

Fur seal odour encodes colony membership, mother-offspring similarity, relatedness and genetic quality

Stoffel, M.A., Caspers, B.A., Forcada, J., Giannakara, A., Baier, M.C., Eberhart-Phillips, L.J., Müller, C. & Hoffman, J.I.

This is a script providing the code for all major analyses in our paper, following the order of analyses in the results section. Some large functions are outsourced into a package that can be loaded into R with the following code.

```
library(devtools)
# install_github()
```

Loading data, standardisation and transformation

Loading the raw scent data (aligned by algorithm) and a factor data frame containing identities for colony membership (colony), mother-offspring pairs (family) and mothers and pups, respectively (age)

```
scent_raw <- as.data.frame(t(read.csv("scent_raw.csv", row.names = 1)))
factors <- read.csv("factors.csv", row.names=1)
names(factors) <- c("colony", "family")
factors$age <- c(rep(1,41), rep(2,41))
```

Standardising observations by total, such that within every observation compounds add up to 100 % (Thus averaging out absolute concentration differences between samples)

```
scent_stand <- as.data.frame(t(apply(scent_raw, 1, function(x) (x/sum(x)) * 100)))
```

Log(x+1) transformation of the standardised scent data.

```
scent <- log(scent_stand + 1)
```

The scent matrix contains 82 observations and 213 compounds (retention times are column names) in total

```
dim(scent)
```

```
## [1] 82 213
```

```
head(scent[1:6])
```

```
##      8.061111111      8.23 8.307142857 8.394 8.47375 8.516153846
## M10 0.000000 0.000000 0.000000      0 0.000000 0.6562090
## M12 0.000000 0.000000 0.4864961      0 0.000000 0.0000000
## M14 3.222626 1.665421 0.000000      0 0.000000 0.0000000
## M15 0.000000 0.000000 0.000000      0 0.000000 0.0000000
## M16 0.000000 0.000000 0.6849915      0 1.008018 0.5654895
## M17 2.330450 0.000000 0.000000      0 0.000000 0.0000000
```

```
head(factors)
```

```
##      colony family age
## M10      2      10  1
## M12      2      12  1
## M14      2      14  1
## M15      1      15  1
## M16      2      16  1
## M17      2      17  1
```

Differences in olfactory fingerprints between the two colonies

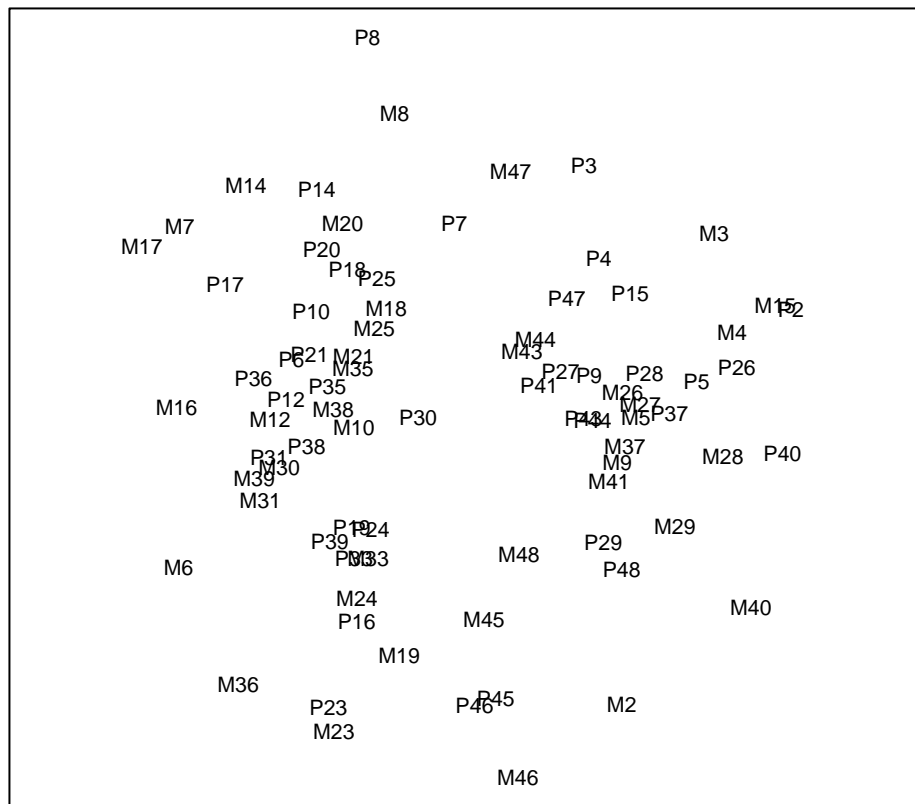
```
library(vegan)
library(MASS)
```

Plot nMDS

```
scent_mds <- isoMDS(vegdist(scent))
```

```
## initial value 28.002906
## iter   5 value 21.594484
## final  value 21.345037
## converged
```

```
ordiplot(scent_mds, type = "t", ylab = "", xlab = "", axes=FALSE, frame.plot=TRUE)
```



Analysis of Similarities (ANOSIM) tests for group differences based on a Bray-curtis similarity matrix.

