

iMKT Pipeline

Brief intro about why we developed the package. The importance of detecting adaptation, and the many MKT derived methodologies. Take from paper.

Explain also that iMKT faces this variety of methodologies, and is able to compute several tests in order to detect adaptation in diverse situations.

In addition, it also provides a way to quantify negative selection, which may overlap with signals of positive selection in the target region, causing the failing of certain MK tests.

This vignette contains three different sections:

- Loading the package and checking test data
- Performing MKT derived methodologies calculation
- Conclusion: comparison of the different MK estimates

Loading the package and checking test data

First of all, install (if this is not done yet) and load the package. Notice that iMKT package includes two sample dataframes named myDafData and myDivergenceData which are the ones used in this tutorial and correspond to **Chr2L RAL and simulans??**. Therefore, it is possible to replicate the vignettes in order to better understand all the package functionalities.

```
## Load package
# install.packages("devtools")
# devtools::install_github("sergihervas/iMKT")
library(iMKT)

## Sample daf data
head(myDafData)
#>      daf      Pi      P0
#> 1 0.025 22490 17189
#> 2 0.075  3217  4780
#> 3 0.125  1616  2874
#> 4 0.175   999  2088
#> 5 0.225   754  1685
#> 6 0.275   679  1443

## Sample divergence data
myDivergenceData
#>      mi      Di      m0      D0
#> 1 2598805 54641 620019 52537
```

The iMKT package includes several functions, classified as follows:

- MKT derived methodologies calculation
 - standardMK(): Standard MKT
 - FWW(): FWW correction

- DGRP(): DGRP correction
- asymptoticMK(): Asymptotic MKT
- iMK(): integrative MKT
- completeMKT(): perform all previous tests
- iMK using PopFly and PopHuman data
 - loadPopFly(): load PopFlyData
 - loadPopHuman(): load PopHumanData
 - PopFlyAnalysis(): perform any test using PopFlyData
 - PopHumanAnalysis(): perform any test using PopHumanData
- Miscelaneous
 - checkInput(): check data before performing analyses
 - themePublication(): output plots and tables styling

Each function has an associated help page with its description, details about its parameters, usage, examples and so on. Remember you can access it writting `?` and the function name (or `?library::function`) in your console (*example*: `?iMKT::standardMK`).

This vignette focuses on the first category of functions: “MKT derived methodologies calculation”. Specifically, it contains examples of each function using the sample data described above. For details about functions from “iMKT using PopFly and PopHuman data” category check the corresponding vignette. The functions from the third category are used within other functions and do not produce analyses output.

Performing MKT derived methodologies calculation

The diverse functions from this category have two common input parameters which are required to perform the corresponding test:

- daf: data frame containing DAF, Pi and P0 values (myDafData)
- divergence: data frame containing divergent and analyzed sites for selected (i) and neutral (0) classes (myDivergenceData)

The output of each function always contains the corresponding alpha estimate, together with specific values and details of the selected methodology.

Standard MKT

Brief theoretical description about MKT

The **standardMK()** function uses daf and divergence input parameters and returns as output a list containing:

- alpha symbol: estimate of alpha using the standard MKT
- Fisher exact test P-value: p-value obtained using the Fisher exact test on a 2x2 contingency table (MKT table)
- MKT table: table containing the number of polymorphic and divergent sites for neutral and selected classes.

- ```
standardMK(daf=myDafData, divergence=myDivergenceData)
#> $alpha.symbol
#> [1] 0.2364499
#>
#> `$Fishers exact test P-value`
#> [1] 1.480943e-183
#>
#> `$MKT table`
#>
#>
#>
#> Polymorphism Divergence
#> -----
#> Neutral class 45101 52537
#> Selected class 35816 54641
#>
#> `$Divergence metrics`
#>
#>
#> Ka Ks omega omegaA omegaD
#> -----
#> 0.0210254 0.0847345 0.2481331 0.058671 0.189462
```

Alpha estimates can be biased by the segregation of slightly deleterious substitutions. One method to partially control this effect is to remove low frequency polymorphisms from the analysis, as proposed by Fay et al. (2001). Using this correction, all polymorphic sites from both neutral and selected classes which have a derived allele frequency lower than the pre-defined cutoff are removed for the calculation of alpha.

