Vignette for package `resamplediversity`

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This vignette documents workflow and results from our paper[1].

Installing the package `resamplediversity`

Our package has one dependency, a package called `adegenet`. On Windows, you can install the package with the following command

```
install.packages("adegenet")
install.packages(file.choose(), repos = NULL)
```

whereupon a window will pop-up. You can now select the package binary (.zip). Instead of `file.choose()` you can specify a (full) path to the .zip file. If you are using R GUI, you can install by clicking `Packages > Install package(s) from local zip file` and navigate to the downloaded file (make sure you have `adegenet` installed). Ultimately, you can build from the **source** tarball on any operating system platform. Source is available on request from package author (`tomaz.skrbinsek@gmail.com`) or maintainer (`roman.lustrik@gmail.com`).

Contact us if you have a problem installing the package.

Analysis workflow

The Dinaric bears are used as the reference population. We can make a quick summary of the data and look at the loci that were used.

```
data(dinaric.genotypes)
```

```
summary(dinaric.genotypes)


 # Total number of genotypes:  513

 # Population sample sizes:

513

 # Number of alleles per locus:
```

```
L01 L02 L03 L04 L05 L06 L07 L08 L09 L10 L11 L12 L13 L14 L15
  6   9   9   7   7   7   6  10  10   6   8  10   6   8   7
L16 L17 L18 L19 L20
  8   7   6  10   7

 # Number of alleles per population:
  1
136

 # Percentage of missing data:
[1] 0.019

 # Observed heterozygosity:
 L01  L02  L03  L04  L05  L06  L07  L08  L09  L10  L11  L12
0.77 0.73 0.76 0.80 0.65 0.63 0.76 0.77 0.82 0.66 0.62 0.70
 L13  L14  L15  L16  L17  L18  L19  L20
0.68 0.72 0.78 0.79 0.80 0.57 0.87 0.76

 # Expected heterozygosity:
 L01  L02  L03  L04  L05  L06  L07  L08  L09  L10  L11  L12
0.76 0.71 0.74 0.79 0.69 0.64 0.76 0.78 0.84 0.65 0.66 0.72
 L13  L14  L15  L16  L17  L18  L19  L20
0.68 0.74 0.77 0.81 0.80 0.59 0.85 0.78
```

```
locNames(dinaric.genotypes)
```

```
    L01     L02     L03     L04     L05     L06     L07
"Cxx20"  "G10B"  "G10C"  "G10D"  "G10J"  "G10L"  "G10M"
    L08     L09     L10     L11     L12     L13     L14
 "G10P"  "G10X"   "G1A"  "Mu05"  "Mu09"  "Mu10"  "Mu11"
    L15     L16     L17     L18     L19     L20
 "Mu15"  "Mu23"  "Mu50"  "Mu51"  "Mu59"  "Mu61"
```

To compare genetic diversity indices between two populations, we need to have a common set of loci and provide correction for unequal sample sizes. The latter is especially important for estimates of allelic richness, as this parameter is heavily dependent on sample size (rare alleles will not make it when sample size is low). Expected heterozygosity is much more robust.
We first need a list of common markers. Let's look at a table of diversity parameters from different brown bear populations around the world (see table 1):

```
data(bear.diversity)
bear.diversity
```

Let's compare genetic diversity between Dinaric bears and bears in Kluane, Yukon. They were studied in study 2:

Table 1: Table of brown bear diversity data from a number of studies around the world.

