`polyDFE v1.11`
User Manual

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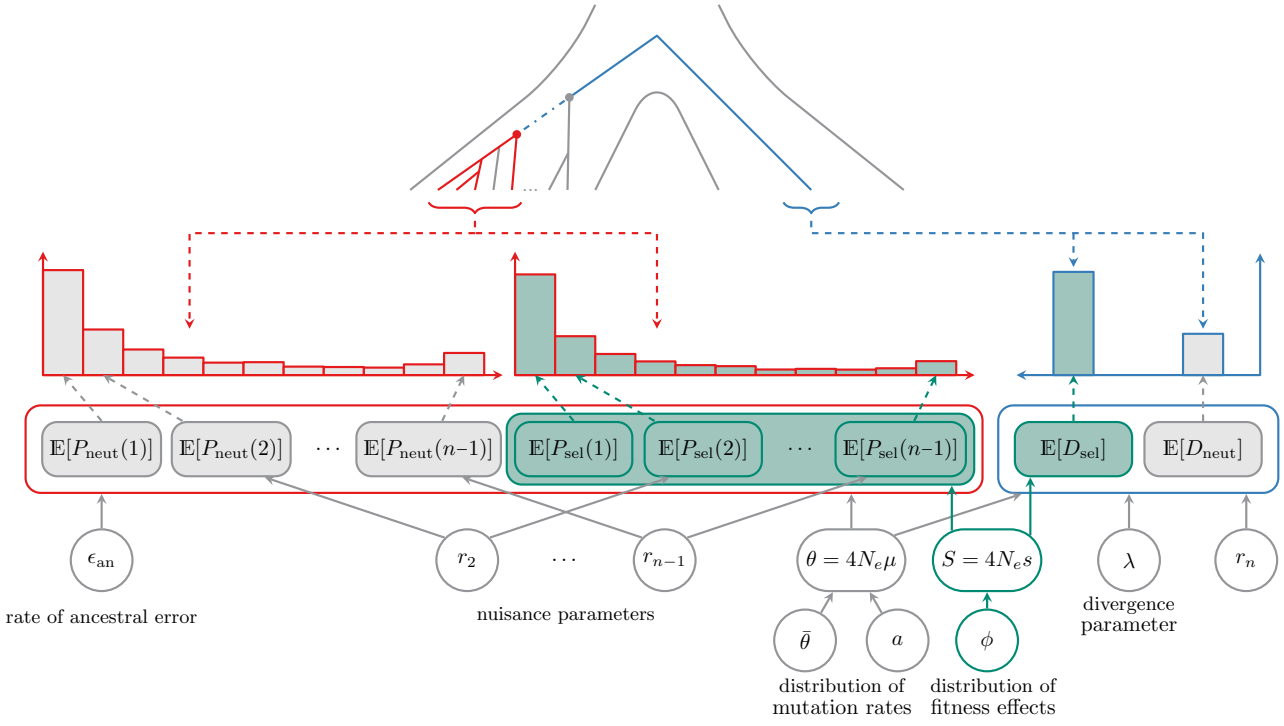
`polyDFE` infers the distribution of fitness effects (DFE) of new mutations from polymorphism, and, if available, can also incorporate divergence data obtained from an outgroup. The polymorphism data is provided as an unfolded site frequency spectrum (SFS). Note that, in order to obtain an unfolded SFS, at least one outgroup is needed. However, including the divergence data when inferring the DFE can lead to bias in the estimated parameters (see Tataru et al. (2017) for further discussion).

Once the DFE is obtained, `polyDFE` also calculates α, the rate of adaptive molecular evolution, commonly defined as the proportion of fixed adaptive mutations among all non-synonymous substitutions. This code implements the program described in Tataru et al. (2017).



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1 Authors

polyDFE has been developed and implemented by Paula Tataru and Marco A.P. Franco.

2 License

polyDFE is open source, distributed under the GNU General Public License, version 3. See LICENSE.txt for details.

3 Requirements

polyDFE is implemented in C and uses the GSL library. This should be downloaded from https://www.gnu.org/software/gsl/ and compiled accordingly. polyDFE has been tested using GSL-1.16.

4 Installation

The simplest way to compile polyDFE is

1. cd to the directory containing the makefile and type

   ```
   make all
   ```

   to compile the code.

