

MAPpoly training section

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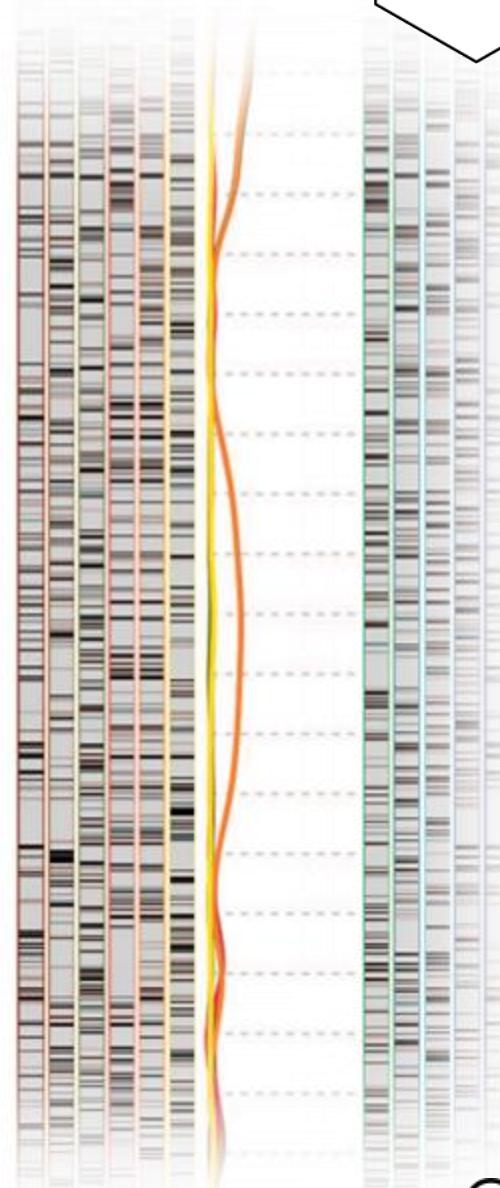
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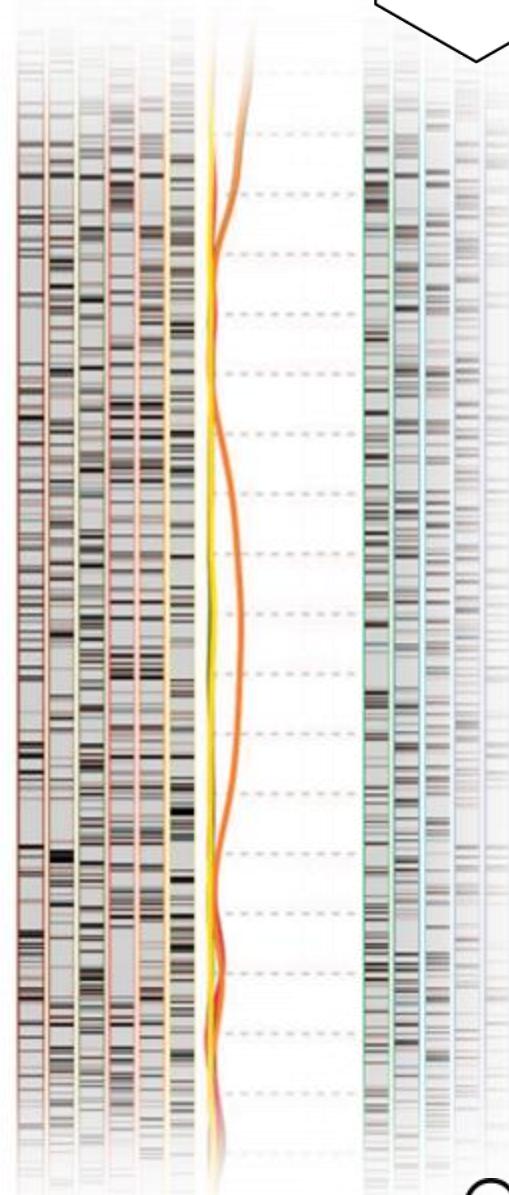
Outline

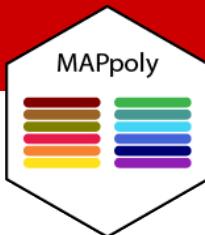
- Installation
- Reading/import datasets
- Filtering
- Two-point analysis
- Grouping
- Ordering
- Phasing
- Modeling errors/genotype probabilities
- Final checking
- Map summary/results
- Genotype probabilities
- Preferential pairing/haplotype probabilities
- Hexaploid scripts/results



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Initial notes about MAPpoly

- Primary intention was implementing a program to construct genetic maps in polyploids with high ploidy levels (i.e. > 4: sugarcane, sweetpotato, etc.)
- Linkage Analysis and Haplotype Phasing in Experimental Autopolyploid Populations with High Ploidy Level Using Hidden Markov Models ([Mollinari and Garcia, 2019](#))
- Obtain conditional probabilities for QTL analysis
- It does not model **double reduction**: double reduced markers/individuals are treated as missing data



BILL &
MELINDA
GATES
foundation



Installation

- From CRAN (stable version)



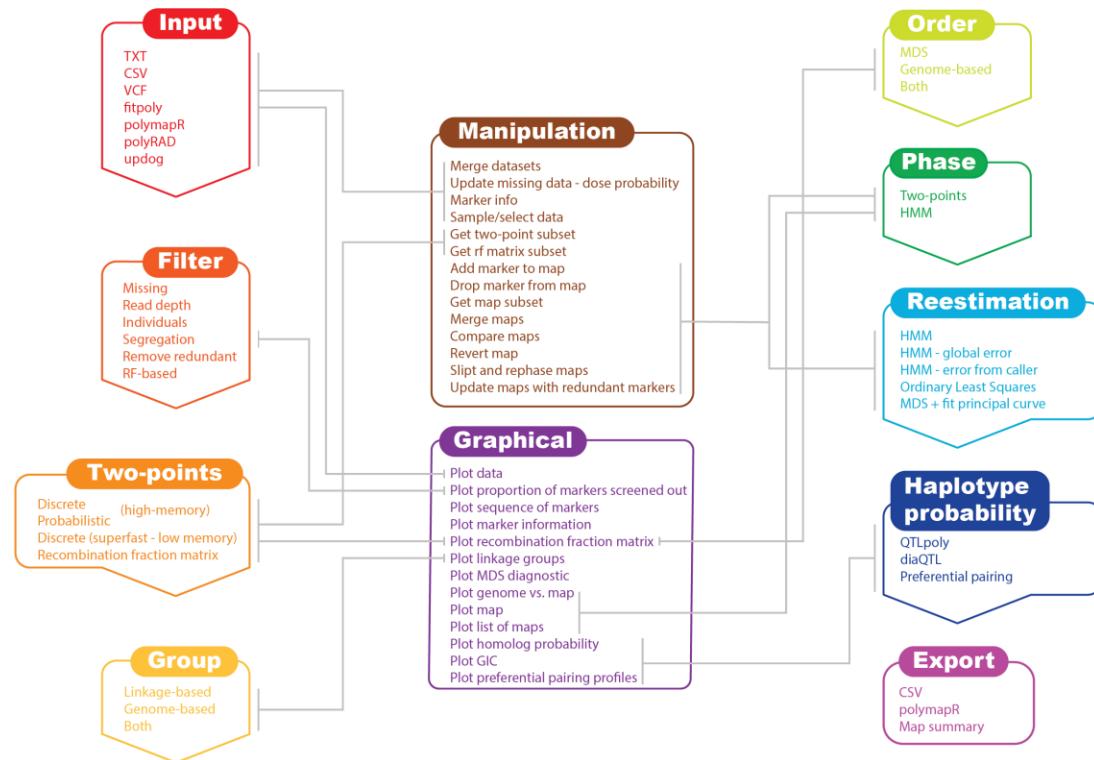
```
install.packages("mappoly")
```

- From GitHub (development version)



```
install.packages("devtools")
devtools::install_github("mmollina/mappoly", dependencies=TRUE)
```

MAPpoly workflow

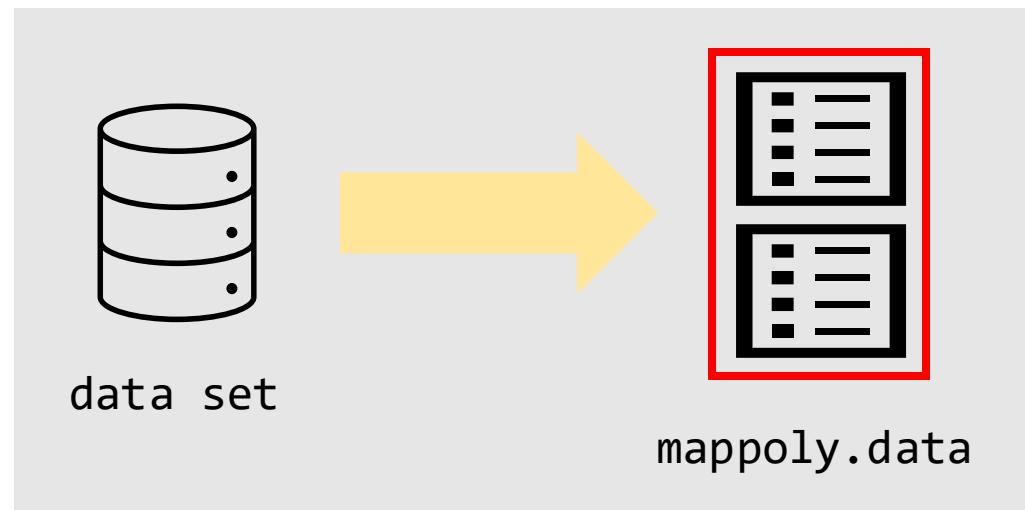


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Reading/importing data sets

Supported datasets

- MAPpoly files
 - Discrete dosage
 - Dosage probability
- CSV files
- [fitPoly](#) files
 - Dosage Probability
- VCF files
 - Discrete dosage
 - Dosage probability



Supported R objects

- [updog](#) objects
- [polyRAD](#) objects
- [polymapR](#)
 - datasets
 - maps

Reading/importing datasets

- MAPpoly data reading functions

1. MAPpoly

```
dat.mappoly <- read_geno(file.in = 'path_to_input_file')
```

2. MAPpoly with genotype probabilities

```
dat.mappoly.prob <- read_geno_prob(file.in = 'path_to_input_file')
```

3. CSV

```
dat.csv <- read_geno_csv(file.in = 'path_to_input_file', ploidy = 6)
```

4. fitPoly, supports genotype probabilities

```
dat.fitpoly <- read_fitpoly(file.in = 'path_to_input_file', ploidy = 6,  
                             parent1 = 'P1', parent2 = 'P2')
```

5. VCF files, supports genotype probabilities (PL field)

```
dat.vcf <- read_vcf(file.in = 'path_to_input_file', ploidy = 6,  
                     parent1 = 'P1', parent2 = 'P2',  
                     min.gt.depth = 0, min.av.depth = 0)
```

Reading/importing datasets

- MAPpoly data importing functions

1. updog

```
dat.updog <- import_from_updog(object = input_multidog_object)
```

2. polyRAD

```
dat.polyRAD <- polyRAD::Export_MAPpoly(object = 'polyrad_object')
```

3. polymapR

Importing data

```
dat.polymapr <- import_data_from_polymapR(input.data = polymapR_screened_data,
                                              ploidy = 6, parent1 = 'P1',
                                              parent2 = 'P2')
```

Importing maps

```
map.polymapr <- import_phased_maplist_from_polymapR(maplist = phased_maplist,
                                                       mappoly.data = mappoly.data)
```

Reading/importing datasets

- Example: reading fitPoly files

```
## Downloading data from GitHub
library("mappoly")
setwd("SCRI/MAPpoly/tetra")
address <- "https://github.com/mmollina/SCRI/raw/main/data/fitpoly_tetra_call/B2721_scores.zip"
download.file(url = address, destfile = "B2721_scores.zip")
unzip(tempfl, files = "B2721_scores.dat")
## Reading fitPoly data
dat <- read_fitpoly(file.in = "B2721_scores.dat",
                     ploidy = 4,
                     parent1 = "Atlantic",
                     parent2 = "B1829",
                     verbose = TRUE)
source("get_solcap.snp_pos.R")
plot(dat)
print(dat, detailed = TRUE)
```



Genotype calling using fitPoly:

https://github.com/mmollina/SCRI/blob/main/data/fitpoly_tetra_call/B2721_fitpoly_call.R

Adding *Solanum tuberosum* genome v4.03 information:

https://github.com/mmollina/SCRI/blob/main/MAPpoly/get_solcap.snp_pos.R



Inspecting data loaded from fitPoly files

This is an object of class 'mappoly.data'

