TARDiS

Temporal And diveRsity Distribution Sampler for Phylogenetics

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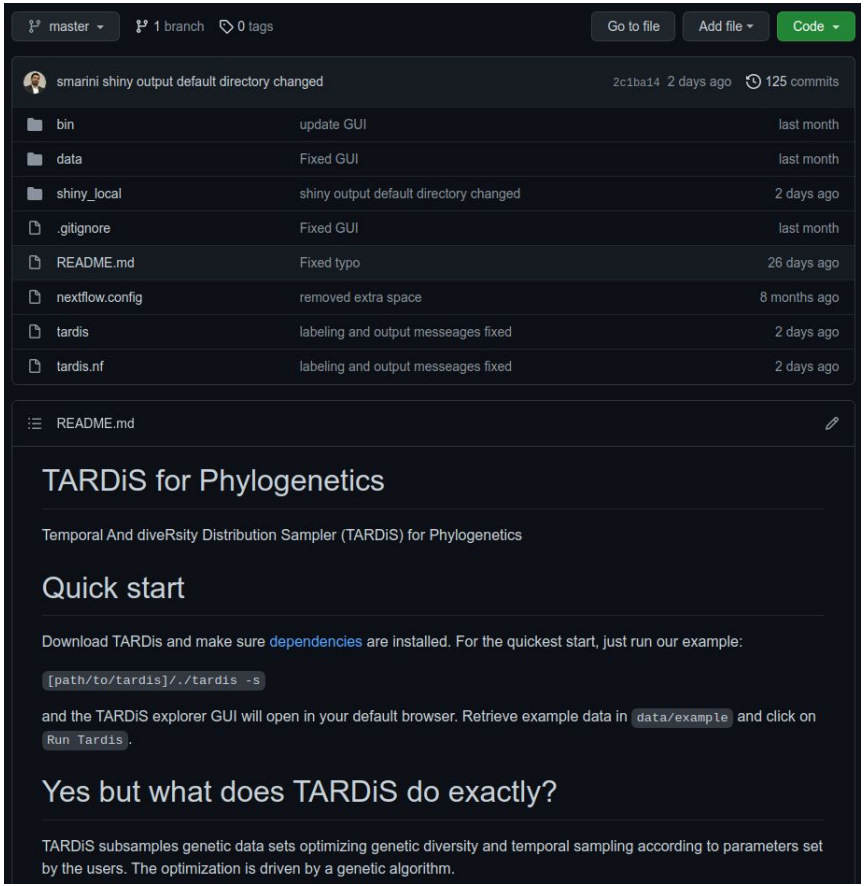
TARDiS

- Method to **subsample** biosequences
  - In: many sequences
  - Out: fewer sequences
- Optimizing:
  - Genetic diversity
  - Temporal distribution
- Based on genetic algorithm
  - R
  - Python
  - Nextflow
- Usage
  - GUI
  - Command line, job allocation on HPC

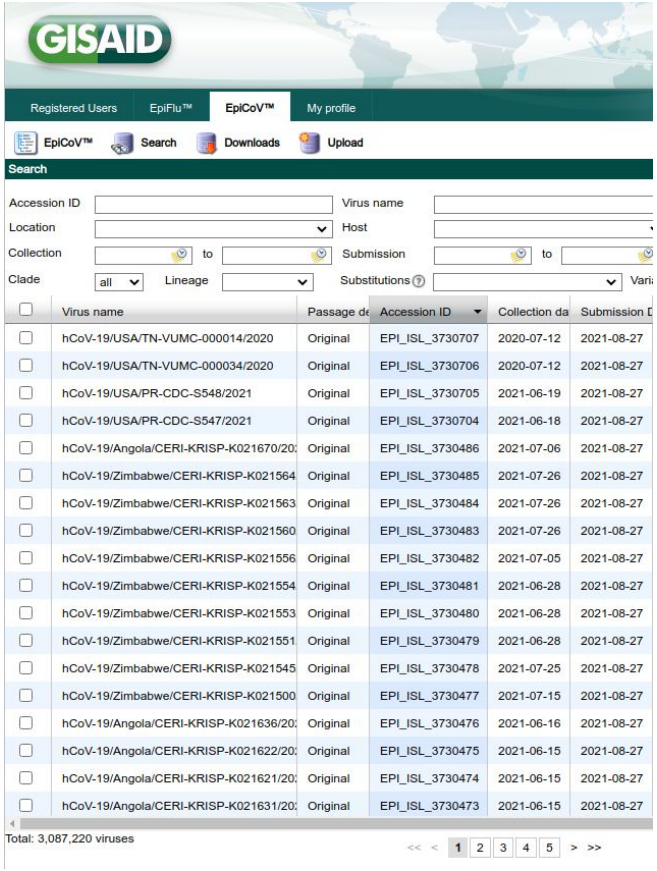
https://github.com/smarini/tardis-phylogenetics



WHY TARDiS

- Too much information
  - GISAID 3+ million viruses in late Aug 2021

- Bias! Genomes are not collected uniformly (of course)
  - Geographical
  - Temporal
  - ...



WAIT WHY DON'T WE BRUTE FORCE THIS?

