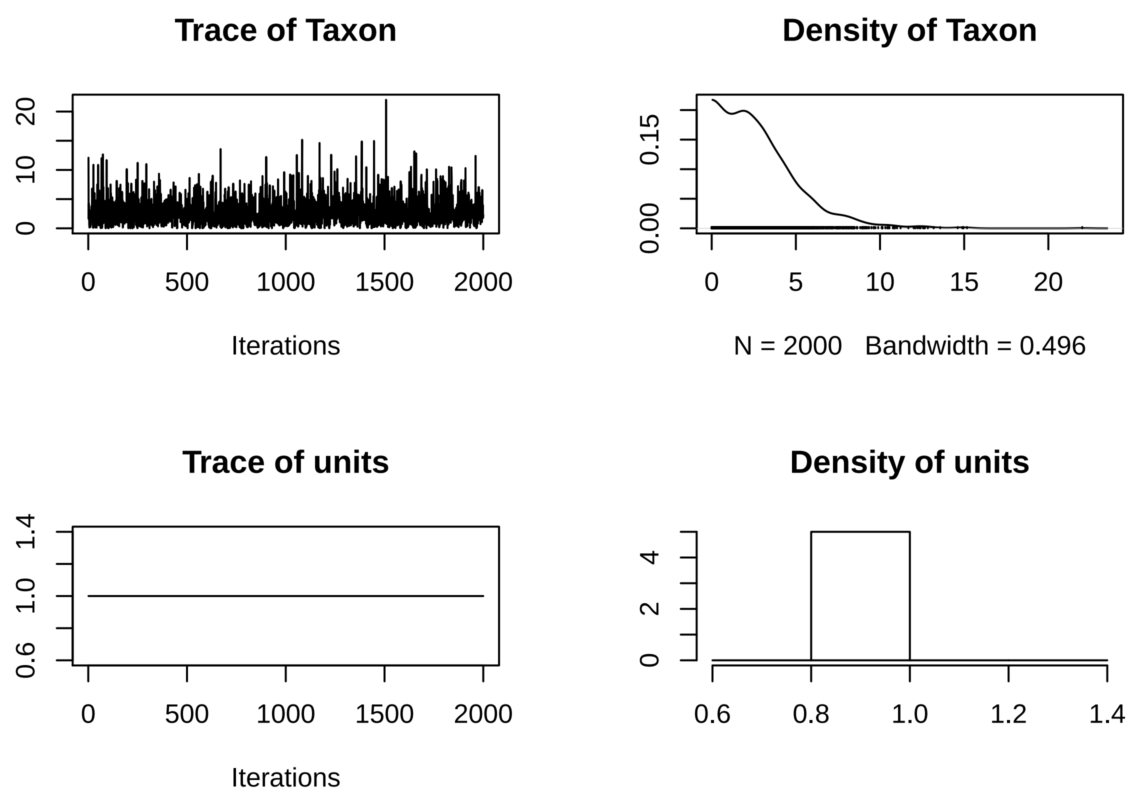
Loop over multiple trees, saving the last iteration of 10,000 iterations for each tree -100

```
m1.bin.multiphyMCMC <- m1.bin.start

for(i in 1:length(tres)){
  IN.tree <- inverseA(tres[[i]],nodes="TIPS")
  start <- list(Liab = m1.bin.multiphyMCMC$Liab[1,],
                R=list(R1=matrix(ncol=1,nrow=1,m1.bin.multiphyMCMC$VCV[1,2])),
                G=list(G1=matrix(ncol=1,nrow=1,m1.bin.multiphyMCMC$VCV[1,1])))

  m1.bin.multiphyMCMC <- MCMCglmm(FemState.bin ~ Range,
                                  random = ~ Taxon,
                                  rcov = ~ units,
                                  ginverse=list(Taxon=inv.phylo$Ainv),
                                  family ="categorical", data = isch_dat, prior = prior.1,
                                  pl=TRUE,slice=TRUE, nitt=10000, thin=1,
                                  burnin=9999, start=start, verbose=F)
  if(i>100){
    m1.bin.start$VCV[i-100,] <- m1.bin.multiphyMCMC$VCV[1,]
    m1.bin.start$Sol[i-100,] <- m1.bin.multiphyMCMC$Sol[1,]
    m1.bin.start$Liab[i-100,] <- m1.bin.multiphyMCMC$Liab[1,]
  }
}
```

Visual check:

```
plot(m1.bin.start)
```


Trace of (Intercept)
Iterations
Density of (Intercept)
N = 2000 Bandwidth = 0.1997
Trace of Range
Iterations
Density of Range
N = 2000 Bandwidth = 2.847e−08