Ploidy level: 4
 No. individuals: 156
 No. markers: 6531
 Missing data under 0.95 prob. threshold: 5.26%
 Redundant markers: 7.92%

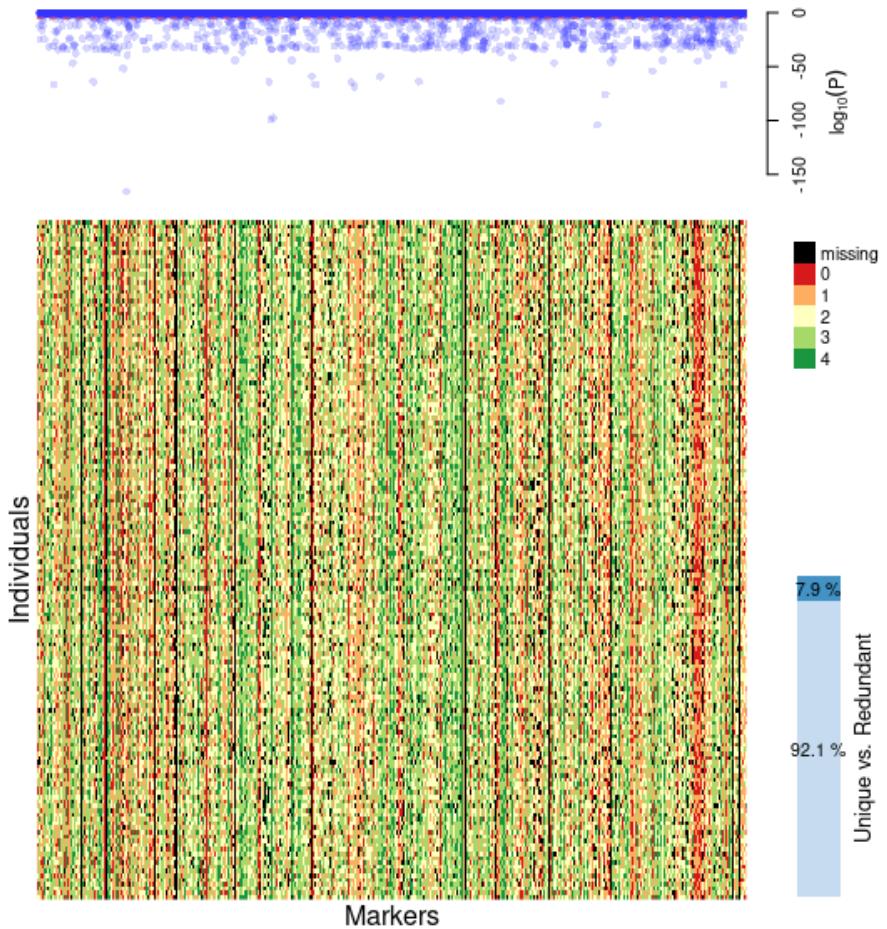
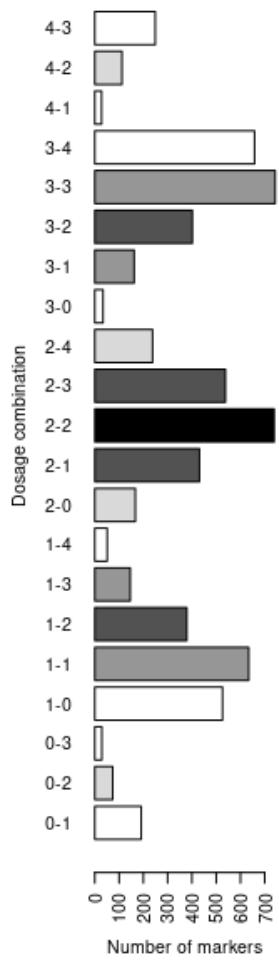
No. markers per sequence:

seq	No.mrk
1	661
2	613
3	538
4	672
5	485
6	531
7	543
8	469
9	496
10	364
11	470
12	415

Markers with no sequence information: 274

No. of markers per dosage combination in both parents:

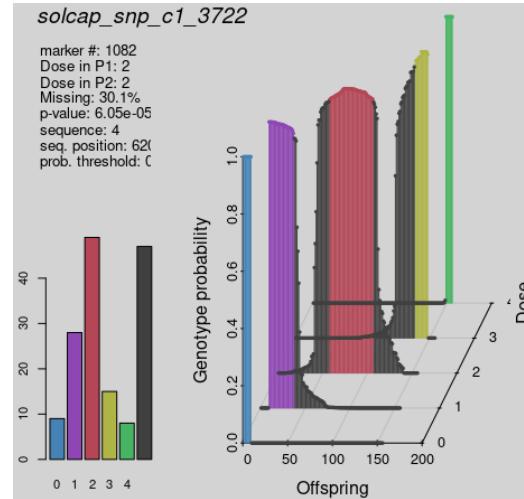
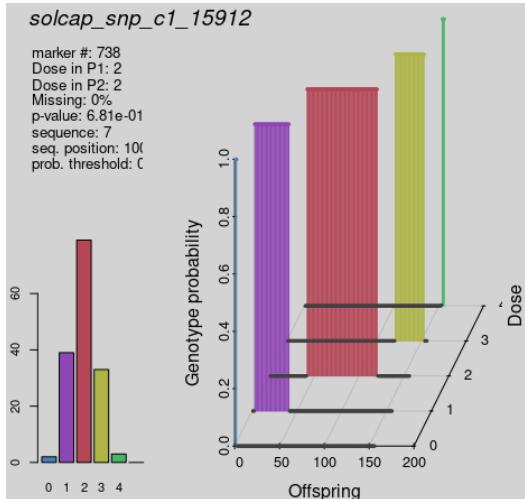
P1	P2	freq
0	1	191
0	2	74
0	3	29
1	0	526
1	1	634
1	2	379
1	3	146
1	4	51
2	0	167
2	1	431
2	2	739
2	3	538
2	4	238
3	0	34
3	1	162
3	2	402
3	3	743
3	4	658
4	1	28
4	2	112
4	3	249



Inspecting specific marker

```
plot_mrk_info(dat, 738)
print_mrk(dat, 738)
```

```
plot_mrk_info(dat, "solcap_snp_c1_3722")
print_mrk(dat, "solcap_snp_c1_3722")
```



solcap_snp_c1_15912

dosage P1: 2
dosage P2: 2

dosage distribution

0	1	2	3	4	mis
2	39	79	33	3	0

expected polysomic segregation

0	1	2	3	4
0.02777778	0.22222222	0.50000000	0.22222222	0.02777778

solcap_snp_c1_3722

dosage P1: 2
dosage P2: 2

dosage distribution

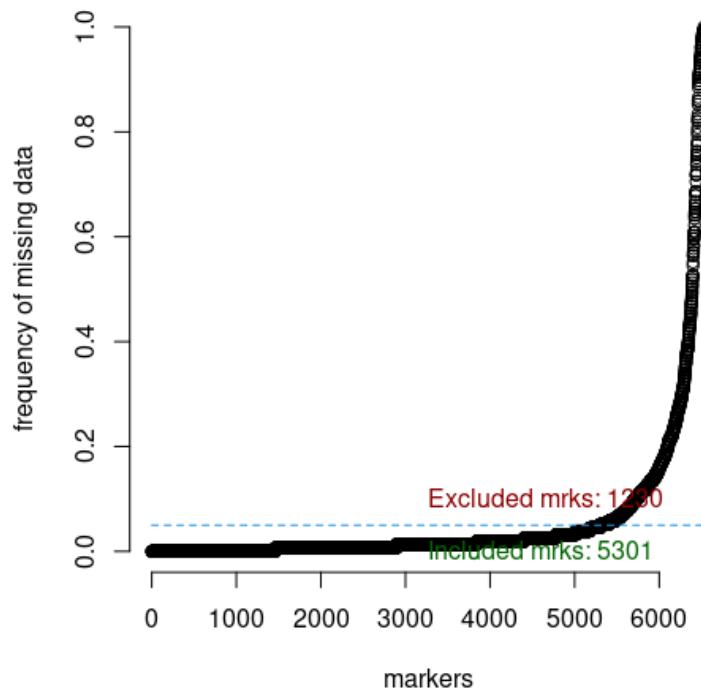
0	1	2	3	4	mis
9	28	49	15	8	47

expected polysomic segregation

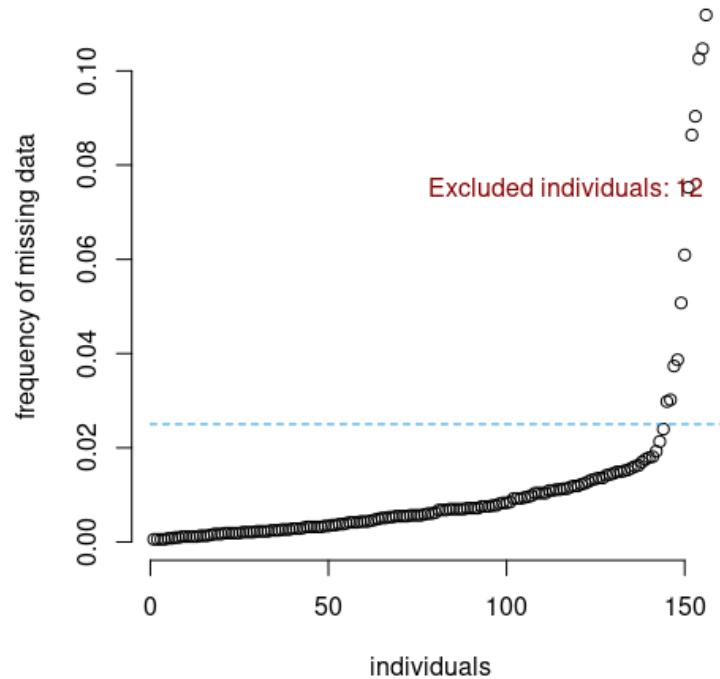
0	1	2	3	4
0.02777778	0.22222222	0.50000000	0.22222222	0.02777778

Filtering by missing data

```
dat <- filter_missing(input.data = dat,  
                      type = "marker",  
                      filter.thres = 0.05)
```

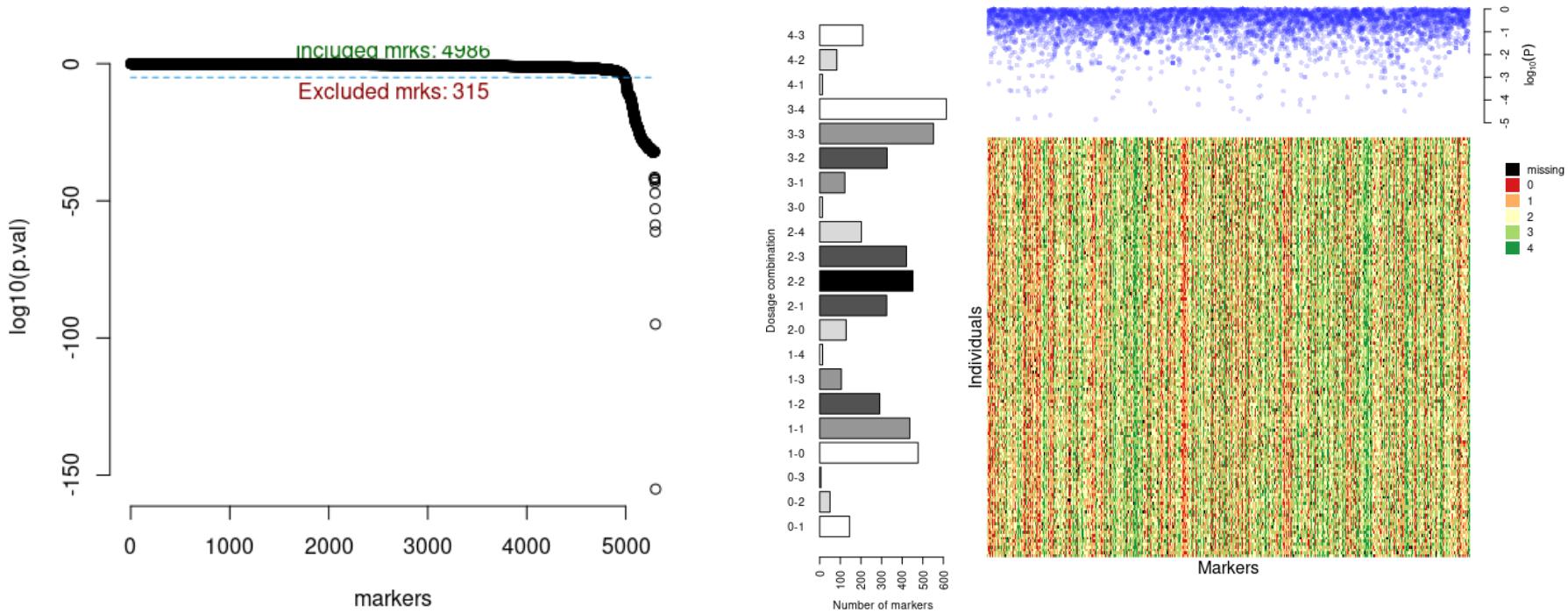


```
dat <- filter_missing(input.data = dat,  
                      type = "individual",  
                      filter.thres = 0.025)
```

