RClone quickmanual: one population

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"Eager Beginners" Manual for RClone package

RClone data format:	one population	

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 multipopulations 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 without clones.

B. RClone data format: one population

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

If you have several populations in your dataset, go to other vignette RClone_qmsevpops.

C. General format

If you have haploid data, you can skip to 4, For GenClone users or 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 simple case: you already have a one-allele per column table

```
data(posidonia)
sort_all(posidonia)
```

2, The classic infile you could have: one locus per column

```
#Let's create your example table:
test <- matrix("232/231", ncol = 2, nrow = 2)
colnames(test) <- paste("locus", 1:2, sep = "_")

#Use :
data1 <- convert_GC(as.data.frame(test), 3, "/")</pre>
```

data1

locus_1_1	$locus_1_2$	$locus_2_1$	locus_2_2
231	232	231	232
231	232	231	232

We used "3" because this is the length of the allele (with 3 numbers). For allele separation, we used "/" because, of course, it was the separator.

3, You already work with Adegenet

Similar to case number 2, except you have to export your genind data into table first:

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

test <- genind2df(data1)
data2 <- convert_GC(test, 3, "/")
#only if yours alleles are of length "3"</pre>
```

4, For GenClone users

Warning: your infile file must include all the informations available, as locus names and ploidy level (which is not mandatory for *GenClone*).

```
#This is nearly a GenClone file, type:
write.table(infile, "infile.csv", col.names = FALSE, row.names = FALSE, sep = ";")

#Now you have a formatted GenClone file:
res <- transcript_GC("infile.csv", ";", 2, 7, 3)
posidonia <- res$data_genet
coord_posidonia <- res$data_coord</pre>
```

You might need to edit your "infile.txt" into "infile.csv" and check if there's "." and not "," for geographic coordinates, and use ";" as separator element.

- "2" is for the ploidy level; should have been "1" for haploid data;
- "7" here is the number of loci;
- "3" is for allele length. Posidonia alleles are always of length "3".

D. Description of data set

D.1 Discrimination of MLG

List unique alleles per locus:

Basic commands:

data(posidonia)

list_all_tab(posidonia)

or, for haploid data:

list_all_tab(haplodata, haploid = TRUE)

Results:

list_all_tab(posidonia)

locus_1	$locus_2$	$locus_3$	locus_4	$locus_5$	locus_6	locus_7
137	182	212	234	165	170	178
139	174	222	242	159	168	180
161	188	210	236	163	172	
151	180	208				
157		216				
159		226				
171		218				

List MLG:

Basic commands:

MLG_tab(posidonia)

or, for haploid data:

MLG_tab(haplodata)

Results:

MLG_tab(posidonia)

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

Allelic frequencies:

Basic commands:

```
freq_RR(posidonia)
```

or, for haploid data:

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

Options:

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

Results:

```
freq_RR(posidonia)
```

locus	allele	freq_ramet	freq_genet	freq_RR
locus_1	137	0.1375	0.1607143	0.1666667
$locus_1$	139	0.0250	0.0357143	0.0370370
$locus_1$	151	0.1500	0.2142857	0.2222222
$locus_1$	157	0.3375	0.2857143	0.2777778
$locus_1$	159	0.0250	0.0357143	0.0370370
$locus_1$	161	0.3125	0.2500000	0.2407407
$locus_1$	171	0.0125	0.0178571	0.0185185

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

On loci

Basic commands:

```
sample_loci(posidonia, nbrepeat = 1000)
```

or, for haploid data:

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

Options:

Results:

