

iMKT using PopFly or PopHuman data

Brief intro about lots of data right now. Then, PopFly and PopHuman as great databases.

Therefore, iMKT package includes some functions which allow an easy retrieval and analysis of population genetics information stored in these genome browsers. Specifically, the functions permit the download of population genetics parameters computed for every gene annotation in several populations for both model species *Drosophila melanogaster* and *Homo sapiens*.

The examples in this vignette focus only on PopFly data and analysis, but the same process could be performed using human data and functions (**PopHumanData**, **loadPopHumandata()**, **PopHumanAnalysis()**), following the same steps described here. Recombination rate values of human data are retrieved from Bh  rer et al. 2017 Nature Commun. and correspond to the sex average estimates.

Loading the row data

The first step is to load the information into your working environment. This would allow a manual examination of the data before starting any analyses. However, this step can be skipped, and the main data object is loaded into the workspace the first time that the analysis function is called. To download PopFly data use the **loadPopFly()** function, without any argument.

Keep in mind that you are downloading the complete gene information of several populations (16 *D. melanogaster* in this case and 26 in the case of *H. sapiens*), and this process could take a while.

Once the function finishes, the **PopFlyData** object is loaded into the workspace. This object can be manually examined or used when performing the main iMKT analyses.

```
library(iMKT)
loadPopFly()
#> Loading PopFly data into your workspace.
#> This process may take several seconds to complete, please be patient.
ls() ## new object created
#> [1] "PopFlyData"
names(PopFlyData)
#> [1] "Pop"           "Name"          "Start"         "End"           "Chr"
#> [6] "p0"           "pi"            "d0"            "di"            "m0"
#> [11] "mi"           "alpha"         "fisher_pval"   "DoS"           "KaKs"
#> [16] "DAF0f"        "DAF4f"         "cM_Mb"
```

Each row of the **PopFlyData** dataframe contains information regarding one gene annotation in one single population. In total, 16 populations and 13745 genes are included. Metrics for each gene contain information about segregating, divergent and analyzed sites and the Derived Allele Frequency (DAF) for neutral (4fold) and putatively selected (0fold) sites; together with some neutrality tests statistics (Standard MKT, Direction of Selection, Ka/Ks) and gene-associated recombination rate estimates (from Comeron et al.).

Once the data is loaded into the workspace, analyses can be performed using the function **PopFlyAnalysis()**.

Performing PopFly Analyses

The **PopFlyAnalysis()** function allows performing any MK test using a subset of PopFly data defined by custom genes and populations lists. It uses the previously loaded dataframe (PopFlyData). In addition to the **genes** and **populations** lists, the function also has the following parameters:

- **recomb**: group genes according to recombination values (must specify number of bins). Options are: TRUE/FALSE. Recombination values (cM/Mb) from Comeron et al. 2012.
- **bins**: number of recombination bins to compute (mandatory if recomb = TRUE)
- **test**: which test to perform. Options include: standardMK (default), DGRP, FWW, asymptoticMK, iMK
- **xlow**: lower limit for asymptotic alpha fit (default=0)
- **xhigh**: higher limit for asymptotic alpha fit (default=1)

Custom genes must be listed using FlyBase IDs (FBgn...), and the available populations from PopFly are: AM, AUS, CHB, EA, EF, EG, ENA, EQA, FR, RAL, SA, SD, SP, USI, USW, ZI.

Hence, using the parameters recomb and bins, the function allows deciding whether to analyze genes grouped by recombination bins or not.

Example 1

In this first example, the analysis is focused in two genes and 2 populations, without considering gene's recombination context, and using DGRP methodology.

The function groups polymorphism and divergence values of the custom genes, creating a new “concatenated gene” for each population of interest and performs the test defined. Then, it returns a list of lists with the default test output (DGRP in this case) for each population (RAL and ZI).

