RClone quickmanual: several populations

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"Eager Beginners" Manual for RClone package

RCione data f	tormat: severa	l populations

A. Introduction to RClone

RClone is a R package version of GenClone program (Arnaud-Haond & Belkhir 2007): to analyse data (SSR, SNP, ...), test for clonality and describe spatial clonal organisation.

Major improvements are multi-populations handling and definition of MLLs (Multilocus Lineages, i.e. slightly distinct Multi Locus Genotypes) through simulations.

RClone allows:

- 1. Description of data set
- discrimination of MLG (MultiLocus Genotypes);
- test for reliability of data (in terms of loci and sampling).
- 2. Determination of MLL (MultiLocus Lineages)
- psex/psex Fis with pvalue computation;
- genetic distance matrix computation and threshold definition.
- 3. Genotypic diversity and evenness indices calculation
- Simpson complement;
- Shannon-Wiener diversity and evenness indices;
- Hill's Simpson reciprocal;

- Pareto index.
- 4. Spatial organisation of MLG/MLL
- spatial autocorrelation methods;
- clonal subrange estimation;
- Aggregation and Edge Effect indices estimation.

Some of these analysis can be applied to dataset with no repeated MLG, regardless of the reproductive system (sexual, partially asexual or strictly asexual).

B. RClone data format: several population

RClone functions works on diploid/haploid, one or several populations dataset.

If you have only one population in your dataset, go to other vignette RClone_quickmanual.

C. General format

If you have haploid data, you can skip to D. Description of data set.

To use RClone functions, your data table must look like:

library(RClone)
data(posidonia)

Po15_1	Po15_2	Po4-3_1	Po4-3_2	Po5-10_1	Po5-10_2	Po5-39_1	Po5-39_2
137	161	182	188	212	216	234	234
139	171	182	182	222	226	234	242
161	161	182	182	210	216	234	234
161	161	182	182	210	216	234	234
161	161	182	182	210	216	234	234
161	161	182	182	210	216	234	234
161	161	182	182	210	216	234	234
161	161	182	182	210	216	234	234
137	157	182	188	208	210	234	234
137	157	174	180	208	210	234	234

There is only one allele per column and, per locus, alleles are sorted by increasing order.

This is **mandatory** for all *RClone* functions.

As formatting can be source of error, we included functions to help formatting your diploid data:

1, The classic infile you could have, one locus per column

data(zostera)
head(zostera)

population	x	У	GA35	GA2	GA17H	GA23	GA12	GA19	GA20	GA16	GA17D
SaintMalo	0.0	18.0	185187	102116	131135	167169	131131	148148	162162	168168	197197
SaintMalo	0.0	15.5	185187	102116	131135	167169	131131	148148	162162	168168	197197
SaintMalo	0.0	3.5	187187	102116	127133	161169	131131	148148	162162	168168	197197
SaintMalo	2.0	3.5	187187	102116	133135	159161	131131	148148	162162	168168	197197
SaintMalo	6.5	18.0	187187	102116	135141	169169	131137	148150	162162	168168	197197
SaintMalo	6.5	10.0	187187	116116	133133	167167	131131	148148	162162	168168	195197

Zostera data is composed of:

```
popvec <- zostera[,1] #futur vecpop
coord_zostera <- zostera[,2:3] #futur coordinates
zostera <- zostera[,4:ncol(zostera)] #dataset
zostera <- convert_GC(zostera, 3) #We used "3" because this is the length of each allele.</pre>
```

head(zostera)

GA35_1	GA35_2	GA2_1	GA2_2	GA17H_1	GA17H_2	GA23_1
185	187	102	116	131	135	167
185	187	102	116	131	135	167
187	187	102	116	127	133	161
187	187	102	116	133	135	159
187	187	102	116	135	141	169
187	187	116	116	133	133	167

2, The simple case: you already have a one-allele per column table

Just remove the pop/coords informations as above and sort your alleles:

```
sort_all(zostera)
```

3, You already work with Adegenet

Similar to case number 1, except you have to export your genind data into table first:

```
#library(adegenet)
#with data1, a genind object from Adegenet:

test <- genind2df(data1)</pre>
```

^{*} a first column with population indication;

^{*} a second and third columns with x/y coordinates;

^{*} a genotypic dataset.

```
data2 <- convert_GC(test, 3, "/")
#only if yours alleles are of length "3"</pre>
```

D. Description of data set

D.1 Discrimination of MLG

List unique alleles per locus:

Basic commands:

```
list_all_tab(zostera, vecpop = popvec)
```

or, for haploid data:

```
list_all_tab(haplodata, haploid = TRUE, vecpop = haplovec)
```