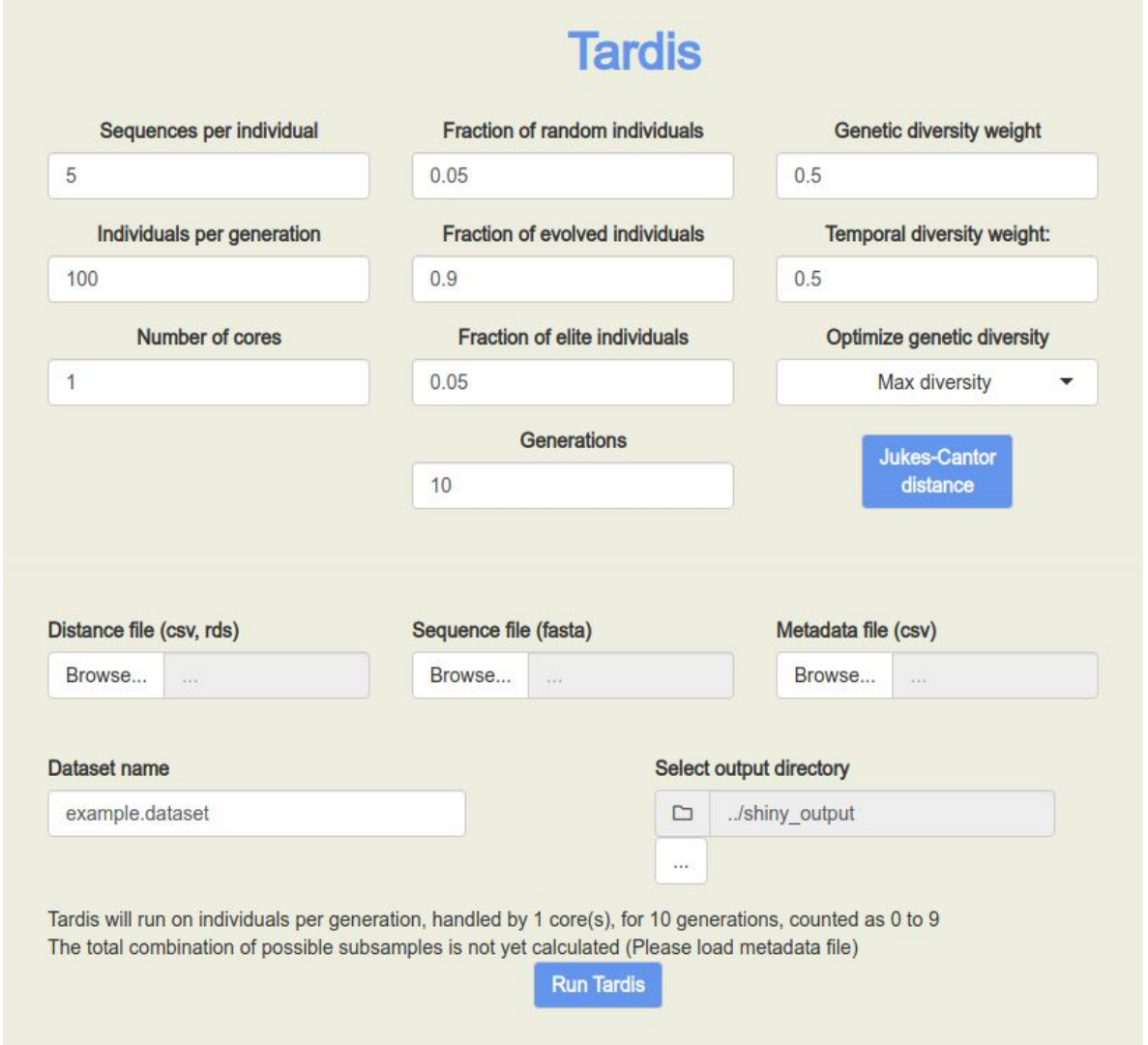
- Select 5 sequences from 100 → 75,287,520
- Select 10 sequences from 1000 → 2.6×10^{23}
- Select 100 sequences from 1000 → 6.4×10^{139}
- Select 150 sequences from 5000 → 1.3×10^{292}
- Recent TARDiS runs
  - Selected 150 sequences from 50000 → 5.61×10^{448}
  - Selected 1000 sequences from 15000 → 4.9×10^{1593}

Atoms in the universe

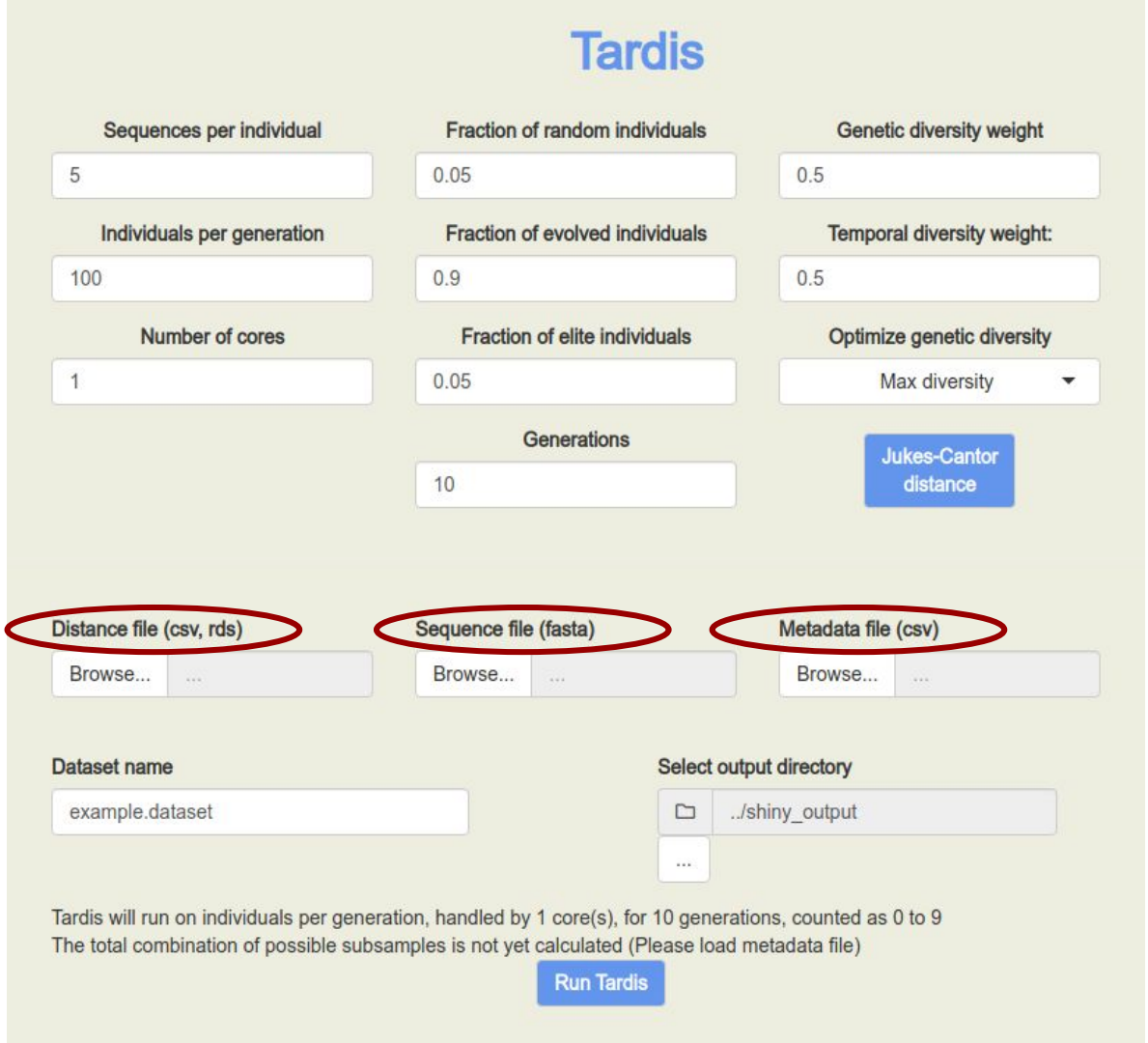
~10^{80}

NGC4651, Jay GaBany, cc-by-sa-3.0

./tardis -s


Tardis
Sequences per individual
5
Fraction of random individuals
0.05
Genetic diversity weight
0.5
Individuals per generation
100
Fraction of evolved individuals
0.9
Temporal diversity weight:
0.5
Number of cores
1
Fraction of elite individuals
0.05
Optimize genetic diversity
Max diversity
Generations
10
Jukes-Cantor distance
Distance file (csv, rds)
Browse...
Sequence file (fasta)
Browse...
Metadata file (csv)
Browse...
Dataset name
example.dataset
Select output directory
../shiny_output
...
Tardis will run on individuals per generation, handled by 1 core(s), for 10 generations, counted as 0 to 9
The total combination of possible subsamples is not yet calculated (Please load metadata file)
Run Tardis