2. You can remove the program binaries and object files from the source code directory by typing

   ```
   make clean
   ```

If the GSL library is not installed in the default directory, than the installation path needs to be specified in the makefile by updating USR_INC and USR_LIB and uncommenting the lines at the top of the makefile:

```
#############################################################
# for non-default GSL installation
#############################################################
# USR_INC := -I<ROOT PATH TO GSL 1.16>/include/
# USR_LIB := -L<ROOT PATH TO GSL 1.16>/lib/
#############################################################
```

5 Running polyDFE

Running polyDFE with the argument -h will print out the usage (required and optional arguments):

```
$ ./polyDFE -h
./polyDFE v1.11
Usage: ./polyDFE -d data_file [-m model(A, B, C, D) [K]] [-t]
        {-s m_neut L_neut m_sel L_sel n -i init_file ID ||
        [-o optim(bfgs, conj_pr, conj_fr)] [-k kind(s, j, s+j)] [-r range_file ID]
                [-i init_file ID [-j]] [-e] [-w] [-g grouping_file ID]
                [-p optim_file ID] [-b [basinhop_file ID]]
                [-l min] [-v verbose(0, 1, frequency)]}
```

polyDFE runs in two modes: -s, used for simulating data under the hierarchical probabilistic model, and -o, used for estimating the DFE and α. If neither -s or -o are given, then -o bfgs is used by default (see -o for details). If both -s and -o are given, polyDFE will terminate with an error. Optional arguments are given between [].

5.1 Specifying data file

Regardless of the mode `polyDFE` is ran in, the file containing the data has to be specified using the following argument:

- `-d data_file`: path to the data file.

  `data_file` is where `polyDFE` will write the simulated data, if `-s` is used, or will read the input data, if `-o` is used.

  The file can contain any number of empty lines and comment lines that start with `#`. Excluding these, the structure of `data_file` is

1	m_{neut} m_{sel} n						
2	p_{neut}(1)	p_{neut}(2)	...	p_{neut}(n-1)	l_{neut}	d_{neut}	$l_{d,neut}$
...	...						
m_{neut}+1	p_{neut}(1)	p_{neut}(2)	...	p_{neut}(n-1)	l_{neut}	d_{neut}	$l_{d,neut}$
m_{neut}+2	p_{sel}(1)	p_{sel}(2)	...	p_{sel}(n-1)	l_{sel}	d_{sel}	$l_{d,sel}$
...	...						
m_{neut}+m_{sel}+1	p_{sel}(1)	p_{sel}(2)	...	p_{sel}(n-1)	l_{sel}	d_{sel}	$l_{d,sel}$

  where

  - m_{neut} and m_{sel} are the number of fragments that are assumed to be evolving neutrally or under selection, respectively;
  - n is the ingroup sample size (number of sequences);
  - $p_z(i)$ is the i^{th} entry in the SFS within the fragment, for sites that are assumed to be evolving neutrally, $z = \text{neut}$, or under selection, $z = \text{sel}$;
  - l_z is the total number of sites within the fragment used for the SFS (number of successfully sequenced sites within the ingroup) that are assumed to be evolving neutrally, $z = \text{neut}$, or under selection, $z = \text{sel}$;
  - d_z is the total number of fixed mutations in the ingroup relative to the outgroup (divergence counts) within the fragment, that are assumed to be evolving neutrally, $z = \text{neut}$, or under selection, $z = \text{sel}$;
  - $l_{\text{d},z}$ is the total number of sites within the fragment used for the divergence counts (number of successfully sequenced sites within the ingroup and outgroup) that are assumed to be evolving neutrally, $z = \text{neut}$, or under selection, $z = \text{sel}$; for a given fragment, l_z and $l_{\text{d},z}$ could be different if certain sites are successfully sequenced in the ingroup, but not in the outgroup.

  The divergence data (last two columns, d_z and $l_{\text{d},z}$) can be absent from the file. If it is absent, it should be absent for all fragments.

  `polyDFE` uses data divided into fragments in order to model variability in mutation rates, as described in Tataru et al. (2017). If only one neutral and one selected fragments are provided, than variability in mutation rates is not modeled.

  The provided files `input/example_1`, `input/example_2` and `input/example_3` are examples of `data_file`.

5.2 Specifying DFE model

Regardless of the mode `polyDFE` is ran in, the assumed DFE model has to be specified using the following argument:

- `-m model(A, B, C, D) [K]`: assumed DFE model.

  `polyDFE` assumes that the DFE ϕ takes one of four specific functional forms, encoded `A, B, C,` and `D`, described below

  - `A`: the DFE is given by a reflected displaced Gamma distribution, parameterized by $\bar{S}$, b and $S_{\max}$, with density

$$\phi_A(S; \bar{S}, b, S_{\max}) = \begin{cases} f_\Gamma(S_{\max} - S; S_{\max} - \bar{S}, b) & \text{if } S \leq S_{\max} \\ 0 & \text{otherwise} \end{cases}$$

    where $\bar{S}$ is the mean of the DFE, b is the shape of the Gamma distribution, $S_{\max}$ is the maximum value that S can take, and $f_\Gamma(x; m, b)$ is the density of the Gamma distribution with mean m and shape b.

- `B`: the DFE is given by a mixture of a Gamma and discrete distributions, parameterized by S_d, b, p_b and S_b, where

$$\phi_B(S; S_d, b, p_b, S_b) = \begin{cases} (1-p_b) f_\Gamma(-S; -S_d, b) & \text{if } S \leq 0 \\ p_b & \text{if } S = S_b \\ 0 & \text{otherwise} \end{cases}$$

  where S_d is the mean of the DFE for $S \leq 0$, b is the shape of the Gamma distribution, p_b is the probability that $S > 0$, and S_b is the shared selection coefficient of all positively selected mutations, and $f_\Gamma(x; m, b)$ is the density of the Gamma distribution with mean m and shape b.

