

recon_v2.2.py

Reconstruction of Estimated Communities from Observed Numbers

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1. Introduction

1.1. Overview

Recon is an algorithm for generating a description of an overall population from a sample (see section 1.2, Terminology). More precisely, Recon uses the distribution of species counts in a sample to estimate the distribution of species counts in the population from which the sample was drawn. This is useful for large and complex populations, in which samples are likely to misrepresent the distribution of species in the overall population.

1.2. Terminology

A *species* is group (class) made up of one or more *individuals*. The number of individuals of a given species is that species' *size*. A *sample* is a set of individuals drawn randomly from an *overall* or *parent* population. Species represented in the population that are not represented in the sample are called *missing species*. (For historical reasons, in the code and below, *clone* is used interchangeably for *species*.)

Diversity refers to any of a set of measures of the frequency distribution in the population. These measures can be thought of as effective numbers of species in the population. The *Hill numbers* (qD ; read e.g. "D-one" for 1D and "D-infinity" for $^\infty D$) are a family of diversity measures defined by the parameter q , which determines the degree to which diversity measures are weighted toward larger species. Many Hill numbers correspond to familiar measures. For example, 0D is *species richness*, a diversity measure that weights all species equally (and so is just a count of the number of different species present, regardless of their relative frequencies). $^\infty D$ is the reciprocal of the Berger-Parker Index, the effective number of species if all species were the size of the largest species. Simple mathematical transformations of 1D and 2D correspond to Shannon entropy (information theory) and the Gini-Simpson Index (economics), respectively.

1.3. Features

Key features of Recon include that it (i) assumes nothing about the shape of the frequency distribution of species in the population (e.g. exponential, power, arbitrary, etc.), (ii) avoids (over)fitting of sampling noise, (iii) scans many starting points in an attempt to find a global best fit, (iv) outputs 95% confidence intervals for the number of species of a given size in the population, and (v) outputs power tables useful in designing experiments for comparing overall populations based on samples.

Please see the license (Section 10) for rules of use.

1.4. Citing Recon

Please cite Recon as follows:

Kaplinsky J. and Arnaout R.(2016) Robust Estimates of Overall Immune-Repertoire Diversity from High-Throughput Measurements on Samples. *Nat. Communications*, In Press.

2. Installation

2.1. Availability

Recon is available on GitHub (<https://github.com/ArnaoutLab/Recon>) subject to the terms in the license (Section 10). It is contained in the file `recon_v2.2.py` (although note the requirements in section 2.2 below).

2.2. Requirements

Producing fits, diversity measures, and power tables requires:

- Python 2.7 or later (<https://www.python.org>)
- The SciPy Python library (<http://scipy.org>)

Producing plots additionally requires:

- d3.js (<http://d3js.org>)
- wkhtmltopdf (<http://wkhtmltopdf.org>)
- cpdf (<http://community.coherentpdf.com>)
- the file `style.css` (included in the Recon distribution)
- the JavaScript file `plot_clone_size_distribution_ref.js` (also included in the Recon distribution)

2.3. Platforms

Recon has been tested on Macintosh OS X (v10.8-11) and several other Unix systems.

2.4. Latest version

The latest version as of this writing is 2.2 (`recon_v2.2.py`).

3. Operation

This section describes the various modes for running Recon. The main use is to produce a description of an overall population from a sample (section 3.1). it can be used to build an error-bar profile (section 3.2; necessary for calculating error bars and power tables), output diversity measures (section 3.3), produce power tables (section 3.4), and generate plots (section 3.5).

3.1. Reconstruction (-R, --run_recon)

3.1.1. Description

Given a set of observations of species frequencies in a sample as input, the -R option outputs a set of parameters that describe the modified maximum-likelihood species frequency distribution in the parent population (see below), without overfitting.