Summary:

```r
summary(m1.bin.start)
```

```
##
##  Iterations = 1:2000
##  Thinning interval  = 1
##  Sample size  = 2000
##
##  DIC: 40.87433
##
##  G-structure:  ~Taxon
##
##       post.mean l-95% CI u-95% CI eff.samp
## Taxon     2.946 1.38e-05    7.511     2000
##
##  R-structure:  ~units
##
##       post.mean l-95% CI u-95% CI eff.samp
## units         1        1        1        0
##
##  Location effects: FemState.bin ~ Range
##
##              post.mean   l-95% CI   u-95% CI eff.samp pMCMC
## (Intercept) -1.212e+00 -3.097e+00  4.650e-01     2000 0.171
## Range        2.764e-07  5.267e-08  5.196e-07     2000 0.008 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Hypothesis test: what is the posterior probability that the probability of polymorphism does not increase with distribution range

```
PMCMC <- sum(m1.bin.start$Sol[,'Range'] <= 0)/length((m1.bin.start$Sol[,'Range'] <= 0))
PMCMC
```

```
## [1] 0.004
```

Posterior mean and HPD interval for the effect of an increase of 1 million Km2 on the probability of being polymorphic

```
PM <- plogis(mean(m1.bin.start$Sol[,1]) + mean(m1.bin.start$Sol[,2])* 1e6) -
      plogis(mean(m1.bin.start$Sol[,1]))
PM
```

```
## [1] 0.05245875
```

```
lwr <- plogis(mean(m1.bin.start$Sol[,1]) + HPDinterval(m1.bin.start$Sol[,2])[1]* 1e6) -
      plogis(mean(m1.bin.start$Sol[,1]))
lwr
```

```
## [1] 0.009444574
```

```
upr <- plogis(mean(m1.bin.start$Sol[,1]) + HPDinterval(m1.bin.start$Sol[,2])[2]* 1e6) -
      plogis(mean(m1.bin.start$Sol[,1]))
upr
```

```
## [1] 0.1041674
```

Obtain estimate using "predict" function

```
Pred <- predict.MCMCglmm(object =  m1.bin.start, newdata = isch_dat, type = "response",
                         interval = "confidence")
HPDinterval(m1.bin.start$Sol[,2])
```

```
##             lower        upper
## var1 5.267299e-08 5.195734e-07
## attr(,"Probability")
## [1] 0.95
```

Make the fitted data frame

```
Pdat <- cbind(isch_dat, Pred)

for(i in 1:nrow(Pdat)){
  if(Pdat$FemState.bin[i] == "P"){
    Pdat$P[i] <- 1
  }
  else {Pdat$P[i] <- 0}
}
```

Run 2

Start run

```
m2.bin.start <- MCMCglmm(FemState.bin ~ Range,
                         random = ~ Taxon,
                         rcov = ~ units,
                         ginverse=list(Taxon=inv.phylo$Ainv),
                         family ="categorical", data = isch_dat,
                         prior = prior.1, pl=TRUE,slice=TRUE,
                         nitt=2000, burnin=0, thin=1,verbose = F)
```

Loop over multiple trees, saving the last iteration of 10,000 iterations for each tree -100

```
m2.bin.multiphyMCMC <- m2.bin.start

for(i in 1:length(tres)){
  IN.tree <- inverseA(tres[[i]],nodes="TIPS")
  start <- list(Liab = m2.bin.multiphyMCMC$Liab[1,],
                R=list(R1=matrix(ncol=1,nrow=1,m2.bin.multiphyMCMC$VCV[1,2])),
                G=list(G1=matrix(ncol=1,nrow=1,m2.bin.multiphyMCMC$VCV[1,1])))

  m2.bin.multiphyMCMC <- MCMCglmm(FemState.bin ~ Range,
                                  random = ~ Taxon,
                                  rcov = ~ units,
                                  ginverse=list(Taxon=inv.phylo$Ainv),
                                  family ="categorical", data = isch_dat, prior = prior.1,
                                  pl=TRUE,slice=TRUE, nitt=10000, thin=1,
                                  burnin=9999, start=start, verbose=F)
  if(i>100){
    m2.bin.start$VCV[i-100,]<- m2.bin.multiphyMCMC$VCV[1,]
    m2.bin.start$Sol[i-100,]<- m2.bin.multiphyMCMC$Sol[1,]
    m2.bin.start$Liab[i-100,]<- m2.bin.multiphyMCMC$Liab[1,]
  }
}
```

Put them into a list

