iMKT Pipeline

A major issue in population genetics is the accurate detection of the impact of natural selection along the genome. In this regard, several tests have been developed in the last years, being the McDonald and Kreitman Test (Standard MKT, McDonald & Kreitman 1991) the most used method. Several modifications to the Standard MKT have been applied over the last years, leading to a battery of MKT-derived methods.

The Integrative McDonald and Kreitman Test (iMKT) package allows computing the McDonald and Kreitman test on polymorphism and divergence genomic data provided by the user or automatically downloaded from PopFly (Hervas et al. 2017) or PopHuman (Casillas et al. 2018).

It includes five MKT derived tests: Standard MKT, FWW correction (Fay et al. 2001), DGRP correction (Mackay et al. 2012), asymptotic MK (Messer & Petrov 2013), and the novel iMKT approach; which allow inferring the rate of adaptive evolution (α), as well as the fraction of strongly deleterious (d), weakly deleterious (b), and neutral (f) sites, using user custom input data.

In this document, we show how to install the package and how to analyze the sample data using different MKT-derived methods. Finally, we briefly discuss the results obtained with each of the available tests. This vignette contains three different sections:

- Loading the package and checking test data
- Performing MKT analyses
- Conclusion: comparison of the different MKT 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 the chromosome arm 2R of the North American Raleigh population (RAL, North Carolina, $n = 205$), using *D. simulans* as outgroup species to estimate divergence metrics. 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:

- Calculation of MKT-derived methods
  - standardMKT(): Standard MKT
  - FWW(): FWW correction
  - DGRP(): DGRP correction
  - asymptoticMKT(): Asymptotic MKT
  - iMKT(): integrative MKT
  - completeMKT(): perform all previous tests
- iMKT using PopFly and PopHuman data
  - loadPopFly(): load PopFlyData
  - loadPopHuman(): load PopHumanData
  - PopFlyAnalisys(): perform any test using PopFlyData
  - PopHumanAnalysis(): perform any test using PopHumanData
- Miscelanious
  - 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. Rembember you can access it writting ? and the function name (or ?library::function) in your console (*example*: ?iMKT::standardMKT).

This vignette focuses on the first category of functions: "Calculation of MKT-derived methods". 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 analyses

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 **standardMKT()** 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.
- Divergence metrics: table containing estimates of Ka, Ks, omega, omegaA, omegaD.

```
standardMKT(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
```

FWW correction

Alpha estimates can be biased by the segregation of slighlty deleterious substitutions. One method to partially controll 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.

The **FWW()** function uses daf and divergence input parameters, along with a default list of cutoffs (0, 0.05, 0.1) and returns as output a list containing:

- 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.