- `C`: the DFE is given by a mixture of a Gamma and Exponential distributions, parameterized by S_d, b, p_b and S_b, where

$$\phi_C(S; S_d, b, p_b, S_b) = \begin{cases} (1-p_b) f_\Gamma(-S; -S_d, b) & \text{if } S \leq 0 \\ p_b \, f_e(S; S_b) & \text{if } S > 0 \end{cases}$$

  where S_d is the mean of the DFE for $S \leq 0$, b is the shape of the Gamma distribution, p_b is the probability that $S > 0$, S_b is the mean of the DFE for $S > 0$, and $f_\Gamma(x; m, b)$ is the density of the Gamma distribution with mean m and shape b, while $f_e(x; m)$ is the density of the Exponential distribution with mean m.

- `D`: the DFE is given as a discrete DFE, where the selection coefficients can take one of S_i distinct values, $1 \leq i \leq$ `K`, where each value S_i has probability p_i, with

$$\sum_{i=0}^{K} p_i = 1$$

  The value of `K` needs to be provided in the command line for model `D`.

  Note that the optimization of the parameters for this model is difficult, due to the fact that the probabilities p_i have to sum to 1.

If `-m` is not given, model `C` is used by default.

5.3 Specifying DFE and other additional parameters

When simulating data (mode `-s`), the DFE and other additional parameters have to be specified using `-i`. This can also optionally be used when inferring the parameters (mode `-o`). This way, the user can control which parameters should be estimated and which should be held fixed, and can also provide the initial values of the parameters used during the optimization. Note that if using `-m D`, `-i` is no longer optional, as it contains the information about the selection coefficients S_i.

- `-i init_file ID`: path to a file containing the values of the DFE and other parameters, and id.

  `init_file` can contain any number of empty lines and comment lines that start with `#`. Excluding these, the structure of each line is

`id`	`[0|1]` ϵ_{an}	`[0|1]` ϵ_{cont}	`[0|1]` λ	`[0|1]` $\bar{\theta}$	`[0|1] a <DFE parameters>`	`[0|1]` $r_2 \ldots r_n$

  Each line starts with a positive numerical id. This is used such that multiple configurations of the parameters can be stored in the same file. When running `polyDFE`, the desired line for initializing the parameters is specified through the id by setting `ID` to the right value.

  The id is followed by the values of the parameters, in the order ϵ_{an}, ϵ_{cont}, λ, $\bar{\theta}$, a, the DFE parameters (see `-m` for details), and the r_i, $2 \leq i \leq n$, parameters, where n is the ingroup sample size.

  All parameters are preceded by a flag, which is either 0 or 1. In the `-s` mode, the flag is not used, but this becomes important for the `-o` mode, where the flag indicates if a parameter should be estimated (flag 0) or should be kept fixed to the value provided in `init_file` (flag 1). All r_i parameters share one flag. The r_i parameters can also be provided for $i > n$, but those values will be ignored. This is useful if the same line in `init_file` is used for multiple data sets that have different sample sizes.

When `-m D` is used, only the probabilities p_i are estimated, while the selection coefficients S_i are fixed to the given values.

For details of the meaning of each parameter, please see Tataru et al. (2017), noting that ϵ_{an} is simply referred to as ϵ.

The parameter ϵ_{cont} is deprecated in v1.1 and is no longer used. For backward compatibility, it is still present in the `init_file`.

The a parameter is directly linked to variability of mutation rates. If it is wished for mutation rates to be assumed constant, than a can be fixed to -1. If both m_{neut} and m_{sel} provided in the input datafile (through argument `-d`) are equal to 1 (i.e. the data is not divided into fragments), then the mutation variability is not estimated by default. When mutation variability is allowed, than the observed data (counts) are assumed to follow a negative binomial distribution, otherwise they are assumed to follow a Poisson distribution. See Tataru et al. (2017) for more details on mutation variability.

The provided files `input/init_model_A.txt`, `input/init_model_BandC.txt` and `input/init_model_D.txt` give examples of the structure of the `init_file` for the different DFE models.

5.4 Simulating data

The simulation mode (`-s`) requires the following argument:

- `-s m_neut L_neut m_sel L_sel n`: simulate data.

  When using `-s`, simulated data will be printed in `data_file` with m_{neut} = `m_neut`, m_{sel} = `m_sel`, $l_{\text{neut}} = l^d_{\text{neut}}$ = `L_neut` and $l_{\text{sel}} = l^d_{\text{sel}}$ = `L_sel`, for all simulated fragments. For details on m_z and l_z, with $z \in \{\text{neut}, \text{sel}\}$, see `-d`.