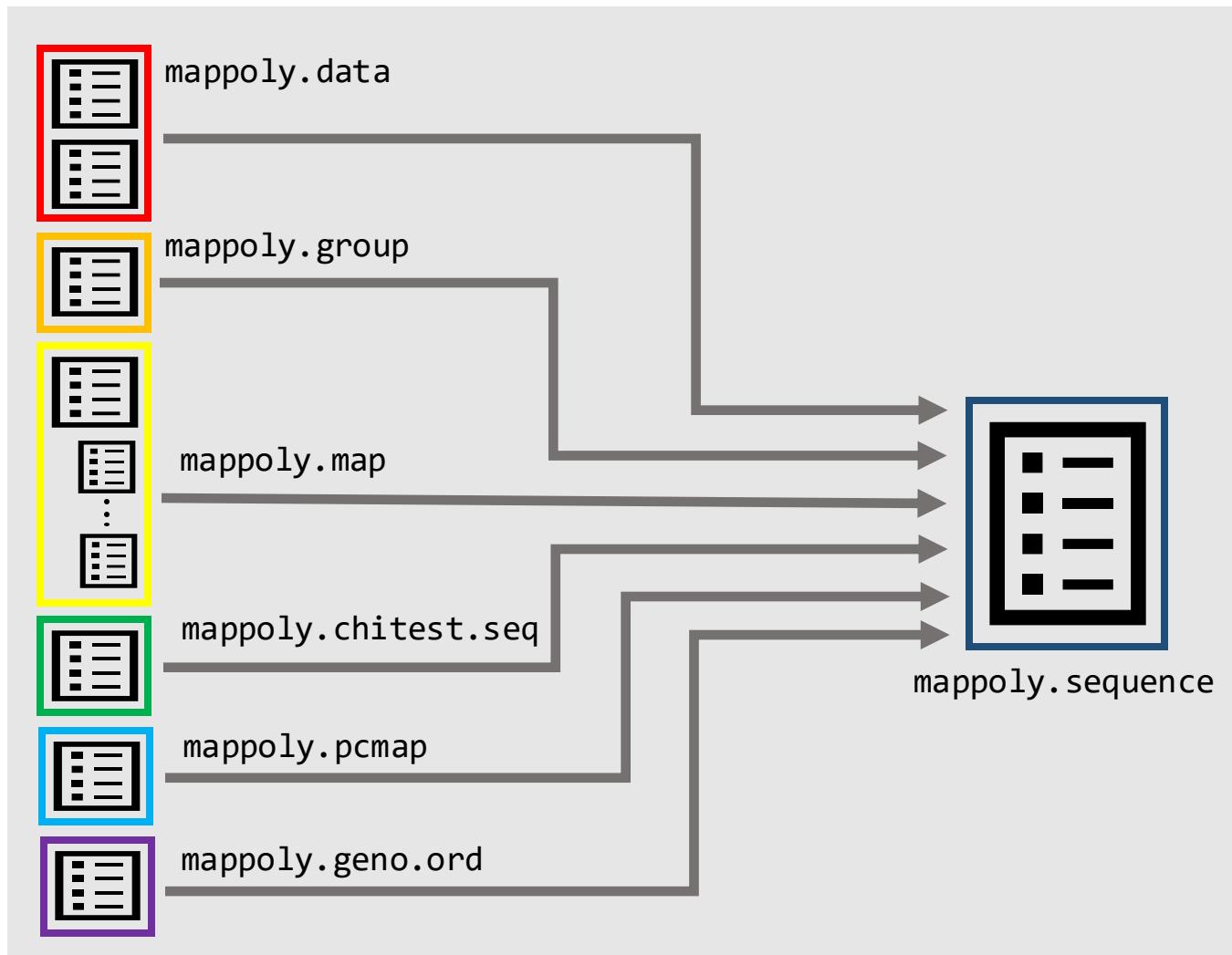


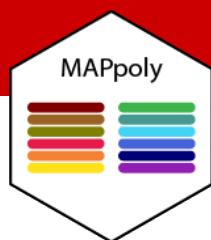
Filtering by segregation distortion

```
s <- filter_segregation(input.data = dat, chisq.pval.thres = 0.05/dat$n.mrk)
s <- make_seq_mappoly(s)
plot(s)
```



Making a sequence of markers

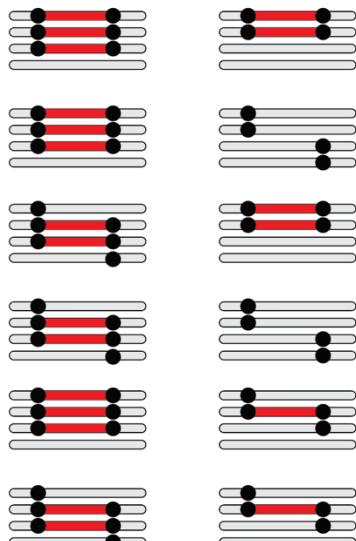




Two-point analysis

```
## Two-point analysis
nc <- parallel::detectCores() - 1
tpt10 <- est_pairwise_rf(s10, ncpus = nc, est.type = "disc")
tpt10$pairwise[90:92]
round(tpt10$pairwise[[91]], 2)
plot(tpt10, first.mrk = 1, second.mrk = 2074)
```

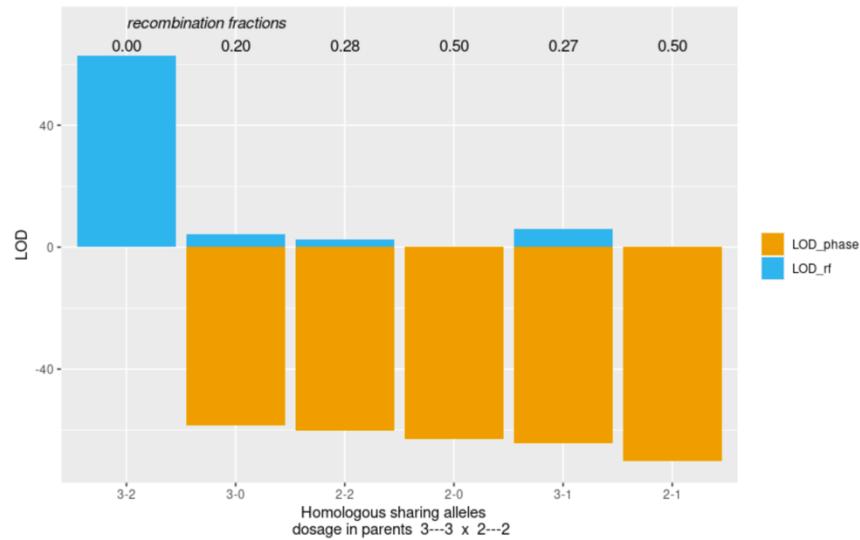
Atlantic B1829-5



1 2074

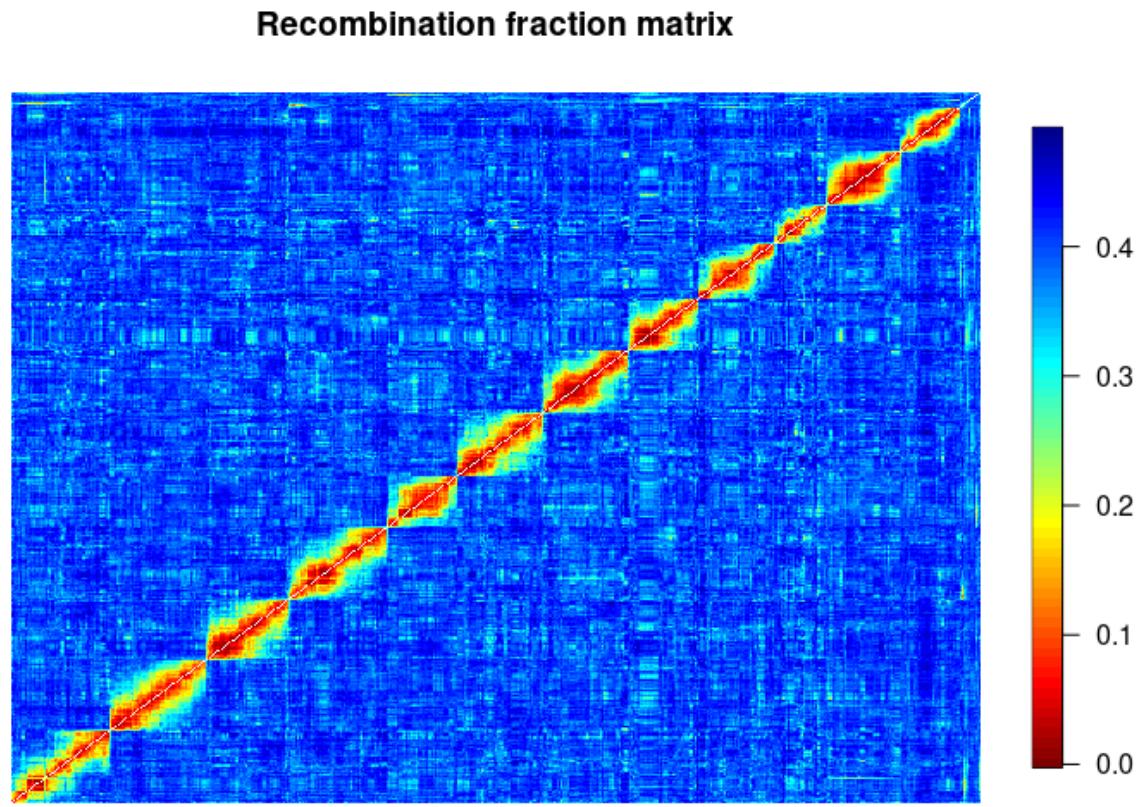
1 2074

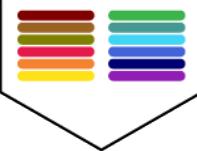
	LOD_ph	rf	LOD_rf
3-2	0.00	0.00	62.86
3-0	-58.46	0.20	4.40
2-2	-60.17	0.28	2.69
2-0	-62.86	0.50	0.00
3-1	-64.29	0.27	5.89
2-1	-70.18	0.50	0.00



Recombination fraction matrix

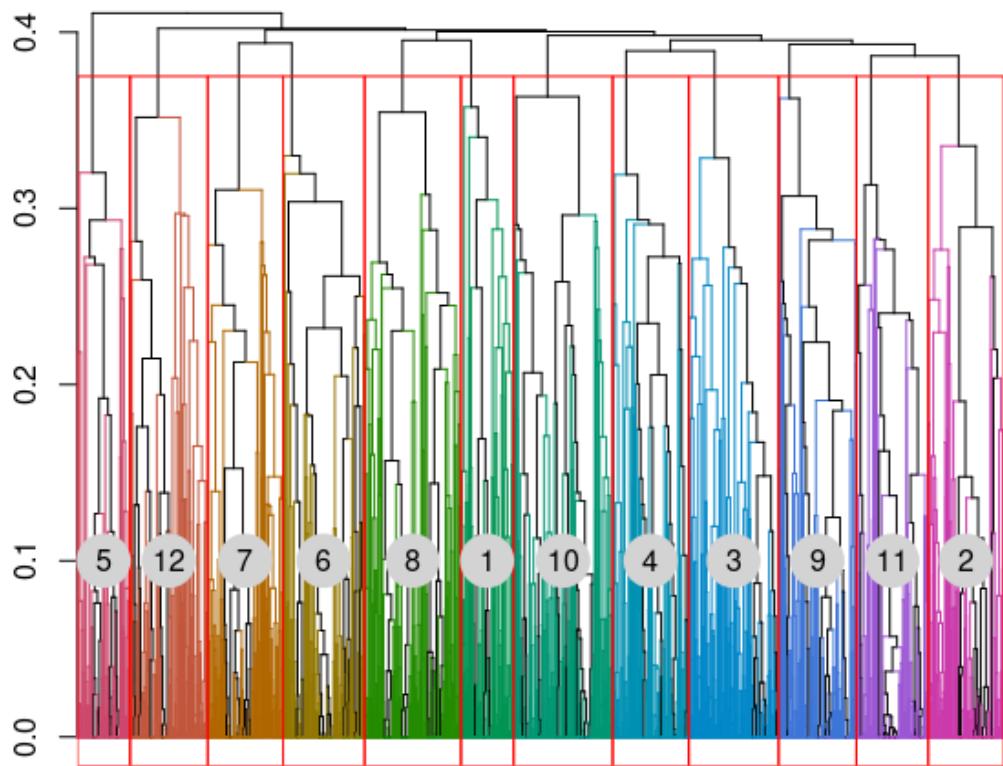
```
m <- rf_list_to_matrix(tpt, ncpus = nc)
gen.order <- get_genomic_order(s)
s.gen.order <- make_seq_mappoly(gen.order)
plot(m, ord = s.gen.order$seq.mrk.names, fact = 10)
```