- Results: alpha estimates (and their associated Fisher exact test P-value) for each cutoff.
- Divergence metrics: global metrics (Ka, Ks, omega) and estimates by cutoff (omegaA, omegaD)
- MKT tables: tables containing the number of polymorphic and divergent sites for neutral and selected classes for each cutoff.

3

```

#> $`Divergence metrics`$`Estimates by cutoff`
#>
#> omegaA.symbol omegaD.symbol
#> Cutoff = 0 0.05867104 0.1894620
#> Cutoff = 0.05 0.13422877 0.1139043
#> Cutoff = 0.1 0.14387102 0.1042621
#>
#>
#> $`MKT tables`
#> $`MKT tables`$`Cutoff = 0`
#>
#>
#> Table: cutoff
#>
#>
#> Polymorphism Divergence
#> -----
#> Neutral class 45101 52537
#> Selected class 35816 54641
#>
#> $`MKT tables`$`Cutoff = 0.05`
#>
#>
#> Table: cutoff
#>
#>
#> Polymorphism Divergence
#> -----
#> Neutral class 27912 52537
#> Selected class 13326 54641
#>
#> $`MKT tables`$`Cutoff = 0.1`
#>
#>
#> Table: cutoff
#>
#>
#> Polymorphism Divergence
#> -----
#> Neutral class 23132 52537
#> Selected class 10109 54641

```

By default the argument **listCutoffs** uses a list of cutoffs with the following values: 0, 0.05, 0.1. Moreover, the function has an optional argument, **plot**, which is set to **FALSE** by default. This parameters can be customized, like in the following example, where we use a list of 4 cutoffs (0.05, 0.15, 0.25, 0.35) and set the plot argument to **TRUE**.

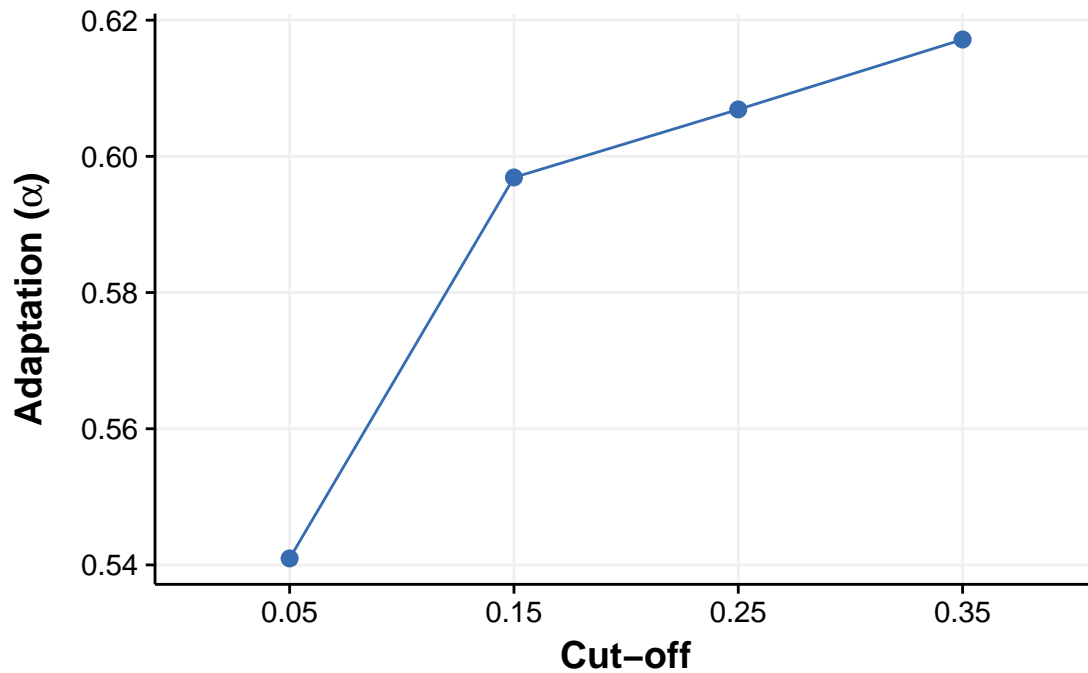
The output in this case contains a **Graph** which shows the adaptation value (alpha) obtained using each cutoff.

```

FWW(daf=myDafData, divergence=myDivergenceData, listCutoffs=c(0.05, 0.15,0.25,0.35), plot=TRUE)
#> $Results
#>
#> alpha.symbol Fishers exact test P-value
#> Cutoff = 0.05 0.5409548 0
#> Cutoff = 0.15 0.5969015 0
#> Cutoff = 0.25 0.6068868 0
#> Cutoff = 0.35 0.6171609 0