Results:

```
list_all_tab(zostera, vecpop = popvec)
```

#SaintMalo

$locus_1$	$locus_2$	$locus_3$	locus_4	$locus_5$	locus_6	locus_7	locus_8	locus_9
185 187 189	102 116	131 127 133 135 137 119	167 161 159 169 163	131 137	148 150	162	168	197 195

#Arcouest

locus_1	locus_2	locus_3	locus_4	locus_5	locus_6	locus_7	locus_8	locus_9
187	102	131	161	131	150	162	168	197
189	116	129	169		148	156	166	
185	108	141	167			160		
	118	133				166		
	120	143				164		
		135						

List MLG:

Basic commands:

```
MLG_tab(zostera, vecpop = popvec)
```

or, for haploid data:

```
MLG_tab(haplodata, vecpop = haplovec)
```

Results:

```
MLG_tab(zostera, vecpop = popvec)[[1]]
#SaintMalo
```

unit_1	unit_2	unit_3	unit_4	unit_5
1	2			
3				
4	11			
5	7	8	9	12
6				

Allelic frequencies:

Basic commands:

```
freq_RR(zostera, vecpop = popvec)
```

or, for haploid data:

```
freq_RR(haplodata, haploid = TRUE, vecpop = haplovec)
```

Options:

```
freq_RR(zostera, vecpop = popvec) #on ramets
freq_RR(zostera, vecpop = popvec, genet = TRUE) #on genets
freq_RR(zostera, vecpop = popvec, RR = TRUE) #Round-Robin methods
```

Results:

```
freq_RR(zostera, vecpop = popvec)[[1]]
#SaintMalo
```

locus	allele	$freq_ramet$	$freq_genet$	$\rm freq_RR$
locus_1	185	0.0517241	0.0588235	0.0588235
$locus_1$	187	0.9137931	0.8823529	0.8823529
$locus_1$	189	0.0344828	0.0588235	0.0588235
$locus_2$	102	0.5000000	0.5000000	0.5000000
$locus_2$	116	0.5000000	0.5000000	0.5000000
$locus_3$	119	0.0172414	0.0294118	0.0294118
$locus_3$	127	0.0689655	0.1176471	0.1176471

D.2 Tests for reliability of loci and subsampling of individuals

On loci

Basic commands:

```
sample_loci(zostera, vecpop = popvec, nbrepeat = 1000)
```

or, for haploid data:

```
sample_loci(haplodata, haploid = TRUE, vecpop = haplovec, nbrepeat = 1000)
```

Options:

Results:

```
res <- sample_loci(zostera, vecpop = popvec, nbrepeat = 1000, He = TRUE)
names(res)</pre>
```

> [1] "SaintMalo" "Arcouest"

names(res\$SaintMalo)

names(resvigncont2\$res2_SU1\$SaintMalo)

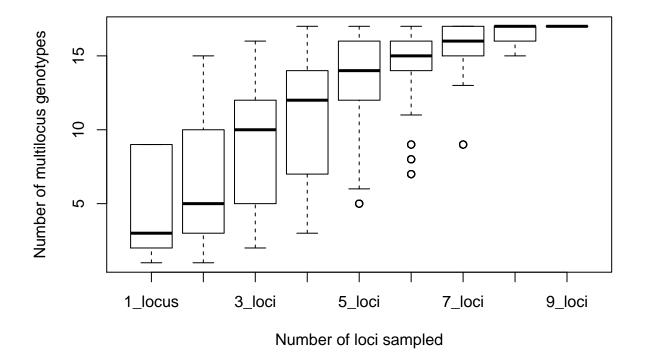
```
#Results: MLG
res$Arcouest$res_MLG
```

nb_loci	min	max	$mean_MLG$	SE
1	1	8	3.861	0.0819780
2	1	12	6.997	0.1021539
3	2	14	9.800	0.0873248
4	4	16	11.757	0.0678864
5	7	16	13.157	0.0555647
6	11	16	14.299	0.0414405
7	13	16	15.061	0.0307933
8	14	16	15.576	0.0210396
9	16	16	16.000	0.0000000

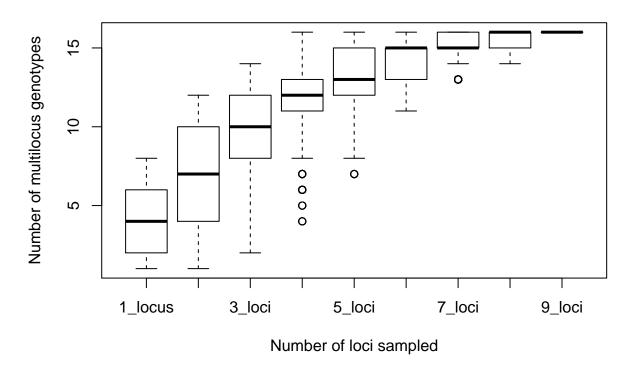
```
#Results: alleles
res$Arcouest$res_alleles
```

```
#Results: raw data
#res$Arcouest$raw_He
#res$Arcouest$raw_MLG
#res$Arcouest$raw_all
```