```
mh.list <- mcmc.list(m1.bin.start$Sol, m2.bin.start$Sol)
```

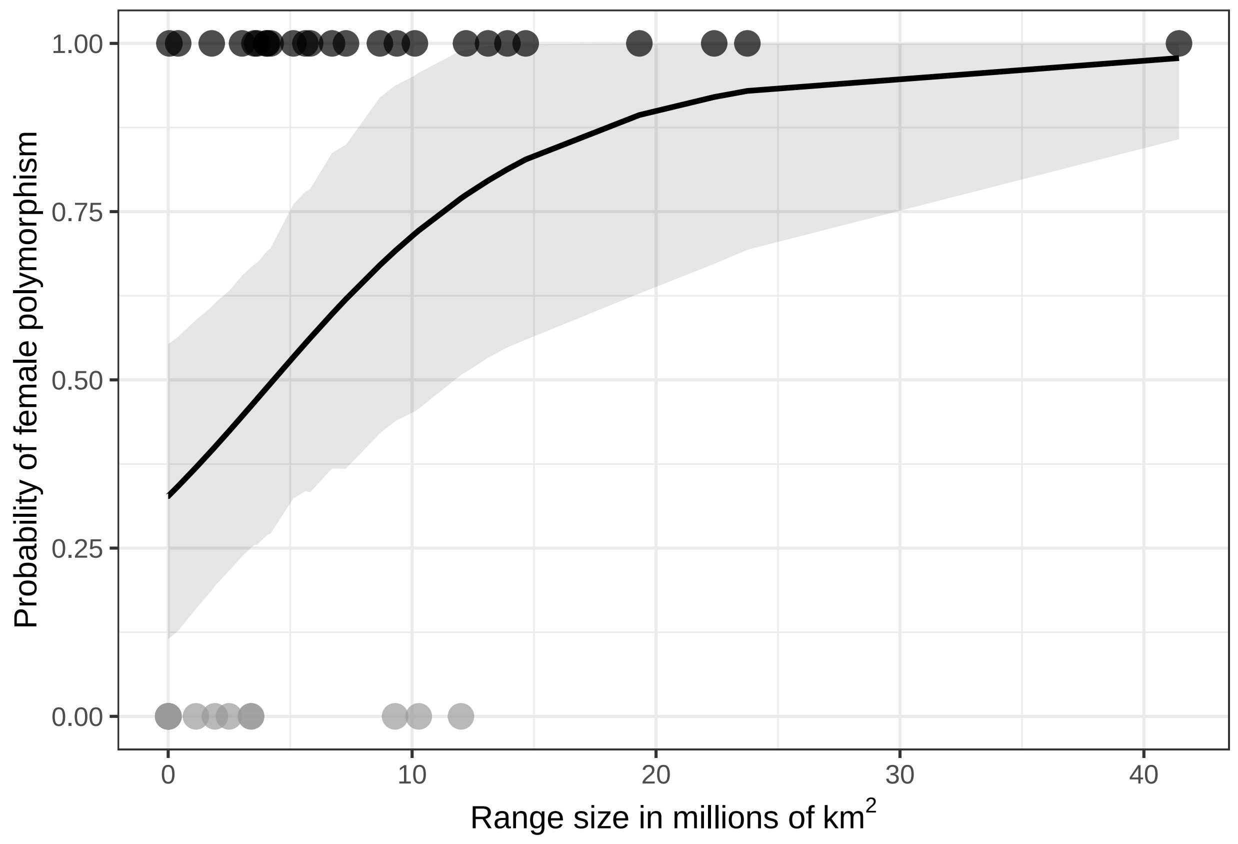
3) Plotting

Call “ggplot2” for plotting, “wesanderson” for colour scheme and “scales” for formatting axis scales

```
require(ggplot2)
require(wesanderson)
require(scales)
```

Plot probability of polymorphism against range size

```r
p1 <- ggplot(data = Pdat, aes(x=Range, y = fit))+
  geom_line(size=1, show.legend = NA, color = "black")+
  theme_bw(base_size = 12)+
  geom_ribbon(aes(ymax = upr, ymin = lwr, x = Range), fill = "black", color=NA,
              alpha = 0.1)+
  geom_point(data = Pdat, aes(color = FemState.bin, fill = FemState.bin, y = P),
             alpha = 0.7 , size = 4, show.legend = FALSE )+
  ylab(label = "Probability of female polymorphism")+
  xlab(label=c(expression("Range size in millions of" ~km^2))) +
  scale_color_manual(breaks = c("M", "P"),
                     values = c("grey60", "black")) +
  scale_fill_manual(breaks = c("M", "P"),
                    values = c("grey60", "black")) +
  scale_x_continuous(labels = number_format(scale = 1e-6))
  theme(panel.grid.major = element_blank(), panel.grid.minor = element_blank())