Explain output of DGRP function.

```
PopFlyAnalysis(genes=c("FBgn0000055", "FBgn0003016"), pops=c("RAL", "ZI"), recomb=F, test="DGRP")
#> [1] "Population = RAL"
#> [1] "Population = ZI"
#> $`Population` = RAL`
#> $`Population` = RAL`$Results
#>
#> alpha.symbol Fishers exact test P-value
#> Cutoff = 0 -1.19780220 0.03712979
#> Cutoff = 0.05 -0.04395604 1.00000000
#> Cutoff = 0.1 -0.04395604 1.00000000
#>
#> $`Population` = RAL`$`Divergence metrics`
#> $`Population` = RAL`$`Divergence metrics`$`Global metrics`
#>
#> Ka Ks omega
#> 1 0.003767024 0.05226481 0.07207573
#>
#> $`Population` = RAL`$`Divergence metrics`$`Estimates by cutoff`
#>
#> omegaA.symbol omegaD.symbol
#> Cutoff = 0 -0.086332464 0.15840819
#> Cutoff = 0.05 -0.003168164 0.07524389
#> Cutoff = 0.1 -0.003168164 0.07524389
#>
#>
```

```

#> $`Population` = RAL`$`MKT tables`
#> $`Population` = RAL`$`MKT tables`$`Number of segregating sites by DAF category - Cutoff = 0`
#>
#>
#> Table: cutoff
#>
#>
#>      DAF.below.cutoff  DAF.above.cutoff
#> -----
#> Neutral class         0                63
#> Selected class        0                40
#>
#> $`Population` = RAL`$`MKT tables`$`Number of segregating sites by DAF category - Cutoff = 0.05`
#>
#>
#> Table: cutoff
#>
#>
#>      DAF.below.cutoff  DAF.above.cutoff
#> -----
#> Neutral class         20                43
#> Selected class        34                 6
#>
#> $`Population` = RAL`$`MKT tables`$`Number of segregating sites by DAF category - Cutoff = 0.1`
#>
#>
#> Table: cutoff
#>
#>
#>      DAF.below.cutoff  DAF.above.cutoff
#> -----
#> Neutral class         20                43
#> Selected class        34                 6
#>
#> $`Population` = RAL`$`MKT tables`$`MKT standard table`
#>
#>
#>
#>      Polymorphism  Divergence
#> -----
#> Neutral class     63          45
#> Selected class    40          13
#>
#>
#> $`Population` = RAL`$`Fractions
#>      0      0.05      0.1
#> d 0.8415918 0.84039746 0.84039746
#> f 0.1584082 0.07524389 0.07524389
#> b 0.0000000 0.08435865 0.08435865
#>
#>
#> $`Population` = ZI`
#> $`Population` = ZI`$Results
#>      alpha.symbol Fishers exact test P-value
#> Cutoff = 0      -0.5279503                0.2545795
#> Cutoff = 0.05   -0.1180124                0.8623250
#> Cutoff = 0.1    -0.1180124                0.8623250
#>

```

```

#> $`Population` = ZI`$`Divergence metrics`
#> $`Population` = ZI`$`Divergence metrics`$`Global metrics`
#>           Ka           Ks           omega
#> 1 0.003765933 0.04524362 0.08323677
#>
#> $`Population` = ZI`$`Divergence metrics`$`Estimates by cutoff`
#>           omegaA.symbol omegaD.symbol
#> Cutoff = 0      -0.043944879      0.12718165
#> Cutoff = 0.05  -0.009822973      0.09305974
#> Cutoff = 0.1   -0.009822973      0.09305974
#>
#>
#> $`Population` = ZI`$`MKT tables`
#> $`Population` = ZI`$`MKT tables`$`Number of segregating sites by DAF category - Cutoff = 0`
#>
#>
#> Table: cutoff
#>
#>           DAF.below.cutoff  DAF.above.cutoff
#> -----
#> Neutral class              0              161
#> Selected class             0              82
#>
#> $`Population` = ZI`$`MKT tables`$`Number of segregating sites by DAF category - Cutoff = 0.05`
#>
#>
#> Table: cutoff
#>
#>           DAF.below.cutoff  DAF.above.cutoff
#> -----
#> Neutral class             108             53
#> Selected class            77             5
#>
#> $`Population` = ZI`$`MKT tables`$`Number of segregating sites by DAF category - Cutoff = 0.1`
#>
#>
#> Table: cutoff
#>
#>           DAF.below.cutoff  DAF.above.cutoff
#> -----
#> Neutral class             108             53
#> Selected class            77             5
#>
#> $`Population` = ZI`$`MKT tables`$`MKT standard table`
#>
#>
#>           Polymorphism  Divergence
#> -----
#> Neutral class          161          39
#> Selected class          82          13
#>
#>
#> $`Population` = ZI`$`Fractions
#>           0      0.05      0.1

