CEGA v1.2 User Manual

Shilei Zhao, Lianjiang Chi, Hua Chen

October 18, 2023

Contents

1 Introduction

CEGA is designed to detect natural selection using multilocus or genomic polymorphism and divergence data from two species. It can detect positive selection in a specific species lineage or balancing selection in one or both species. CEGA is especially useful for investigating natural selection in noncoding regions. CEGA implements a two-step maximum likelihood estimation of parameters. In the first step, the software estimates the global model parameters N_0, N_1, N_2 and T. After the global parameters are inferred, CEGA implements the second step to estimate the locus-specific parameters λ_1^l and λ_2^l and mutation rate μ^l.

If you have any issues or suggestions with the software, please get in touch with Shilei Zhao at zhaoshilei2018d@big.ac.cn.

If you use CEGA and publish your analysis, please cite the publication:

Zhao, S., Chi, L. & Chen, H. CEGA: a method for inferring natural selection by comparative population genomic analysis across species. Genome Biol 24, 219 (2023). https://doi.org/10.1186/s13059-023-03068-8

2 Installation

CEGA can run on Linux platforms. The CEGA software is freely available on http://github.com/ChenHuaLab/CEGA. Download the file “CEGA-v1.2.tar.gz” from the release CEGA-v1.2.

Implement the following commands (gcc>=4.4):

```
tar -zxvf CEGA-v1.2.tar.gz
cd CEGA-v1.2
make
```

After that, the software can be tested by running with the toy example:

```
./CEGA -i1 ./testdata/testdata_species1.vcf -i2 ./testdata/testdata_species2.vcf -t 10 -o result.out
```

It costs ~10 seconds to complete the program (Intel(R) Xeon(R) Gold 6230 CPU @ 2.10GH), and generates the output file named “result.out”. Note: the number of threads is set with the -t option.

3 Command line arguments

To run CEGA, enter the following command:

```
./CEGA [arguments]
```

Inputs:

-i1 population 1 genetic variant file (.vcf .vcf.gz .tped .hap).

-p1 population 1 position file (format: chr position, split by tab), only required for .hap (-i1) genetic variant file.

-i2 population 2 genetic variant file (.hap .vcf .vcf.gz .tped).

-p2 population 2 position file (format: chr position, split by tab), only required for .hap (-i2) genetic variant file.

-o output file name.

Options:

-N0 (double) initial lower bound upper bound (defalut: 10000.0 100.0 1000000.0).

Set the initial value and range of haploid effective population size for common ancestor species. CEGA will estimate N0 under these constraints. For two species of long-term divergence time, providing additional information on N0 can help to infer global parameters more accurately. Especially, N0 can be fixed by setting the same values of the low bound and up bound.

-N1 (double) initial lower bound upper bound (defalut: 10000.0 100.0 1000000.0).

Similar to -N0. CEGA can infer N1 from data reasonably, and it is not recommended to set the constraints.

-N2 (double) initial lower bound upper bound (defalut: 10000.0 100.0 1000000.0).

Similar to -N0. CEGA can inter N2 from data reasonably, and it is not recommended to set the constraints.

-T (double) initial lower bound upper bound (defalut:10000.0 100.0 10000000.0).

Set the initial value and range of divergence time. CEGA will estimate T under these constraints. For two species of long-term divergence time, providing additional information on T can help to infer global parameters more accurately.

-t (int) thread number (default: 1).

-d (int) filtering windows with s1+s2+s12+D < this value (default: 0).

-mu (double) mutation rate (default:2.5e-8). Unit: per base per generation.

-ws (int) window_size step_size (default: 10000 1000). Unit: bp.

Set the window size and step size (unit: bp). The first window of each chromosome starts from the first SNP.

-wf (file) window file (format: chr start (1-base, include) end (1-base, include) effective_length, split by tab), if input, '-ws' is disable (default: null).