3.1.2. Usage

```
python recon_v2.2.py -R [options...] -o OUTPUT_FILE INPUT_FILE
```

3.1.3. Input

Input is a text file containing the number of individuals of each species seen in the sample (i.e., the species sizes in the sample). The name of this file is supplied as the parameter `INPUT_FILE`.

The default format is a tab-delimited file with lines of the form

```
species name <tab> species size
```

with a newline character delimiting lines. Species size is an integer. Sample data in this format can be found in the file `test_sample_data_4.txt`; in it, species 9_0 has a size of 9 individuals, individual, species 8_0 has 8 individuals, species 1_1833458 has 1 individual, etc.

An alternative format is specified by the `-c (--clone_distribution_in_file)` option (see section 3.1.6 below). This is a tab-delimited format with lines of the form

```
species size <tab> number of species of this size
```

where both values are integers. `test_sample_1.txt`, `test_sample_2.txt`, and `test_sample_3.txt` are all in this format. The file `test_sample_4.txt` contains the same data as `test_sample_1.txt`, only in the default extended format.

Note that files in the default format can be long and therefore take a few seconds to read (`test_sample_data_4.txt` is over two million lines long). The alternative format is much more compact and therefore faster to read. For example, `test_sample_data_1.txt` contains the same information as `test_sample_data_4.txt`, only in this more compact format, and runs in ~2 seconds vs. ~7 seconds for `test_sample_data_4.txt`.

3.1.4. Output

The above command returns a tuple with the following elements, in order:

- a list of weights that, with means, describes the reconstructed parent distribution. Each weight w_i is the fraction of all species in the parent distribution that each contribute a mean m_i number of individuals to the sample;

- a list of means that, with weights, describes the reconstructed parent distribution. Each mean m_i is the mean number of individuals a species of this size contributes to the sample. Means are Poisson parameters;
- an integer of the number of missing species;
- a dictionary of the the species-size distribution in the sample (the observed distribution), where each key is a species size, and the corresponding value is the number of species of that size. If the `-c` option is given, the keys and values should correspond to the left and right columns of input data (a useful check that your data was read in successfully);
- if given (the `-A` option), the true number of species in the parent population;
- a float of the log-likelihood of this fit; and
- a float of the time in seconds for the fit.

See 3.1.6 below for an example.

Recon also writes to **FILE_OUT** a summary of the fit. We refer to files of this type as "fitfiles;" fitfiles are used as input for other Recon functions (below). The last block in the fitfile that is offset by multiple equal signs ("=====") contains the final weights and means (as a single list of weights-then-means; denoted by lines with "**fitted parameters**") and missing species (denoted by lines with "**estimated n0**"). These are the same as in the screen output described above.

3.1.5. Required parameters

<code>-R, --run_recon</code>	Required to run Recon in this mode (i.e. to tell it to reconstruct an overall distribution)
<code>-o OUTPUT_FILE</code>	The filename to be used for output. Note that if this file exists it will not be overwritten; instead recon will exit with an error message.
<code>INPUT_FILE</code>	A text file containing the number of individuals of each species seen in the sample (i.e. the species sizes in the sample). See 3.1.4 above for possible formats for this file.

3.1.6. Optional parameters

<code>-a, --aicc_multiple AICC_MULTIPLE</code>	Sets the multiple of the observed number of datapoints that Recon considers observations. E.g., if only singletons, doublets, and triplets are observed but user believes not seeing quadruplets is evidence of absence (vs. absence of evidence), <code>-a 1.3</code> will tell Recon to consider this as four
--	---

observations for purposes of calculating AICc.

`-c, --clone_distribution_in_file`

Allows recon to read an alternative tab-delimited format with lines of the form

species size <tab> number of species of this size

where both values are integers. test_sample_1.txt, test_sample_2.txt, and test_sample_3.txt are all in this format. As mentioned above, test_sample_4.txt contains the same data as test_sample_1.txt, only in the default extended format.