```

```
#>
#> $Graph
```



```
#>
#> $`Divergence metrics`
#> $`Divergence metrics`$`Global metrics`
#> Ka Ks omega
#> 1 0.02102543 0.0847345 0.2481331
#>
#> $`Divergence metrics`$`Estimates by cutoff`
#> omegaA.symbol omegaD.symbol
#> Cutoff = 0.05 0.1342288 0.11390431
#> Cutoff = 0.15 0.1481110 0.10002208
#> Cutoff = 0.25 0.1505887 0.09754438
#> Cutoff = 0.35 0.1531380 0.09499504
#>
#>
#> $`MKT tables`
#> $`MKT tables`$`Cutoff = 0.05`
#>
#>
#> Table: cutoff
#>
#> Polymorphism Divergence
#> -----
#> Neutral class 27912 52537
#> Selected class 13326 54641
#>
#> $`MKT tables`$`Cutoff = 0.15`
#>
#>
#> Table: cutoff
#>
```

```

#> Polymorphism Divergence
#> -----
#> Neutral class 20258 52537
#> Selected class 8493 54641
#>
#> $`MKT tables`$`Cutoff = 0.25`
#>
#>
#> Table: cutoff
#>
#> Polymorphism Divergence
#> -----
#> Neutral class 16485 52537
#> Selected class 6740 54641
#>
#> $`MKT tables`$`Cutoff = 0.35`
#>
#>
#> Table: cutoff
#>
#> Polymorphism Divergence
#> -----
#> Neutral class 13778 52537
#> Selected class 5486 54641

```

## DGRP correction

To take adaptive and slightly deleterious mutation mutually into account,  $P_n$ , the count of segregating sites in the non-synonymous class, should be separated into the number of neutral variants and the number of weakly deleterious variants,  $P_n = P_n(\text{neutral}) + P_n(\text{weakly del.})$ . If both numbers are estimated, adaptive and weakly deleterious selection can be evaluated independently.

Consider a pair of  $2 \times 2$  contingency tables. The first one corresponds to the standard MKT table with the theoretical counts of segregating sites and divergent sites for each cell.

The second table contains the count of  $P_n$  and  $P_s$  for two-frequency categories: below and over a threshold cutoff.

Add brief explanation about 2nd table!

To estimate  $\alpha$  from the standard MKT table correcting by the segregation of weakly deleterious variants, we have to substitute the  $P_n$  by the expected number of neutral segregating sites,  $P_n(\text{neutral})$ . The correct estimate of  $\alpha$  is then  $\alpha = 1 - (P_n(\text{neutral})/P_s)(D_s/D_n)$ .

The **DGRP()** function behaves similar to the **FWW()** function. It takes the same input argument and returns the same output but containing also estimates on the **fractions of negative selection** (d: strongly deleterious, f: neutral and b: weakly deleterious).

```

DGRP(daf=myDafData, divergence=myDivergenceData)
#> $Results
#> alpha.symbol Fishers exact test P-value
#> Cutoff = 0 0.2364499 1.480943e-183
#> Cutoff = 0.05 0.4249071 0.000000e+00
#> Cutoff = 0.1 0.4125636 0.000000e+00