	Population	N	Study	A	SEA	He	SEHe
1	Carpathians - Romania (1)	16	5	7.78	0.81	0.81	0.01
2	Carpathians - Romania (2)	109	10	8.46	0.57	0.80	0.01
3	Alaska Range, Alaska	28	1			0.78	
4	Kluane, Yukon	50	2	7.38	0.56	0.76	0.02
5	Richardson Mountains, NWT	119	2	7.50	0.63	0.76	0.03
6	Brooks Range, Alaska	148	2	7.63	0.50	0.75	0.02
7	Croatia (Dinara-Pindos NW)	156	9	7.58	0.54	0.74	0.03
8	Slovenia (NW Dinaric Mountains)	513	0	6.68	0.41	0.73	0.02
9	Greece(Dinara-Pindos SE)	49	8	6.33	0.42	0.76	0.02
10	Carphatians - Northern Slovakia	71	10	6.08	0.29	0.71	0.02
11	Scandinavia - NN	29	3	5.59	0.40	0.69	0.02
12	Flathead River, BC/MT	40	2	6.50	0.71	0.69	0.03
13	Carpathians - Central Slovakia	96	10	6.00	0.25	0.70	0.03
14	Scandinavia - NS	108	3	6.18	0.35	0.69	0.03
15	West Slope, Alberta	41	2	6.38	0.56	0.68	0.04
16	Kuskoskwim Range, Alaska	55	2	6.13	0.44	0.68	0.03
17	Scandinavia - M	88	3	5.94	0.40	0.68	0.02
18	Scandinavia - S	155	3	5.47	0.33	0.68	0.02
19	East Slope, Alberta	45	2	7.00	0.82	0.67	0.06
20	Carpathians - Eastern Slovakia	16	10	5.23	0.22	0.65	0.03
21	Paulatuk Alaska	58	2	5.75	0.88	0.65	0.65
22	Admiralty Island, Alaska	30	1			0.63	
23	Coppermine, NWT	36	2	5.75	1.03	0.61	0.07
24	Pakistan	28	4	3.92	0.38	0.58	0.04
25	Yellowstone, MT/WY	57	2	4.38	0.60	0.55	0.08
26	Cantabrian (Spain) - W	39	7	3.44	0.30	0.48	0.05
27	Baranof and Chiegagof Is, Alaska	35	1			0.49	
28	Apennines	17	5	2.44	0.24	0.44	0.07
29	Gobi (Mongolia)	8	6	2.00		0.29	
30	Cantabrian (Spain) - E	8	7	1.75	0.17	0.28	0.06
31	Kodiak Island, Alaska	34	2	2.13	0.35	0.27	0.10

```
data(included.studies)
```

```
bear.diversity[4, ]

     Population  N Study   A  SEA   He  SEHe
4 Kluane, Yukon 50     2 7.4 0.56 0.76 0.025

included.studies[included.studies$ID == 2, ]

  ID               Reference       GeoArea
2  2 Paetkau et al., 1998b North America
```

```
2 Exploration of variation in genetic diversity across the range North American brown bear
  NP LocUsed LocCommon
2 11       8         8
```

Looking at the original paper by Paetkau et al., the common markers between both populations are G10B, G10C ,G10D, G10L, G10M, G10P, G10X, G1A. We look at the markers in the reference genotypes:

```
locNames(dinaric.genotypes)
```

```
    L01     L02     L03     L04     L05     L06     L07
"Cxx20"  "G10B"  "G10C"  "G10D"  "G10J"  "G10L"  "G10M"
    L08     L09     L10     L11     L12     L13     L14
 "G10P"  "G10X"   "G1A"  "Mu05"  "Mu09"  "Mu10"  "Mu11"
    L15     L16     L17     L18     L19     L20
 "Mu15"  "Mu23"  "Mu50"  "Mu51"  "Mu59"  "Mu61"
```

Genetic diversity study of this population included samples of 50 individuals. We need to subset the locus panel using generic names of loci:

```
loci_na <- c("L02", "L03", "L04", "L06", "L07",
             "L08", "L09", "L10")
```

We will resample Dinaric genotypes multiple times to the same sample size that was used the Kluane population study (50 samples) using the sampe panel of loci to get comparable genetic diversity indices. This will take a while and produce a lot of relatively useless output from each subsample (omitted here)

```
resampled.ar <- subsample.gen(genotypes = dinaric.genotypes,
                              nboots = 1000,
                              nsamps = 50,
                              loci = loci_na)
```

Look at the results:

```
resampled.ar
```

```
    A SEA   He  SEHe   Ho  SEHo
1 6.1 0.7 0.73 0.026 0.74 0.031
```

Now we can calculate diversity ratios between the Dinaric bear population and Kluane bears.

```
calcDivRat(ref = 6.12, SEref = 0.7, obs = 7.38,
           SEobs = 0.56, type = "A") #allelic richness ratio

   Ar SEAr
1 1.2 0.17

calcDivRat(ref = 0.73, SEref = 0.026, obs = 0.76,
           SEobs = 0.025, type = "He") #heterozygosity ratio
```

```
  Her SEHer
1   1  0.05
```

We can see that allelic richness is 21% higher in Kluane than in Dinaric Mountains, and heterozgosity 4%.
We can now batch-run the corrections for the entire set of North American populations studied by Paetkau et al. using the same locus set:

```
na.pops <- bear.diversity[bear.diversity$Study == 1 |
bear.diversity$Study == 2, ]
```

Table 2: North American populations of brown bears studied by Paetkau et al.