```
res <- sample_loci(posidonia, nbrepeat = 1000, He = TRUE) #time consuming
names(res)</pre>
```

> NULL

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

nb_loci	\min	max	$mean_MLG$	SE
1	3	13	6.265	0.1046505
2	7	21	14.265	0.1362400
3	11	26	20.142	0.0966083
4	19	27	23.566	0.0617532
5	22	28	25.443	0.0460312
6	25	28	26.856	0.0311164
7	28	28	28.000	0.0000000

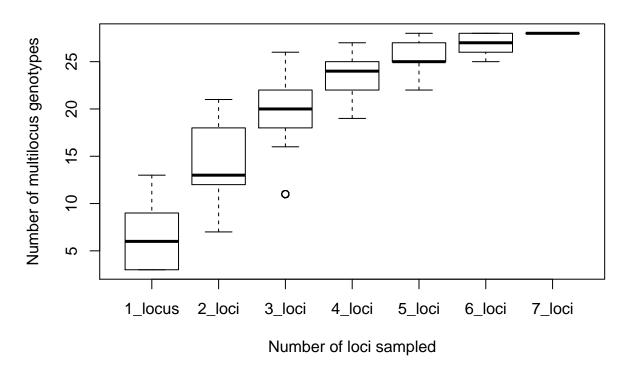
#Results: alleles res\$res_alleles

nb_loci	min	max	$mean_all$	SE	Не	SE
1	2	7	4.092	NA	0.5491902	NA
2	5	14	8.329	132.25780	0.5492449	1.2174962
3	8	18	12.416	88.28636	0.5503377	0.8028116
4	11	21	16.531	70.20927	0.5504794	0.6456283
5	15	24	20.699	60.66198	0.5504022	0.5523189
6	22	27	24.895	54.60655	0.5521684	0.4933410
7	29	29	29.000	NA	0.5513110	NA

#Results: raw data

#res\$raw_He
#res\$raw_MLG
#res\$raw_all

Genotype accumulation curve



Same on units

Basic commands:

```
sample_units(posidonia, nbrepeat = 1000)
```

or, for haploid data:

```
sample_units(haplodata, haploid = TRUE, 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 Determination of MLL

E.1 psex/psex Fis with pvalue computation

pgen, psex and p-values

Basic commands:

```
pgen(posidonia)
psex(posidonia)
```

or, for haploid data:

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

Options: (idem on psex and pgen)

```
#allelic frequencies computation:
psex(posidonia) #psex on ramets
psex(posidonia, genet = TRUE) #psex on genets
psex(posidonia, RR = TRUE) #psex with Round-Robin method
#psex computation
psex(posidonia) #psex with one psex per replica
psex(posidonia, MLGsim = TRUE) #psex MLGsim method
#pvalues:
psex(posidonia, nbrepeat = 100) #with p-values
psex(posidonia, nbrepeat = 1000, bar = TRUE) #with p-values and a progression bar
```

Results:

pgen	genet	psex	pvalue
2.20e-06			
0.00e+00			
4.77e-05			
4.77e-05	3	0.00190284159898287	0.392857142857143
4.77e-05	3	$1.76851132496336\mathrm{e}\text{-}06$	0
4.77e-05	3	$1.06767920426143\mathrm{e}\text{-}09$	0

res[[2]] #sim psex values

```
    [1] 2.682915e-03 1.351209e-03 3.404466e-03 1.543552e-03 4.299086e-03
    [6] 6.265958e-03 9.866499e-03 1.920650e-03 2.045403e-03 5.527621e-04
    [11] 6.364326e-04 1.374158e-03 5.837434e-03 3.624390e-03 2.895358e-03
    [16] 5.969326e-03 9.347855e-04 7.666523e-04 6.671097e-05 2.522795e-03
    [21] 5.676186e-03 1.297853e-03 1.105800e-03 5.573546e-03 2.807860e-03
    [26] 4.025514e-03 1.851704e-03 5.309521e-03
```

Fis, pgen Fis, psex Fis and p-values

Not for haploid data!

 \mathbf{Fis}

Basic commands:

```
Fis(posidonia)
```

Options:

Results:

```
Fis(posidonia, RR = TRUE)[[2]]
```

locus	Hobs	Hatt	Fis
locus_1	0.6666667	0.7994410	0.1660839
$locus_2$	0.5185185	0.5024949	-0.0318882
$locus_3$	0.8846154	0.8099548	-0.0921788
$locus_4$	0.2962963	0.2620545	-0.1306667
$locus_5$	0.3214286	0.5512987	0.4169611
$locus_6$	0.6400000	0.6555102	0.0236613
$locus_7$	0.3571429	0.3818182	0.0646259

pgen Fis, psex Fis and p-values

Basic commands: (idem for pgen_Fis and psex_Fis)

```
pgen_Fis(posidonia)
```

Options:

```
#allelic frequencies:
psex_Fis(posidonia) #psex Fis on ramets
psex_Fis(posidonia, genet = TRUE) #psex Fis on genets
psex_Fis(posidonia, RR = TRUE) #psex Fis with Round-Robin method
#psex computation
psex_Fis(posidonia) #psex Fis, one for each replica
psex_Fis(posidonia, MLGsim = TRUE) #psex Fis with MLGsim method
#pvalues
psex_Fis(posidonia, nbrepeat = 100) #with p-values
psex_Fis(posidonia, nbrepeat = 1000, bar = TRUE) #with p-values and a progression bar
```