./tardis -s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Run Tardis

INPUT

Sequences data set

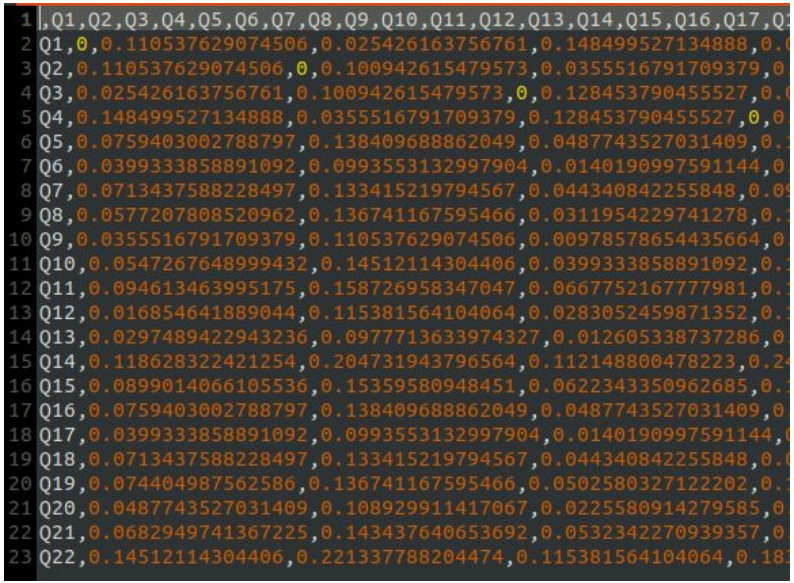
- fasta
- DNA
- **Aligned**

```
>Q1
GAAAAAAAAAAAAAAAAAAACTTTAAAATCTGTGTGGCTGTCACTCGGCTGCATGCTTAGTGCACTCACGCAGTATAATTA
ATAACTAATTACTGTCGTTGACAGGACACGAGTAACTCGTCTATCTTCTGCAGGCTGCTTACGGTTTCGTCCGTGTTGCA
GCCGATCATCAGCACATCTAGGTTTTGTCCGGGTGTGACCGAAAGGTAAGATGGAGAGCCTTGTCCCTGGTTTCAACGAG
AAAACACACGTCCAACTCAGTTTGCCTGTTTTACAGGTTCGCGACGTGCTCGTACGTGGCTTTGGAGACTCCGTGGAGGA
GGTCTTATCAGAGGCACGTCAACATCTTAAAGATGGCACTTGTGGCTTAGTAGAAGTTGAAAAAGGCGTTTTGCCTCAAC
TTGAACAGCCCTATGTGTTCATCAAACGTTCGGATGCTCGAACTGCACCTCATGGTCATGTTATGGTTGAGCTGGTAGCA
GAACTCGAAGGCATTCAGTACGGTCGTAGTGGTGAGACACTTGGTGTCCTTGTCCCTCATGTGGGCGAAATACCAGTGGC
TTACCGCAAGGTTCTTCTTCGTAAGAACGGTAATAAAGGAGCTGGTGGCCATAGTTACGGCGCCGATCTAAAGTCATTTG
CCCCCCGCGACGAGCTTGGCAGTGATCGAGGGGAAGATTTTCAAGATTTATGGAACACTAAACATAGCAGTGGTGTTACC
>Q2
GAAATGTTCTCTAAACGAACTTTAAAATCTGTGTGGCTGTCACTCGGCTGCATGCTTAGTGCACTCACGCAGTATAATTA
ATAACTAATTACTGTCGTTGACAGGACACGAGTAACTCGTCTATCTTCTGCAGGCTGCTTACGGTTTCGTCCGTGTTGCA
GCCGATCATCAGCACATCTAGGTTTTGTCCGGGTGTGACCGAAAGGTAAGATGGAGAGCCTTGTCCCTGGTTTCAACGAG
AAAACACACGTCCAACTCAGTTTGCCTGTTTTACAGGTTTGCGACGTGCTCGTACGTGGCTTTGGAGACTCCGTGGAGGA
GGTCTTATCAGAGGCACGTCAACATCTTAAAGATGGCACTTGTGGCTTAGTAGAAGTTGAAAAAGGCGTTTTGCCTCAAC
TTGAACAGCCCTATGTGTTCATCAAACGTTCGGATGCTCGAACTGCACCTCATGGTCATGTTATGGTTGAGCTGGTAGCA
GAACTCGAAGGCATTCAGTACGGTCGTAGTGGTGAGACACTTGGTGTCCTTGTCCCTCATGTGGGCGAAATACCAGTGGC
TTACCGCAAGGTTCTTCTTCGTAAGAACGGTAATAAAGGAGCTGGTGGCCATAGTTACGGCGCCGATCTAAAGTCATTTG
TTACCGCAAGGTTCTTCTTCGTAAGAACGGTAATAAAGGAGCTGGTGGCCATAGTTACGGCGCCGATCTAAAGTCATTTG
>Q3
GATCTGTTCTCTAAACGAACTTTAAAATCTGTGTGGCTGTCACTCGGCTGCATGCTTAGTGCACTCACGCAGTATAATTA
ATAACTAATTACTGTCGTTGACAGGACACGAGTAACTCGTCTATCTTCTGCAGGCTGCTTACGGTTTCGTCCGTGTTGCA
GCCGATCATCAGCACATCTAGGTTTCGTCCGGGTGTGACCGAAAGGTAAGATGGAGAGCCTTGTCCCTGGTTTCAACGAG
AAAACACACGTCCAACTCAGTTTGCCTGTTTTACAGGTTCGCGACGTGCTCGTACGTGGCTTTGGAGACTCCGTGGAGGA
GGTCTTATCAGAGGCACGTCAACATCTTAAAGATGGCACTTGTGGCTTAGTAGAAGTTGAAAAAGGCGTTTTGCCTCAAC
TTGAACAGCCCTATGTGTTCATCAAACGTTCGGATGCTCGAACTGCACCTCATGGTCATGTTATGGTTGAGCTGGTAGCA
GAACTCGAAGGCATTCAGTACGGTCGTAGTGGTGAGACACTTGGTGTCCTTGTCCCTCATGTGGGCGAAATACCAGTGGC
```

INPUT

Distance matrix

- csv | rds (R)
- colnames = rownames = aligned sequence fasta header
- Distance
  - measure of similarity [0, ∞]
  - identical seqs → dist 0
  - Hamming, Chebyshev, Patristic, Blast E-value, …
  - Default: Jukes-Cantor