```

```

#>
#> $`Divergence metrics`
#> $`Divergence metrics`$`Global metrics`
#> Ka Ks omega
#> 1 0.02102543 0.0847345 0.2481331
#>
#> $`Divergence metrics`$`Estimates by cutoff`
#> omegaA.symbol omegaD.symbol
#> Cutoff = 0 0.05867104 0.1894620
#> Cutoff = 0.05 0.10543351 0.1426996
#> Cutoff = 0.1 0.10237067 0.1457624
#>
#>
#> $`MKT tables`
#> $`MKT tables`$`Number of segregating sites by DAF category - Cutoff = 0`
#>
#>
#> Table: cutoff
#>
#> DAF.below.cutoff DAF.above.cutoff
#> -----
#> Neutral class 0 45101
#> Selected class 0 35816
#>
#> $`MKT tables`$`Number of segregating sites by DAF category - Cutoff = 0.05`
#>
#>
#> Table: cutoff
#>
#> DAF.below.cutoff DAF.above.cutoff
#> -----
#> Neutral class 17189 27912
#> Selected class 22490 13326
#>
#> $`MKT tables`$`Number of segregating sites by DAF category - Cutoff = 0.1`
#>
#>
#> Table: cutoff
#>
#> DAF.below.cutoff DAF.above.cutoff
#> -----
#> Neutral class 21969 23132
#> Selected class 25707 10109
#>
#> $`MKT tables`$`MKT standard table`
#>
#>
#> Polymorphism Divergence
#> -----
#> Neutral class 45101 52537
#> Selected class 35816 54641
#>
#>
#> $Fractions

```

```

#> 0 0.05 0.1
#> d 0.810538 0.81053943 0.8105391
#> f 0.189462 0.14269958 0.1457624
#> b 0.000000 0.04676099 0.0436985

```

Again, by default the argument **listCutoffs** uses a list of cutoffs with the following values: 0, 0.05, 0.1, and the argument **plot** is set to **FALSE**. These parameters can be customized, like in the following example, where we use a list of 4 cutoffs (0.05, 0.15, 0.25, 0.35) and set the plot argument to **TRUE**.

The output in this case contains two **Graphs** which show the adaptation value (alpha) and the negative selection fractions obtained using each cutoff.

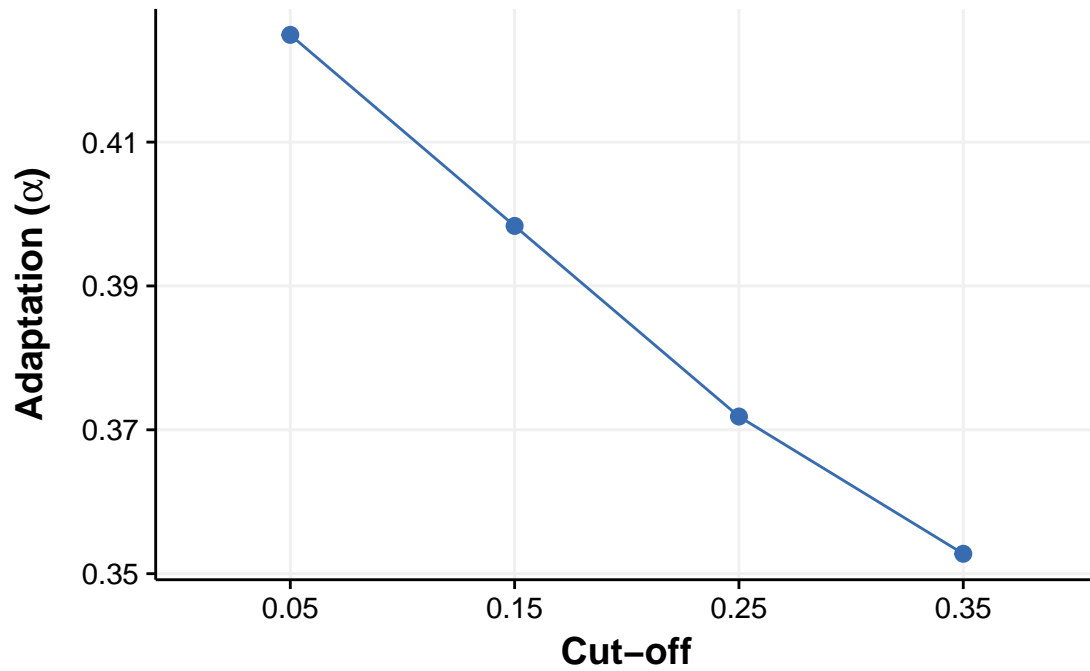
```

DGRP(daf=myDafData, divergence=myDivergenceData, listCutoffs=c(0.05, 0.15,0.25,0.35), plot=TRUE)
#> $Results
#> alpha.symbol Fishers exact test P-value
#> Cutoff = 0.05 0.4249071 0
#> Cutoff = 0.15 0.3983440 0
#> Cutoff = 0.25 0.3718449 0
#> Cutoff = 0.35 0.3527647 0
#>
#> $Graph

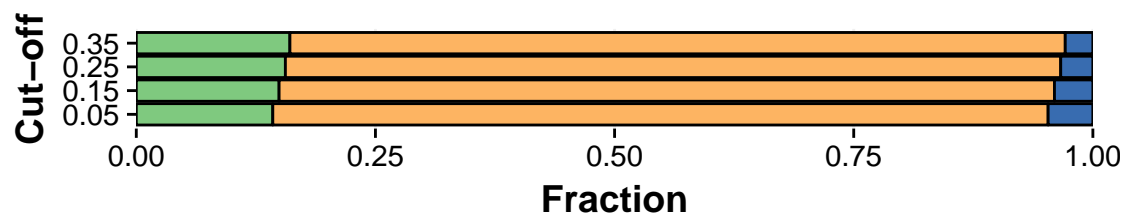
```