Results:

pgenFis	genet	psexFis	pvalue
1.05 e-05			
0.00e+00			
4.39e-05			
4.39e-05	3	0.00175402908240928	0.258064516129032
4.39e-05	3	1.50248895374508e-06	0
4.39 e - 05	3	$8.36013934496707 \mathrm{e}\text{-}10$	0

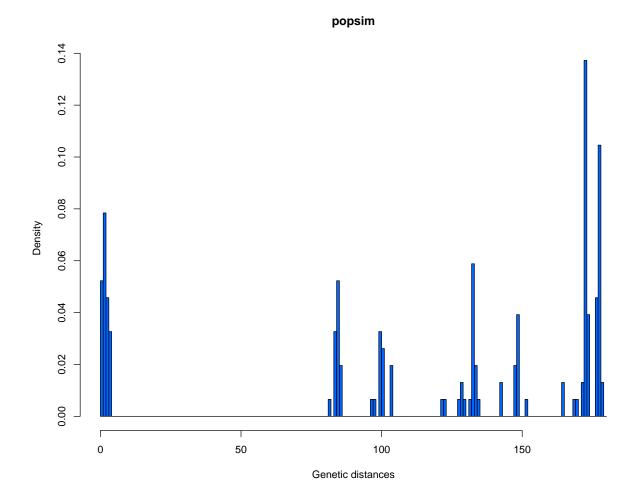
res[[2]] #sim psex Fis values

```
    [1] 0.0040481045 0.0031602068 0.0092107387 0.0005867821 0.0078841578
    [6] 0.0016065540 0.0008205260 0.0037157779 0.0069945737 0.0013747738
    [11] 0.0025227684 0.0012591533 0.0131772838 0.0011010652 0.0016714224
    [16] 0.0036430883 0.0043642467 0.0009267953 0.0146375958 0.0097961140
    [21] 0.0056357471 0.0049308171 0.0105839008 0.0018554896 0.0057345994
    [26] 0.0180426243 0.0025966226 0.0045779356 0.0036178632 0.0088153811
    [31] 0.0076859203
```

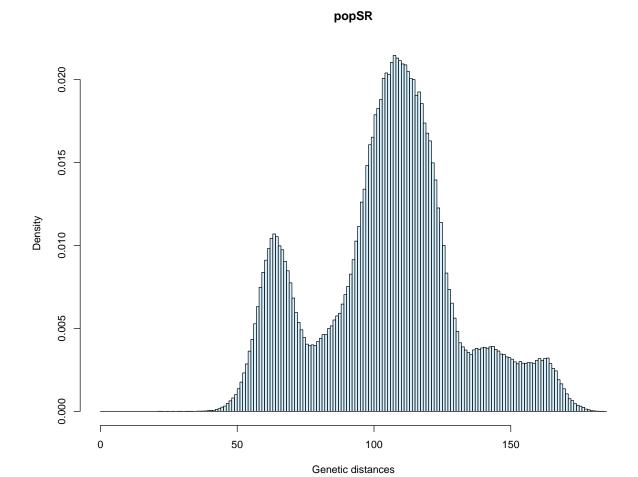
E.2 Tests for MLLs occurrence and assessment of their memberships

Genetic distance matrix computation and threshold definition

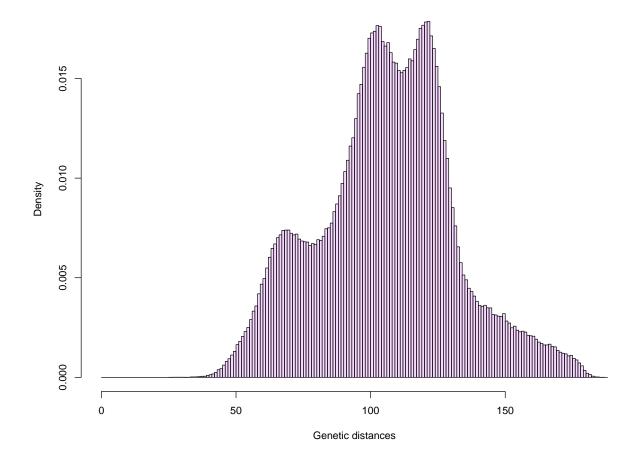
On a theoretical diploid population with c = 0.9999 (c, clonality rate).