`-d, --bin_size BIN_SIZE`

Average number of observations per individual (default: 1). In many circumstances, each individual in the sample will be observed and counted once. However, there are cases where each individual in the sample will be observed and counted multiple times. **BIN_SIZE** allows for this possibility.

`-l, --parameter_limit PARAMETER_LIMIT`

The maximum number of parameters that the algorithm will use to fit the data (default, 20). Recon will continue adding parameters until the AICc indicates that additional parameters are not justified. In practice the limit of 20 is essentially never reached.

`-s, --noise_test_ratio_threshold NOISE_TEST_RATIO_THRESHOLD`

Sets the noise threshold ratio. Recon will reject fitting a population of clones that is so small that its contribution to the sample is comparable to the sampling noise from other populations that are present.

NOISE_TEST_RATIO_THRESHOLD is the factor by which the expected contribution of a population of clones must rise above the sampling noise (i.e. standard deviation from Poisson sampling in the contribution to the count of singletons) from the next largest population. Empirically, the default of 3.0 produces good results for fitting distributions of the sort reported in the paper (see section 1.4).

The noise threshold ratio corresponds to a false-positive rate for fitting a clone population to sampling noise. If the noise threshold ratio is set higher than 3.0 then Recon will be less sensitive to noise (lower false positive rate), but will fit fewer missing species (higher false negative rate). Conversely, if it is set lower, it will be more sensitive to noise (higher false positive rate), but fit more missing species (lower false negative rate).

The exact false positive rate that 3.0 corresponds to depends on the mean parameter of the smallest fitted clone size. The false positive rate for accepting the presence of a small clone size can be found by considering the CDF of a Poisson distribution. Specifically, it will be

$\text{gammmaincc}(\text{NOISE_TEST_RATIO_THRESHOLD} * m, m)$,
 where gammmaincc is the complementary incomplete gamma function and m is mean parameter of the smallest fitted clone size (see section 3.1.7 for some example means).

At a noise threshold ratio of 3.0, this gives:

m	false positive rate
1e-1	0.45
1e-2	0.11
1e-3	1.8e-2
1e-4	2.6e-3
1e-05	3.3e-4
1e-06	4.0e-05

Typical values of m for which noise is a limiting factor are 1e-3 or below. At a noise threshold ratio of 3.0 this therefore corresponds to false positive rates of less than 2%.

**-t --threshold
THRESHOLD**

Allows you to modify **THRESHOLD**, the clone size above which sampling error is considered small, which means that Recon will assume the frequency of species of this size or greater in the population is the same as the frequency in the sample. It defaults to 30, which usually gives good results: this is because if, in a sample from a well mixed population, species A is seen 30 times in a sample it is very unlikely that there is another species B which is the same size as A in the parent population but is missing or very poorly represented in the sample. Smaller values will give faster run times but less accurate results.

3.1.7. Example

Command:

```
python recon_v2.2.py -R -t 30 -c -o test_sample_1_fitfile.txt
test_sample_1.txt
```

Output:

```
([0.88205137420509439, 0.11794862579490561], [0.306026605847127,
1.0723167141789034], 5224621, {1: 1833459, 2: 405423, 3: 86822,
4: 18467, 5: 3694, 6: 626, 7: 128, 8: 20, 9: 1}, None, -
1572523.5668443954, 2.2577288150787354)
```

As described above, this output consists of:

list of weights `[0.88205137420509439, 0.11794862579490561]`

list of means	[0.306026605847127, 1.0723167141789034]
number of missing species	5224621
observed (input) distribution	{1: 1833459, 2: 405423, 3: 86822, 4: 18467, 5: 3694, 6: 626, 7: 128, 8: 20, 9: 1}
	Note this means that in the observed (input) data, 1,833,459 species were represented by a single individual each, 405,423 species were represented by two individuals each, and so on.
true number of species in the parent population	None (meaning none was given; this would have been supplied as part of the command using the <code>-a</code> option)
log-likelihood of this fit	-1572523.5668443954
seconds elapsed	2.2577288150787354

3.2. Error bars (`-e`, `--make_errorBars`)

3.2.1. Description

This mode generates an error bar parameter file from a set of fits on data for which the number of missing species is known (i.e., validation datasets). This file is needed for D-number tables (section 3.3) and power tables (section 3.4).