Genotype accumulation curve



Genotype accumulation curve



Same on units

Basic commands:

```
sample_units(zostera, vecpop = popvec, nbrepeat = 1000)
```

or, for haploid data:

```
sample_units(haplodata, haploid = TRUE, vecpop = haplovec, nbrepeat = 1000)
```

This sub-sampling analysis deliver basic estimates of richness and diversity for an increasing number of sampling units.

They can be used to standardise estimates of populations with different sampling effort.

E. Discrimination of clonal lineages

E.1 psex/psex Fis with pvalue computation

pgen, psex and p-values

Basic commands:

```
pgen(zostera, vecpop = popvec)
psex(zostera, vecpop = popvec)
```

or, for haploid data:

```
pgen(haplodata, haploid = TRUE, vecpop = haplovec)
psex(haplodata, haploid = TRUE, vecpop = haplovec)
```

Options: (idem on psex and pgen)

Results:

```
res <- psex(zostera, vecpop = popvec, RR = TRUE, nbrepeat = 1000)
res$Arcouest[[1]]
#if nbrepeat != 0, res contains a table of psex values and a vector of sim-psex values</pre>
```

pgen	genet	psex	pvalue
0.0001692 0.0000416 0.0000900 0.0000416 0.0000000 0.0001800	2	0.00124641891362029	0.00571428571428571

```
res$Arcouest[[2]] #a part of sim-psex values
```

```
> [1] 0.005507812 0.080799100 0.073342047 0.080799100 0.019908965
```

Fis, pgen Fis, psex Fis and p-values

Not for haploid data!

 \mathbf{Fis}

Basic commands:

> [6] 0.008798312 0.002194897 0.003586792 0.046359147 0.116134553

```
Fis(zostera, vecpop = popvec)
```

Options:

```
Fis(zostera, vecpop = popvec) #Fis on ramets

Fis(zostera, vecpop = popvec, genet = TRUE) #Fis on genets

Fis(zostera, vecpop = popvec, RR = TRUE) #Fis with Round-Robin methods

#RR = TRUE contains two results: a table with allelic frequencies

#and a table with Fis results
```

Results:

```
Fis(zostera, vecpop = popvec, RR = TRUE) $Arcouest[[2]]
```

locus	Hobs	Hatt	Fis
locus_1	0.2666667	0.3300242	0.1919786
$locus_2$	0.7500000	0.6995968	-0.0720461
$locus_3$	0.6250000	0.7721774	0.1906005
$locus_4$	0.6250000	0.5383065	-0.1610487
$locus_5$	0.0000000	0.0000000	NaN
$locus_6$	0.2000000	0.1862069	-0.0740741
locus_7	0.2857143	0.3772941	0.2427280
$locus_8$	0.1875000	0.1754032	-0.0689655
$locus_9$	0.0000000	0.0000000	NaN

pgen Fis, psex Fis and p-values

Basic commands: (idem for pgen_Fis and psex_Fis)

```
pgen_Fis(zostera, vecpop = popvec)
```

Options:

Results:

```
res <- psex_Fis(zostera, vecpop = popvec, RR = TRUE, nbrepeat = 1000)
res$Arcouest[[1]]
#if nbrepeat != 0, res contains a table of psex values and a vector of sim-psex Fis values</pre>
```

pgenFis	genet	psexFis	pvalue
0.0001459 0.0000587 0.0000708 0.0000587 0.0000003 0.0001417	2	0.00175772228659339	0.0154639175257732

res\$Arcouest[[2]] #a part of sim psex Fis values

```
> [1] 0.046691095 0.065845604 0.047964606 0.030472230 0.029045389
```

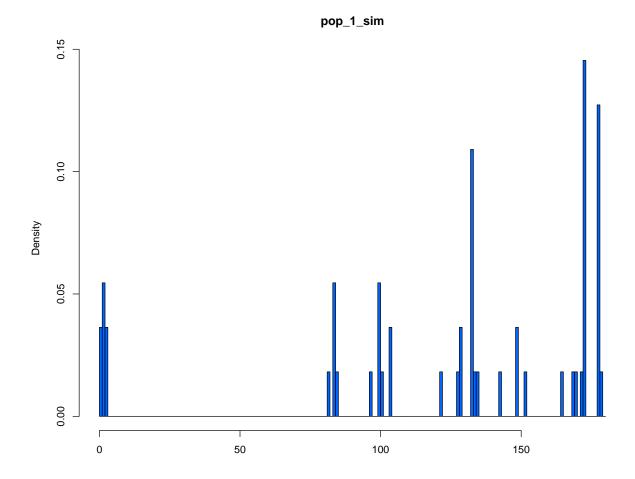
E.2 Tests for MLLs occurrence and assessment of their memberships