5.4.1 Examples

When simulating data, `polyDFE` throws a warning about overwriting the file and requires input from the user to confirm the action:

```
$ ./polyDFE -d input/example_1 -m A -s 500 2000 500 6000 20 -i input/init_model_A.txt 1
---- Running command
---- ./polyDFE -d input/example_1 -m A -s 500 2000 500 6000 20 -i input/init_model_A.txt 1
Warning: simulating data, input/example_1 will be overwritten.
Do you want to continue with simulation? (Y/N): Y
```

This is to ensure that no files containing data are overwritten by mistake.

The provided files `input/example_1`, `input/example_2` and `input/example_3` are obtained by calling

```
$ ./polyDFE -d input/example_1 -m A   -s 500  2000 500  6000 20 -i input/init_model_A.txt 1
$ ./polyDFE -d input/example_2 -m C   -s  50 20000  50 60000 20 -i input/init_model_BandC.txt 1
$ ./polyDFE -d input/example_3 -m D 5 -s 100 10000 100 30000 20 -i input/init_model_D.txt 1
```

The simulated `data_file` contains information at the beginning about the DFE model used for the simulation, together with the values of all parameters.

5.5 Estimating DFE and α

The estimation (optimization) mode (`-o`) allows for the estimation of DFE and α. For this, next to the arguments described above, it allows for a series of optional arguments, which control the optimization, both how the parameters are estimated, but also when a set of parameters found is considered to be optimal.

5.5.1 Controlling the optimization procedure

One of the key issues in inferring the parameters is to ensure that the likelihood function is properly optimized over the space of parameters. `polyDFE` implements multiple steps to ensure, as much as possible, that good parameters are found. These can be customized by the user using the following arguments:

- `-o (bfgs, conj_pr, conj_fr)`: optimization method to use for estimating the parameters.

  `polyDFE` uses `GSL` to run a local optimization algorithm and find estimates of the parameters. `GSL` implements multiple optimization algorithms. Generally, optimization is more efficient if it also uses the derivative of the function to be optimized. Therefore, in polyDE we have chosen to use three of the algorithms offered by `GSL`, which all rely on the function (in this case, the data likelihood) derivatives. These are:

  - `bfgs`: the Broyden-Fletcher-Goldfarb-Shanno (BFGS) algorithm.
  - `conj_pr`: the Polak-Ribiere conjugate gradient algorithm;
  - `conj_fr`: the Fletcher-Reeves conjugate gradient algorithm.

  For details on the different optimization algorithms, please see the `GSL` manual. When `-o` is not given, `-o bfgs` is used by default. `polyDFE` has been tested using the BFGS algorithm.

  Note that the optimization of the parameters for model `D` (see `-m` for details) is difficult, due to the fact that the probabilities p_i are constrained to sum to 1, while all of the above algorithms are unconstrained.

- `-p optim_file ID`: path to a file containing parameters that control the optimization algorithm, and id.

  `optim_file` can contain any number of empty lines and comment lines that start with `#`. Excluding these, the structure of each line is

id	ϵ_{abs}	step size	tolerance	max iterations

  Each line starts with a positive numerical id. This is used such that multiple configurations for the optimization algorithms can be stored in the same file. When running `polyDFE`, the desired line for the range of the parameters is specified through the id by setting `ID` to the right value.

  The `optim_file` is used to control the behavior of the optimization algorithms from `GSL`, specified using `-o`:

  - ϵ_{abs}: this the absolute tolerance value that the gradient of the function to be optimized (here, the likelihood) is tested against. When the gradient is smaller than this values, than `GSL` considers that it has successfully found an optimum. If this value is too large, it will lead to an early termination of the algorithm. If the value is too small, it might lead to a longer running time of the algorithm without, potentially, much improvement on the function. However, it is always better to have an absolute tolerance value that is too small than too large. If a set of values found is a true optimum, than the gradient should be 0. Default value: 0.00001.
  - step size: the `GSL` optimization algorithms use a line search and the step size determines the size of the first step performed in the search. Default value: 2.
  - tolerance: specifies the accuracy of the line search, with the precise meaning depending on the algorithm used. Default value: 0.1.
  - max iterations: the `GSL` optimization algorithms are iterative. If the number of performed iterations reaches max iter, than the algorithm is stopped, regardless of the gradient value. Default value: 1500.

  For more details on the above, please consult the `GSL` manual.

  The provided file `input/params.optim` is an example of an `optim_file`.

- `-l min`: limit running time to `min`.

  To control the running time of `polyDFE`, one run of the optimization algorithm is stopped if it has used more than `min`. By default, `min` is set to 300 (5h). Note that if `min` is set too low, there is a high chance that the optimization algorithm will terminate without finding good optimal parameters. Setting `min` to a negative number will allow the optimization algorithm to run until it finishes, without a limited running time.

- `-e`: automatically estimate the initial value of the parameters.

  The optimization algorithms require an initial value for the parameters to be optimized. This can either be provided using `-i` if the user has a good idea for such values, or can, by using `-e`. If `-e` is used without `-i`, then all the parameters (expect for ϵ_{cont}, which, by default, is not estimated and set to 0) will be automatically initialized and then optimized. However, if `-i` and `-e` are used together, the initial value

of the parameters is estimated automatically only for those parameters that are flagged with 0 and are consequently optimized.