p1
```



Monomorphic: light grey
Polymorphic: dark grey

Call plotting packages

```r
require(gridExtra)
require(gtable)
require(ggtree)
```

Plot range size per species and probablity of polymorphism against range size

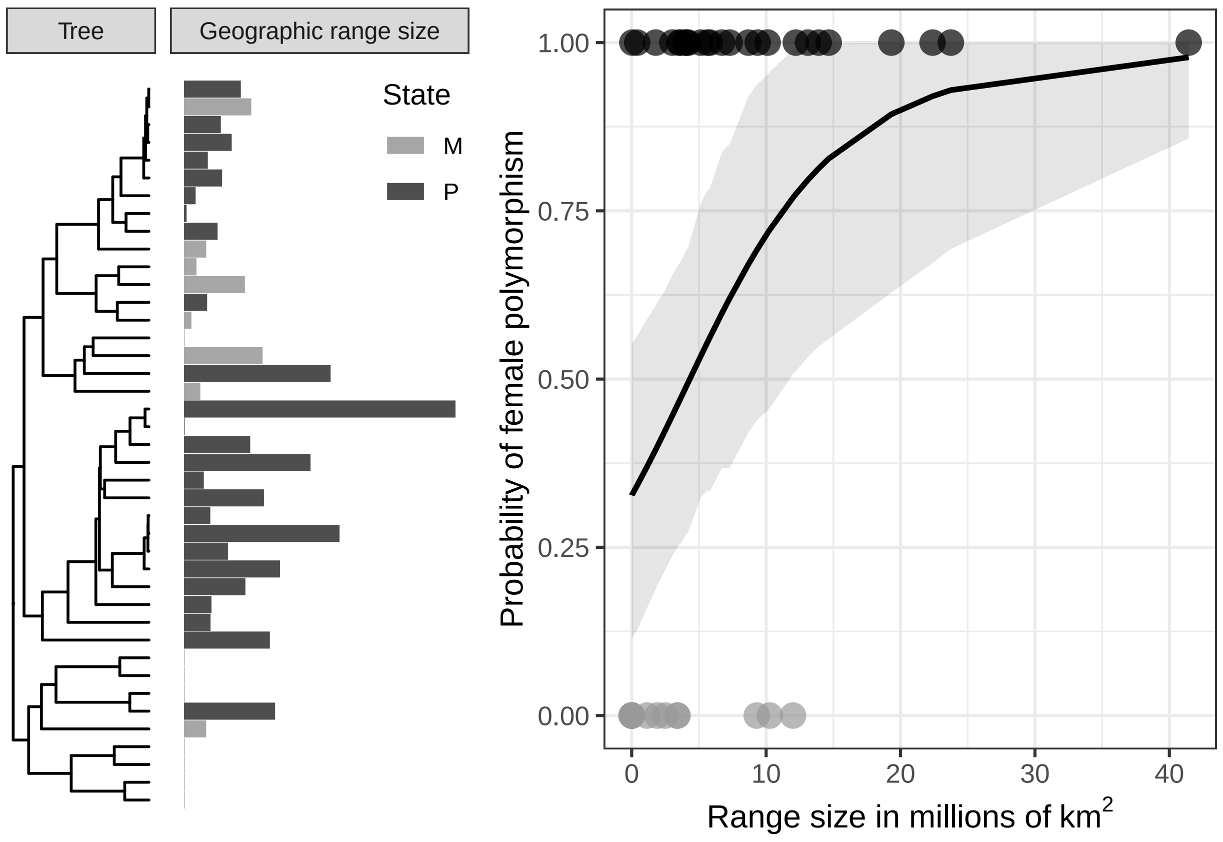
```r
tre1 <- read.nexus(file ="StarBEAST2/summary.tree")
range_dat <- data.frame(Species = isch_dat[,1],  State = isch_dat[,3], Range =isch_dat$Range)

for (i in 1:length(range_dat$Species)){
  if (range_dat$Range[i] < 100000){
    range_dat$Range[i] <- 100000
  }
}
p <- ggtree(tre1)
```

```
## Warning: `data_frame()` is deprecated as of tibble 1.1.0.
## Please use `tibble()` instead.
## This warning is displayed once every 8 hours.
## Call `lifecycle::last_warnings()` to see where this warning was generated.
```

```r
p2 <- facet_plot(p, panel="Geographic range size", data=range_dat, geom=geom_segment, mapping = aes(x=0

gt = ggplot_gtable(ggplot_build(p2))
gt$layout$l[grep('panel-1', gt$layout$name)]
```

```
## [1] 5
```

```r
gt$widths[5] = 0.5*gt$widths[5]
lay <- rbind(c(1,1,2,2,2))
Fig5 <- grid.arrange(gt, p1, ncol = 2, layout_matrix = lay)
```



Save plot as a PDF

```
ggsave("Fig5.pdf", plot = Fig5, device = NULL, path = "Figures",
       scale = 1, width = 7, height = 5, dpi = 600, limitsize = TRUE,
       units = "in")
```

Diagnostics

a) Gelman-Rubin statistic

Gelman-rubin diagnostic of convergence

```
GR <- gelman.diag(mh.list,confidence = 0.95, transform=TRUE, autoburnin=FALSE,
                  multivariate=T)
GR
```