3.2.2. Usage

```
python recon.py -e -o OUTPUT_FILE ERROR_BAR_FIT_DIRECTORY
```

3.2.4. Input

A directory that contains fits for distributions with known numbers of species (and therefore known numbers of missing species).

3.2.4. Output

An error bar parameter file.

3.2.5. Required parameters

<code>-e</code> , <code>--make_errorBars</code>	Tells recon to make an error-bar parameter file.
<code>-o</code> , <code>--file_out</code> <code>OUTPUT_FILE</code>	The name of the new error-bar parameter file
<code>ERROR_BAR_FIT_</code>	The name of a directory that contains the fits with known missing species. (The known missing species are encoded in

DIRECTORY the weights and means of the population.)

3.2.6. Example

```
python recon.py -e error_bar_parameters.txt Test_dir
```

3.3. Diversity measures (-D, --make_table_of_D_numbers)

3.3.1. Description

Given a fitfile (either the output from section 3.1 above or any population description in that format), outputs a table of diversity measures as Hill numbers (see section 1.2, Terminology). Note that measures for any Hill number are obtainable, but the appropriate Hill number(s) must have been provided when making the `ERROR_BAR_PARAMETER_FILE` that Recon uses for outputting diversity (section 3.2 above).

3.3.2. Usage

```
python recon_v2.2.py -D [options...] -b ERROR_BAR_PARAMETER_FILE -o
OUTPUT_FILE INPUT_FILE [INPUT_FILE_2 INPUT_FILE_3 ...]
```

3.3.3. Input

<code>INPUT_FILE</code>	A fitfile, which can be either the output from section 3.1 above or any population description in that format
<code>ERROR_BAR_PARAMETER_FILE</code>	A text file that Recon uses to calculate error bars produced by the <code>-e (--make_error_bars)</code> option

3.3.4. Output

A table of Hill numbers for the reconstructed distribution, one row for each input file (see `INPUT_FILE` below). Columns prefixed "`obs_`" show the Hill numbers from the observed data in the sample (sample diversities). Columns prefixed "`est_`" show the Hill numbers Recon has estimated for the population (overall diversities). The difference between `est_0D` and `obs_0D` is n_0 , the estimated number of missing species. Columns with the suffixes "+" and "-" indicate upper and lower error-bar limits, respectively.

3.3.5. Required parameters

<code>-D,</code> <code>--make_table_of_D_numbers</code>	Tells Recon to make a table of D numbers
<code>-b</code> <code>ERROR_BAR_PARAMETERS</code>	<code>ERROR_BAR_PARAMETERS</code> is a file that contains parameters for constructing error bars on fits. The supplied file <code>error_bar_params.txt</code> can be used. Alternatively, Recon can generate a custom error-bar parameter file from a set of gold-standard fits (see section 3.2 above).
<code>-o, --file_out</code> <code>OUTPUT_FILE</code>	The desired name of the output file.
<code>INPUT_FILE</code> <code>[INPUT_FILE_2</code> <code>INPUT_FILE_3 ...]</code>	The input file(s). Multiple files should be separated by spaces; each will generate one row in the output table. Each input file is fitfile (see section 3.3.3).