	Population	N	Study	A	SEA	He	SEHe
3	Alaska Range, Alaska	28	1			0.78	
4	Kluane, Yukon	50	2	7.38	0.56	0.76	0.02
5	Richardson Mountains, NWT	119	2	7.50	0.63	0.76	0.03
6	Brooks Range, Alaska	148	2	7.63	0.50	0.75	0.02
12	Flathead River, BC/MT	40	2	6.50	0.71	0.69	0.03
15	West Slope, Alberta	41	2	6.38	0.56	0.68	0.04
16	Kuskoskwim Range, Alaska	55	2	6.13	0.44	0.68	0.03
19	East Slope, Alberta	45	2	7.00	0.82	0.67	0.06
21	Paulatuk Alaska	58	2	5.75	0.88	0.65	0.65
22	Admiralty Island, Alaska	30	1			0.63	
23	Coppermine, NWT	36	2	5.75	1.03	0.61	0.07
25	Yellowstone, MT/WY	57	2	4.38	0.60	0.55	0.08
27	Baranof and Chicgagof Is, Alaska	35	1			0.49	
31	Kodiak Island, Alaska	34	2	2.13	0.35	0.27	0.10

The batch run will TAKE A LONG TIME and produce a lot of useless output on screen. I reduced the number of resamples (`nboots`) to 100 to keep the computation time reasonable. In a real study, you would want `nboots` to be at least 1000.

```
adjusted_na <- runall(N = na.pops$N,
                      genotypes = dinaric.genotypes,
                      loci = loci_na,
                      nboots = 100)
```

Results are presented below.

```
#these are resampled value for the reference population, hence
prefix "ref".
names(adjusted_na) <- paste("ref", names(adjusted_na),
                            sep = "")
pops.adjusted_na <- cbind(na.pops, adjusted_na)
```

```
pops.adjusted_na

                         Population   N Study   A  SEA   He
3              Alaska Range, Alaska  28     1  NA   NA 0.78
4                     Kluane, Yukon  50     2 7.4 0.56 0.76
5         Richardson Mountains, NWT 119     2 7.5 0.63 0.76
6               Brooks Range, Alaska 148     2 7.6 0.50 0.75
12            Flathead River, BC/MT  40     2 6.5 0.71 0.69
15               West Slope, Alberta  41     2 6.4 0.56 0.68
16         Kuskoskwim Range, Alaska  55     2 6.1 0.44 0.68
19              East Slope, Alberta  45     2 7.0 0.82 0.67
21                  Paulatuk Alaska  58     2 5.8 0.88 0.65
22         Admiralty Island, Alaska  30     1  NA   NA 0.63
23                   Coppermine, NWT  36     2 5.8 1.03 0.61
25                Yellowstone, MT/WY  57     2 4.4 0.60 0.55
27 Baranof and Chicgagof Is, Alaska  35     1  NA   NA 0.49
31            Kodiak Island, Alaska  34     2 2.1 0.35 0.27
    SEHe refNsamp refA refSEA refHe refSEHe refHo refSEHo
3     NA       28  5.8  0.67  0.72   0.026  0.74   0.038
4  0.025       50  6.1  0.70  0.73   0.026  0.74   0.031
5  0.030      119  6.4  0.71  0.74   0.025  0.74   0.027
6  0.019      148  6.5  0.72  0.74   0.025  0.74   0.025
12 0.027       40  6.0  0.68  0.73   0.026  0.74   0.032
15 0.036       41  6.0  0.69  0.73   0.026  0.74   0.032
16 0.026       55  6.2  0.71  0.73   0.025  0.74   0.029
19 0.062       45  6.1  0.70  0.73   0.026  0.75   0.032
21 0.650       58  6.2  0.70  0.73   0.026  0.74   0.031
22    NA       30  5.9  0.67  0.73   0.026  0.75   0.034
23 0.073       36  6.0  0.69  0.73   0.026  0.74   0.033
25 0.081       57  6.2  0.70  0.73   0.026  0.74   0.029
27    NA       35  6.0  0.69  0.73   0.026  0.74   0.035
31 0.098       34  6.0  0.70  0.73   0.026  0.75   0.034
```