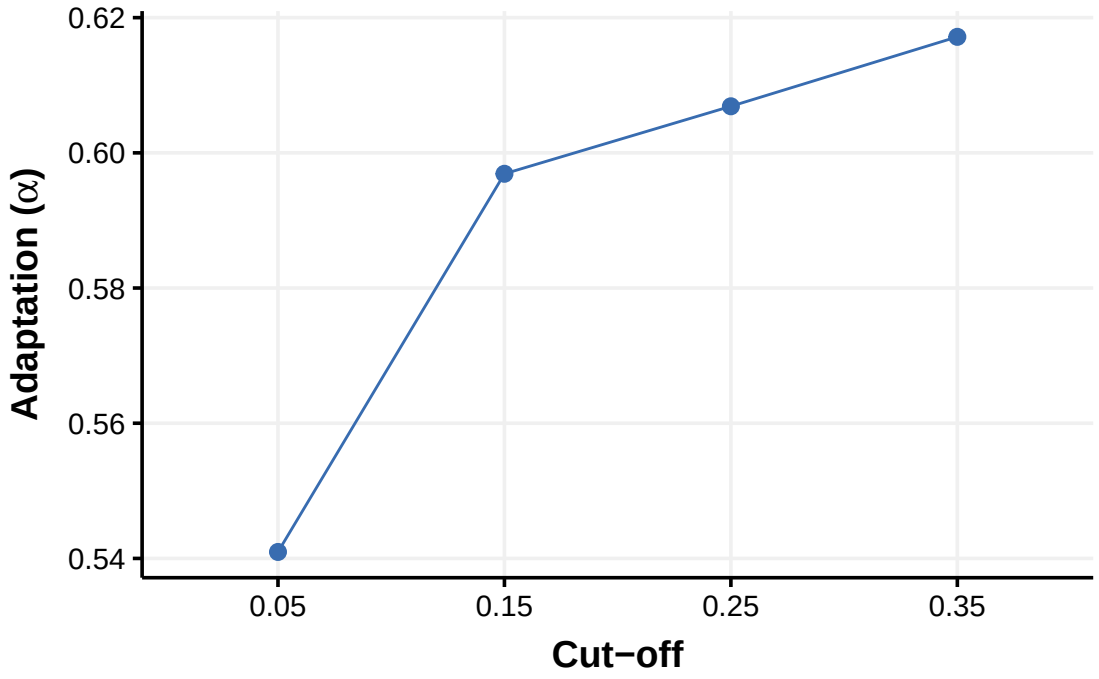
```
FWW(daf=myDafData, divergence=myDivergenceData)
#> $Results
#>                alpha.symbol Fishers exact test P-value
#> Cutoff =  0       0.2364499              1.480943e-183
#> Cutoff =  0.05    0.5409548               0.000000e+00
#> Cutoff =  0.1     0.5798139               0.000000e+00
#>
#> $`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    0.13422877     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, Pn , 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, Pn = Pn(neutral) + Pn(weakly del.). If both numbers are estimated, adaptive and weakly deleterious selection can be evaluated independently.

Consider a pair of 2×2 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 Pn and Ps 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 Pn by the expected number of neutral segregating sites, Pn(neutral). The correct

estimate of alpha is then alpha = 1 - (Pn (neutral)/Ps)(Ds/Dn).

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).

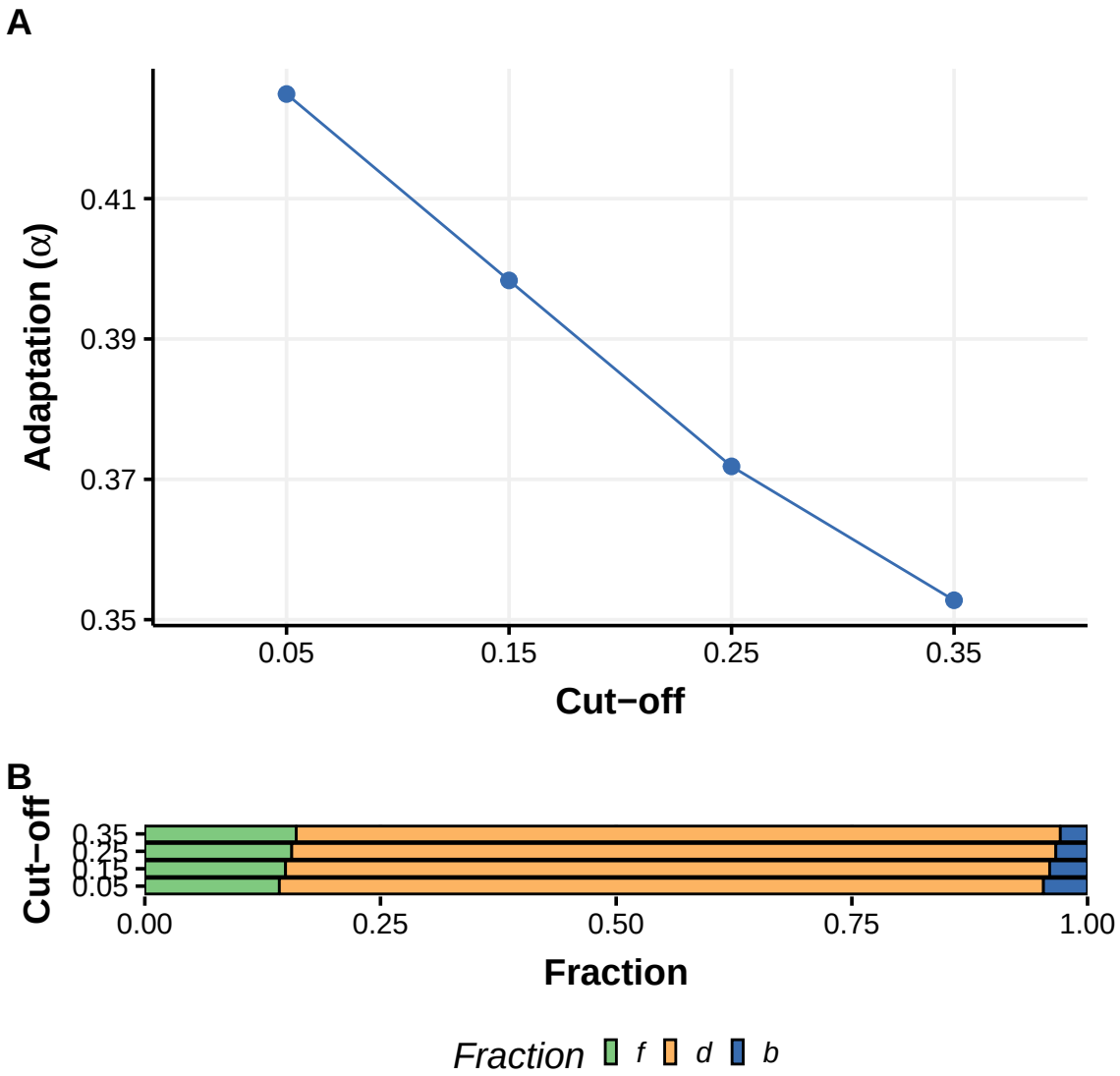
```
DGRP(daf=myDafData, divergence=myDivergenceData)
#> $Results
#>                alpha.symbol Fishers exact test P-value
#> Cutoff =  0       0.2364499              1.480943e-183
#> Cutoff =  0.05    0.4249071               0.000000e+00
#> Cutoff =  0.1     0.4125636               0.000000e+00
#>
#>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




```
#>
#> $`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 **asymptoticMKT()** 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).