```
## Potential scale reduction factors:
##
##             Point est. Upper C.I.
## (Intercept)          1       1.01
## Range                1       1.00
##
## Multivariate psrf
##
## 1
```

b) Autocorrelation

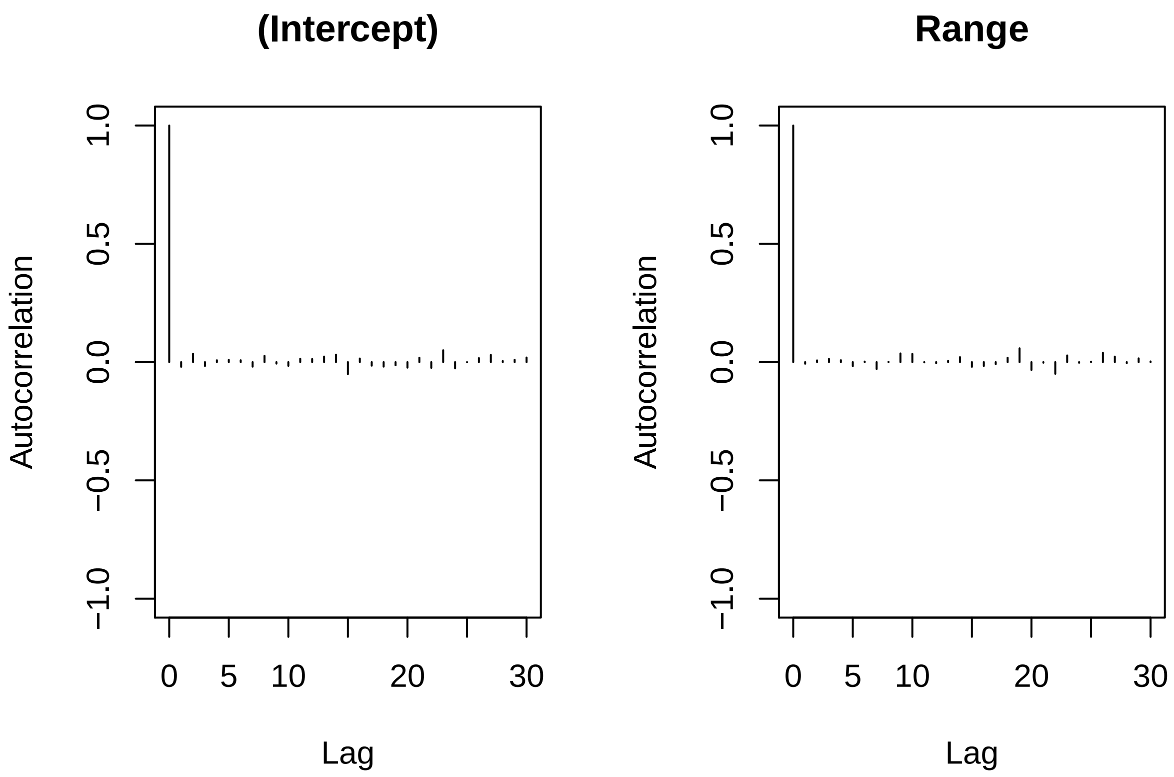
Calculate autocorrelation between draws

```
diag(autocorr(m1.bin.start$Sol)[2, , ])
```

```
##  (Intercept)        Range
## -0.019830998 -0.006898415
```

Present autocorrelation in plot form

```
autocorr.plot(m1.bin.start$Sol)
```



c) Effective sample size

Calculate effective sample size

```
effectiveSize(m1.bin.start$Sol)
```

```
## (Intercept)       Range
##        2000        2000
```

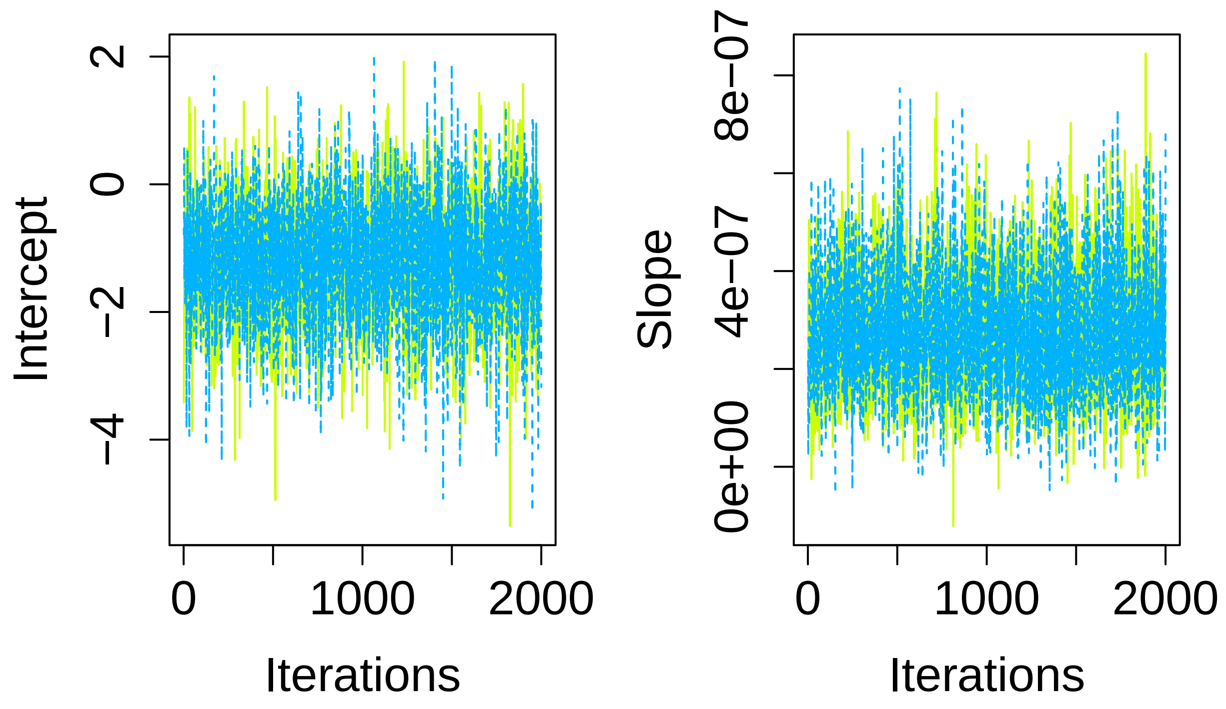
d) Visual diagnostic of trace convergence and mixing

Prepare data for plotting multiple traces