**A**



**B**



*Fraction* ■ *f* ■ *d* ■ *b*

```
#>
#> $`Divergence metrics`
#> $`Divergence metrics`$`Global metrics`
#> Ka Ks omega
#> 1 0.02102543 0.0847345 0.2481331
#>
#> $`Divergence metrics`$`Estimates by cutoff`
#> omegaA.symbol omegaD.symbol
#> Cutoff = 0.05 0.10543351 0.1426996
#> Cutoff = 0.15 0.09884233 0.1492908
#> Cutoff = 0.25 0.09226702 0.1558661
#> Cutoff = 0.35 0.08753258 0.1606005
#>
#>
#> $`MKT tables`
#> $`MKT tables`$`Number of segregating sites by DAF category - Cutoff = 0.05`
#>
#>
#> Table: cutoff
```

```

#>
#>
#> DAF.below.cutoff DAF.above.cutoff
#> -----
#> Neutral class 17189 27912
#> Selected class 22490 13326
#>
#> $`MKT tables`$`Number of segregating sites by DAF category - Cutoff = 0.15`
#>
#>
#> Table: cutoff
#>
#> DAF.below.cutoff DAF.above.cutoff
#> -----
#> Neutral class 24843 20258
#> Selected class 27323 8493
#>
#> $`MKT tables`$`Number of segregating sites by DAF category - Cutoff = 0.25`
#>
#>
#> Table: cutoff
#>
#> DAF.below.cutoff DAF.above.cutoff
#> -----
#> Neutral class 28616 16485
#> Selected class 29076 6740
#>
#> $`MKT tables`$`Number of segregating sites by DAF category - Cutoff = 0.35`
#>
#>
#> Table: cutoff
#>
#> DAF.below.cutoff DAF.above.cutoff
#> -----
#> Neutral class 31323 13778
#> Selected class 30330 5486
#>
#> $`MKT tables`$`MKT standard table`
#>
#>
#> Polymorphism Divergence
#> -----
#> Neutral class 45101 52537
#> Selected class 35816 54641
#>
#>
#> $Fractions
#> 0.05 0.15 0.25 0.35
#> d 0.81053943 0.81053552 0.8105368 0.81054057
#> f 0.14269958 0.14929076 0.1558661 0.16060050
#> b 0.04676099 0.04017372 0.0335971 0.02885892

```

## Asymptotic MKT

Petrov reference + explanation

This function is adapted from the code developed in “Haller BC, Messer PW. asymptoticMK: A Web-Based Tool for the Asymptotic McDonald-Kreitman Test. G3 (Bethesda). 2017 May 5;7(5):1569-1575”, stored in: <http://github.com/MesserLab/asymptoticMK>. The main adaptation we did is that the function presented here only fits an exponential model, removing the linear fitting performed initially in the cases where it was not possible to fit an asymptotic curve.