Filtering by pairwise recombination fraction

```
gr <- group_mappoly(m, expected.groups = 12,
                      comp.mat = TRUE)
gr
```



This is an object of class 'mappoly.group'

Criteria used to assign markers to groups:

- Number of markers: 4489

- Number of linkage groups: 12

- Number of markers per linkage groups:

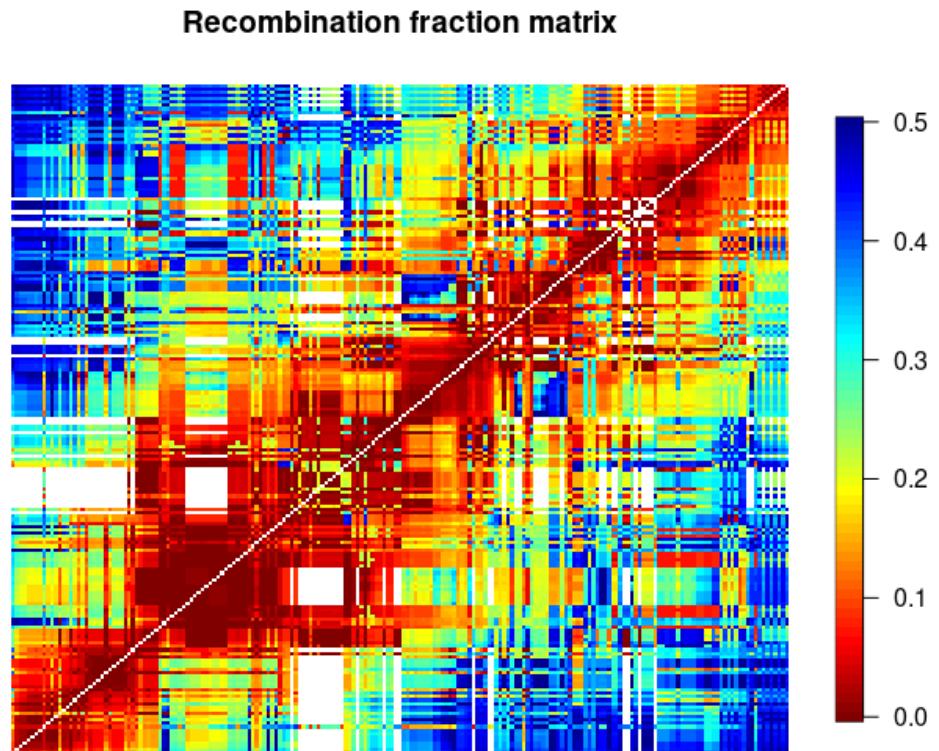
group	n.mrk
1	255
2	366
3	435
4	369
5	256
6	395
7	365
8	467
9	377
10	480
11	348
12	376

	10	9	7	5	12	3	6	4	11	1	8	2	NoChr
1	201	1	2	2	5	3	1	9	3	7	3	11	7
2	2333	3	2	0	1	0	2	1	6	1	6	9	
3	1	11	381	7	3	5	6	9	0	6	3	2	1
4	3	0	1	315	3	5	6	8	1	4	5	5	13
5	1	0	2	3	234	2	1	2	0	1	4	2	4
6	3	2	1	0	1	366	2	3	6	5	0	4	2
7	2	3	1	3	0	1	345	2	3	2	1	0	2
8	4	5	2	2	2	2	5	428	2	5	3	5	2
9	4	6	4	6	1	0	5	3	329	5	1	2	11
10	3	5	7	6	2	1	1	5	2	423	2	7	16
11	4	7	2	3	3	1	0	4	0	2	301	9	12
12	0	1	1	1	0	5	2	9	0	6	8	337	6

Ordering within a specific linkage group

Genome order

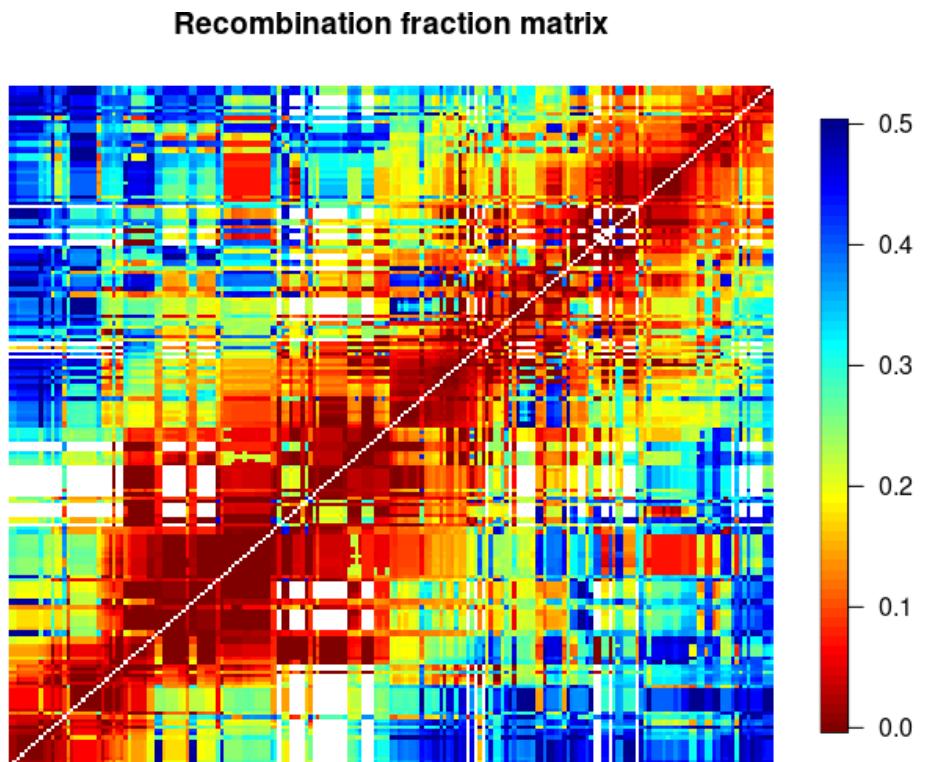
```
s1 <- make_seq_mappoly(gr, arg = 1,  
                        genomic.info = 1)  
tpt1 <- make_pairs_mappoly(tpt, s1)  
m1 <- make_mat_mappoly(m, s1)  
##Genomic order  
g.o1 <- get_genomic_order(s1)  
s1.g <- make_seq_mappoly(g.o1)  
plot(g.o1)  
plot(m1, ord = s1.g$seq.mrk.names,  
     fact = 3)
```



Ordering within a specific linkage group

MDS order

```
mds.o1 <- mds_mappoly(m1, n = c(201,47))
plot(mds.o1)
s1.mds <- make_seq_mappoly(mds.o1)
plot(m1, ord = s1.mds$seq.mrk.names)
```



Phasing and HMM estimation of distance

Phasing and HMM estimation of distance

Tetraploid

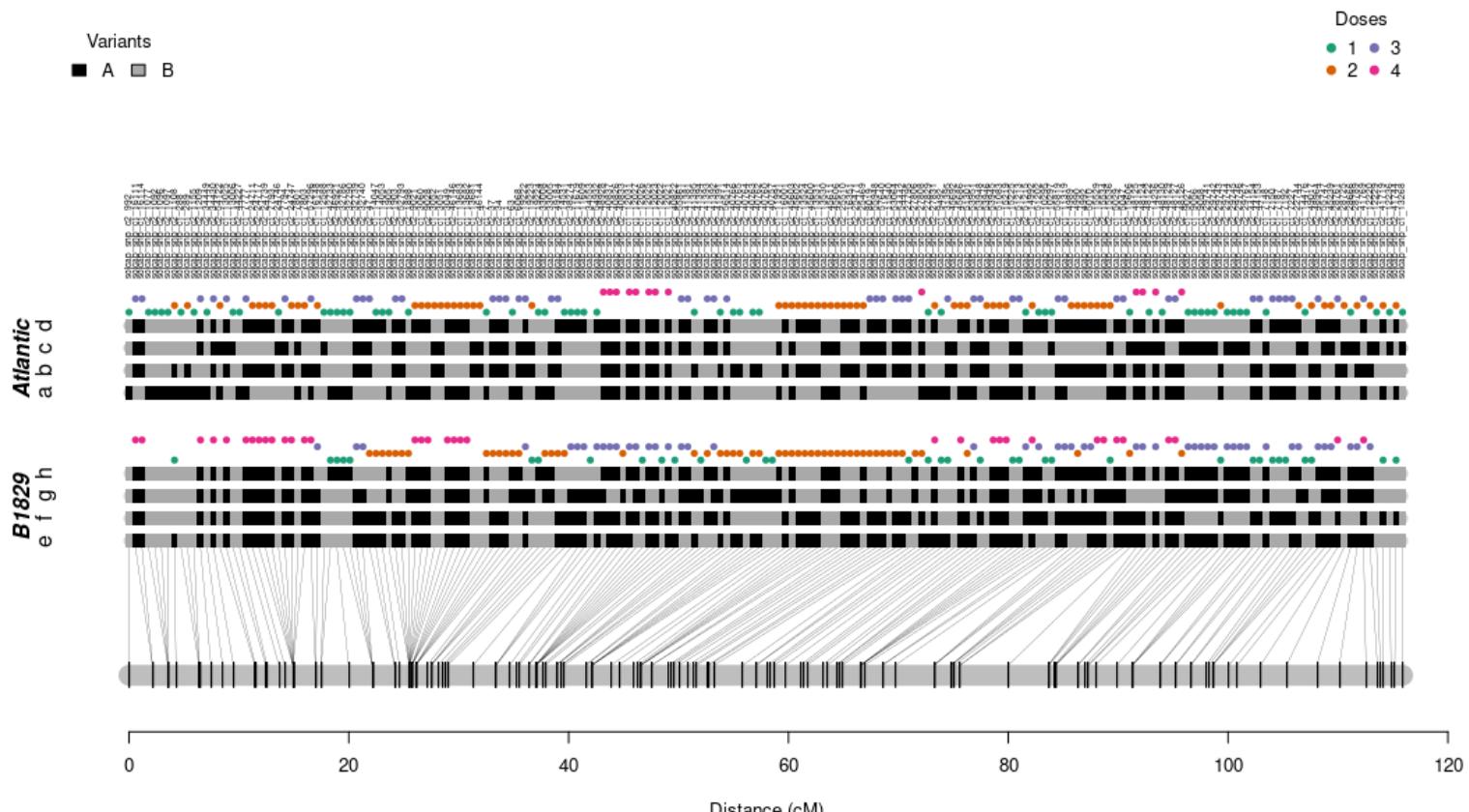
Argument	Value
start.set	3 – 10
thres.twopt	10
thres.hmm	10 – 50
extend.tail	30 – 100
phase.number.limit	20
sub.map.size.diff.limit	2 (thousands per LG) ... 10 (hundred(s) per LG)

Hexaploid

Argument	Value
start.set	3 – 5
thres.twopt	10
thres.hmm	10 – 50
extend.tail	50 – 200
phase.number.limit	20
sub.map.size.diff.limit	2 (thousands per LG) ... 10 (hundred(s) per LG)