The argument -wf is a substitute for -ws. Set the windows by providing a detailed window information file. The window information file provides effective window sizes, denoting the remaining genomic length after filtering. See details on the file format in the “Input Files” section.

-wf_g (file) window file to specify neutral genome region for estimating global parameters, format same to '-wf' (default: null).

Set the subset of the genomic regions applied in estimating the global parameters. If not input, CEGA will estimate the global parameters using the complete genomic information in input files. See details on the file format in the “Input Files” section.

-LRT (int)1: implement CEGA-LRT, 0: implement CEGA-λ (default:0)

CEGA provides two methods to do significant test: the distribution of λ (CEGA-λ), and the likelihood ratio test (CEGA-LRT). By default, CEGA implement CEGA-λ.

4 Input Files

4.1 vcf format

Use -vcf to specify a .vcf file (see details on https://github.com/samtools/hts-specs). A .vcf file contains three parts in the following order: (1) Meta-information lines (lines beginning with "##"). (2) One header line (line beginning with "#CHROM"). (3) Data lines contain marker and genotype data (one variant per line). The first nine columns contain information about the locus, and the file is organized in the following way:

<chr#> <physical position > <id > <reference allele > <alternate allele > <quality > <filter > <info > <format > <individual 1 genotype > ... <individual N genotype >

For example:

```
##fileformat=VCFv4.2
##FORMAT=<ID=GT,Number=1,Type=Integer,Description="Genotype">
##FORMAT=<ID=GP,Number=G,Type=Float,Description="Genotype Probabilities">
##FORMAT=<ID=PL,Number=G,Type=Float,Description="Phred-scaled Genotype Likelihoods">
#CHROM   POS ID   REF ALT QUAL    FILTER  INFO    FORMAT  sample1 sample2  sample3
chr1  108 .   .   .   .   .   .   .   0/0   0/0   0/0
chr1  167 .   .   .   .   .   .   .   1/1   ./.   1/1
chr1  306 .   .   .   .   .   .   .   1/1   1/0   0/1
chr1  336 .   .   .   .   .   .   .   0/0   0/0   0/0
```

represents three diploid samples with variant information at four loci. The symbol "." is used to denote missing data. Columns in the red box are necessary for CEGA. The genetic data is not required to be phased.

CEGA accepts any chromosome symbols, such as chr1, 1, chromosome1, etc. Note that the format needs to be unified in all input files. Sex chromosomes need to run separately due to the different effective population sizes. For autosomes, it is recommended to run together to estimate the global parameters more precisely.

4.2 tped format

Use -tped to specify a .tped (transposed PLINK file, see details on http://pngu.mgh.harvard.edu/) file containing genetic variant information. The file is organized in the following way:

<chr#> <id > <genetic position > <physical position > <haploid copy 1> ... <haploid copy N>

For example,

```
chr1 rs108     0.000108 108  0    0    0    0    0    0
chr1 rs167     0.000167 167  1    1    9    9    1    1
chr1 rs306     0.000306 306  1    1    1    0    0    1
chr1 rs336     0.000336 336  0    0    0    0    0    0
```

represents six haploid samples with variant information at four loci. Any symbols except for "0" and "1" are interpreted as missing data.

4.3 hap and pos format

Use -hap to specify a .hap file containing genetic variant information. A .hap file organizes variant data with rows representing a single haploid copy from an individual and columns representing consecutive loci delimited by whitespace. For example,

```
0 1 1 0
0 1 1 0
0 1 1 0
0 1 0 0
0 1 0 0
0 1 1 0
```

represents six haploid samples with variant information at four loci. Any symbols except for "0" and "1" are interpreted as missing data.

Note that for .hap genetic variant file, CEGA expects an additional position file to provide physical position information. The position file contains two columns representing chromosome and physical position delimited by whitespace. The columns in the .hap file exactly correspond to rows in the position file.

For example,

```
chr1 108
chr1 167
chr1 306
chr1 336
```

represents the positions of four SNPs in the aforementioned .hap example file.