The **asymptoticMK()** function uses the common daf and divergence parameters along with two arguments which define the lower and higher limit for the asymptotic alpha fit (xlow and xhigh). These two optional parameters are set to 0 and 1 by default, although it is recommended to use a higher limit of 0.9 in order to remove possible biases due to polarization error.

The function's output is a table with: the model type (exponential) along with the fitted function values (a, b, c), the asymptotic alpha estimate with its corresponding lower and higher confidence interval values, and the original alpha estimate (using the standard MKT methodology and the polymorphic sites within the xlow and xhigh cutoffs).

```
asymptoticMK(daf=myDafData, divergence=myDivergenceData, xlow=0, xhigh=0.9)
#> model a b c alpha_asymptotic CI_low
#> 1 exponential 0.6258904 -1.395108 18.96187 0.6258904 0.6041863
#> CI_high alpha_original
#> 1 0.647166 0.2157308
```

## iMK

Asymptotic explanation + Sergi slightly deleterious approach

The **iMK()** function takes the default input parameters (daf and divergence), the xlow and xhigh arguments (presented in the **asymptoticMK()** function) and it also has the optional argument **plot**, set as **FALSE** by default. However, in this example we use **plot=TRUE** to display the graphical results.

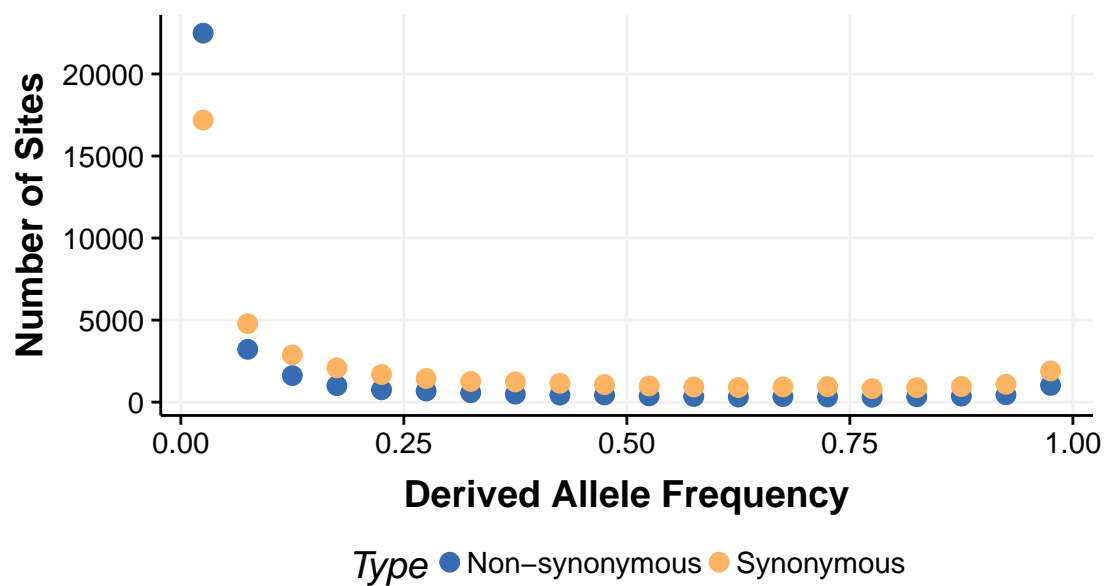
The output of the function contains:

- Asymptotic MK table: table corresponding to the asymptoticMK() function output.
- Fractions of sites: negative selection fractions (d: strongly deleterious, f: neutral and b: weakly deleterious).
- Graphs: 3 plots showing: (A) the distribution of alleles frequencies for neutral and selected sites, (B) the asymptotic alpha estimate with xlow, xhigh, original alpha and asymptotic alpha marks, and (C) the negative selection fractions.