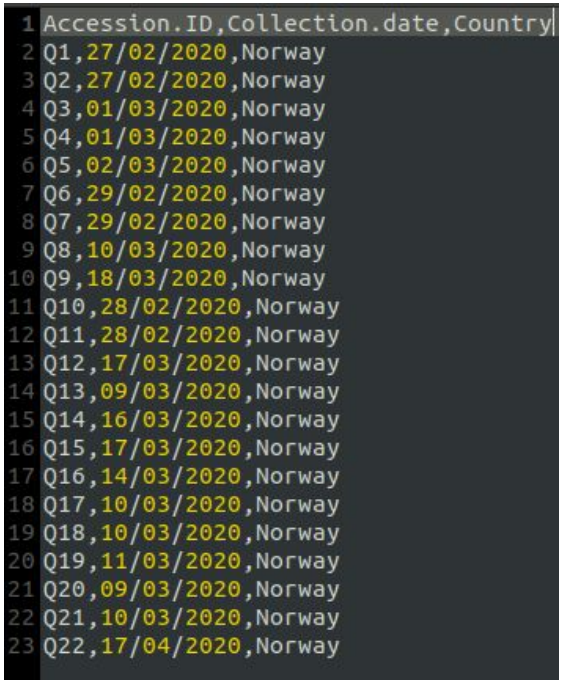


```
,Q1,Q2,Q3,Q4,Q5,Q6,Q7,Q8,Q9,Q10,Q11,Q12,Q13,Q14,Q15,Q16,Q17,Q
Q1,0,0.110537629074506,0.025426163756761,0.148499527134888,0.
Q2,0.110537629074506,0,0.100942615479573,0.0355516791709379,0
Q3,0.025426163756761,0.100942615479573,0,0.128453790455527,0.
Q4,0.148499527134888,0.0355516791709379,0.128453790455527,0,0
Q5,0.0759403002788797,0.138409688862049,0.0487743527031409,0.
Q6,0.0399333858891092,0.0993553132997904,0.0140190997591144,0
Q7,0.0713437588228497,0.133415219794567,0.044340842255848,0.0
Q8,0.0577207808520962,0.136741167595466,0.0311954229741278,0.
Q9,0.0355516791709379,0.110537629074506,0.00978578654435664,0
Q10,0.0547267648999432,0.14512114304406,0.0399333858891092,0.
Q11,0.094613463995175,0.158726958347047,0.0667752167777981,0.
Q12,0.016854641889044,0.115381564104064,0.0283052459871352,0.
Q13,0.0297489422943236,0.0977713633974327,0.012605338737286,0
Q14,0.118628322421254,0.204731943796564,0.112148800478223,0.2
Q15,0.0899014066105536,0.15359580948451,0.0622343350962685,0.
Q16,0.0759403002788797,0.138409688862049,0.0487743527031409,0
Q17,0.0399333858891092,0.0993553132997904,0.0140190997591144,
Q18,0.0713437588228497,0.133415219794567,0.044340842255848,0.
Q19,0.074404987562586,0.136741167595466,0.0502580327122202,0.
Q20,0.0487743527031409,0.108929911417067,0.0225580914279585,0
Q21,0.0682949741367225,0.143437640653692,0.0532342270939357,0
Q22,0.14512114304406,0.221337788204474,0.115381564104064,0.18
```

INPUT

Metadata

- csv, 1 seq = 1 line
- Mandatory columns
  - “Accession.ID” = fasta header
  - “Collection.date” = dd/mm/yyyy
  - Any other column is ignored



```
Accession.ID,Collection.date,Country
Q1,27/02/2020,Norway
Q2,27/02/2020,Norway
Q3,01/03/2020,Norway
Q4,01/03/2020,Norway
Q5,02/03/2020,Norway
Q6,29/02/2020,Norway
Q7,29/02/2020,Norway
Q8,10/03/2020,Norway
Q9,18/03/2020,Norway
Q10,28/02/2020,Norway
Q11,28/02/2020,Norway
Q12,17/03/2020,Norway
Q13,09/03/2020,Norway
Q14,16/03/2020,Norway
Q15,17/03/2020,Norway
Q16,14/03/2020,Norway
Q17,10/03/2020,Norway
Q18,10/03/2020,Norway
Q19,11/03/2020,Norway
Q20,09/03/2020,Norway
Q21,10/03/2020,Norway
Q22,17/04/2020,Norway
```

How many cores do we use?