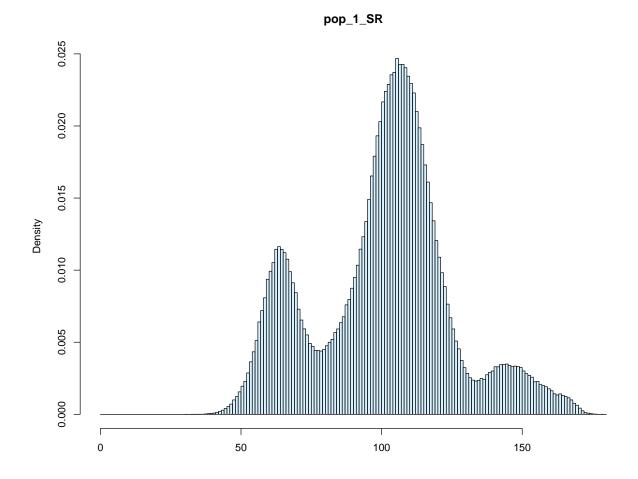
Genetic distance matrix computation and threshold definition

On a theoretical diploid population with c = 0.9999 (c, clonality rate):

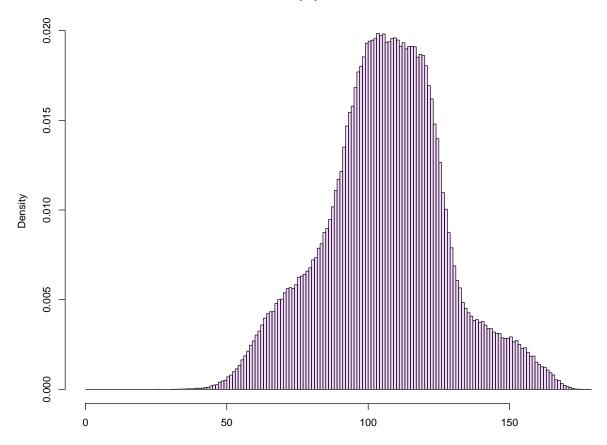
```
data(popsim)
vecsim <- c(rep(1,50), rep(2,50))</pre>
```

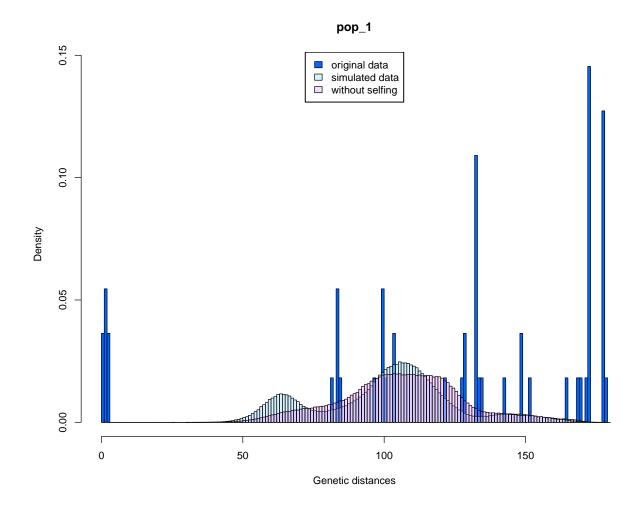
> [6] 0.009682317 0.101570928 0.200020973 0.004458226 0.033269017