```
colnames(m1.bin.start$Sol)[1] <- "Intercept"

for (i in 1:length(colnames(m1.bin.start$Sol))){
  for (j in 1:2){
    temp <- eval(parse(text=paste("m", j,".bin.start$Sol[,",i,"]", sep = "")))
    assign(paste("trace_","mcmc", j, colnames(m1.bin.start$Sol)[i], sep=""), temp)
  }
}

temp_list = mcmc.list()
for (i in 1:length(colnames(m1.bin.start$Sol))){
  for (j in 1:2){
    temp_list[[j]] <-  eval(parse(text=paste("trace_mcmc", j ,
                                             colnames(m1.bin.start$Sol)[i], sep = "")))
    assign(paste("trace_", colnames(m1.bin.start$Sol)[i], sep = ""), temp_list)
  }
}
```

Plot traces

```
ylabs <- c("Intercept", "Slope")
par(mfrow=c(1,2))
for (i in 1:2){
  temp <-  eval(parse(text=paste("trace_", colnames(m1.bin.start$Sol)[i], sep = "")))
  traceplot(temp,col = rainbow(3, start=0.2, end=0.9), ylab = ylabs[i], cex.lab=1.5, cex.axis=1.5)
}
```

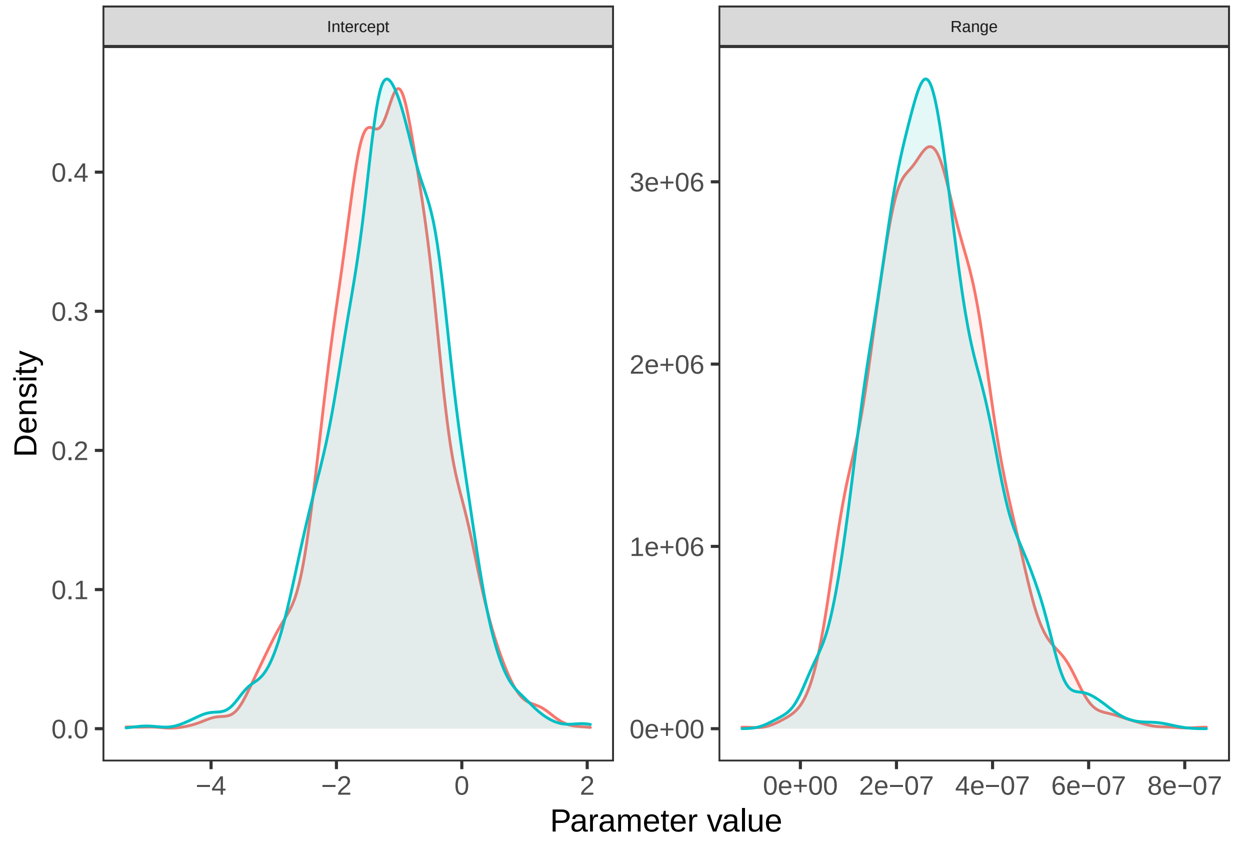


Prepare data for plotting multiple density curves