```
asymptoticMKT(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 0.6043894
#>     CI_high alpha_original
#> 1 0.6477918      0.2157308
```

iMKT

The integrative MKT combines the approach developed by Messer & Petrov (2013) to estimate the fraction of adaptive substitutions (α) and an adaptation of the theoretical framework established by Mackay et al. (2012) to quantify the fraction of putatively selected sites that are under purifying selection pressures.

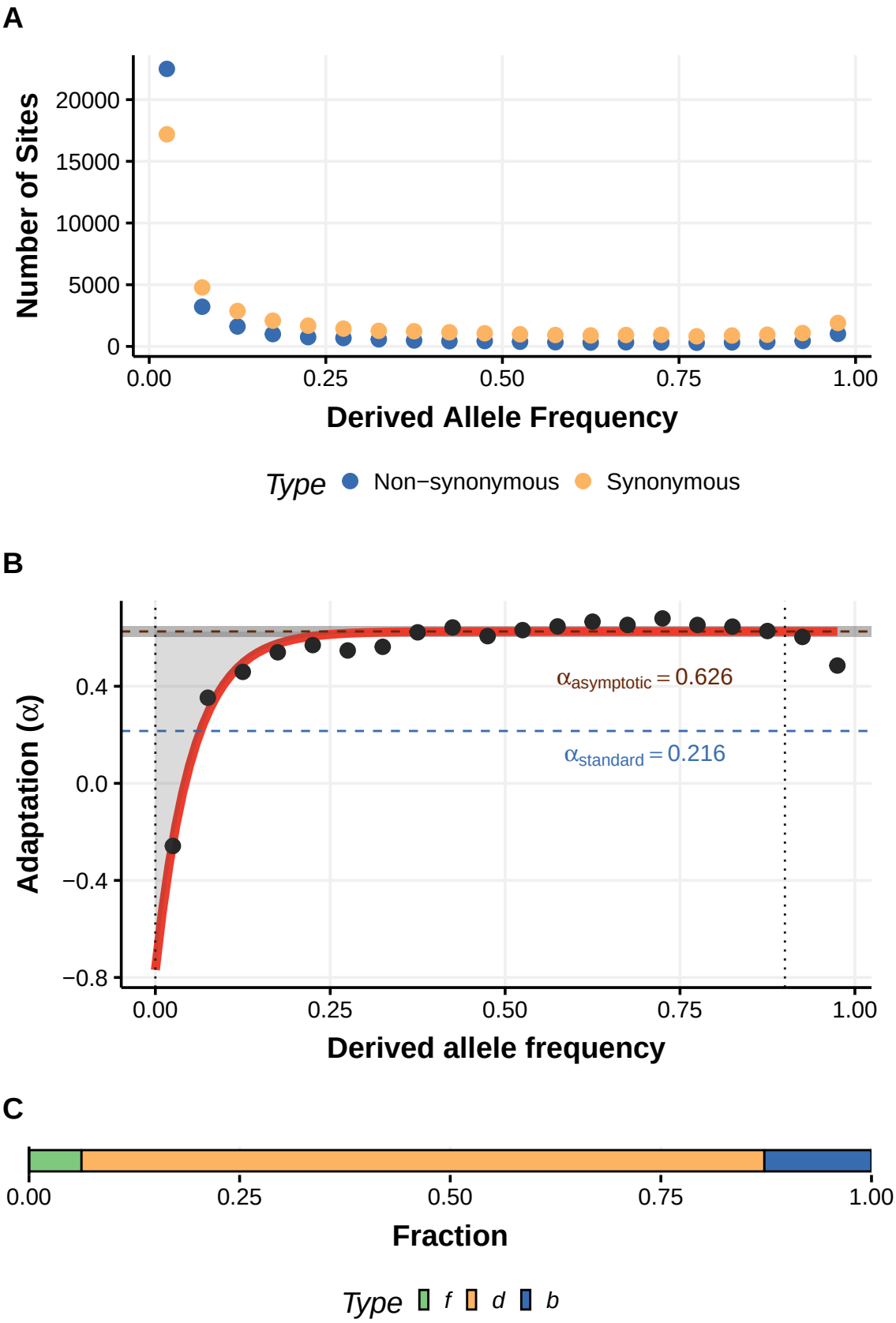
The **iMKT()** function takes the default input parameters (daf and divergence), the xlow and xhigh arguments (presented in the **asymptoticMKT()** function) and it also has the optional argument **plot**, set as **FALSE** by default. However, in this example we use **plot=TRUE** to display the graphical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

The output of the function contains:

- Asymptotic MK table: table corresponding to the asymptoticMKT() 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.