3.3.6. Optional parameters

<code>-Q HILL_NUMBER</code> <code>[HILL_NUMBER_2</code> <code>HILL_NUMBER_3 ...]</code>	Hill-number parameter(s) for table (i.e., q). Note that the error-bar parameters file must be run for whatever Hill numbers are desired for the power table. Multiple parameters should be separated by spaces.
---	--

3.3.7. Example

```
python recon_v2.2.py -D -Q 0 1 inf -b error_bar_parameters.txt -o
test_D_number_table.txt test_sample_1_fitfile.txt
test_sample_2_fitfile.txt test_sample_3_fitfile.txt
```

This will output a file called `test_D_number_table.txt` with a header containing some reference information:

```
# python recon_v2.2.py -D -Q 0 inf -b error_bar_parameters.txt -o
test_D_number_table.txt test_sample_1_fitfile.txt test_sample_2_fitfile.txt
test_sample_3_fitfile.txt
# infiles = ['test_sample_1_fitfile.txt', 'test_sample_2_fitfile.txt',
'test_sample_3_fitfile.txt']
# observed_threshold = 30
# precomputed_error_bar_file = error_bar_parameters.txt
```

and the following lines (for clarity, only the first five columns are shown here and the values are have been truncated to two decimal points):

sample_name	obs_0.0D	est_0.0D	est_0.0D-	est_0.0D+
test_sample_1_fitfile.txt	2348640.0	7573260.81	5755678.21	inf
test_sample_2_fitfile.txt	2500.0	3013.53	2709.16	3513.20
test_sample_3_fitfile.txt	336.0	472.77	412.73	580.95

3.4. Power tables (**-p**, **--make_power_table**)

3.4.1. Description

Generates a power table with the minimum sample size required to be able to detect differences of a given magnitude in a given Hill number for two populations. That is, if you have two populations, and want to be able detect a difference in 1D of x%, the table tells you how big your samples have to be.

3.4.2. Usage

```
python recon.py -p [-q HILL_NUMBER -m MIN_NUMBER_OF_DOUBLET] -o
FILE_OUT ERROR_BAR_PARAMETERS
```

3.4.3. Input

An error-bar parameters file (**ERROR_BAR_PARAMETERS**) and the name of the output file that will contain the power table.

3.4.4. Output

A power table in which the rows and columns show the minimum fold differences that can be detected for different population sizes. Recon produces a single power table for each Hill number, so building tables for e.g. $q=0$, $q=1$, and $q=2$ requires running Recon with the **-p** option separately with **-q 0** (the default), **-q 1**, and **-q 2**.

If the output file already exists, Recon will issue a warning and print the output to stdout without overwriting the existing file.

3.4.5. Required parameters

-o, --file_out OUTPUT_FILE	The desired name of the output file.
ERROR_BAR_PARAMETERS	A file that contains parameters for constructing error bars on fits. The supplied file error_bar_params.txt can be used. Alternatively, Recon can generate an error bar parameter file from a set of gold standard fits (see section 3.3).

3.4.6. Optional parameters

-C NUMBER_OF_SPECIES [NUMBER_OF_SPECIES_2 NUMBER_OF_SPECIES_3...]	The (space-delimited) rough number of species in the overall population to consider; used as the columns of the power table. Default is 1e4 3e4 1e5 3e5 1e6 3e6 (10,000, 30,000, 100,000, 1 million, 3 million).
--	---

<code>-F FOLD_DIFFERENCES</code> <code>[FOLD_DIFFERENCES_2</code> <code>FOLD_DIFFERENCES_3...]</code>	The rows of the table. Default is 1.1 1.2 1.3 1.4 1.5 2.0 5.0 (i.e., 10%, 20%, 30%, 40%, 50%, 2x, and 5x).
<code>-q, --q HILL_NUMBER</code>	The Hill number for which the power calculation is carried out (default: 0)
<code>-m, --min_number_of_doublets</code> <code>MIN_NUMBER_OF_DOUBLET</code>	An additional statistical minimum required for good results. The default of 100 should be good for most purposes.