The parameters $\bar{\theta}$, a, λ and r_i can be estimated deterministically. The rest of the parameters are estimated using a grid search approach. However, for the DFE model `D`, the probabilities p_i are set to be uniform when `-e` is used.

If `-i` is not used, `-e` is used by default.

- `-j`: use jointly both the provided initial values and automatically estimated values of parameters.

  When `-j` is used together with `-i`, then the optimization algorithm is ran twice, once where the initial value of the parameters is the one given in the `init_file`, and a second time where the automatic estimation from `-e` is used. At the end, the parameters that have the best likelihood, found from one of the two runs, will be reported.

- `-k kind(s, j, s+j)`: kind of likelihood optimization to use for estimating the parameters.

  The likelihood computation is split in two parts: the neutral likelihood for the neutral SFS, which does not require the DFE, and the selected likelihood for the selected SFS, which requires the DFE. Many DFE inference methods first infer all the parameters but the DFE from the neutral SFS, and then conditional on those, they infer the DFE from the selected SFS. By default, `polyDFE` infers all of the parameters jointly by using both the neutral and selected SFS, which is computationally more costly as many parameters have to be optimized at the same time. The type of likelihood optimiztion can be controlled through `-k`:

  - `s`: `polyDFE` is ran in a `single` mode, where the neutral likelihood is first optimized, followed by the optimization of the selected likelihood;
  - `j`: `polyDFE` is ran in a `joint` mode, where the joint likelihood is optimized;
  - `s+j`: `polyDFE` is ran in a `single + joint` mode, where first the `single` mode is used and the parameters found are used as initial values in the following `joint` mode.

  By default, `polyDFE` uses `-k j`. Note that using `-k s` might lead to parameters that are not optimal.

- `-r range_file ID`: path to a file containing the allowed range of all parameters, and id.

  `range_file` can contain any number of empty lines and comment lines that start with `#`. Excluding these, the structure of each line is

id	k	$\epsilon_{\text{an}}^{\text{min}}$ $\epsilon_{\text{an}}^{\text{max}}$	$\epsilon_{\text{cont}}^{\text{min}}$ $\epsilon_{\text{cont}}^{\text{max}}$	λ^{min} λ^{max}	$\bar{\theta}^{\text{min}}$ $\bar{\theta}^{\text{max}}$	<DFE parameters>	r^{min} r^{max}

  Each line starts with a positive numerical id. This is used such that multiple configurations of the parameters can be stored in the same file. When running `polyDFE`, the desired line for the range of the parameters is specified through the id by setting `ID` to the right value.

  The id is followed by k, which controls the transformation of the parameters (see below) and the range within each parameter should be estimated. Some parameters are intrinsically constrained (for example, p_b for DFE models `B` and `C` is, by construction, constrained to be between 0 and 1), while many parameters have to be positive (or negative, for example, S_d for DFE models `B` and `C`). In order to treat all parameters in the same way and to also allow the user to narrow the range in which the optimum parameters are searched, all parameters are then constrained to the range provided by the user through the `range_file`. However, the optimization algorithms `polyDFE` uses (see `-o` for details) are unconstrained, in that the parameters can take any value in the interval $(-\infty, +\infty)$. In order to change the original constrained optimization problem to an unconstrained one, `polyDFE` transforms the parameters from the range provided by the user to the interval $(-\infty, +\infty)$. For this, a generalized logistic function is used. This function is parameterized by a growth rate (or steepness of curve) k, which controls the spread of the transformation: the smaller the k, the more quicker the transformed values move from $-\infty$ to $+\infty$. `polyDFE` has been tested with a value of $k = 0.01$. If the optimization algorithm cannot find a good solution (i.e. the gradient is small, see `-p` for details), the k parameter can be changed in the hope of better performance.

  Note that, in order for the optimization algorithm to successfully find the optimum parameters (the parameters that have the largest likelihood), the ranges should be large enough to ensure that they cover the optimum values. If a parameter is inferred to be very close to one of the borders of its range, than the range should be expanded.

  If initial values of the parameters provided through `-i` or as calculated when `-e` is provided are not within the ranges, the program will automatically update the ranges.

If ranges are not provided, than `polyDFE` sets automatic ranges that are very large.

The parameter ϵ_{cont} is deprecated in v1.1 and is no longer used. For backward compatibility, it is still present in the `range_file`.

The provided files `input/range_model_A.txt`, `input/range_model_BandC.txt` and `input/range_model_D.txt` are examples of `range_file`.

- `-g grouping_file ID`: path to a file containing grouping information for the r_i parameters, and id.

  `grouping_file` can contain any number of empty lines and comment lines that start with `#`. Excluding these, the structure of each line is

  ```
  id        G        i1    i2    ...    iG
  ```

  Each line starts with a positive numerical id. This is used such that multiple configurations of the grouping can be stored in the same file. When running `polyDFE`, the desired line for the range of the parameters is specified through the id by setting `ID` to the right value.