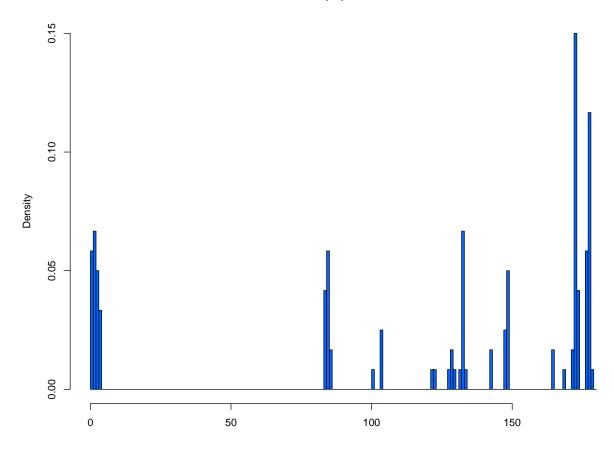


pop_1_SRWS

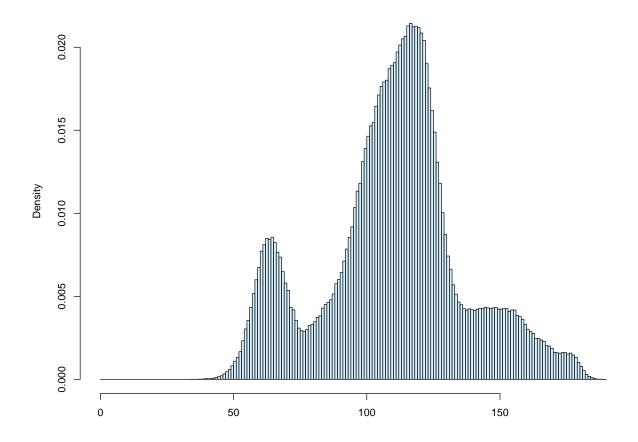


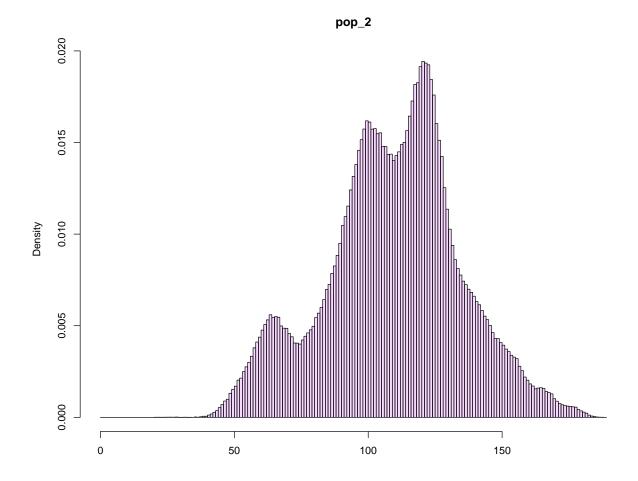


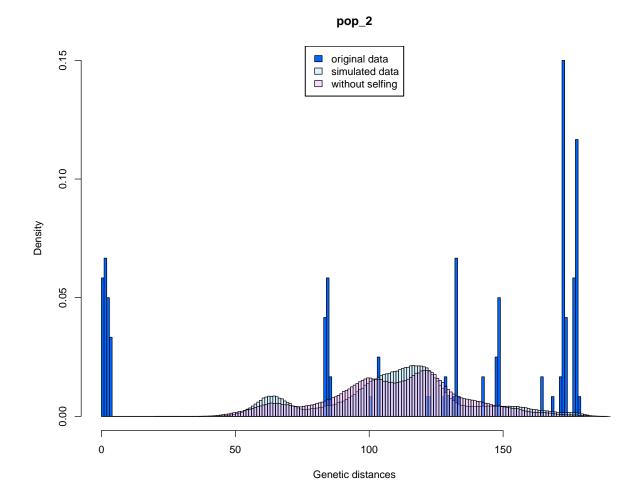












```
table(respop[[1]]$distance_matrix)
>
    1
            3 82 84 85 97 100 101 104 122 128 129 133 134 135 143 149
            2
                1
                     3
                                          2
                                                       2
                             1
                                 3
                                      1
                                              1
                                                  1
> 152 165 169 170 172 173 178 179
        1
                     1
                         8
\#alpha2 = 3
#creating MLL list:
MLLlist <- MLL_generator(popsim, vecpop = vecsim, alpha2 = c(3,0))</pre>
##This will create a list of MLL (alpha2 = 3) and MLG (alpha2 = 0) !
res <- genet_dist(popsim, vecpop = vecsim, alpha2 = c(3,0))</pre>
MLLlist <- MLL_generator2(list(res[[1]]$potential_clones,</pre>
```

For haploid data, theoretical example:

#determining alpha2

res[[2]]\$potential_clones), MLG_list(popsim, vecpop = vecsim), vecpop = vecsim)

F. Genotypic diversity, richness and evenness indices calculation

F.1 Classic genotypic indices

Basic commands:

```
clonal_index(zostera, vecpop = popvec)
```

or, with MLL:

```
clonal_index(popsim, vecpop = vecsim, listMLL = MLLlist)
```

or, for haploid data:

```
clonal_index(haplodata, vecpop = vechaplo)
```

Results:

```
clonal_index(zostera, vecpop = popvec)
```

	N	G	R	H"	J'	D	V	Hill
SaintMalo	29	17	0.5714286	2.671294	0.9428497	0.9507389	0.8559028	20.300000
Arcouest	30	16	0.5172414	2.268605	0.8182264	0.8413793	0.3918367	6.304348