We can now calculate diversity ratios:

```
Ar.na <- with(pops.adjusted_na,
              calcDivRat(ref = refA, SEref = refSEA, obs = A,
                         SEobs = SEA, type = "A"))
Her.na <-  with(pops.adjusted_na,
                calcDivRat(ref = refHe, SEref = refSEHe,
                           obs = He, SEobs = SEHe, type = "He"))
pops.adjusted_na.out <- cbind(pops.adjusted_na, Ar.na, Her.na)
pops.adjusted_na.out[, c("Population","Ar","SEAr","Her","SEHer")]

                         Population   Ar  SEAr  Her SEHer
3              Alaska Range, Alaska   NA    NA 1.08    NA
4                     Kluane, Yukon 1.20 0.165 1.04 0.050
5         Richardson Mountains, NWT 1.16 0.161 1.03 0.054
6               Brooks Range, Alaska 1.17 0.150 1.02 0.043
12            Flathead River, BC/MT 1.08 0.170 0.95 0.050
```

```
15              West Slope, Alberta 1.06 0.154 0.93 0.060
16         Kuskoskwim Range, Alaska 0.99 0.134 0.93 0.048
19              East Slope, Alberta 1.16 0.191 0.92 0.091
21                 Paulatuk Alaska 0.93 0.177 0.89 0.889
22         Admiralty Island, Alaska   NA    NA 0.87    NA
23                 Coppermine, NWT 0.96 0.206 0.84 0.105
25               Yellowstone, MT/WY 0.71 0.127 0.75 0.114
27 Baranof and Chicgagof Is, Alaska   NA    NA 0.67    NA
31            Kodiak Island, Alaska 0.36 0.072 0.37 0.135
```

To compare Cantabrian bears to the populations in North America, we also calculate reference-population calibrated ratios for this population, and we have comparable genetic diversity indices even if different locus panels and different sample sizes were used. Result is presented in table 3.

```
cant.pops <- bear.diversity[bear.diversity$Study == 7, ]
loci_cant <- c("L02", "L03", "L04", "L05", "L06", "L08", "L09",
               "L10", "L11", "L12", "L13", "L18", "L19", "L20")
adjusted_cant <- runall(N = cant.pops$N,
                        genotypes = dinaric.genotypes,
                        loci = loci_cant,
                        nboots = 100)
names(adjusted_cant) <- paste("ref", names(adjusted_cant),
                              sep = "")
pops.adjusted_cant <- cbind(cant.pops, adjusted_cant)

Ar.cant <- with(pops.adjusted_cant,
                calcDivRat(ref = refA, SEref = refSEA, obs = A,
                           SEobs = SEA, type = "A"))
Her.cant <- with(pops.adjusted_cant,
                 calcDivRat(ref = refHe, SEref = refSEHe,
                            obs = He, SEobs = SEHe, type = "He"))
pops.adjusted_cant.out <- cbind(pops.adjusted_cant, Ar.cant,
                                Her.cant)

pops.comparison <- rbind(
          pops.adjusted_na.out,
          pops.adjusted_cant.out)

pops.comparison[, c("Population","Ar","SEAr","Her","SEHer")]
```

Look at the population comparison with comparable diversity indices:

Table 3: reference-population calibrated diversity ratios for North American and Cantabrian (Spain) populations.