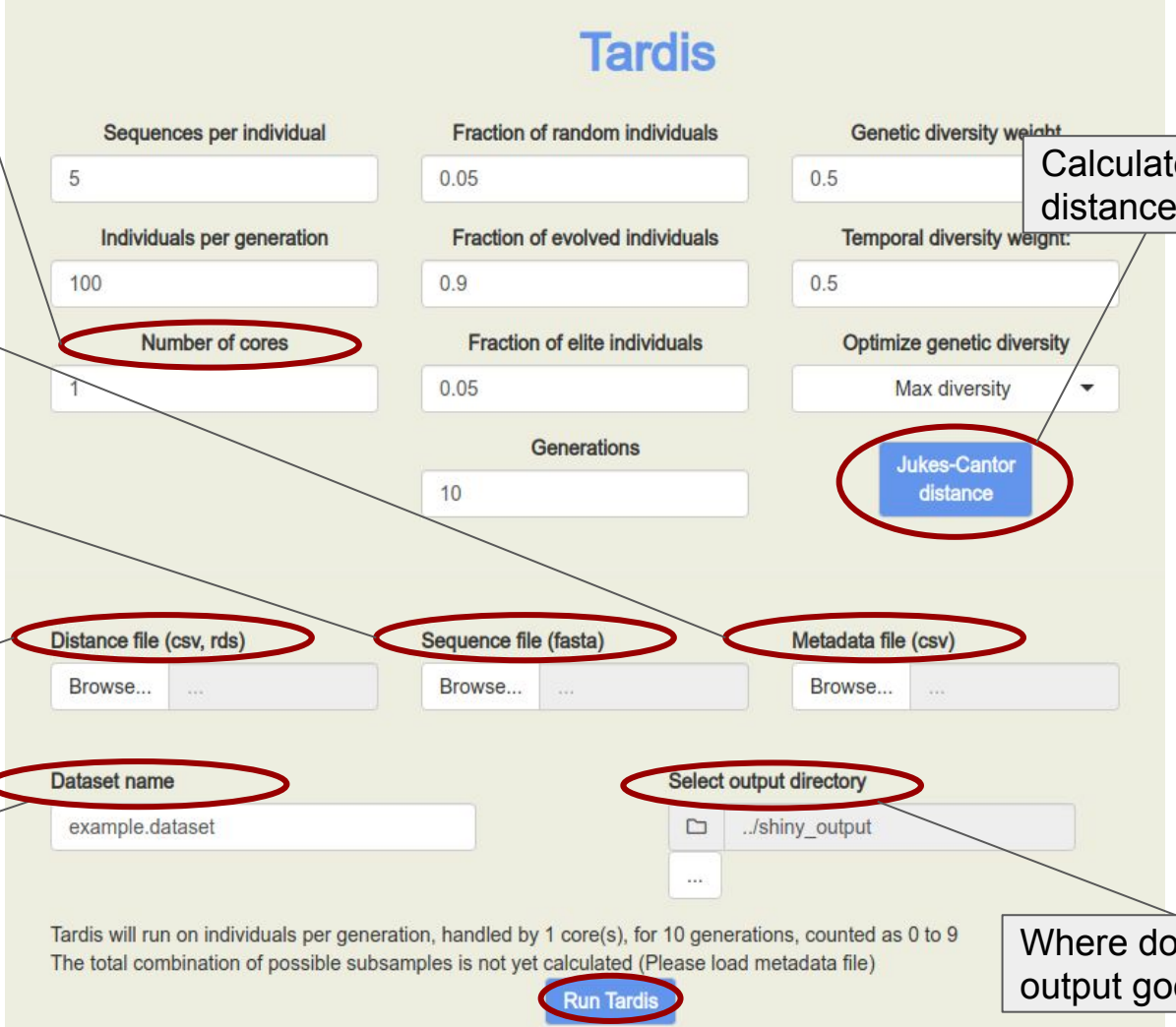
./tardis -s

Load metadata file

Load sequence file

Load distance file

How do we call the output?



Calculate the distance

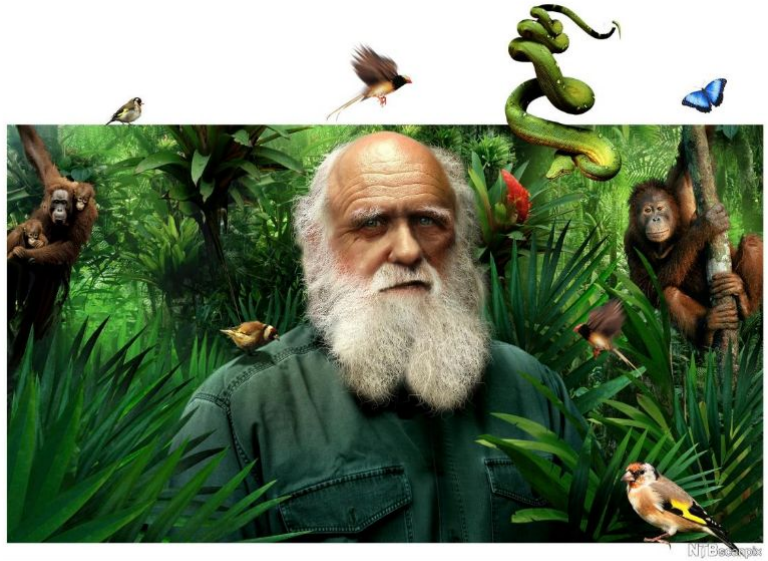
Where do the output goes?

PRINCIPLES OF GENETIC ALGORITHMS

- Artificial intelligence

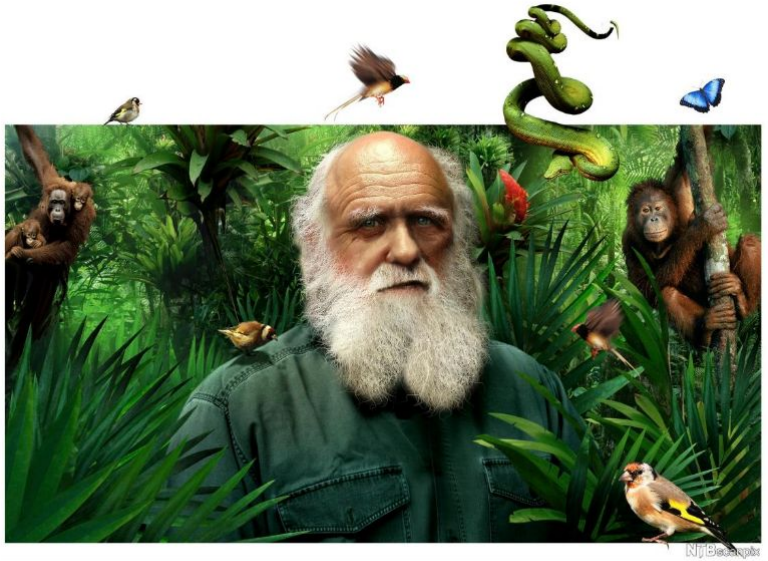
  (Biomimetic systems)

- Solve a problem with a Darwinian evolutionary approach
- Literally evolve the best solution
  - is the **most fit** to solve the problem
  - evolves from a population of solutions



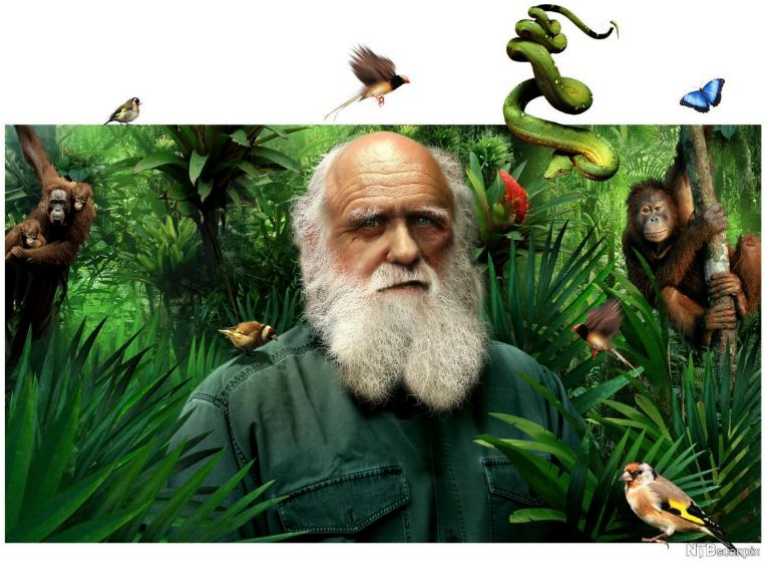
PRINCIPLES OF GENETIC ALGORITHMS

- Set a problem
  - Find the best sequence subsample in terms of **genetic diversity** and **temporal distribution**
- Set a fitness function
  - subset *a* > subset *b*?
  - Measure average genetic distance
  - Measure how sequences are distributed over the timeline (we want a uniform distribution)
  - Fitness is **number**
- Generate a random pop of **solutions**
  - Each solution = a subset
  - Think of solutions as animals in an environment (our problem)
    - Some are fitter than other (fitness function)
    - We can rank solutions based on fitness