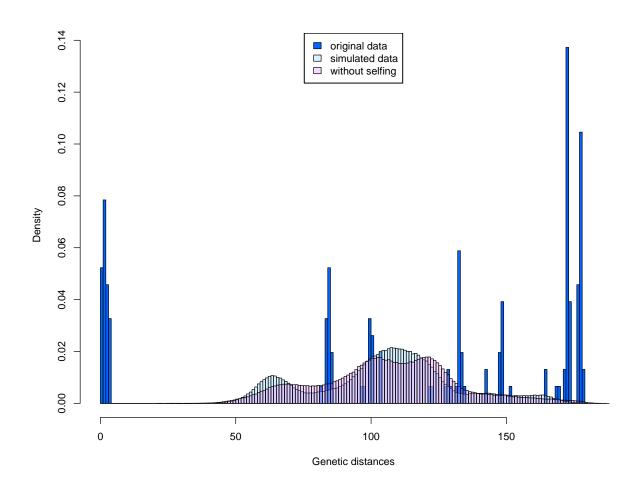


```
p2 <- hist(ressim$distance_matrix, freq = FALSE, col = rgb(0.7,0.9,1,0.5), main = "popSR", xlab = "Genetic distances", breaks = seq(0, max(ressim$distance_matrix)+1, 1))
```



popSRWS





```
#determining alpha2
table(respop$distance_matrix)
>
                4 82 84
                           85
                               86
                                    97
                                        98 100 101 104 122 123 128 129 130
            7
                5
    8 12
                    1
                        5
                             8
                                 3
                                     1
                                         1
                                             5
                                                 4
                                                     3
                                                                      2
> 132 133 134 135 143 148 149 152 165 169 170 172 173 174 177 178 179
                                     2
\#alpha2 = 4
```

```
#creating MLL list:
MLLlist <- MLL_generator(popsim, alpha2 = 4)
#or
res <- genet_dist(popsim, alpha2 = 4)
MLLlist <- MLL_generator2(res$potential_clones, MLG_list(popsim))</pre>
```

For haploid data, theoretical example:

```
respop <- genet_dist(haplodata, haploid = TRUE)
ressim <- genet_dist_sim(haplodata, haploid = TRUE, nbrepeat = 1000)
MLLlist <- MLL_generator(haplodata, haploid = TRUE, alpha2 = 4)
#or</pre>
```

```
res <- genet_dist(haplodata, haploid = TRUE, alpha2 = 4)
MLLlist <- MLL_generator2(res$potential_clones, haploid = TRUE, MLG_list(haplodata))</pre>
```

F. Genotypic diversity, richness and evenness indices calculation

F.1 Classic genotypic indices

Basic commands:

```
clonal_index(posidonia)
```

or, with MLL:

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

or, for haploid data:

```
clonal_index(haplodata)
```

Results:

clonal_index(posidonia)

	N	G	R	Н"	J'	D	V	Hill
MLG	40	28	0.6923077	3.149621	0.9452064	0.9705128	0.7921811	33.91304

F.2 Pareto index

Basic commands:

```
Pareto_index(posidonia)
```

or, with MLL:

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

or, for haploid data:

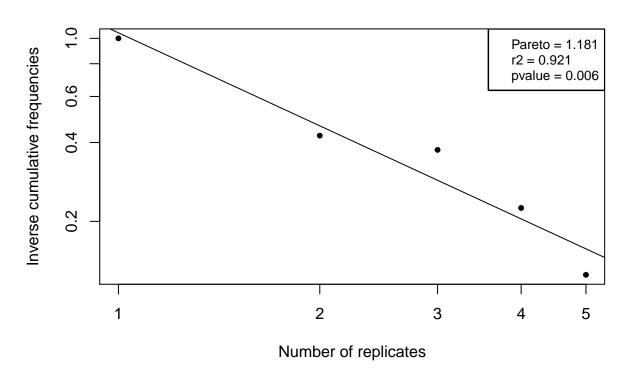
```
Pareto_index(haplodata)
```

 ${\bf Options:}$

```
Pareto_index(posidonia, graph = TRUE) #classic graphic
Pareto_index(posidonia, legends = 2, export = TRUE) #export option
Pareto_index(posidonia, full = TRUE) #all results
```

```
res <- Pareto_index(posidonia, full = TRUE, graph = TRUE, legends = 2)
```