3.4.7. Example

```
python recon_v2.2.py -p -C 1e4 1e5 1e6 -F 1.5 5 10 -q 1 -o
test_power_table.txt error_bar_parameters.txt
```

This outputs a file called `test_power_table.txt` with the following contents (header information followed by the actual table):

```
# Wed Apr 20 16:10:29 2016
# python recon_v2.2.py -p -C 1e4 1e5 1e6 -F 1.5 5 10 -q 1 -o
test_power_table.txt error_bar_parameters.txt
error_bar_file = test_power_table.txt
number_of_error_bars = 2.0
min_number_of_doublets = 100
fraction_small_clones = 0.1
q = 1

      10000      100000      1000000
1.5    14142    44721    141421
5.0    14142    44721    141421
10.0   14142    44721    141421
```

3.5. Resampling (`-r, --resample`)

3.5.1. Description

This allows resampling of a fit (model). The output gives the maximum likelihood observed species size distribution of samples from the model. This is the distribution that Recon attempts to make as close as possible to the observed species size distribution. The closeness of the fits can be compared to measure the goodness of fit.

3.5.2. Usage

```
python recon_v2.2.py -r -o OUTPUT_FILE INPUT_FILE
```

3.5.3. Input

A Recon fit file such as those produced by the **-R** option (see section 3.1).

3.5.4. Output

A list of species sizes up to the threshold that was used in the original fit is outputted, together with a count of species for each size. Output is written to **OUTPUT_FILE**.

3.5.5. Required parameters

OUTPUT_FILE	The desired name of the output file.
INPUT_FILE	A file that contains model fitted parameters, for example as output from a previously completely Recon fit (see section 3.1)

3.5.6. Example

```
python recon_v2.2.py -r -o test_sample_1_resample.txt
test_sample_1_fitfile.txt
```

The output file will consist of two (tab-delimited) columns with a headerline preceded by a hash tag (#). The first column lists the clone size, and the second column the number of clones:

```
#clone_size    no_clones
1              1833117
2              406083
3              86315
4              18638
5              3722
6              651
7              99
8              13
9              2
```

Note how this resampled distribution compares to the observed data, which can be found in the third line of the input file, `test_sample_1_fitfile.txt`:

```
observed_clone_size_distribution = {1: 1833459, 2: 405423, 3:
86822, 4: 18467, 5: 3694, 6: 626, 7: 128, 8: 20, 9: 1}
```

In columnar form:

```
1          1833459
2          405423
3          86822
4          18467
5          3694
```

6	626
7	128
8	20
9	1

one can see close agreement between the observed data and the resampled data, supporting this being a good fit. However, comparison is even easier with plots (the `-x` mode; section 3.6 below).

3.6. Plotting (`-x`, `--make_resample_plot`)

3.6.1. Description

Plots a resampled fit (see section 3.5) against the input data. Note the additional requirements for plotting (see section 2.2 above). Note clones larger than the threshold used for the fit (see `-t`, `--threshold` option in section 3.1.6) will still be plotted, but because these are data that have been added back in, these points will usually be spot-on.

3.6.2. Usage

```
python recon_v2.2.py -x [options...] -o PLOT_FILE -b
ERROR_BAR_PARAMETER_FILE INPUT_FILE
```

3.6.3. Input

A Recon fitfile and an error-bar parameter file.

3.6.4. Output

A plotfile containing the data to be plotted, and a PDF of the plot. These are described in more detail in section 3.6.7, below.

3.6.5. Required parameters

<code>-o PLOT_FILE</code>	The desired name of the output file that will contain the data to be plotted.
<code>-b ERROR_BAR_PARAMETER _FILE</code>	A file that contains parameters for constructing error bars on fits. The supplied file <code>error_bar_params.txt</code> can be used. Alternatively, Recon can generate an error bar parameter file from a set of gold standard fits (see section 3.3).