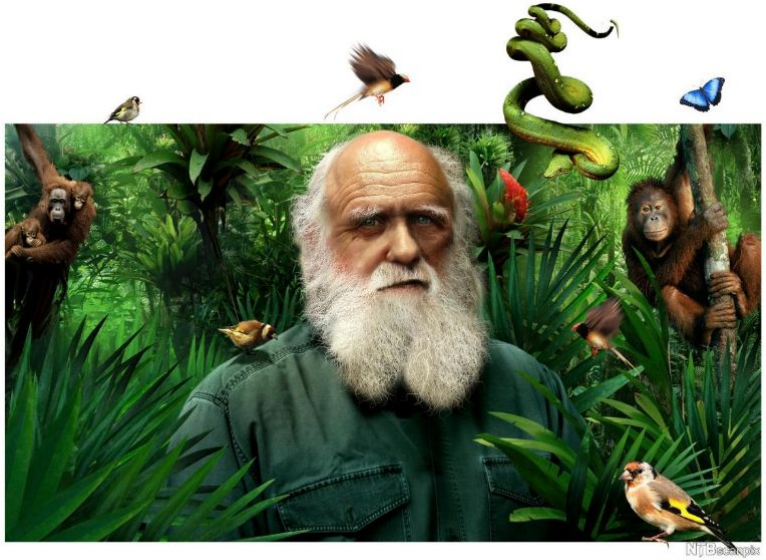
PRINCIPLES OF GENETIC ALGORITHMS

- Select solutions for mating
  - Solutions are merged to generate new ones
- Selection depends of fitness
  - Fitter solution, higher the probability of being selected for mating
- Mating example
  - Subset of 5 sequences out of 100 total sequences → Q1, ..., Q100
  - A = seq(Q1, Q2, Q5, Q50, Q81)
  - B = seq(Q1, Q2, Q9, Q8, Q15)
  - A mates B → C = seq(Q1, Q2, Q8, Q10, Q81)



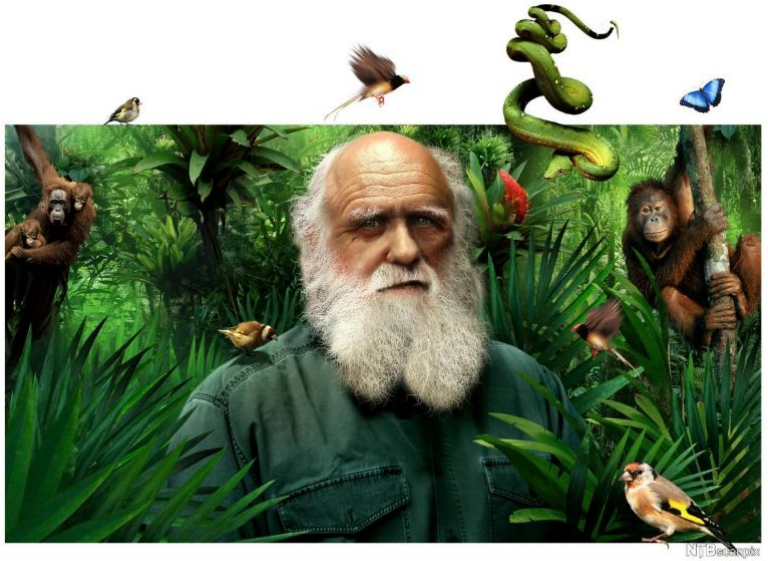
PRINCIPLES OF GENETIC ALGORITHMS

- Selection depends of fitness
  - Fitter solution, higher the probability of being selected for mating
- Start with random generated pop
- Iteratively, solutions are selected to fill a new generation
- We expect that, based on Darwinian principles, our populations will get better at solving the problem



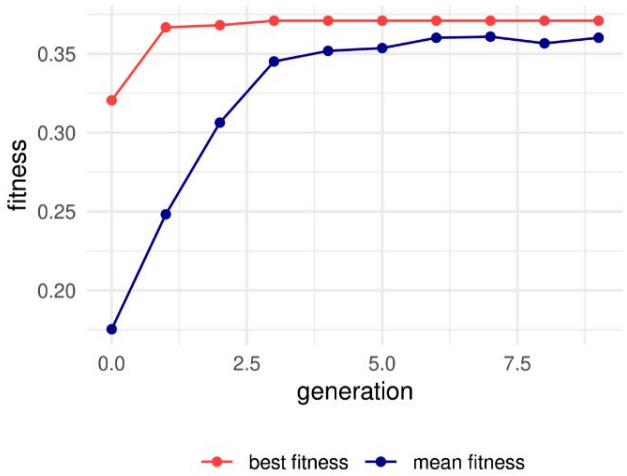
PRINCIPLES OF GENETIC ALGORITHMS

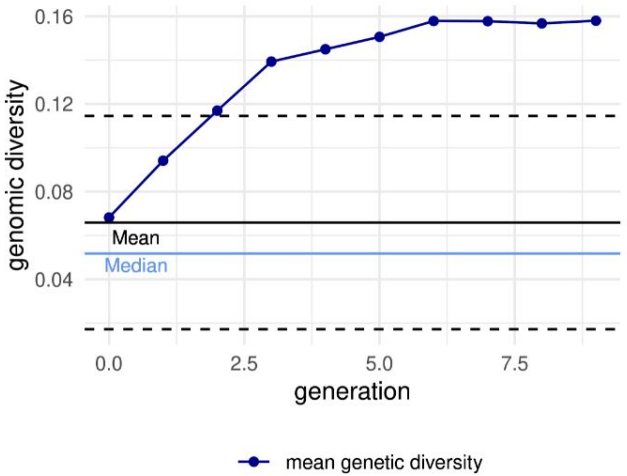
- Streetlight regulation
- Airlines Revenue Management
- Computer-automated design
- Trade & finance strategies
- Bioinformatics (e.g. motif discovery)
- Code-breaking and cryptography
- Container loading optimization
- Design of water distribution systems
- Logistics
- …

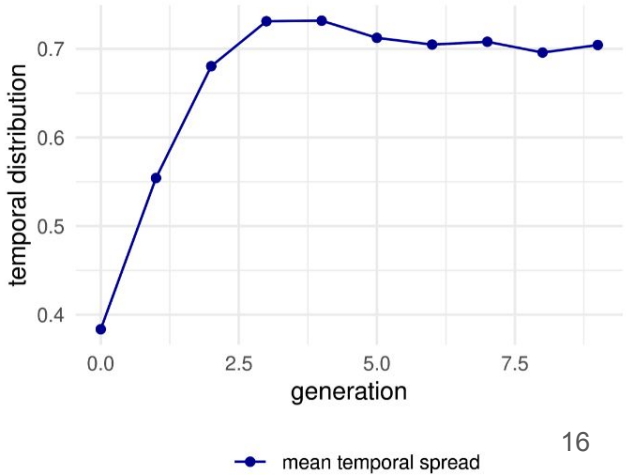


OUTPUT

- Subsample fasta
- Per-generation fitness stats
  - General
  - Temporal distribution
  - Genetic diversity