  The `grouping_file` is used to provide information on grouping the r_i parameters. By default, each entry $1 \leq i < n$ in the SFS, and the divergence count, have one value of r_i associated to it. If n is very large, this leads to a lot of parameters that need to be estimated. One could expect that the distortion that is incurred on the counts that are modeled using the r_i parameters could be similar for neighboring values (for example, $i-1$, i and $i+1$). Then only one r value can be estimated for those counts that share a similar distortion.

  The grouping given in `grouping_file` will result in r values corresponding to the SFS entries as follows (where one interval represents one range for the SFS entries):

  $$\begin{array}{cccccc} r_1 & r_2 & r_3 & \dots & r_{G+1} \\ [1] & [2, i_1] & [i_1+1, i_2] & \dots & [i_G, n'] \end{array}$$

  where n' is either n, if divergence data is used in the estimation (see `-w` for details), or $n-1$ otherwise. For identifiability reasons, r_1 is always set to 1 and not estimated.

  The provided file `input/params.grouping` is an example of a `grouping_file`.

- `-b [basinhop_file ID]`: path to a file containing parameters that control the basin-hopping algorithm, and id.

  `basinhop_file` can contain any number of empty lines and comment lines that start with `#`. Excluding these, the structure of each line is

  ```
  id   max same   max iterations   temperature   step   accept rate   interval   factor
  ```

  Each line starts with a positive numerical id. This is used such that multiple configurations for the basin-hopping algorithm can be stored in the same file. When running `polyDFE`, the desired line for the range of the parameters is specified through the id by setting `ID` to the right value.

  By default, `polyDFE` runs the optimization algorithm once (or twice, see `-j` for details). However, the optimization algorithms are local, in that they only find an optimum that is in the neighborhood of the initial values of the parameters. To look more thoroughly for a global optimum, `polyDFE` allows the user to run the basin-hopping algorithm (Purisima and Scheraga, 1987; Wales and Doye, 1997; Wales and Scheraga, 1999). `polyDFE` contains a reimplementation of the basin-hopping algorithm found in the `Python` library, `scipy` (Jones et al., 2001–; Wales and Doye, 1997).

  Basin-hopping attempts to find the global optimum by iterating the following steps

  1. perturbing randomly the current value of the parameters;
  2. running the local optimization algorithm (as chosen through `-o`) using the new values as initial values;
  3. accepting or rejecting the found optimum based on the likelihood and temperature.

  Basin-hopping has been shown to be very efficient for a wide variety of problems in physics and chemistry. Unfortunately, there is no way to determine if the true global optimum has actually been found, and therefore it is left to the user to ensure that. The algorithm is controlled through

- max same: If after max same iterations, the basin-hopping algorithm does not find an improved set of parameters, than the algorithm stops. Default value: 50.
- max iterations: The basin-hopping algorithm is ran for a maximum number of iterations. Default value: 500.
- temperature: The temperature is used in the metropolis criterion when accepting or rejecting the new values. For best results, the temperature should be comparable to the typical difference in likelihood between local optima. Default value: 1.
- step: The step controls how far away from the current value the perturbated value is. This is crucial for the algorithm's performance. Ideally, it should be comparable to the typical separation between local optima of the likelihood. The algorithm implemented in `polyDFE` will automatically adjust the step, but it make take many iterations to find an optimal value. Default value: 50.
- accept rate: The target accept rate (percentage of new values that are accepted in step 3) for when adjusting the step. Default value: 0.5.
- interval: Every interval iterations, the basin-hopping algorithm adjusts the step. Default value: 10.
- factor: When the step is adjusted, it is done with this factor. Default value: 0.9.

`-b` can be used without `basinhop_file ID`. If only `-b` is given, then `polyDFE` runs basin-hopping with the default parameter values given above.

The provided file `input/params.basinhop` is an example of a `basinhop_file`.

5.5.2 Excluding divergence data

By default `polyDFE` performs the inference using all available data, including divergence data if this is present in the data file given through `-d data_file`. Divergence data can be excluded from the analysis by using the argument:

- `-w`: do not use divergence data.

  When `-w` is used, then the DFE and other parameters are estimated without using divergence data, as described in Tataru et al. (2017). If the divergence data is absent from the `data_file`, `-w` will be used by default.

5.5.3 Additional arguments

- `-t`: the total running time of `polyDFE` will be measured and printed on the standard output at the end.
- `-v verbose(0, 1, frequency)`: verbose frequency.