```
colnames(m2.bin.start$Sol)[1] <- "Intercept"

reshaping <- function(x) {
  y <- rbind(t(x))
  result <- setNames(as.data.frame.table(y),c("variable","run","value"))
}

for (i in 1:2){
  temp <- eval(parse(text=paste("m", i, ".bin.start$Sol", sep = "")))
  reshape <- reshaping(temp)
  reshape$run <- as.factor(rep(i, nrow(reshape)))
  assign(paste("reshape", i, sep = ""), reshape)
}

plot_df <- rbind(reshape1,reshape2)
```

Plot by each parameter

```
p <- ggplot(plot_df, aes(x= value, fill = run, colour = run)) +
  geom_density(alpha = 0.1) +
  theme_bw(base_size = 12)+
  theme(legend.position = "none", strip.text.x = element_text(size = 6),
        panel.grid.major = element_blank(), panel.grid.minor = element_blank())+
  labs(x="Parameter value", y = "Density") +
  facet_wrap(~variable, scales = "free", ncol = 3, nrow = 5)

p
```



Run with log

Start run

```
m3.bin.start <- MCMCglmm(FemState.bin ~ log(Range),
                         random = ~ Taxon,
                         rcov = ~ units,
                         ginverse=list(Taxon=inv.phylo$Ainv),
                         family ="categorical", data = isch_dat,
                         prior = prior.1, pl=TRUE,slice=TRUE,
                         nitt=2000, burnin=0, thin=1,verbose = F)
```

Loop over multiple trees, saving the last iteration of 10,000 iterations for each tree -100

```
m3.bin.multiphyMCMC <- m3.bin.start

for(i in 1:length(tres)){
  IN.tree <- inverseA(tres[[i]],nodes="TIPS")
  start <- list(Liab = m3.bin.multiphyMCMC$Liab[1,],
                R=list(R1=matrix(ncol=1,nrow=1,m3.bin.multiphyMCMC$VCV[1,2])),
                G=list(G1=matrix(ncol=1,nrow=1,m3.bin.multiphyMCMC$VCV[1,1])))

  m3.bin.multiphyMCMC <- MCMCglmm(FemState.bin ~ log(Range),
                                  random = ~ Taxon,
                                  rcov = ~ units,
                                  ginverse=list(Taxon=inv.phylo$Ainv),
                                  family ="categorical", data = isch_dat, prior = prior.1,
                                  pl=TRUE,slice=TRUE, nitt=10000, thin=1,
                                  burnin=9999, start=start, verbose=F)
  if(i>100){
    m3.bin.start$VCV[i-100,]<- m3.bin.multiphyMCMC$VCV[1,]
    m3.bin.start$Sol[i-100,]<- m3.bin.multiphyMCMC$Sol[1,]
    m3.bin.start$Liab[i-100,]<- m3.bin.multiphyMCMC$Liab[1,]
  }
}
```

Hypothesis test: what is the posterior probability that the probability of polymorphism does not increase with distribution range

```
PMCMC <- sum(m3.bin.start$Sol[,2] <= 0)/length((m3.bin.start$Sol[,2] <= 0))
PMCMC
```

```
## [1] 0.009
```

Posterior mean and HPD interval for the effect of an increase of 1 million Km2 on the probability of being polymorphic

```
PM <- plogis(mean(m3.bin.start$Sol[,1]) + mean(m3.bin.start$Sol[,2])) -
  plogis(mean(m3.bin.start$Sol[,1]))
PM
```

```
## [1] 0.01130726
```

```
lwr <- plogis(mean(m3.bin.start$Sol[,1]) + HPDinterval(m3.bin.start$Sol[,2])[1]) -
  plogis(mean(m3.bin.start$Sol[,1]))
lwr
```

```
## [1] 0.001593481
```

```
upr <- plogis(mean(m3.bin.start$Sol[,1]) + HPDinterval(m3.bin.start$Sol[,2])[2]) -
  plogis(mean(m3.bin.start$Sol[,1]))
upr
```

```
## [1] 0.02338128
```

Obtain estimate using “predict” function

```
Pred3 <- predict.MCMCglmm(object =  m3.bin.start, newdata = isch_dat, type = "response",
                          interval = "confidence")
HPDinterval(m3.bin.start$Sol[,2])
```