Pareto distribution



G. Spatial components of clonality

G.1 Spatial autocorrelation

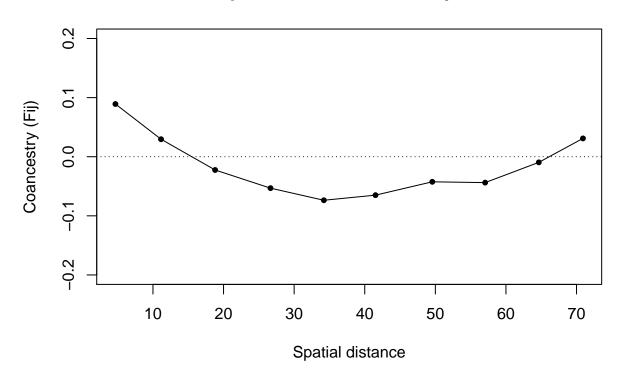
Basic commands:

```
autocorrelation(posidonia, coords = coord_posidonia, 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:
data(posidonia)
data(coord_posidonia)
#kinship distances:
autocorrelation(posidonia, coords = coord_posidonia, Loiselle = TRUE)
autocorrelation(posidonia, coords = coord_posidonia, Ritland = TRUE)
#ramets/genets methods:
autocorrelation(posidonia, coords = coord_posidonia, Loiselle = TRUE) #ramets
autocorrelation(posidonia, coords = coord_posidonia, Loiselle = TRUE,
                    genet = TRUE, central_coords = TRUE)
                                            #genets, central coordinates of each MLG
autocorrelation(posidonia, coords = coord_posidonia, Loiselle = TRUE,
                genet = TRUE, random_unit = TRUE) #genets, one random unit per MLG
autocorrelation(posidonia, coords = coord_posidonia, Loiselle = TRUE,
                genet = TRUE, weighted = TRUE) #genets, with weighted matrix on kinships
#distance classes construction:
autocorrelation(posidonia, coords = coord_posidonia, 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(posidonia, coords = coord_posidonia, Loiselle = TRUE,
                    vecdist = distvec) #custom distance vector
autocorrelation(posidonia, coords = coord_posidonia, Loiselle = TRUE,
                    class1 = TRUE, d = 7) #7 equidistant classes
autocorrelation(posidonia, coords = coord_posidonia, Loiselle = TRUE,
                    class2 = TRUE, d = 7)
                    #7 distance classes with the same number of units in each
#graph options:
autocorrelation(posidonia, coords = coord_posidonia, Ritland = TRUE, graph = TRUE)
                                                                     #displays graph
autocorrelation(posidonia, coords = coord_posidonia, Ritland = TRUE, export = TRUE)
                                                                     #export graph
```

#pvalues computation

autocorrelation(posidonia, coords = coord_posidonia, Ritland = TRUE, nbrepeat = 1000)

Spatial aucorrelation analysis



names(res)

- > [5] "Matrix_kinship_results" "Class_kinship_results"
- > [7] "Class_distance_results"

res\$Main_results #enables graph reproduction

dist_min	$\operatorname{dist}_{-\operatorname{max}}$	$dist_mean$	$\ln({\rm dist_mean})$	nb_pairs	$mean_Ritland$	pval_kin
0.50000	7.51665	4.683712	1.544091	97	0.0891802	0.000
7.61577	15.20691	11.148114	2.411270	157	0.0296031	0.000
15.23975	22.80351	18.807914	2.934278	119	-0.0224115	0.390
22.94014	30.41381	26.648255	3.282724	110	-0.0531668	0.000
30.50000	38.00329	34.206496	3.532416	121	-0.0736379	0.000
38.02959	45.59879	41.524146	3.726275	64	-0.0650049	0.000
46.09772	53.08484	49.568560	3.903357	34	-0.0424233	0.144
53.53737	60.66144	57.055830	4.044030	29	-0.0438132	0.154
61.00205	68.00184	64.657149	4.169099	31	-0.0095349	0.800
68.52919	76.04111	70.912179	4.261442	18	0.0309692	0.106