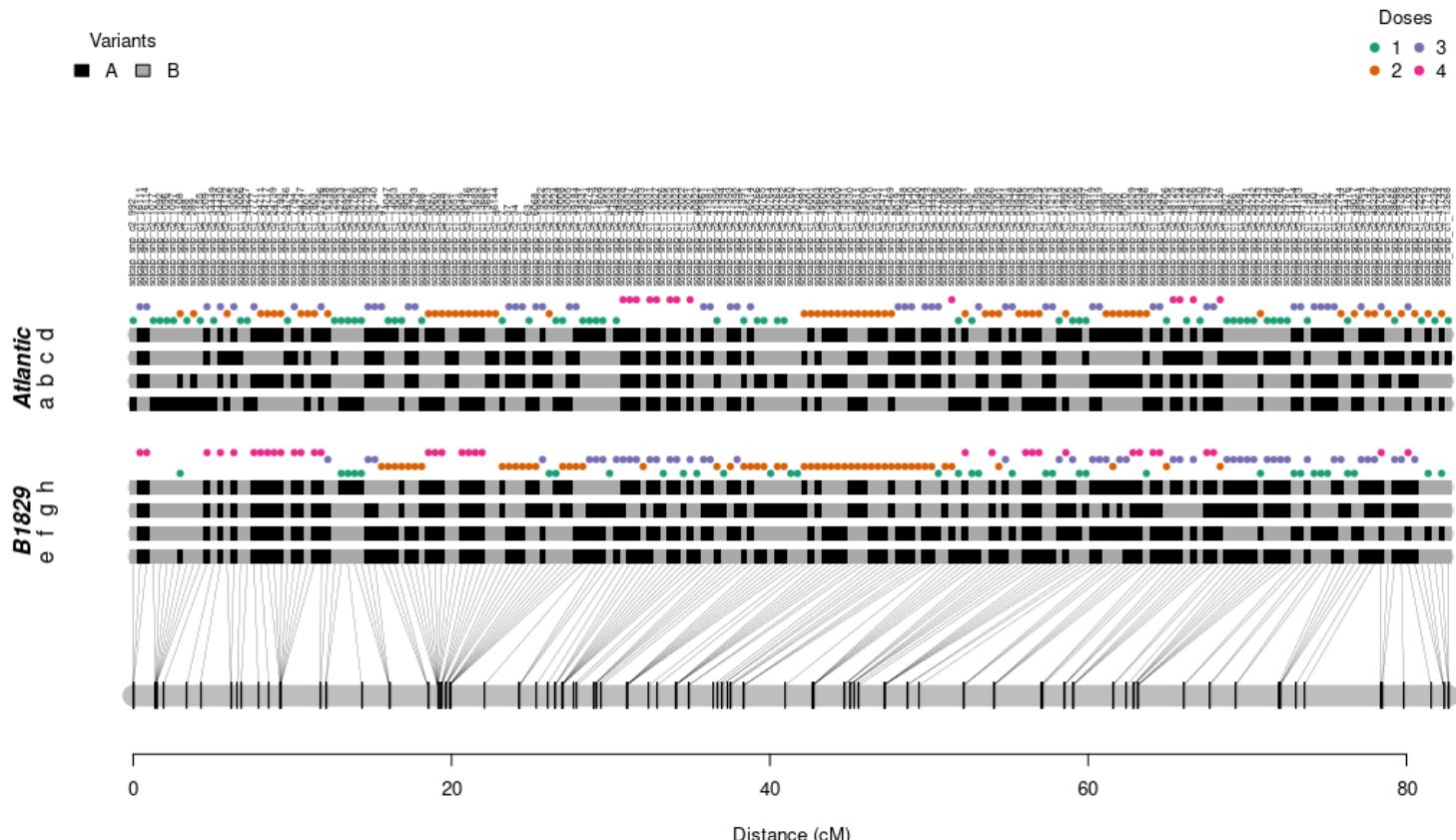
Resulting map

```
plot(lg1.genotype.map, mrk.names = TRUE, cex = 0.5, P = "Atlantic", Q = "B1829")
```



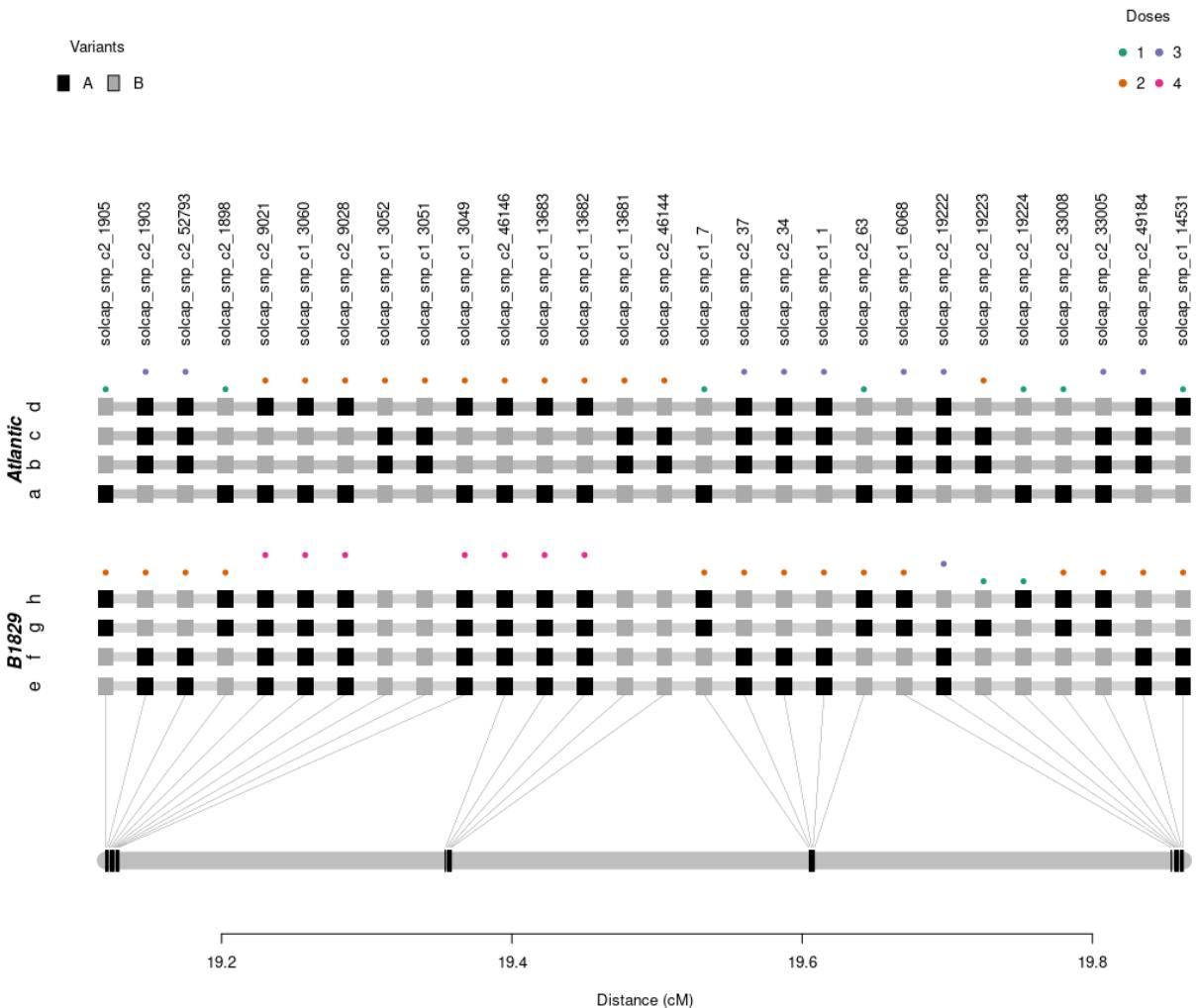
Resulting map – error modeling

```
lg1.geno.map.err <- est_full_hmm_with_global_error(lg1.geno.map, error = 0.05, tol = 10e-4)
plot(lg1.geno.map,err, mrk.names = TRUE, cex = 0.5, P = "Atlantic", Q = "B1829")
```



Resulting map: 20-30 cM segment

```
plot(lg1.geno.map.err,
     mrk.names = TRUE,
     left.lim = 19,
     right.lim = 20,
     P = "Atlantic",
     Q = "B1829")
```



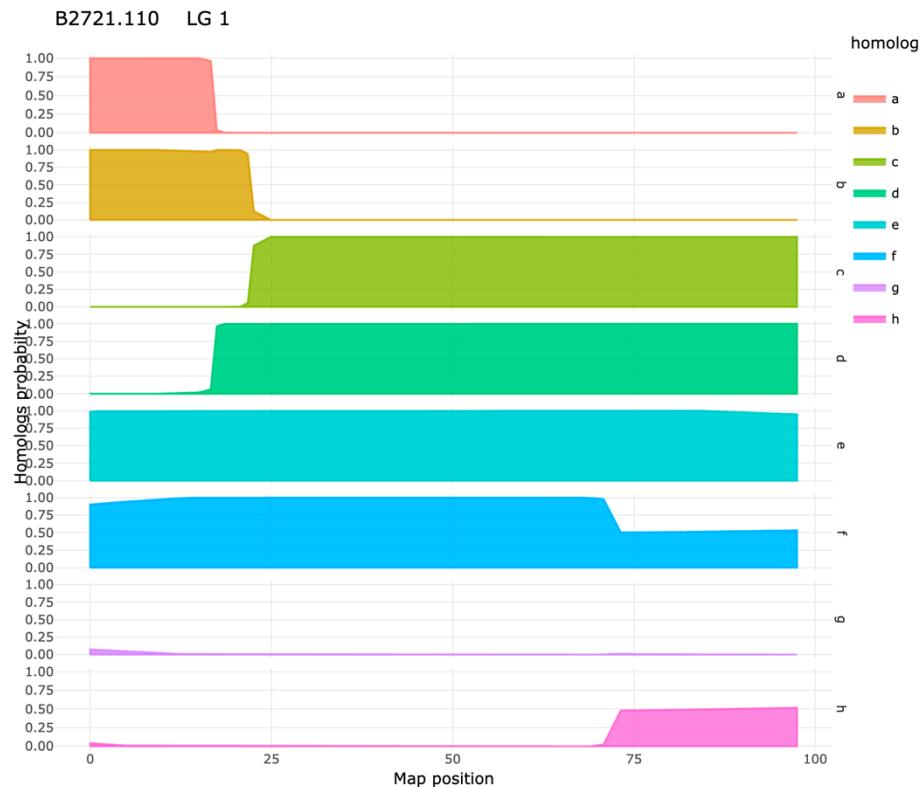
Probabilistic haplotype reconstruction

```
g.lg10 <- calc_genoprob(lg10.geno.map, step = 1)
h.lg10 <- calc_homoprob(g.lg10)
plot(h.lg10, ind = "B2721.110")
```



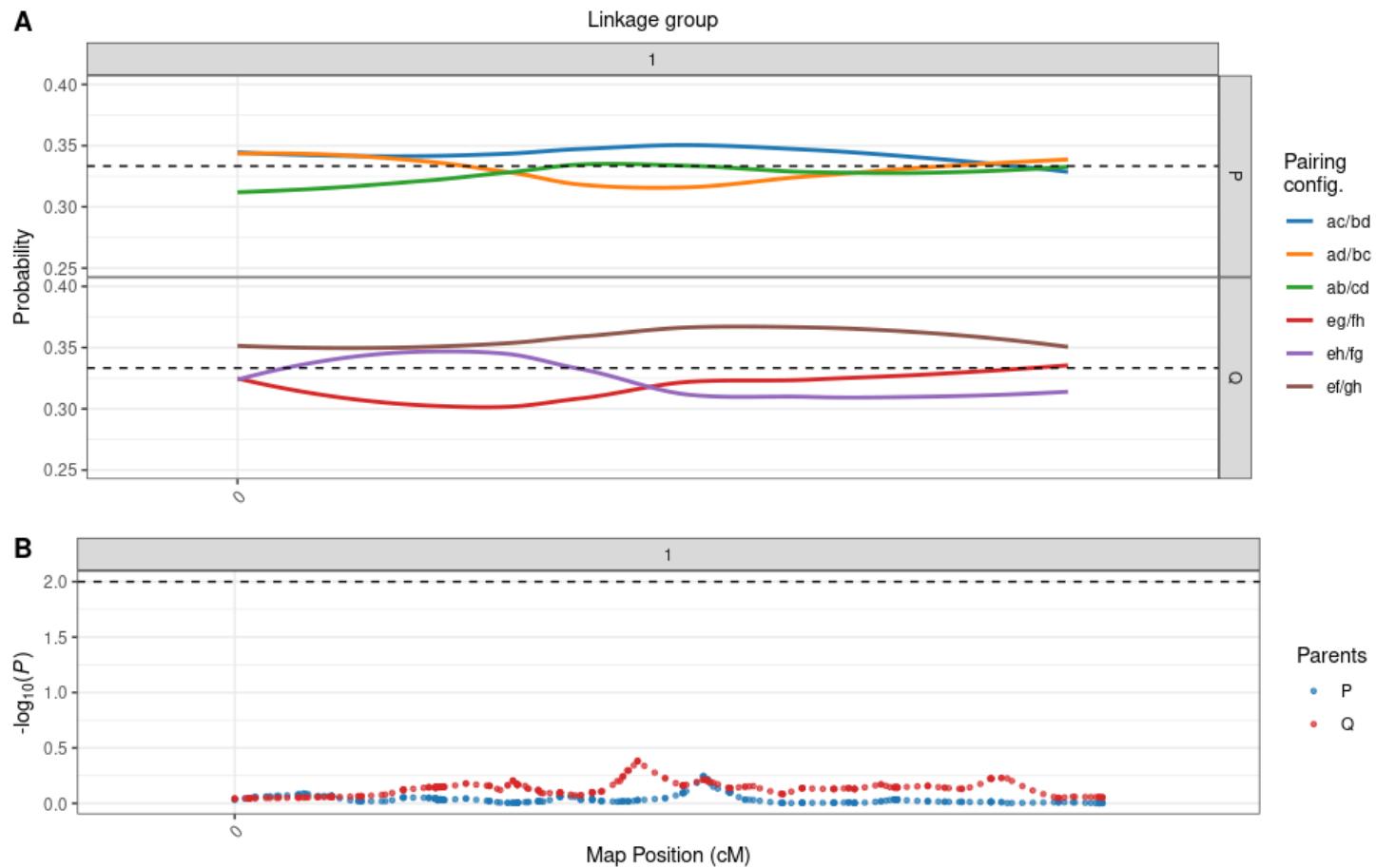
Probabilistic haplotype reconstruction

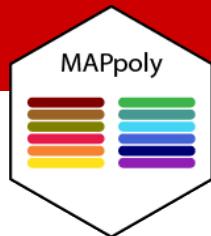
```
g.lg10.err <- calc_genoprob_error(lg10.geno.map.err, step = 1, error = 0.05)
h.lg10.err <- calc_homoprob(g.lg10.err)
plot(h.lg10.err, ind = "B2721.110")
```