```

```
#> d 0.8728183 0.87282798 0.87282798
#> f 0.1271817 0.09305974 0.09305974
#> b 0.0000000 0.03411227 0.03411227
```

Example 2

In this second example, genes from RAL population are grouped in 2 recombination bins, using recombination values from Comeron et al (ref). The test used is iMK, with xlow and xhigh values set to 0 and 0.9, respectively.

In this case, the function creates two different “concatenated” genes containing the same number of genes each (6 in this case), grouping again polymorphism and divergence values. These aggregations are made according to the gene associated recombination rate estimates. The function returns a list of lists with the default test output (iMKT in this case) for each population (RAL) and recombination bin (1 and 2).

Explain iMK function output.

```
geneList <- c("FBgn0053196", "FBgn0086906", "FBgn0261836", "FBgn0031617", "FBgn0260965",
              "FBgn0028899", "FBgn0052580", "FBgn0036181", "FBgn0263077", "FBgn0013733",
              "FBgn0031857", "FBgn0037836")
PopFlyAnalysis(genes=geneList , pops=c("RAL"), recomb=T, bins=2, test="iMK", xlow=0, xhigh=0.9)
#> [1] "Population = RAL"
#> [1] "Recombination bin = 1"
#> [1] "Recombination bin = 2"
#> $`Population = RAL`
#> $`Population = RAL`$`Recombination bin = 1`
#> $`Population = RAL`$`Recombination bin = 1`$`Asymptotic MK table`
#>      model      a      b      c alpha_asymptotic CI_low CI_high
#> 1 exponential 0.4626 -0.8511 14.8205      0.4626 -0.9265 55.216
#>   alpha_original
#> 1      0.2489
#>
#> $`Population = RAL`$`Recombination bin = 1`$`Fractions of sites`
#>   Type Fraction
#> 1      d 0.6515607
#> 2      f 0.3484393
#> 3      b 0.0000000
#>
#> $`Population = RAL`$`Recombination bin = 1`$`Recombination bin Summary`
#>   numGenes minRecomb medianRecomb meanRecomb maxRecomb
#> 1      6 0.6230327      1.216535      1.20733 1.759126
#>
#>
#> $`Population = RAL`$`Recombination bin = 2`
#> $`Population = RAL`$`Recombination bin = 2`$`Asymptotic MK table`
#>      model      a      b      c alpha_asymptotic CI_low CI_high
#> 1 exponential 0.6632 -0.5395 7.3407      0.6628 -0.3272 0.7304
#>   alpha_original
#> 1      0.426
#>
#> $`Population = RAL`$`Recombination bin = 2`$`Fractions of sites`
#>   Type Fraction
```

```

#> 1      d 0.727223
#> 2      f 0.272777
#> 3      b 0.000000
#>
#> $`Population = RAL`$`Recombination bin = 2`$`Recombination bin Summary`
#>   numGenes minRecomb medianRecomb meanRecomb maxRecomb
#> 1         6 2.277748      3.518252   3.398377  4.104627
rm(geneList)

```