3.6.6. Optional parameters

<code>-X, --x_max X_MAX</code>	The upper limit of the x-axis in the plot. The default is set dynamically based on the data, up to a limit of 40. (The user can override this limit using this option.)
--------------------------------	---

<code>-Y, --y_max Y_MAX</code>	The upper limit of the y-axis in the plot. The default is set dynamically based on the data.
<code>-q, --q HILL_NUMBER</code>	The Hill number for which the power calculation is carried out (default: 0)
<code>-m, --min_number_of_doublets MIN_NUMBER_OF_DOUBLET</code>	An additional statistical minimum required for good results. The default of 100 should be good for most purposes.

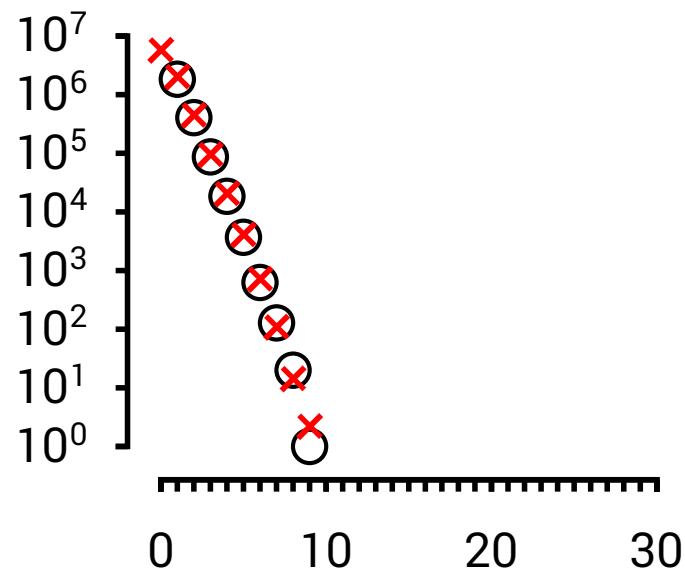
3.6.7. Example

```
python recon_v2.2.py -x --x_max 30 -o test_sample_1_plotfile.txt
-b error_bar_parameters.txt test_sample_1_fitfile.txt
```

Outputs a plotfile `test_sample_1_plotfile.txt` that contains the observed and refitted data side by side:

clone_size	sample	fit	lower_limit	upper_limit
0	0.0	5224621.0	3407038.40444	inf
1	1833459	1833117		
2	405423	406083		
3	86822	86315		
4	18467	18638		
5	3694	3722		
6	626	651		
7	128	99		
8	20	13		
9	1	2		

as well as a PDF file with the same prefix as the input file, i.e., `test_sample_1_fitfile.pdf`.



In this PDF, the x-axis is the clone size and the y-axis is the number of clones of that size. The black circles represent the observed data and red x's represent the resampled fit. "X marks the spot" represents a good fit. Note there is no circle for zero; the red x at zero is Recon's estimate of the number of missing species (i.e., the number of species that appear zero times in the sample).

3.7. Workflow

3.7.1. Typical case

Most commonly the goal will be to estimate the diversity (by the desired Hill number) of the overall populations from which a set of samples were drawn. For this, the workflow is to perform reconstruction on all samples (section 3.1); then, for each sample, plot a resampling from the model against the data to confirm goodness of fit (section 3.6); and finally to use pre-computed error bars to calculate diversity measures for all samples (section 3.3).

3.7.2. Custom error bars

If you want to explore diversity measures for q s other than the ones in the pre-computed error bars, you should first make a new error-bar parameter file (section 3.2) with the desired q s. If you want an error-bar profile based on your own custom set of distributions, first make those distributions; then perform reconstruction on them (section 3.1), placing the resulting fit files in their own directory; and then generate an error-bar parameter file based on those new fits (section 3.2).

3.7.3. Power tables

Power tables are most useful when planning experiments. Generating them is usually a standalone workflow that starts with the appropriate error-bar parameters (section 3.2) and involves just generating power tables for the desired q , total diversity, and sample size (section 3.4).

4. Contact information

Questions, comments, and other correspondence should be addressed to Ramy Arnaout at rarnaout@gmail.com.

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