Preferential pairing profiles

```
p1 <- calc_prefpair_profiles(g1)
plot(p1, min.y.prof = 0.25, max.y.prof = 0.40)
```





Parallel map construction – genomic order

```
#### Functions ####
phasing_and_hmm_rf <- function(X){
  dir.create("map_output", showWarnings = FALSE)
  fl <- paste0("output_map_ch_", X$seq$sequence[1], ".txt")
  fl <- file.path("map_output", fl)
  sink(fl)
  map <- est_rf_hmm_sequential(input.seq = X$seq,
                                 start.set = 3,
                                 thres.twopt = 10,
                                 thres.hmm = 50,
                                 extend.tail = 30,
                                 twopt = X$tpt,
                                 verbose = TRUE,
                                 phase.number.limit = 20,
                                 sub.map.size.diff.limit = 5)
  sink()
  return(map)
}
error_model <- function(X, error = 0.05, tol = 10e-4){
  x <- est_full_hmm_with_global_error(input.map = X,
                                        error = error,
                                        tol = tol,
                                        verbose = FALSE)
  return(x)
}

#### Correspondence with genome
z<-as.numeric(colnames(gr$seq.vs.grouped.snp)[1:12])

#### Assembling linkage groups (order based on genome)
LGS<-vector("list", 12)
for(ch in 1:12){
  cat("\n ~~~~~ ch:", ch, "...\\n")
  lg <- which(z==ch)
  s.temp<-make_seq_mappoly(gr, lg, genomic.info = 1)
  tpt.temp <- make_pairs_mappoly(tpt, s.temp)
  s.tempfilt <- rf_snp_filter(tpt.temp, 5, 5, 0.15, c(0.05, 1))
  m.temp <- make_mat_mappoly(m, s.tempfilt)
  g.o <- get_genomic_order(s.temp)
  s.g <- make_seq_mappoly(g.o)
  tpt.temp <- make_pairs_mappoly(tpt, input.seq = s.g)
  LGS[[ch]] <- list(seq = s.g, tpt = tpt.temp)
}
```

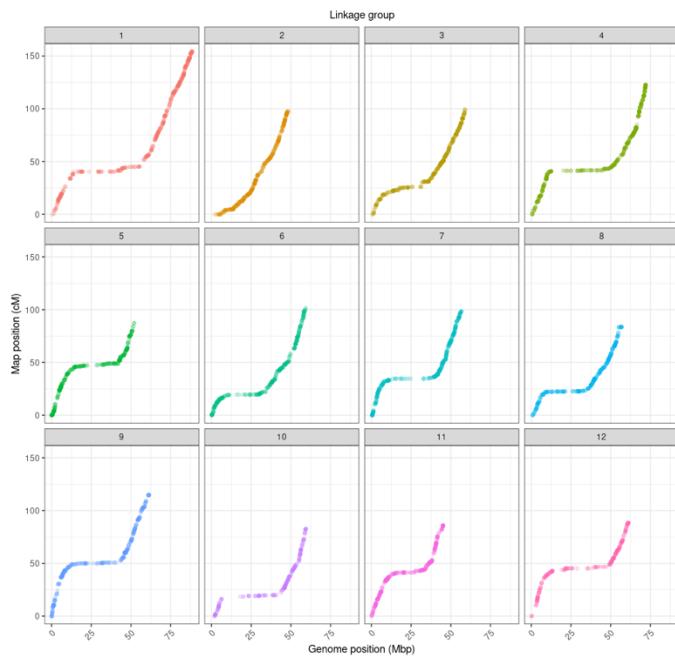
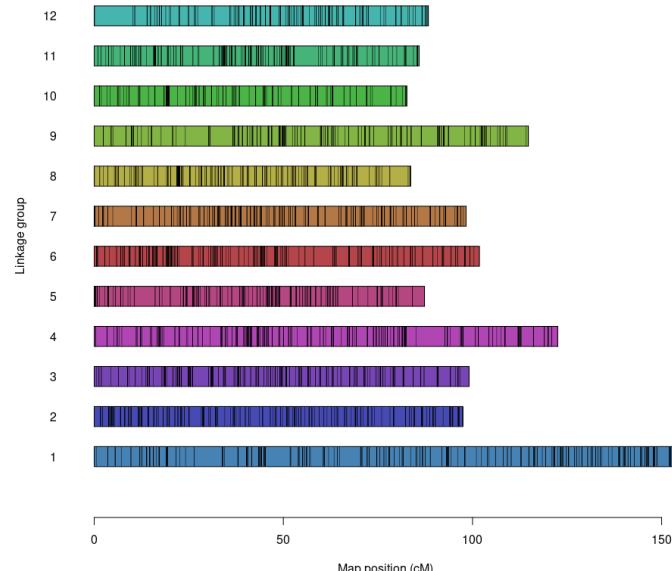
```
#### Parallel map construction
cl <- parallel::makeCluster(12)
parallel::clusterEvalQ(cl, require(mappoly))
parallel::clusterExport(cl, "dat")
# ~12.5 minutes
MAPs.geno <- parallel::parLapply(cl, LGS, phasing_and_hmm_rf)
# ~2.5 minutes
MAPs.geno.err <- parallel::parLapply(cl, MAPs.geno,
                                         error_model)
# ~22 seconds
genoprob <- parallel::parLapply(cl,
                                  MAPs.geno.err,
                                  calc_genoprob_error,
                                  step = 1,
                                  error = 0.05)
parallel::stopCluster(cl)
```

```
#### Map results
map.out <- plot_map_list(MAPs.geno.err, col = "ggstyle")
map.out
plot_genome_vs_map(MAPs.geno.err, same.ch.lg = TRUE)
summary_maps(MAPs.geno.err)
export_map_list(MAPs.geno.err, file = "output_map.csv")
```

```
#### Preferential pairing
pp <- calc_prefpair_profiles(genoprob)
print(pp)
head(pp$prefpair.psi)
plot(pp, P = "Atlantic", Q = "B1829")
```

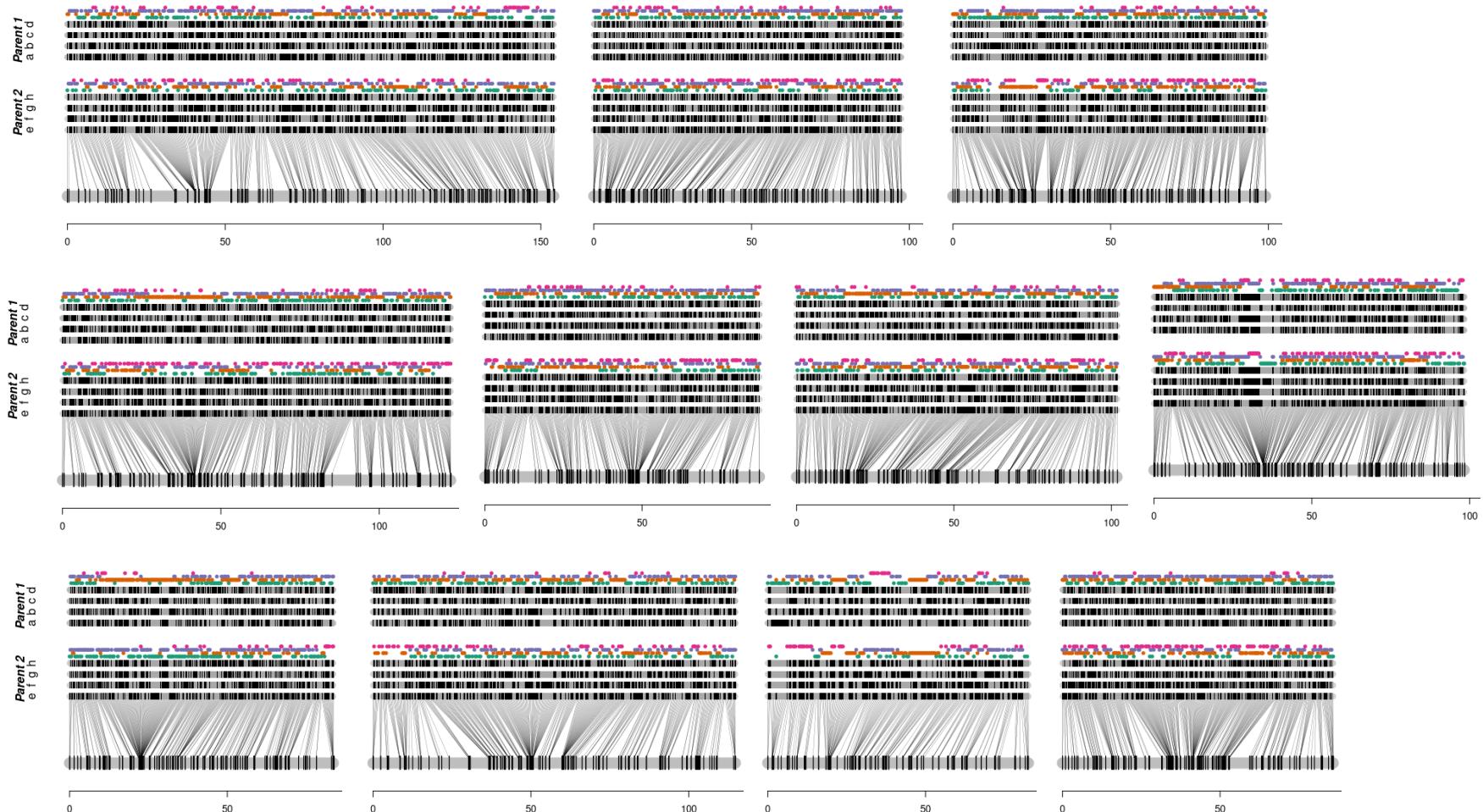
```
#### Haplotype probabilities
hp <- calc_homoprob(genoprob)
print(hp)
plot(hp, ind = 2, lg = 1)
plot(hp, ind = 2, lg = 1:12, use.plotly = FALSE)
```