	Population	Ar	SEAr	Her	SEHer
3	Alaska Range, Alaska			1.07	
4	Kluane, Yukon	1.21	0.17	1.04	0.05
5	Richardson Mountains, NWT	1.15	0.16	1.03	0.05
6	Brooks Range, Alaska	1.16	0.15	1.02	0.04
12	Flathead River, BC/MT	1.08	0.17	0.95	0.05
15	West Slope, Alberta	1.06	0.15	0.93	0.06
16	Kuskoskwim Range, Alaska	0.99	0.13	0.93	0.05
19	East Slope, Alberta	1.16	0.19	0.92	0.09
21	Paulatuk Alaska	0.93	0.18	0.89	0.89
22	Admiralty Island, Alaska			0.87	
23	Coppermine, NWT	0.97	0.21	0.84	0.10
25	Yellowstone, MT/WY	0.71	0.13	0.75	0.11
27	Baranof and Chicgagof Is, Alaska			0.67	
31	Kodiak Island, Alaska	0.36	0.07	0.37	0.14
26	Cantabrian (Spain) - W	0.60	0.07	0.67	0.07
30	Cantabrian (Spain) - E	0.38	0.05	0.41	0.09

Results from the paper

Only resampling reference population corrections are done. You can calculate Ar and Her on your own as an exercise (see the example in previous section).

North America[2, 3]

`Nsamples_usa` is a vector of the number of samples.

```
loci_nor <- c("L02", "L03", "L04", "L06",
              "L07", "L08", "L09", "L10")
Nsamples_nor <- c(28, 50, 119, 148, 40, 41, 55, 45,
                  58, 30, 36, 57, 35, 34)
adjusted_nor <- runall(N = Nsamples_nor,
                       genotypes = dinaric.genotypes,
                       loci = loci_nor,
                       nboots = 1000)
```

```
adjusted_nor

   Nsamp   A  SEA   He  SEHe   Ho  SEHo
1     28 5.8 0.67 0.72 0.026 0.74 0.036
2     50 6.1 0.70 0.73 0.026 0.74 0.031
3    119 6.5 0.72 0.73 0.025 0.74 0.026
4    148 6.6 0.72 0.74 0.025 0.74 0.026
5     40 6.0 0.69 0.73 0.026 0.74 0.033
6     41 6.0 0.69 0.73 0.026 0.74 0.032
```

```
7      55 6.2 0.71 0.73 0.026 0.74 0.030
8      45 6.1 0.70 0.73 0.026 0.74 0.031
9      58 6.2 0.70 0.73 0.026 0.74 0.030
10     30 5.9 0.68 0.73 0.026 0.74 0.035
11     36 6.0 0.69 0.73 0.026 0.74 0.034
12     57 6.2 0.71 0.73 0.026 0.74 0.030
13     35 5.9 0.68 0.73 0.026 0.74 0.034
14     34 6.0 0.69 0.73 0.026 0.74 0.034
```

Scandinavia[4]

```
loci_skandinavija <- c("L02", "L03", "L04", "L05", "L06", "L07",
                       "L08", "L09", "L10", "L11", "L13", "L15",
                       "L17", "L18", "L19", "L20")
Nsamples_skand <- c(108, 29, 155, 88)
adjusted_skand <- runall(N = Nsamples_skand,
                         genotypes = dinaric.genotypes,
                         loci = loci_skandinavija,
                         nboots = 1000)
```

```
adjusted_skand

  Nsamp   A  SEA   He  SEHe   Ho  SEHo
1   108 6.1 0.44 0.73 0.019 0.73 0.023
2    29 5.6 0.42 0.72 0.020 0.73 0.028
3   155 6.2 0.44 0.73 0.019 0.73 0.022
4    88 6.0 0.44 0.73 0.019 0.73 0.024
```

Romania and Ital[5]

```
loci_RO_I <- c("L02","L03","L04","L06","L08",
               "L10","L15","L18","L19")
Nsamples_ROI <- c(16, 17)
adjusted_ROI <- runall(N = Nsamples_ROI,
                       genotypes = dinaric.genotypes,
                       loci = loci_RO_I,
                       nboots = 1000)
```

```
adjusted_ROI

  Nsamp   A  SEA  He SEHe   Ho  SEHo
1    16 5.1 0.56 0.7 0.03 0.73 0.047
2    17 5.2 0.56 0.7 0.03 0.73 0.046
```