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

- > mean_Ritland
- > -0.01602399

res\$Slope_and_Sp_index #gives b and Sp indices

	b	b_log	Sp	Sp_log
obs_value	-0.0007007	-0.0357734	0.0007693	0.0392760
$mean_sim$	0.0000020	0.0000347	-0.0000008	0.0000097
sd_sim	0.0002752	0.0062994	0.0002726	0.0062438
0.95 _inf	-0.0006246	-0.0141703	-0.0004583	-0.0098627
0.95 _sup	0.0004646	0.0100574	0.0006179	0.0141312
0.9 _inf	-0.0004780	-0.0112594	-0.0004014	-0.0087134
0.9 _sup	0.0004031	0.0089617	0.0004759	0.0112549
pval_upper	0.0150000	0.0000000	0.9890000	1.0000000
pval_lower	0.9850000	1.0000000	0.0110000	0.0000000
$pval_2sides$	0.0300000	0.0000000	0.0220000	0.0000000

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

G.2 Clonal subrange

Basic commands:

```
clonal_sub(posidonia, coords = coord_posidonia)
```

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(posidonia, coords = coord_posidonia, class1 = TRUE, d = 7)

#7 equidistant classes

clonal_sub(posidonia, coords = coord_posidonia, class1 = TRUE, d = 7)

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

```
res <- clonal_sub(posidonia, coords = coord_posidonia)
res[[1]] #Global clonal subrange</pre>
```

> [1] 11.6619

res\$clonal_sub_tab #details per class

nb_pairs	$\operatorname{dist_min}$	$\operatorname{dist_max}$	$dist_mean$	Fr	$\log(\text{Fr})$
97	0.5	7.516648	4.683712	0.1649485	-0.7826518
157	7.615773	15.20691	11.14811	0.04458599	-1.350802
119	15.23975	22.80351	18.80791	0	-Inf
110	22.94014	30.41381	26.64826	0	-Inf
121	30.5	38.00329	34.2065	0	-Inf
64	38.02959	45.59879	41.52415	0	-Inf
34	46.09772	53.08484	49.56856	0	-Inf
29	53.53737	60.66144	57.05583	0	-Inf
31	61.00205	68.00184	64.65715	0	-Inf
18	68.52919	76.04111	70.91218	0	-Inf

G.3 Aggregation index

Basic commands:

```
agg_index(posidonia, coords = coord_posidonia)
```

or, with MLL:

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

or, for haploid data:

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

Options:

```
res <- agg_index(posidonia, coords = coord_posidonia, nbrepeat = 1000)
```

res\$results #Aggregation index

Ac	pval	nbrepeat
0.2272127	0	1000

#res\$simulation #vector of sim aggregation index

G.4 Edge Effect

Basic commands:

```
#for posidonia, center of quadra is at 40,10
edge_effect(posidonia, coords = coord_posidonia, center = c(40,10))
```

or, with MLL:

```
edge_effect(popsim, coords = coord_sim, center = c(40,10), listMLL = MLLlist)
```

or, for haploid data:

```
edge_effect(haplodata, coords = coord_haplo, center = c(40,10))
```

Options:

Results:

```
res <- edge_effect(posidonia, coords = coord_posidonia, center = c(40,10), nbrepeat = 1000)
```

res\$results #Aggregation index

Ee	pval_Ee	nbrepeat
0.0778672	0.434	1000

H. BONUS: "Ready to use" Table

Summary function of main results:

Basic commands:

```
genclone(posidonia, coords = coord_posidonia)
```

or, with MLL:

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

or, for haploid data:

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

Options:

Results:

```
GenClone(posidonia, coords = coord_posidonia)
```

N	Lineage	nb_L	nb_all	SE	Fis	pval_2sides	Fis_WR	pval_2sides.1	R
40	MLG	28	4.142857	0.7693093	0.05076926	NA	0.02568129	NA	0.6923077

$Pareto_index$	$Sp_Loiselle$	$pval_2sides$	$\mathrm{Sp}_\mathrm{L}_\mathrm{WR}$	$pval_2sides.1$	$Sp_Ritland$	$pval_2sides.2$
1.180756	0.001230855	NA	0.0012436	NA	0.0007693264	NA

Sp_R_WR	pval_2sides	Н"	J'	D	V	Hill
0.0008031684	NA	3.149621	0.9452064	0.9705128	0.7921811	33.91304