Results

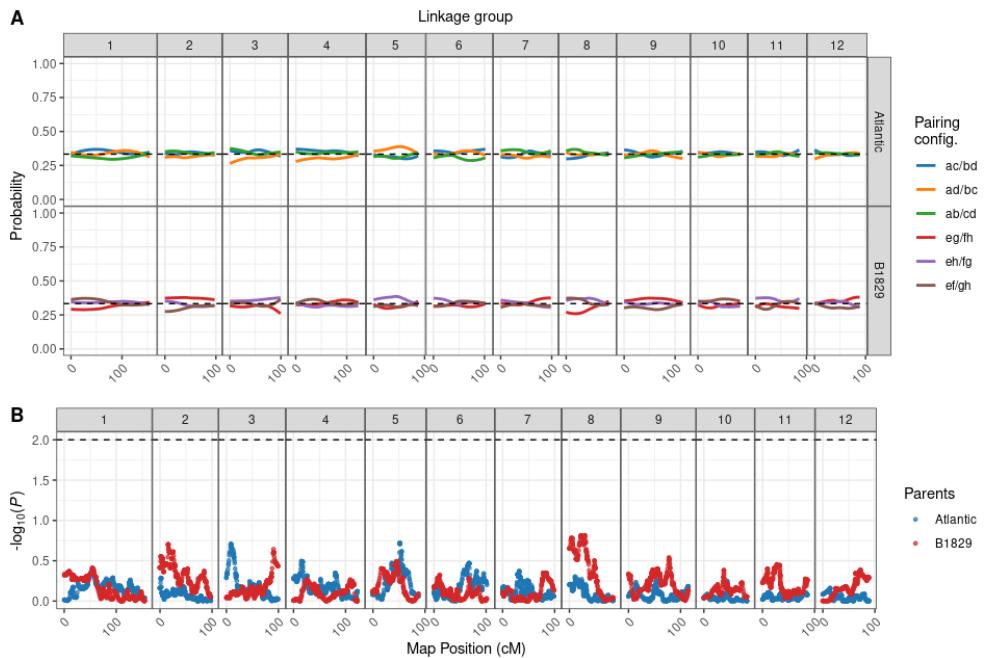
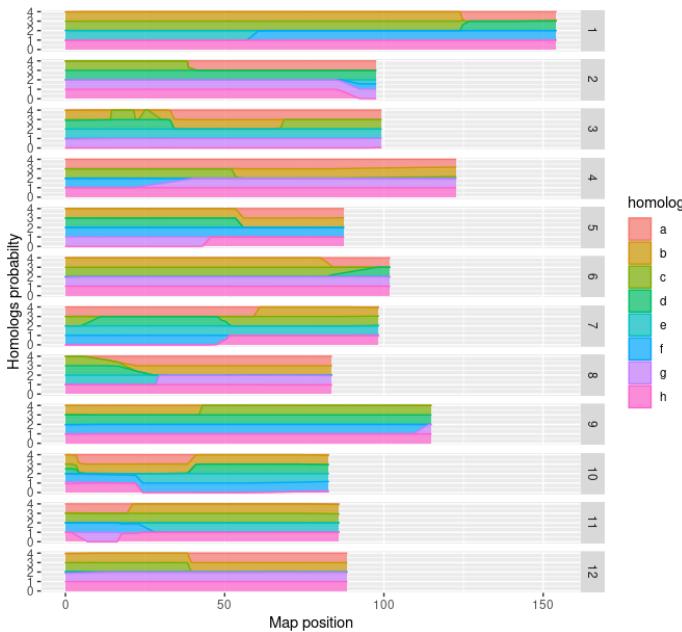


LG	Genomic sequence	Map length (cM)	Markers/cM	Simplex	Double-simplex	Multiplex	Total	Max gap	
1	1	1-NA	155.5	2.89	103	107	202	449	12.84
2	2	2	97.25	4.77	142	132	155	464	2.99
3	3	3	113.91	3.92	160	26	212	446	8.38
4	4	4	121.8	3.92	123	81	235	477	7.71
5	5	5-NA	90.15	3.87	127	56	140	349	5.69
6	6	6	106.18	4.15	72	83	247	441	3.55
7	7	7-NA-4	97.43	4.95	141	74	181	482	4.96
8	8	8-NA-1	93.83	3.88	59	102	165	364	3.32
9	9	9-7-NA	117.73	3.16	81	95	161	372	6.51
10	10	10	97.52	2.47	56	34	131	241	3.68
11	11	11	85.8	4.16	97	52	192	357	5.97
12	12	12	90.81	3.28	121	27	114	298	3.62
13	Total	<NA>	1267.91	3.79	1282	869	2135	4740	5.77

Results – phased map



Results

A**B2721.058**

```
> print(pp)
This is an object of class 'mappoly.prefpair.profiles'
-----
No. positions: 5148 in 12 LGs
No. individuals: 144

Pairing configurations:
Parent 1:
  ac/bd  ad/bc  ab/cd
Parent 2:
  eg/fh  eh/fg  ef/gh
-----
Homolog pairs:
Parent 1:
  ab    ac    ad    bc    bd    cd
Parent 2:
  ef    eg    eh    fg    fh    gh
```

```
> head(pp$prefpair.psi)
      marker pair.conf probability parent LG map.position
1 solcap.snp_c2_36664    ac/bd  0.3339590    P 1      0
2 solcap.snp_c2_36664    ad/bc  0.3417002    P 1      0
3 solcap.snp_c2_36664    ab/cd  0.3243408    P 1      0
4 solcap.snp_c2_36664    eg/fh  0.2865708    Q 1      0
5 solcap.snp_c2_36664    eh/fg  0.3492717    Q 1      0
6 solcap.snp_c2_36664    ef/gh  0.3641575    Q 1      0
```

```
> print(hp)
      marker homolog individual probability map.position LG
1 solcap.snp_c2_36664    a B2721.001  0.5519      0 1
2 solcap.snp_c2_36664    b B2721.001  0.4459      0 1
3 solcap.snp_c2_36664    c B2721.001  0.3628      0 1
4 solcap.snp_c2_36664    d B2721.001  0.6395      0 1
5 solcap.snp_c2_36664    e B2721.001  0.9986      0 1
6 solcap.snp_c2_36664    f B2721.001  0.0275      0 1
7 solcap.snp_c2_36664    g B2721.001  0.9332      0 1
8 solcap.snp_c2_36664    h B2721.001  0.0407      0 1
9 solcap.snp_c2_36664    d B2721.058  0.0199      0 1
10 solcap.snp_c2_36664   e B2721.058  0.9968      0 1
```

On-line resources

- [MAPpoly web page](#)
- [GitHub page for the topics I presented in this workshop](#)
- Tetraploid: [B2721 potato biparental population \(Atlantic x B1829-5\)](#)
- Hexaploid: [BT sweetpotato population \(Beauregard x Tanzania\)](#)
- Detailed analytic procedures for [Pereira et al. \(2020\)](#)

APPENDIX

Hexaploid map – Results

Hexaploid map – Results

- Script for hexaploid
 - <https://github.com/mmollina/SCRI/tree/main/MAPpoly/hexa>
- Complete map
 - Unraveling the Hexaploid Sweetpotato Inheritance Using Ultra-Dense Multilocus Mapping ([Molinari et al., 2020](#)).
- Shiny applications
 - Map: https://gt4sp-genetic-map.shinyapps.io/bt_map/
 - Haplotypes: https://gt4sp-genetic-map.shinyapps.io/offspring_haplotype_BT_population/

Biparental Population - BT

- Beauregard x Tanzania
- 315 individuals
- GBS – GBSpoly protocol (Bode Olukolu – U Tennessee)
- Two reference genomes *I. trifida* and *I. triloba* (Zhangjun Fei's group – BTI Cornell). In the available dataset we used *I. trifida*.
- In this example we used three chromosomes: ch3, ch9 and ch12



Beauregard

X



Tanzania



Biparental Population - BT



X



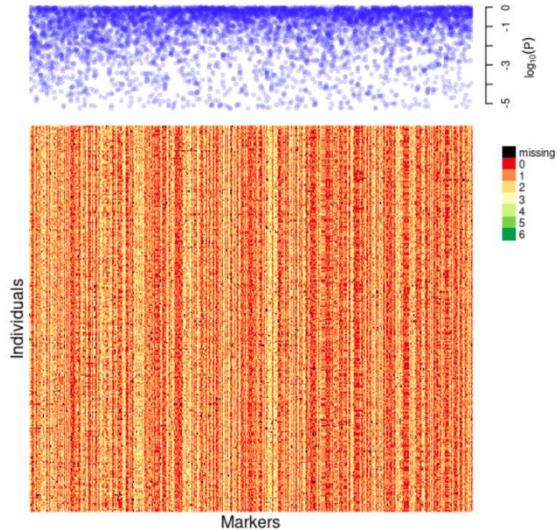
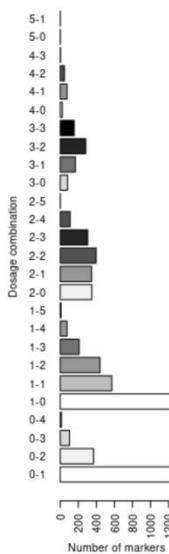
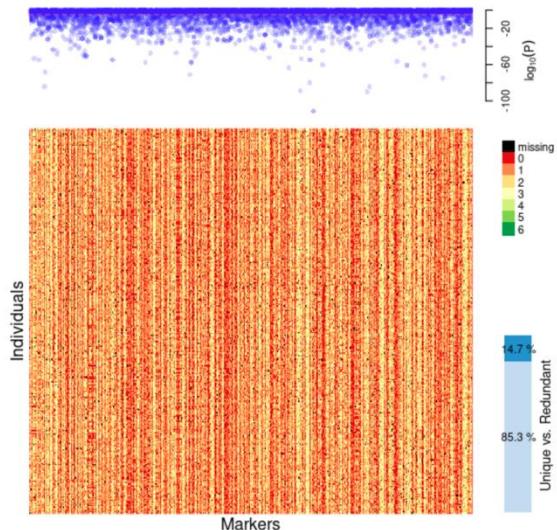
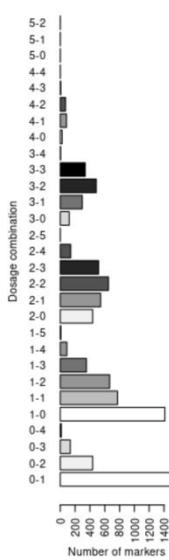
Beauregard

Tanzania





Results for hexaploid sweetpotato BT population



This is an object of class 'mappoly.sequence'

Parameters not estimated

Ploidy level: 6
No. individuals: 303
No. markers: 6602

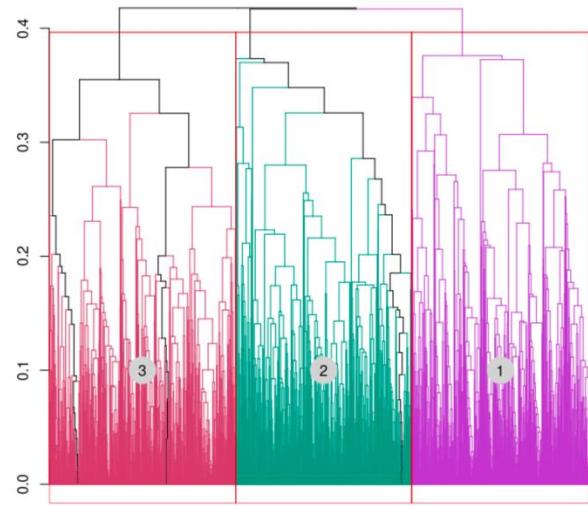
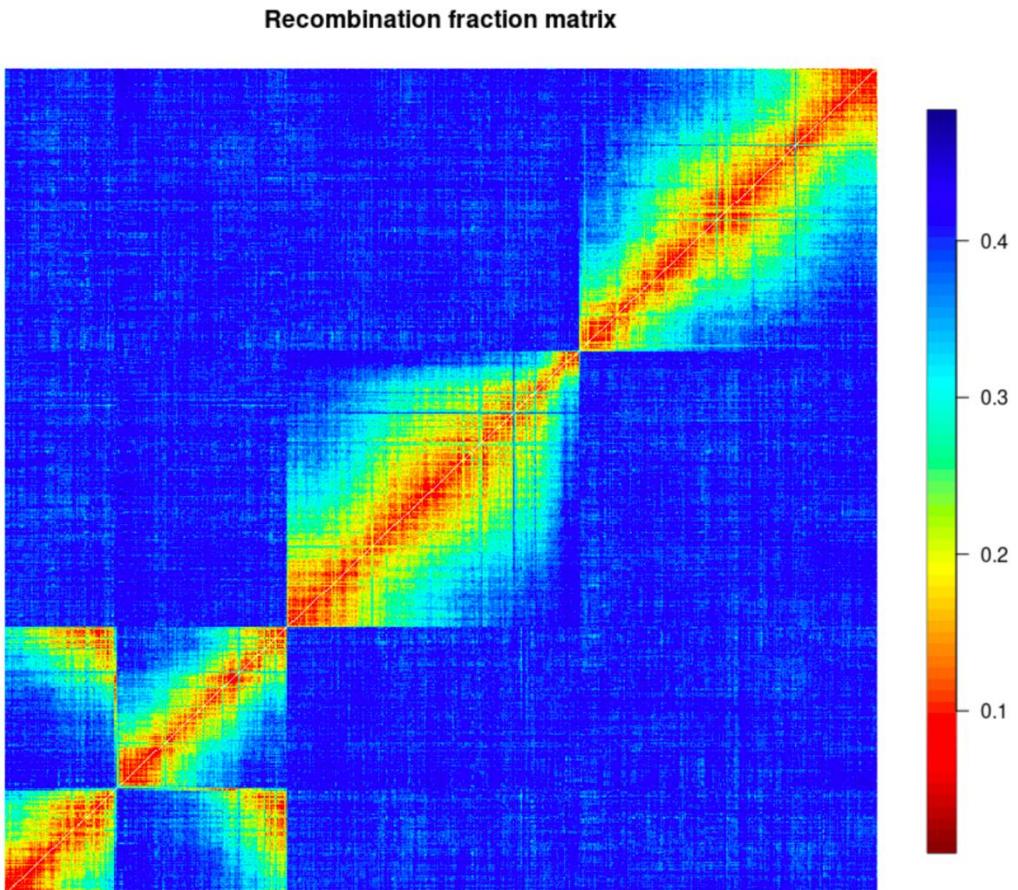
No. markers per sequence:
sequence No.mrk

sequence	No.mrk
12	2249
3	2141
9	2212

No. of markers per dosage in both parents:

dP	dQ	freq
0	1	1226
0	2	368
0	3	102
0	4	13
1	0	1249
1	1	571
1	2	440
1	3	209
1	4	75
1	5	9
2	0	349
2	1	347
2	2	397
2	3	304
2	4	109
2	5	1
3	0	81
3	1	167
3	2	282
3	3	154
4	0	23
4	1	75
4	2	46
4	3	3
5	0	1
5	1	1