Dissimilarity between the two colonies.

```
anosim(dat = scent, grouping = factors$colony,
        distance = "bray", permutations = 1000)
```

```
##
## Call:
## anosim(dat = scent, grouping = factors$colony, permutations = 1000,      distance = "bray")
## Dissimilarity: bray
##
## ANOSIM statistic R: 0.5691
##      Significance: 0.000999
##
## Based on 1000 permutations
```

Dissimilarity between mothers from the two colonies.

```
anosim(dat = scent[factors$age == 1, ], grouping = factors$colony,
       distance = "bray", permutations = 1000)
```

```
##
## Call:
## anosim(dat = scent[factors$age == 1, ], grouping = factors$colony,      permutations = 1000, distance = "bray")
## Dissimilarity: bray
##
## ANOSIM statistic R: 0.5748
##      Significance: 0.000999
##
## Based on 1000 permutations
```

Dissimilarity between pups from the two colonies.

```
anosim(dat = scent[factors$age == 2, ], grouping = factors$colony,
       distance = "bray", permutations = 1000)
```

```
##
## Call:
## anosim(dat = scent[factors$age == 2, ], grouping = factors$colony,      permutations = 1000, distance = "bray")
## Dissimilarity: bray
##
## ANOSIM statistic R: 0.556
##      Significance: 0.000999
##
## Based on 1000 permutations
```

Genetic differentiation of the two colonies was tested in Arlequin (Fst) and Structure (Bayesian structure analysis)

Mother offspring similarity

Overall

```
anosim(dat = scent, grouping = factors$family,
       distance = "bray", permutations = 1000)
```

```
##
## Call:
## anosim(dat = scent, grouping = factors$family, permutations = 1000,      distance = "bray")
## Dissimilarity: bray
##
## ANOSIM statistic R: 0.6723
##      Significance: 0.000999
##
## Based on 1000 permutations
```

Within colony 1 (Special study beach)

```
anosim(dat = scent[factors$colony == 1, ],
       grouping = factors[factors$colony == 1, ]$family,
       distance = "bray", permutations = 1000)
```

```
##
## Call:
## anosim(dat = scent[factors$colony == 1, ], grouping = factors[factors$colony == 1, ]$family, pe
## Dissimilarity: bray
##
## ANOSIM statistic R: 0.5339
##      Significance: 0.000999
##
## Based on 1000 permutations
```

Within colony 2 (Freshwater beach)

```
anosim(dat = scent[factors$colony == 2, ],
       grouping = factors[factors$colony == 2, ]$family,
       distance = "bray", permutations = 1000)
```

```
##
## Call:
## anosim(dat = scent[factors$colony == 2, ], grouping = factors[factors$colony == 2, ]$family, pe
## Dissimilarity: bray
##
## ANOSIM statistic R: 0.4532
##      Significance: 0.000999
##
## Based on 1000 permutations
```

Olfactory similarity vs. geographic distance on special study beach

For the special study beach, exact data of the geographical positions is available.

```
coord <- read.csv("coordinates_beach1.csv", row.names=1)
```

Converting coordinates to pairwise euclidian distance matrix

```
dist_mat <- as.matrix(dist(coord, method = "euclidian"))
```

Constructing bray curtis similarity matrix of all individuals from beach 1

```
scent_bc <- as.matrix(vegdist(as.matrix(scent[factors$colony == 1, ])),
                          method = "bray")
```

Geographic distance vs. olfactory similarity in mothers

```

geo_mum <- dist_mat[1:20, 1:20]
scent_mum <- scent_bc[1:20, 1:20]
vegan::mantel(geo_mum, scent_mum, method = "spearman")

##
## Mantel statistic based on Spearman's rank correlation rho
##
## Call:
## vegan::mantel(xdis = geo_mum, ydis = scent_mum, method = "spearman")
##
## Mantel statistic r: 0.008091
##      Significance: 0.447
##
## Upper quantiles of permutations (null model):
##   90%   95% 97.5%   99%
## 0.198 0.259 0.327 0.381
##
## Based on 999 permutations

```

Geographic distance vs. olfactory similarity in pups

```

geo_pup <- dist_mat[21:40, 21:40]
scent_pup <- scent_bc[21:40, 21:40]
vegan::mantel(geo_pup, scent_pup, method = "spearman")

##
## Mantel statistic based on Spearman's rank correlation rho
##
## Call:
## vegan::mantel(xdis = geo_pup, ydis = scent_pup, method = "spearman")
##
## Mantel statistic r: 0.06039
##      Significance: 0.332
##
## Upper quantiles of permutations (null model):
##   90%   95% 97.5%   99%
## 0.178 0.228 0.275 0.316
##
## Based on 999 permutations

```

Relatedness and overall olfactory similarity

Load pairwise relatedness based on 41 markers

```
relatedness <- as.matrix(read.csv("relatedness.csv", row.names=1))
```