  `-v` controls how often information about the status of the optimization algorithm is printed on the screen. By default, `verbose` is set to 0, and only the initial and final values of the parameters are printed. Otherwise, every `verbose` iterations of the optimization algorithm, information is printed about the current value of the parameters, the corresponding likelihood, gradient and status of the optimization. Apart from the possible status values that `GSL` uses, `polyDFE` uses the additional values:

  - -3: local optimization is stuck in the same parameter values (it cannot further improve on the current values).
  - -4: restarting the local optimization lead to the same parameter values.
  - -5: the local optimization is being restarted; sometimes, when the local optimization cannot further improve on the current values, restarting the optimization can allow it to proceed further. If the local optimization is stuck in the same values, restarting is done at most 3 consecutive times.
  - -6: the local optimization used at least `min` minutes given through `-l`, and has been stopped.
  - -7: the likelihood evaluation returned NAN and the local optimization is therefore stopped. This can happen for certain values of the parameters, where the numerical integration over the DFE fails.
  - -8: a maximum number of likelihood evaluations has been reached, and the local optimization has been stopped. If this status appears, it indicates an issue with the local optimization procedure and the obtained results are not reliable.

5.5.4 What to do when the gradient is large

If inferred parameters are at a true optimum, than the gradient of the corresponding likelihood should be 0. If the gradient is large, this indicates that the optimization failed in finding good values for the parameters. The run of `polyDFE` can be changed in multiple ways to try to address this problem:

- The optimization algorithm terminates with status 0 (see `-v`) if the gradient is smaller than the absolute tolerancevalues, ϵ_{abs}. If this is too large, the optimization algorithm might terminate prematurely. The value can be changed using `-p`.
- The optimization algorithm is ran for a certain number of iterations. If this number is reached, `polyDFE` prints the message "reached maximum number of iterations allowed". If this happens and the gradient is still large, the number of iterations should be increased using `-p`.
- The optimization algorithm has a time limit, after which it is stopped. In this case, `polyDFE` prints the message "reached maximum running time allowed". If this happens and the gradient is still large, the running time allowance should be increased using `-l`.
- If `polyDFE` was ran with `-k s` or `-k s+j`, setting `-k j` might lead to better estimates.
- If the inferred value of one of the parameters is very close to one of the borders of its range, than its range should be consequently increased using `-r`.
- The optimization algorithm's performance is highly dependent on the initial values of the parameters. To investigate a wider range of initial values, the basin-hopping algorithm can be ran using `-b`.

5.5.5 Examples

Inferring a full DFE using model `C` using default arguments:

```
$ ./polyDFE -d input/example_2 > output/example_2_full_C
```

Inferring a full DFE using model `C`, while running the optimization algorithm twice, once initialized with estimated parameters, and once with parameters specified from file:

```
$ ./polyDFE -d input/example_2 -i input/init_model_BandC.txt 1 -v 20 -j > output/example_2_init_C
```

Inferring a deleterious DFE using model `C`:

```
$ ./polyDFE -d input/example_2 -i input/init_model_BandC.txt 2 -v 100 > output/example_2_del
```

Inferring a full DFE using model `C` without mutation variability:

```
$ ./polyDFE -d input/example_2 -i input/init_model_BandC.txt 3 > output/example_2_novar_C
```

Inferring a full DFE using model `C` without nuisance r parameters, and using different kinds of likelihood optimization and parametrization of the optimziation algorithm:

```
$ ./polyDFE -d input/example_2 -i input/init_model_BandC.txt 4 > output/example_2_nonuis_C
$ ./polyDFE -d input/example_2 -i input/init_model_BandC.txt 4 -j -k s \
            -p input/params.optim 4 > output/example_2_nonuis_s_C
$ ./polyDFE -d input/example_2 -i input/init_model_BandC.txt 4 -j -k s+j \
            > output/example_2_nonuis_sj_C
```

The above examples illustrate that using `-k s` can give poor estimates. For the run that was initialized with the values calculated automatically (see `-j`), when `-k s` was used, the likelihood was -4260.4124 with gradient 3.6338, while when `-k s+j` was used, the likelihood was -3924.4526 with gradient 0.0001. In fact, for `-k s`, `polyDFE` finds good estimates only when it was initialized with the values used for simulating the data from `input/init_model_BandC.txt`, while for `-k s+j`, the initial values used do not affect the estimates found. This indicates that `-s` should be used with caution. However, using `-k s+j` might sometimes be just as good as `-k j`, but faster.

Inferring a full DFE using model `A` and 10 basin-hopping iterations:

```
$ ./polyDFE -d input/example_2 -m A -i input/init_model_A.txt 1 -e \
            -b input/params.basinhop 1 -v 200 > output/example_2_full_A
```

For the inference under model A, running the optimization intialized with `-e` lead to a gradient of 2.89626, which is very large. Running 10 iterations of basin-hopping was sufficient to find a set of parameters with a gradient of 0.00021.