F.2 Pareto index

Basic commands:

```
Pareto_index(zostera, vecpop = popvec)
```

or, with MLL:

```
Pareto_index(popsim, vecpop = vecsim, listMLL = MLLlist)
```

or, for haploid data:

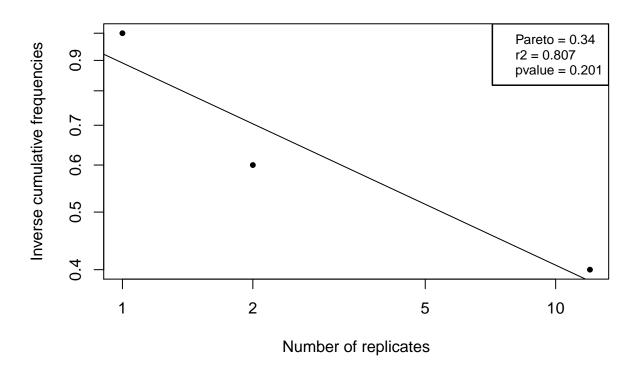
```
Pareto_index(haplodata, vecpop = vechaplo)
```

Options:

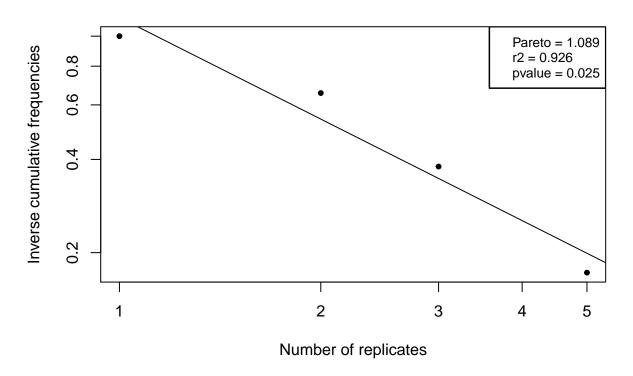
Results:

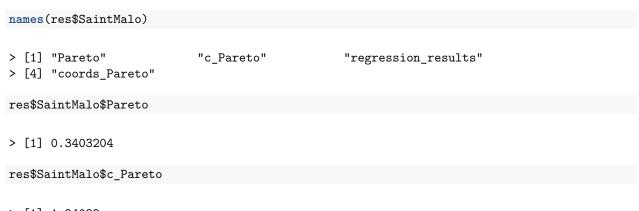
```
res <- Pareto_index(zostera, vecpop = popvec, full = TRUE, graph = TRUE, legends = 2)</pre>
```

Pareto distribution



Pareto distribution





> [1] 1.34032

G. Spatial components of clonality

G.1 Spatial autocorrelation

Basic commands:

```
autocorrelation(zostera, coords = coord_zostera, vecpop = popvec, Loiselle = TRUE)
or, with MLL:
autocorrelation(popsim, coords = coord sim, Loiselle = TRUE, listMLL = MLLlist)
or, for haploid data:
autocorrelation(haplodata, haploid = TRUE, coords = coord_haplo, Loiselle = TRUE)
Lot's of options:
#kinship distances:
autocorrelation(zostera, coords = coord_zostera, vecpop = popvec, Loiselle = TRUE)
autocorrelation(zostera, coords = coord_zostera, vecpop = popvec, Ritland = TRUE)
#ramets/genets methods:
autocorrelation(zostera, coords = coord_zostera, vecpop = popvec, Loiselle = TRUE)
autocorrelation(zostera, coords = coord_zostera, vecpop = popvec,, Loiselle = TRUE,
                    genet = TRUE, central_coords = TRUE)
                                            #genets, central coordinates of each MLG
autocorrelation(zostera, coords = coord_zostera, vecpop = popvec, Loiselle = TRUE,
                    genet = TRUE, random_unit = TRUE)
                                                    #genets, one random unit per MLG
autocorrelation(zostera, coords = coord_zostera, vecpop = popvec, Loiselle = TRUE,
                    genet = TRUE, weighted = TRUE)
                                            #genets, with weighted matrix on kinships
#distance classes construction:
autocorrelation(zostera, coords = coord_zostera, vecpop = popvec, Loiselle = TRUE)
                                                                 #10 equidistant classes
distvec <-c(0,10,15,20,30,50,70,76.0411074)
                                    #with 0, min distance and 76.0411074, max distance
autocorrelation(zostera, coords = coord_zostera, vecpop = popvec, Loiselle = TRUE,
                    vecdist = distvec) #custom distance vector
autocorrelation(zostera, coords = coord_zostera, vecpop = popvec, Loiselle = TRUE,
                    class1 = TRUE, d = 7) #7 equidistant classes
autocorrelation(zostera, coords = coord_zostera, vecpop = popvec, Loiselle = TRUE,
                    class2 = TRUE, d = 7)
                            #7 distance classes with the same number of units in each
#graph options:
autocorrelation(zostera, coords = coord_zostera, vecpop = popvec, Ritland = TRUE,
                                                        graph = TRUE) #displays graph
autocorrelation(zostera, coords = coord_zostera, vecpop = popvec, Ritland = TRUE,
                                                        export = TRUE) #export graph
```