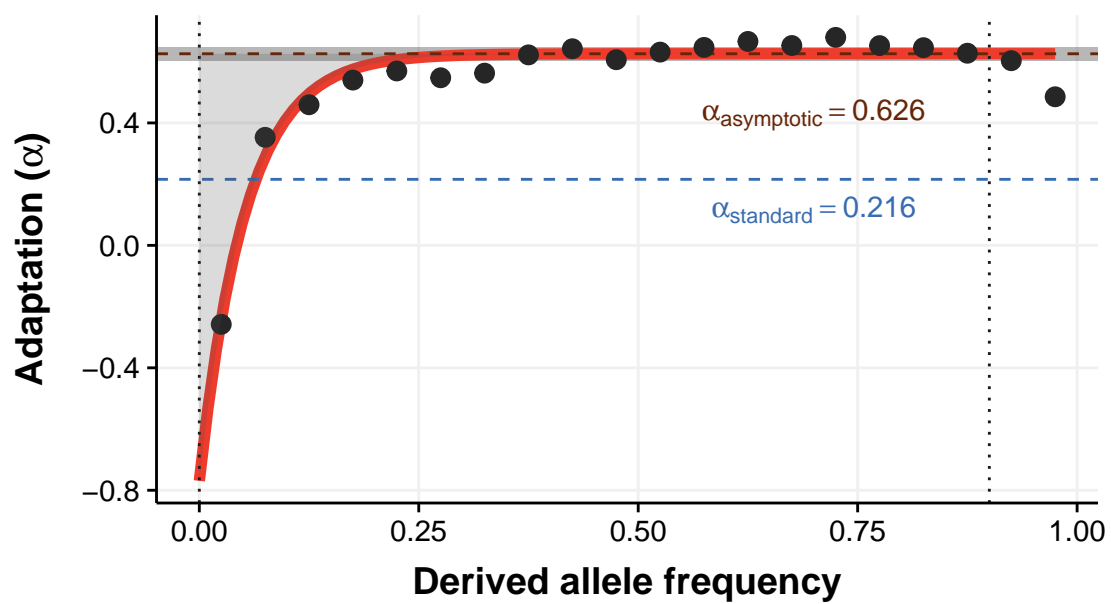
```
iMK(daf=myDafData, divergence=myDivergenceData, xlow=0, xhigh=0.9, plot=TRUE)
#> $`Asymptotic MK table`
#> model a b c alpha_asymptotic CI_low CI_high
#> 1 exponential 0.6259 -1.3951 18.9619 0.6259 0.6043 0.6473
#> alpha_original
#> 1 0.2157
#>
#> $`Fractions of sites`
#> Type Fraction
#> 1 d 0.81053796
```

```
#> 2 f 0.06232362
#> 3 b 0.12713842
#>
#> $Graphs
```

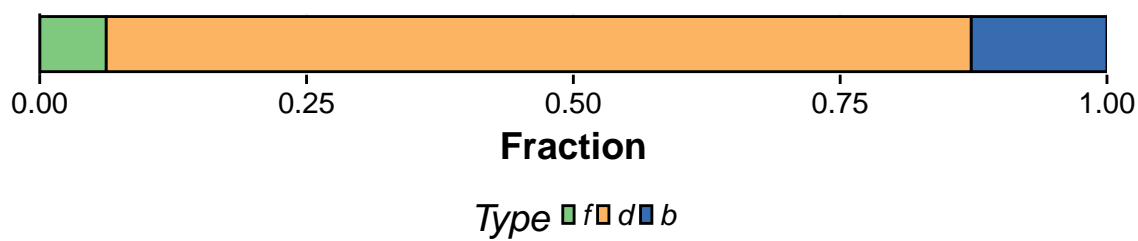
**A**



**B**



**C**



## Conclusion: comparison of the different MK estimates

Summary of each alpha estimate:

| Standard | FWW_0.05 | FWW_0.1 | DGRP_0.05 | DGRP_0.1 | asymptotic_iMK |
|----------|----------|---------|-----------|----------|----------------|
| 0.2365   | 0.5409   | 0.5798  | 0.4249    | 0.4249   | 0.6259         |

Comment results and strengths and weakness of each method.