```
##            lower     upper
## var1 0.04394925 0.5155364
## attr(,"Probability")
## [1] 0.95
```

Make the fitted data frame

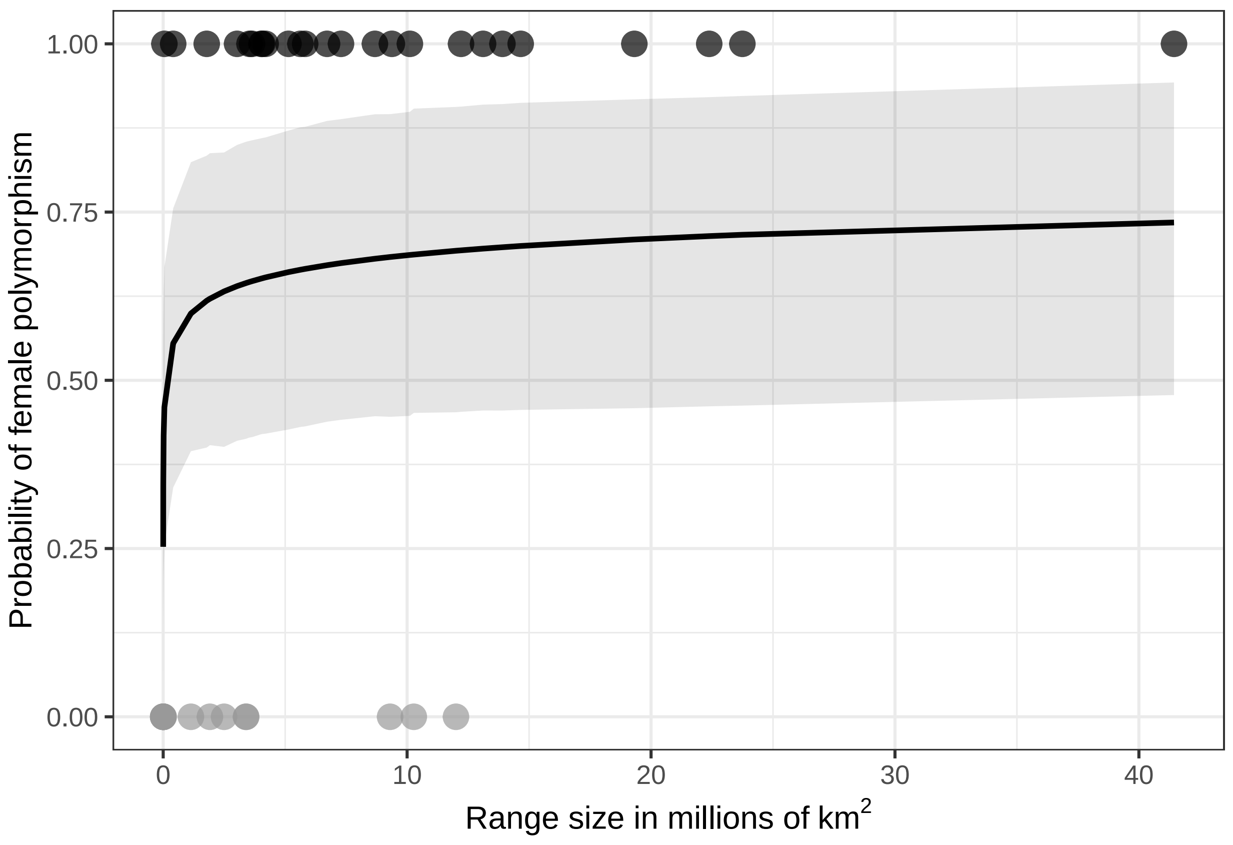
```
Pdat3 <- cbind(isch_dat, Pred3)

for(i in 1:nrow(Pdat3)){
  if(Pdat3$FemState.bin[i] == "P"){
    Pdat3$P[i] <- 1
  }
  else {Pdat3$P[i] <- 0}
}
```

Plot

```
p3 <- ggplot(data = Pdat3, aes(x=Range, y = fit))+
  geom_line(size=1, show.legend = NA, color = "black")+
  theme_bw(base_size = 12)+
  geom_ribbon(aes(ymax = upr, ymin = lwr, x = Range), fill = "black", color=NA,
            alpha = 0.1)+
  geom_point(data = Pdat3, aes(color = FemState.bin, fill = FemState.bin, y = P),
           alpha = 0.7 , size = 4, show.legend = FALSE )+
  ylab(label = "Probability of female polymorphism")+
  xlab(label=c(expression("Range size in millions of" ~km^2))) +
  scale_color_manual(breaks = c("M", "P"),
                     values = c("grey60", "black")) +
  scale_fill_manual(breaks = c("M", "P"),
                    values = c("grey60", "black")) +
  scale_x_continuous(labels = number_format(scale = 1e-6))
theme(panel.grid.major = element_blank(), panel.grid.minor = element_blank())
```

```
## List of 2
##  $ panel.grid.major: list()
##   ..- attr(*, "class")= chr [1:2] "element_blank" "element"
##  $ panel.grid.minor: list()
##   ..- attr(*, "class")= chr [1:2] "element_blank" "element"
##  - attr(*, "class")= chr [1:2] "theme" "gg"
##  - attr(*, "complete")= logi FALSE
##  - attr(*, "validate")= logi TRUE
```

```
p3
```


1.00
0.75
0.50
0.25
0.00
0
10
20
30
40
Probability of female polymorphism
Range size in millions of km^2