```
iMKT(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 0.6045  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
Number of Sites
20000
15000
10000
5000
0
0.00
0.25
0.50
0.75
1.00
Derived Allele Frequency
Type
Non−synonymous
Synonymous
B
Adaptation (α)
0.4
0.0
−0.4
−0.8
αasymptotic = 0.626
αstandard = 0.216
0.00
0.25
0.50
0.75
1.00
Derived allele frequency
C
0.00
0.25
0.50
0.75
1.00
Fraction
Type
f
d
b

Conclusion: comparison of the different MKT estimates

The following table includes the estimates of the rate of adaptive evolution (α) obtained using the same data and each of the MKT methods included in the iMKT package. Results for FWW and DGRP corrections were produced using two different cut-offs (0.05 and 0.1).

Standard	FWW_0.05	FWW_0.1	DGRP_0.05	DGRP_0.1	asymptotic_iMKT
0.2365	0.5409	0.5798	0.4249	0.4126	0.6259

We observe that the Standard MKT tends to underestimate the true rate of adaptation, probably due to the presence of weakly deleterious mutations which reduce the power of the test to detect adaptive evolution.

Besides, we observe an increase of when using any of the other methods Comment results and strengths and weakness of each method. Specifically, FWW correction seems to perform better than DGRP. Finally, the asymptotic method allows to estimate the largest rate of adaptive evolution. As we are analyzing a complete chromosome arm with a large number of segregating sites (in all DAF categories), the asymptotic test is able to remove almost all weakly deleterious mutations from the analysis and thus, we obtain the estimates closest to the true level of adaptation.