Pairwise bray curtis similarity of all individuals

```
scent_bc <- 1-(as.matrix(vegdist(as.matrix(scent))), method = "bray"))
```

Mantel test between relatedness and bray curtis similarity of all individuals

```
vegan::mantel(relatedness, scent_bc, method = "spearman", permutation = 1000)
```

```
##
## Mantel statistic based on Spearman's rank correlation rho
##
## Call:
## vegan::mantel(xdis = relatedness, ydis = scent_bc, method = "spearman",      permutations = 1000)
##
## Mantel statistic r: 0.07231
##      Significance: 0.007992
##
## Upper quantiles of permutations (null model):
##      90%      95%  97.5%      99%
## 0.0352 0.0505 0.0593 0.0691
##
## Based on 1000 permutations
```

Mothers: mantel test between relatedness and bray curtis similarity

```
vegan::mantel(relatedness[factors$age == 1, factors$age == 1],
              scent_bc[factors$age == 1, factors$age == 1],
              method = "spearman", permutation = 1000)
```

```
##
## Mantel statistic based on Spearman's rank correlation rho
##
## Call:
## vegan::mantel(xdis = relatedness[factors$age == 1, factors$age ==      1], ydis = scent_bc[factors$age == 1, factors$age == 1], method = "spearman", permutation = 1000)
##
## Mantel statistic r: 0.05938
##      Significance: 0.097902
##
## Upper quantiles of permutations (null model):
##      90%      95%  97.5%      99%
## 0.0585 0.0749 0.0932 0.1053
##
## Based on 1000 permutations
```

Pups: mantel test between relatedness and bray curtis similarity

```
vegan::mantel(relatedness[factors$age == 2, factors$age == 2],
              scent_bc[factors$age == 2, factors$age == 2],
              method = "spearman", permutation = 1000)
```

```
##
## Mantel statistic based on Spearman's rank correlation rho
##
```

```
## Call:
## vegan::mantel(xdis = relatedness[factors$age == 2, factors$age == 2], ydis = scent_bc[factors$age == 2, factors$age == 2])
##
## Mantel statistic r: 0.02985
##      Significance: 0.24476
##
## Upper quantiles of permutations (null model):
##      90%      95%     97.5%     99%
## 0.0552 0.0713 0.0817 0.1018
##
## Based on 1000 permutations
```

Correlation between Heterozygosity and number of compounds in odour profiles

1. Load standardised multilocus heterozygosity (sMLH) based on 41 markers

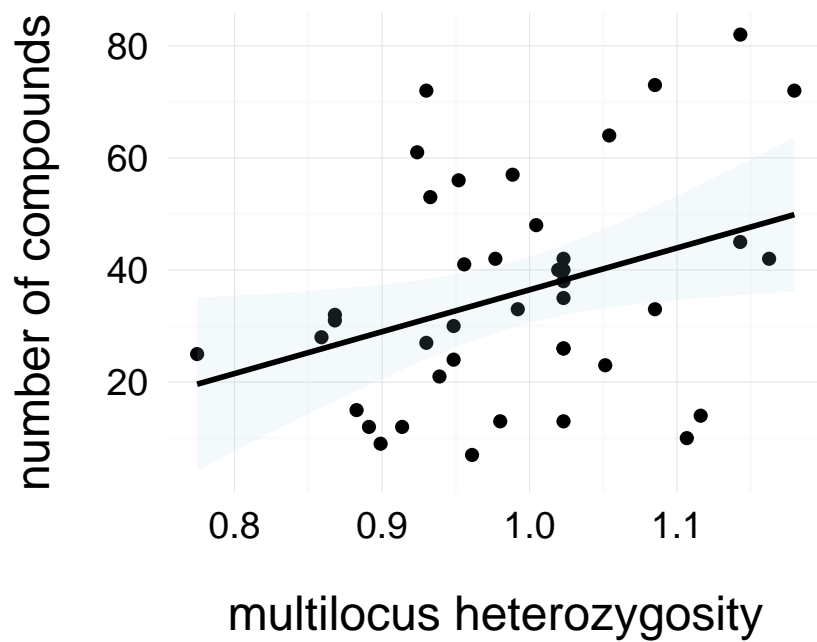
```
heterozygosity <- as.matrix(read.csv("heterozygosity.csv", row.names=1))
```

2. Average number of compounds per individual

```
num_comp <- as.vector(apply(scent, 1, function(x) length(x[x>0])))
```

3. Correlation between Heterozygosity and number of compounds in mothers

```
het_mum <- heterozygosity[factors$age == 1]
num_comp_mum <- num_comp[factors$age==1]
# summary(glm(het_mum ~ num_comp_mum))
```

4. Correlation between Heterozygosity and number of compounds in pups

```
het_pup <- heterozygosity[factors$age == 2]
num_comp_pup <- num_comp[factors$age==2]
# summary(glm(het_pup ~ num_comp_pup))
```

*resampling plot missing
g2 and plot missing*