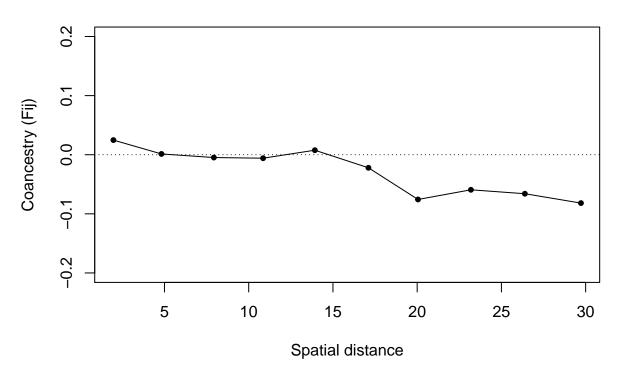
Results:

#pvalues computation

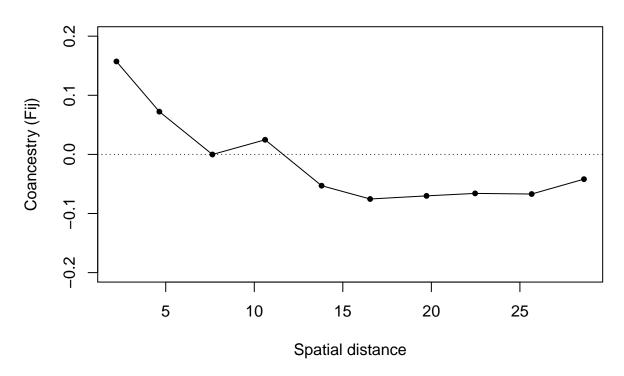
nbrepeat = 1000)

autocorrelation(zostera, coords = coord_zostera, vecpop = popvec, Ritland = TRUE,

Spatial aucorrelation analysis



Spatial aucorrelation analysis



names(res\$Arcouest)

> [7] "Class_distance_results"

${\tt res\$Arcouest\$Main_results} \ \textit{\#enables} \ \textit{graph} \ \textit{reproduction}$

dist_min	dist_max	dist_mean	ln(dist_mean)	nb_pairs	mean_Ritland	pval_kin
1.11803	3.00000	2.215687	0.7955625	23	0.1572378	0.000
3.04138	6.02080	4.641553	1.5350490	35	0.0723040	0.000
6.10328	9.05539	7.636613	2.0329541	62	-0.0001518	0.292
9.12414	12.09339	10.612723	2.3620535	61	0.0247131	0.014
12.16553	15.13275	13.802695	2.6248639	58	-0.0529723	0.022
15.18223	18.06931	16.543032	2.8059650	50	-0.0754410	0.000
18.24829	21.10095	19.726507	2.9819633	54	-0.0701760	0.000
21.18962	24.08319	22.470382	3.1121981	28	-0.0659711	0.034
24.41311	27.22591	25.664587	3.2451121	20	-0.0670280	0.068
27.45906	30.26962	28.617915	3.3540329	15	-0.0418956	0.436

```
apply(res$Arcouest$Main_results, 2, mean)[6] #mean Fij
```

- > mean_Ritland
- > -0.0119381

res\$Arcouest\$Slope_and_Sp_index #gives b and Sp indices

	b	b_log	Sp	Sp_log
obs_value	-0.0069054	-0.0877033	0.0081938	0.1040665
$mean_sim$	0.0000230	0.0002994	-0.0000088	-0.0000719
sd_sim	0.0009620	0.0107159	0.0009555	0.0106701
0.95 _inf	-0.0021680	-0.0232025	-0.0015771	-0.0177145
0.95 _sup	0.0016229	0.0187066	0.0021527	0.0233927
0.9 _inf	-0.0017065	-0.0190059	-0.0014359	-0.0155080
0.9 _sup	0.0014775	0.0162403	0.0017695	0.0190726
pval_upper	0.0000000	0.0000000	1.0000000	1.0000000
pval_lower	1.0000000	1.0000000	0.0000000	0.0000000
$pval_2sides$	0.0000000	0.0000000	0.00000000	0.0000000

```
#raw data:
#res$Arcouest$Slope_resample
#res$Arcouest$Kinship_resample
#res$Arcouest$Matrix_kinship_results
#res$Arcouest$Class_kinship_results
#res$Arcouest$Class_distance_results
```