6 Post-processing using R

polyDFE is accompanied by a series for R functions that allow

- parsing the output of polyDFE for easy manipulation in R
- summarizing the DFE estimated by polyDFE
- calculating α using alternative definitions
- bootstrapping data
- performing model testing
- performing model averaging
- creating `init_file` containing the best found parameters
- creating grouping for the r parameters

6.1 Parsing the output of polyDFE

The function

```
parseOutput(filename, init = FALSE)
```

allows the user to parse the output of polyDFE from multiple runs stored in `filename`. The function returns a list with entries for each separate run of polyDFE found, containing

- the name of the input file used;
- the DFE model used;
- the best likelihood found and the corresponding gradient;
- the values of all parameters (including those that have been fixed using the flag 1 in `init_file`, see `-i` for details);
- which parameters have been estimated;
- the value of n (the sample size of the data);
- expected SFS and, if relevant, divergence counts and misattributed polymorphism;
- estimates of α.

If `init = TRUE`, then the function returns the values for the local optimization where the parameter values found in `init_file` where used as initial values for the parameters during the optimization (see `-i`, `-e` and `-j` for details), regardless if this returned the best likelihood or not.

6.2 Summarizing the DFE

The function

```
getDiscretizedDFE(estimates, sRanges = c(-100, -10, -1, 0, 1, 10))
```

discretizes the DFE found in `estimates`, which is one entry from the list returned by `parseOutput`. The discrete categories are given as a vector in `sRanges`.

6.3 Calculating α

The function

```
estimateAlpha(estimates, supLimit = 0, div = NULL, poly = TRUE)
```

calculates both α_{div} (when `div = NULL`) and α_{dfe} (when `div` is given) (Tataru et al., 2017), using the parameters found in `estimates`, which is one entry from the list returned by `parseOutput`.

Galtier (2016) argues that mutations with positive selection coefficients that are not higher than a certain threshold should, effectively, be treated as neutral mutations when calculating α (see also Tataru et al. (2017)). This threshold can be controlled here by setting `supLimit`.

When used, `div` should contain divergence data, which can be read using the function

```
parseDivergenceData(filename)
```

where `filename` is the same data file used for running `polyDFE`, see -d for details.

As described in Tataru et al. (2017), divergence data can contain misatributed polymorphism. This is the default behavior, but the correction can be turned off by setting `poly = FALSE`.

6.4 Bootstrapping data

The function

```
bootstrapData(inputfile, outputfile = NULL, rep = 1)
```

creates bootstrap data from the data found in `inputfile`, which is the same data file used for running `polyDFE`, see -d for details. The output is written to files with names `<outputfile>_i` for $1 \leq i \leq$ `rep`. By default, `outputfile = NULL`, and the function writes to `<inputfile>_i` instead.

6.5 Model testing

The function

```
compareModels(est1, est2 = NULL, nested = NULL)
```

compares the models found in `est1` and `est2` by calculating the AIC and, where relevant, the LRT.

Both `est1` and `est2` can either be lists returned by `parseOutput`, or file names, as for `parseOutput`. If these contain more then one run of `polyDFE`, then run i from `est1` is compared to run i from `est2`.

The function can automatically detect if models are nested for calculating the LRT, however nestedness can be enforced by setting `nested = TRUE`. Caution should be used when the r parameters have been grouped (see -g for details), as in this case the function cannot correctly detect nestedness.

The function returns a list containing two entries

- `AIC` is a matrix containing the number of estimated parameters, the log likelihood and AIC for `est1` in columns 1 – 3 and for `est2` in columns 4 – 6, for all runs of `polyDFE` found in each row;
- `LRT` is a matrix containing the degrees of freedom in column 1, the likelihood of `est1` in column 2, the likelihood of `est2` in column 3 and the p-value from the LRT test in column 4, for all runs of `polyDFE` found in each row.

If `est2 = NULL`, only the AIC is calculated for `est1`.

6.6 Model averaging

The function

```
getAICweights(estimates)
```

calculates AIC weights (Posada and Buckley, 2004) for the runs of `polyDFE` found in `estimates`, a list as returned by `parseOutput`. The weights can the be used to calculate model avearge for a parameter of interest, such as any DFE parameter, or a discretized DFE (see `getDiscretizedDFE`)), α, or any other calculable quantities.

6.7 Creating `init_file`

The function

```
createInitLines(estimates, outputfile, startingID = 1, fix = c("eps_cont"), groupingDiff = NA)
```

writes the values of the parameters found in `estimates`, a list as returned by `parseOutput`, or a file name, as for `parseOutput`. For each run of `polyDFE`, a new line is appended to `<outputfile>_init` containing the values of the best parameters found in the format described previously (see `-i` for details).

The `ID`s for each line are consecutive and start at `startingID`.

The parameters given in `fix` are flagged with 1, while the rest of the parameters are flagged with 0. If `fix = "all"`, than all parameters are flagged with 1.

Grouping of r parameters (see `-g` for details) can be calculated automatically by setting `groupingDiff`. If the difference between r_i and r_{i+1} is smaller than `groupingDiff`, than they are placed in the same group. The grouping information is appended to `<outputfile>_grouping`, with `ID` matching to the `ID` in `<outputfile>_init`. Automatic grouping should never be used on r parameters that have already been estimated for groups.

6.8 Examples

The accompanying file `example.R` shows how to post-process the `polyDFE` output files generated in 5.4.1.

References

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