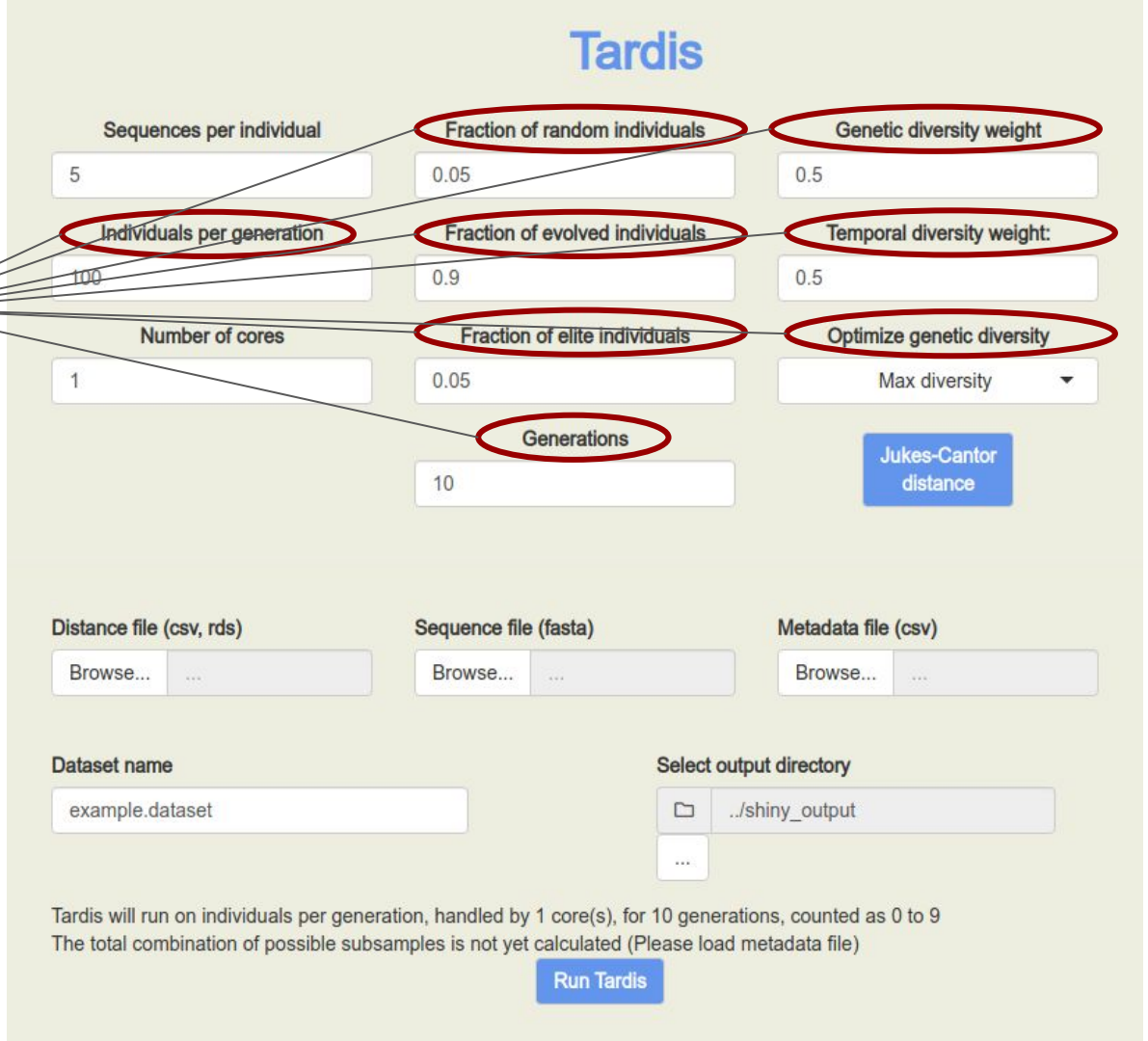






./tardis -s

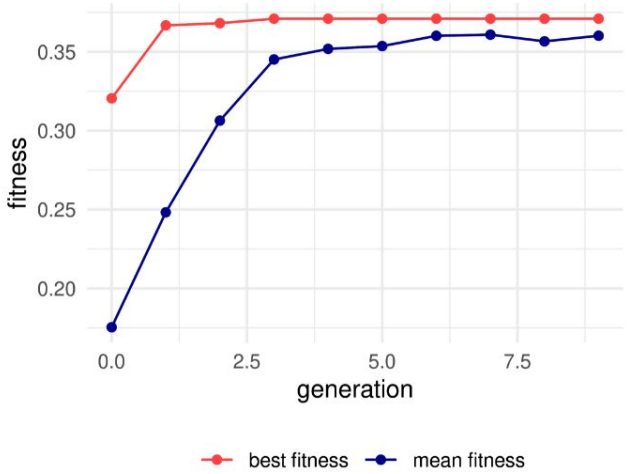
Parameters of the genetic algorithm

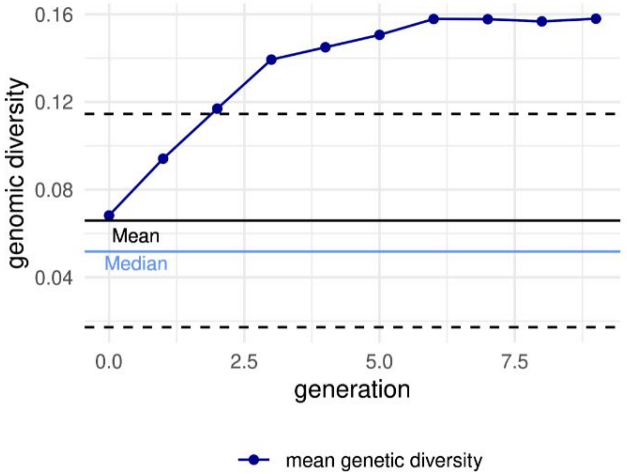


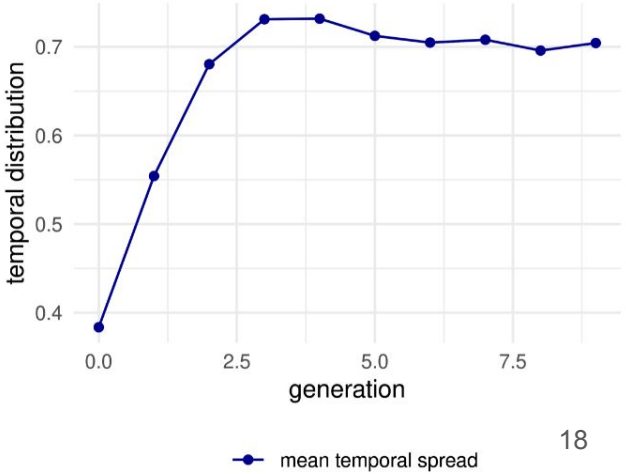
HOW TO DECIDE PARAMETERS?