Recombination fraction matrix and grouping



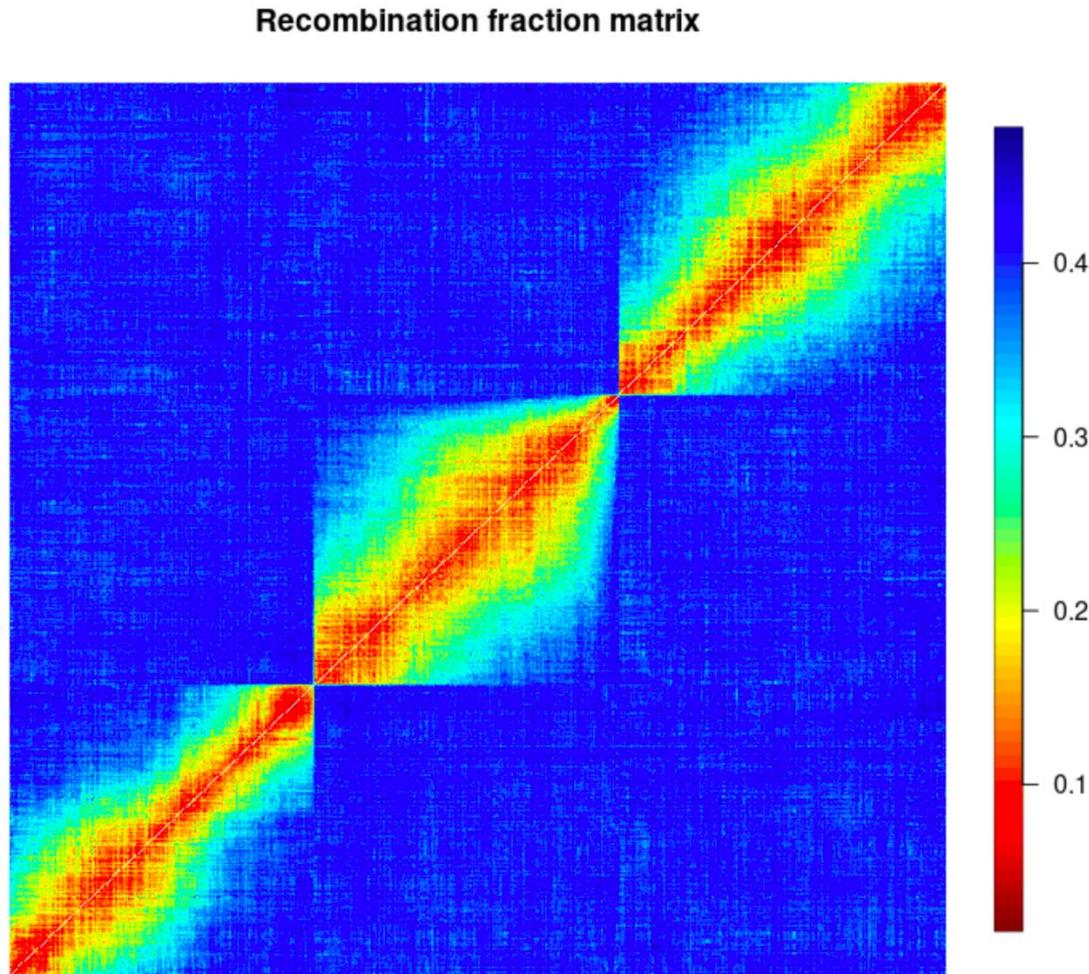
This is an object of class 'mappoly.group'

Criteria used to assign markers to groups:

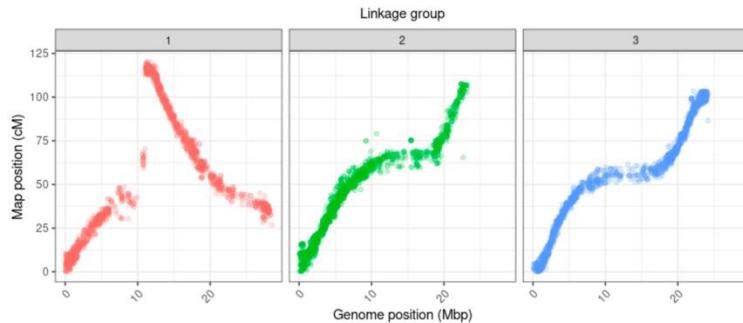
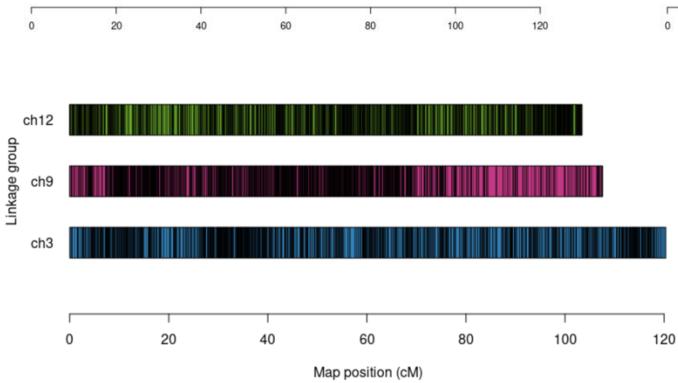
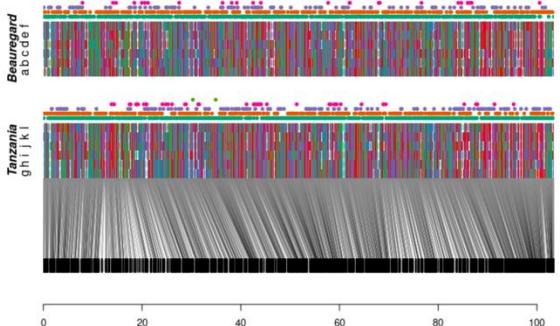
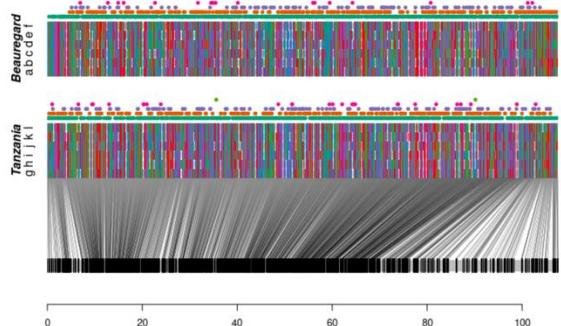
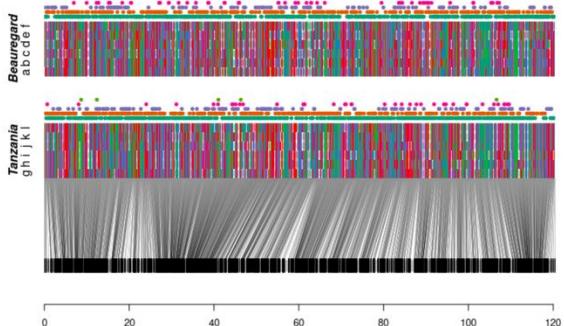
- Number of markers: 6216
- Number of linkage groups: 3
- Number of markers per linkage groups:
group n.mrk
1 2048
2 2022
3 2146

	3	9	12	NoChr
1	1996	30	22	0
2	7	1997	18	0
3	0	2	2144	0

Ordered recombination fraction matrix



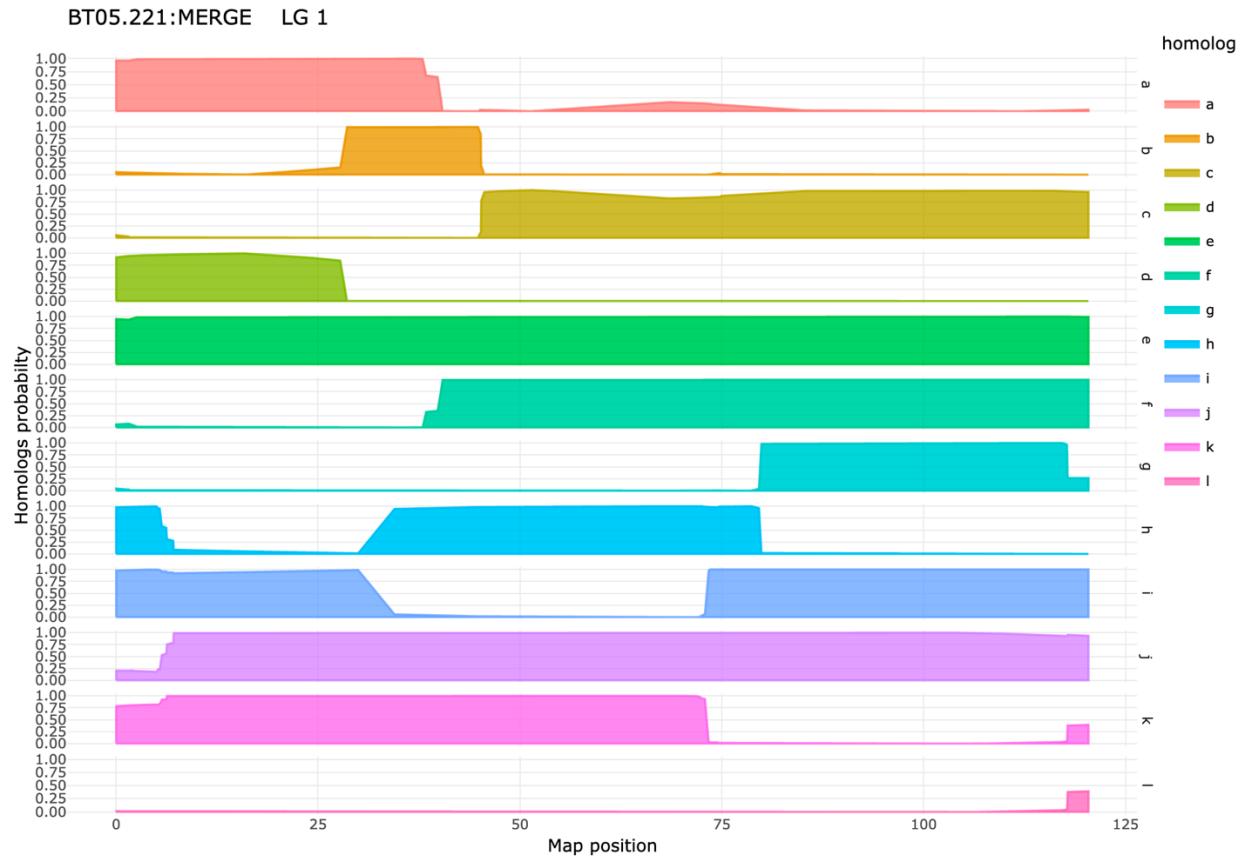
Map results



```
> summary_maps(updated.map)
```

	LG	Genomic sequence	Map length (cM)	Markers/cM	Simplex	Double-simplex	Multiplex	Total	Max gap
1	1		3	120.43	16.33	663	130	801	1967
2	2		9	107.58	19.31	798	184	697	2077
3	3		12	103.47	21.09	785	154	851	2182
4	Total		<NA>	331.48	18.91	2246	468	2349	6226
									0.98

Homolog probabilities



Preferential pairing profiles