G.2 Clonal subrange

Basic commands:

```
clonal_sub(zostera, coords = coord_zostera, vecpop = popvec)
```

or, with MLL:

```
clonal_sub(popsim, coords = coord_sim, listMLL = MLLlist)
```

or, for haploid data:

```
clonal_sub(haplodata, haploid = TRUE, coords = coord_haplo)
```

Options: same distance classes definition as autocorrelation:

```
#custom distance classes

clonal_sub(zostera, coords = coord_zostera, vecpop = popvec, class1 = TRUE, d = 7)

#7 equidistant classes

clonal_sub(zostera, coords = coord_zostera, vecpop = popvec, class1 = TRUE, d = 7)

#7 distance classes with the same number of units in each
```

Results:

```
res <- clonal_sub(zostera, coords = coord_zostera, vecpop = popvec)
res$Arcouest[[1]] #Global clonal subrange
> [1] 19.10497
```

res\$Arcouest\$clonal_sub_tab #details per class

nb_pairs	dist_min	dist_max	dist_mean	Fr	$\log(\text{Fr})$
19	0.7071068	2.915476	1.969185	0.3157895	-0.5006024
49	3.201562	6.184658	4.821457	0.3265306	-0.4860761
49	6.264982	9.219544	7.93246	0.1836735	-0.7359536
74	9.340771	12.36932	10.85383	0.1756757	-0.7552884
77	12.5	15.43535	13.92621	0.1688312	-0.7725474
70	15.5	18.5809	17.10734	0.1285714	-0.8908555
36	18.72165	21.59282	20.04924	0.08333333	-1.079181
31	21.70829	24.69818	23.18994	0	-Inf
25	24.82438	27.85678	26.39228	0	-Inf
5	28.41215	30.99193	29.72322	0	-Inf

G.3 Aggregation index

Basic commands:

```
agg_index(zostera, coords = coord_zostera, vecpop = popvec)
```

or, with MLL:

```
agg_index(popsim, coords = coord_sim, listMLL = MLLlist)
```

or, for haploid data:

```
agg_index(haplodata, coords = coord_haplo)
```

Options:

Results:

```
res <- agg_index(zostera, coords = coord_zostera, vecpop = popvec, nbrepeat = 1000)
```

res\$SaintMalo\$results #Aggregation index

Ac	pval	nbrepeat
0.1965314	0.006	1000

#res\$SaintMalo\$simulation #vector of sim aggregation index

G.4 Edge Effect

Basic commands:

or, with MLL:

```
edge_effect(popsim, coords = coord_sim, center = rep(c(15,10),2), listMLL = MLLlist)
```

or, for haploid data:

```
edge_effect(haplodata, coords = coord_haplo, center = rep(c(15,10),2))
```

Options:

Results:

```
res <- edge_effect(zostera, coords = coord_zostera, vecpop = popvec,
center = rep(c(15,10),2), nbrepeat = 100) #better put 1000 nbrepeat at least
```

res\$SaintMalo\$results #Aggregation index

Ee	pval_Ee	nbrepeat
0.0288465	0.798	1000

H. BONUS: "Ready to use" Table

Summary function of main results:

Basic commands:

```
GenClone(zostera, coords = coord_zostera, vecpop = popvec)
```

or, with MLL:

```
GenClone(popsim, coords = coord_sim, listMLL = MLLlist)
```

or, for haploid data:

```
GenClone(haplodata, haploid = TRUE, coords = coord_haplo)
```

Options:

Results:

```
GenClone(zostera, coords = coord_zostera, vecpop = popvec)
```

	N	Lineage	nb_L	nb_all	SE	Fis	pval_2sides	Fis_WR	pval_2sides.1
SaintMalo	29	MLG	17	2.777778	0.6620208	-0.1350152	NA	-0.03917716	NA
Arcouest	30	MLG	16	3.111111	0.6111111	-0.1017437	NA	-0.2108197	NA

	R	Pareto_index	Sp_Loiselle	pval_2sides	Sp_L_WR	pval_2sides.1	Sp_Ritland
SaintMalo	0.5714286	1.088812	0.008698691	NA	0.00542055	NA	0.008193788
Arcouest	0.5172414	0.3403204	0.007036951	NA	0.003786488	NA	0.003920472

	pval_2sides	Sp_R_WR	pval_2sides.1	Н"	J'	D	V	Hill
SaintMalo	NA	0.004776727	NA	2.671294	0.9428497	0.9507389	0.8559028	20.3
Arcouest	NA	0.001466866	NA	2.268605	0.8182264	0.8413793	0.3918367	6.304348

When a locus is homozygous, it is ignored for Fis and Fis_WR values computation.