Cantabria[6]

```
loci_Cantabria <- c("L02", "L03", "L04", "L05", "L06", "L08",
                    "L09", "L10", "L11", "L12", "L13", "L18",
                    "L19", "L20")
Nsamples_Cant <- c(8, 39)
adjusted_Cant <- runall(N = Nsamples_Cant,
                        genotypes = dinaric.genotypes,
                        loci = loci_Cantabria,
                        nboots = 1000)
```

```
adjusted_Cant

  Nsamp   A  SEA   He  SEHe   Ho  SEHo
1     8 4.6 0.38 0.68 0.026 0.72 0.047
2    39 5.7 0.48 0.71 0.021 0.72 0.029
```

Pakistan[7]

```
loci_Pakistan <- c("L02", "L03", "L04", "L05", "L06", "L09",
                   "L10", "L13", "L15", "L17", "L18", "L19")
Nsamples_Pak <- 28
adjusted_pak <- runall(N = Nsamples_Pak,
                       genotypes = dinaric.genotypes,
                       loci = loci_Pakistan,
                       nboots = 1000)
```

```
adjusted_pak

  Nsamp   A  SEA   He  SEHe   Ho  SEHo
1    28 5.5 0.53 0.72 0.025 0.73 0.034
```

Greece[8]

```
loci_Greece <- c("L03", "L04", "L05", "L08", "L17", "L19")
Nsamples_Greece <- 49
adjusted_Greece <- runall(N = Nsamples_Greece,
                          genotypes = dinaric.genotypes,
                          loci = loci_Greece,
                          nboots = 1000)
```

```
adjusted_Greece

  Nsamp   A  SEA   He  SEHe   Ho  SEHo
1    49 6.5 0.52 0.77 0.024 0.78 0.037
```

Croatia[9]

```
loci_Croatia <- c("L02", "L03", "L04", "L05", "L06", "L08",
                  "L09", "L13", "L17", "L18", "L19")
Nsamples_Croatia <- 156
adjusted_Croatia <- runall(N = Nsamples_Croatia,
                           genotypes = dinaric.genotypes,
                           loci = loci_Croatia,
                           nboots = 1000)
```

```
adjusted_Croatia

  Nsamp   A SEA   He  SEHe   Ho  SEHo
1   156 6.5 0.6 0.73 0.025 0.73 0.029
```

Slovakia and Romania[10]

```
loci_SkRo <- c("L02", "L03", "L04", "L05", "L06", "L07",
               "L08", "L09", "L13", "L17", "L18", "L19")
Nsamples_SkRo <- c(71,96,16,109)
adjusted_SkRo <- runall(N = Nsamples_SkRo,
                        genotypes = dinaric.genotypes,
                        loci = loci_SkRo,
                        nboots = 1000)
```

```
adjusted_SkRo

  Nsamp   A  SEA   He  SEHe   Ho  SEHo
1    71 6.2 0.54 0.73 0.023 0.74 0.029
2    96 6.3 0.54 0.73 0.023 0.74 0.028
3    16 5.5 0.49 0.72 0.025 0.74 0.039
4   109 6.3 0.55 0.74 0.023 0.74 0.028
```

Gobi[11]

```
loci_gobi <- c("L02", "L03", "L04", "L06", "L09", "L10")
Nsamples_gobi <- 8
adjusted_gobi <- runall(N = Nsamples_gobi,
                        genotypes = dinaric.genotypes,
                        loci = loci_gobi,
                        nboots = 1000)
```

```
adjusted_gobi

  Nsamp   A  SEA   He  SEHe   Ho  SEHo
1     8 4.6 0.61 0.68 0.038 0.74 0.067
```

References

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```r
sessionInfo()

R version 2.15.1 (2012-06-22)
Platform: x86_64-pc-mingw32/x64 (64-bit)

locale:
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attached base packages:
[1] stats     graphics  grDevices utils     datasets
[6] methods   base

other attached packages:
[1] adegenet_1.3-4 ade4_1.5-0     MASS_7.3-18
[4] xtable_1.7-0   knitr_0.5

loaded via a namespace (and not attached):
 [1] codetools_0.2-8 digest_0.5.2    evaluate_0.4.2
 [4] formatR_0.4     highlight_0.3.1 parser_0.0-14
 [7] plyr_1.7.1      Rcpp_0.9.10     stringr_0.6
[10] tools_2.15.1
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