Empirically

- Check the graphical output, is a plateau reached?
- What are the limits of the machine I am using?
- “Batches” to split the GA population and process one piece at the time



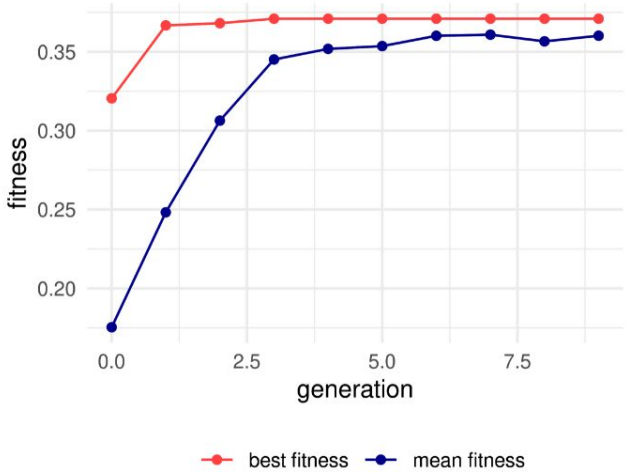


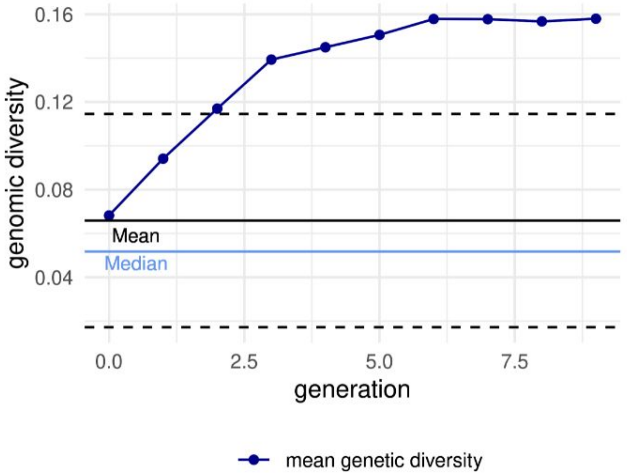


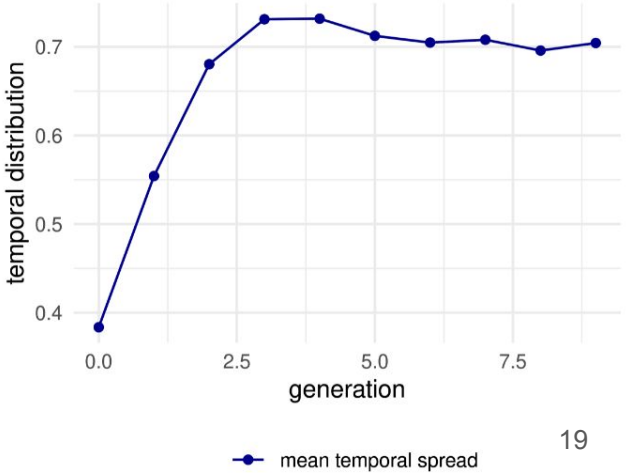
HOW ABOUT REPRODUCIBILITY?

Seed set by default

- seeds.txt file in /data
- Will ensure two runs on the same data set provide the same results
- User can change the file with own seeds







COMMAND LINE

- GUI designed for small datasets/exploration
- For large datasets/memory problems → command line
- Command line
  - Local machine
  - HPC (used on Hipergator with SLURM)
  - Can handle large data splitting the data into batches
- Calculate distance from command line

```
Rscript bin/JC.pairwise.dist.R -i <alignement_file> \

-d <output_distance_file> -c <number_of_cores>
```

- Launch TARDiS from command line

```
./tardis example/example.config
```

EXAMPLE.CONFIG

- Configuration file where parameters are specified

```
params.data_set = "example"
params.nsamples = 4
params.gensize = 100
params.nbatches = 1
params.ncores = 2
params.ngenerations = 10
params.fracnew = 0.14
params.fracevolved = 0.85
params.fracelite = 0.01
params.wdiv = 1
params.wtem = 1
```

NEXTFLOW.CONFIG

- Different NextFlow profiles
- Default is local
- Other can be defined based on need (e.g. memory)

```
profiles {

  local {
    executor.name = "local"
  }

  small {
    executor.name = "slurm"
    process.memory = "10G"
    process.time = "96h"
    process.clusterOptions="--qos=salemi-b"
  }

  medium {
    executor.name = "slurm"
    process.memory = "30G"
  }

  large {
    executor.name = "slurm"
    executor.memory = "128G"
  }
}
```

```
./tardis -p large example/example.config
```

TARDiS OPTIONS

`./tardis -h`

```
Temporal And diveRsity Diistribution Sampler - TARDiS

Usage: tardis [options] CONF

where CONF is the path to the configuration file. The configuration file should be named
DATASET.config, where DATASET is the dataset name. Use -H to see the list of parameters
that can be placed in the configuration file.

Options:

 -s         | Enable Shiny GUI (all other options ignored)
 -g GROUPS  | Enable groups mode
 -b NBATCH  | Number of batches (default: )
 -n NGENS   | Set number of generations for GA (defaut: 10)
 -a AFILE   | Name of alignment file (default: aln.fa) [*]
 -d DFILE   | Name of distances file (default: jc.distance.precalc.rds) [*]
 -m MFILE   | Name of metadata file (default: metadata.csv) [*]
 -o ODIR    | Name of output directory (default: output/dataset_name)
 -t DISTOPT | Genetic diversity optimization, possible values: max, mean, median (default: max)
 -p PROF    | Profile name, possible values: local, small, medium, large (default: local)
 -x         | Enable debug mode (will not delete intermediate files)
 -v         | Display version number and copyright notice
 -H         | Describe format of configuration file

[*] If the default filename is used, the program assumes that the file is in the
    same directory as the configuration file.
```

GROUP OPTION

- Multiple aligned sequences with a group label
- Could be a per-country split, e.g. a single fasta file with
  - 1000 sequences from USA
  - 500 sequences from UK
  - 100 sequences from Italy
- We want an ad-hoc, per group sampling
  - 100 sequences from USA
  - 100 sequences from UK
  - 100 sequences from Italy
- The GA parameters for each group are different (diff search space)

```
./tardis -g <group_parameter_file> -m <group_metadata_file> \

-a <group alignment file>
```

GROUP OPTION

group	params.ncores	params.nsamples	params.gensize	params.nbatches	params.ngenerations	params.fracnew	params.fracevolved	params.fracelite	profile
USA	8	100	1000	1	50	0.3	0.69	0.01	local
UK	4	100	250	1	25				local

- One line per group
- Needs column “group”
- Non-specified parameters are kept at default
- Non-specified groups not subsampled and retained in full for the output
- General output (full set) + per-group output

TARDiS TEAM

Feel free to contact me for help/bugs

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