4.4 window file

Use -wf to specify a window file containing the range and the effective size of local windows. CEGA will detect selection signals for all the local windows. The file is organized in the following way:

<chr#> <start position> <end position> <effective window size>

For example,

```
chr1 1001     11000    8000
chr1 2001     12000    10000
chr1 3001     13000    10000
chr1 4001     14000    10000
chr1 5001     15000    10000
```

represents five overlapping local windows with a window size of 10kb and a step size of 1kb. The effective size of the first window is 8kb, indicating a 2kb length region within chromosome 1: 1001-11000 has been filtered from generating the genetic variant file.

4.5 window file for estimating global parameters

Use -wf_g to specify a window file containing a subset of the input genetic variant information for estimating global parameters. The file is organized in the following way:

<chr#> <start position> <end position> <effective window size>

For example,

```
chr1 10000001     120000000    105000000
chr1 125000001    240000000    110000000
```

represents a 225Mb region with an effective size of 215Mb to estimate the global parameters. Note the windows in this file should not be overlapped.

In practice, the window files in sections 4.4 and 4.5 are more complicated than the above examples.

5 Output File

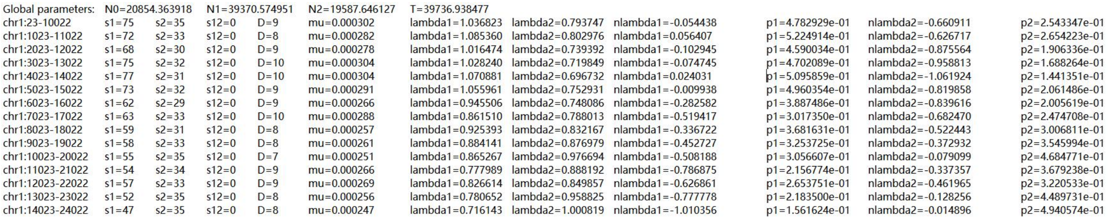
CEGA produces one file as output. The format of output file is depend on the option -LRT.

5.1 CEGA-λ (-LRT 0, default)

If you do the significant test by the distribution of λ, the output file will contain 12 columns organized in the following way:

<window position> <polymorphic sites within species 1> <polymorphic sites within species 2> <shared polymorphic sites of both species 1 and 2> <divergent sites> <mutation rate> <lambda1> <lambda2> <normalized lambda1> <p-value 1> <normalized lambda2> <p-value 2>.

For example:



```
Global parameters:  N0=20854.363918  N1=39370.574951  N2=19587.646127  T=39736.938477
chr1:23-10022     s1=75  s2=35  s12=0  D=9   mu=0.000302  lambda1=1.036823  lambda2=0.793747  nlambda1=-0.054438  p1=4.782929e-01  nlambda2=-0.660911  p2=2.543347e-01
chr1:1023-11022   s1=72  s2=33  s12=0  D=8   mu=0.000282  lambda1=1.085360  lambda2=0.802976  nlambda1=0.056407   p1=5.224914e-01  nlambda2=-0.626717  p2=2.654223e-01
chr1:2023-12022   s1=68  s2=30  s12=0  D=9   mu=0.000278  lambda1=1.016474  lambda2=0.739392  nlambda1=-0.102945  p1=4.590034e-01  nlambda2=-0.875564  p2=1.906336e-01
chr1:3023-13022   s1=75  s2=32  s12=0  D=10  mu=0.000304  lambda1=1.028240  lambda2=0.719849  nlambda1=-0.074745  p1=4.702089e-01  nlambda2=-0.958813  p2=1.688264e-01
chr1:4023-14022   s1=77  s2=31  s12=0  D=10  mu=0.000304  lambda1=1.070881  lambda2=0.696732  nlambda1=0.024031   p1=5.095859e-01  nlambda2=-1.061924  p2=1.441351e-01
chr1:5023-15022   s1=73  s2=32  s12=0  D=9   mu=0.000291  lambda1=1.055961  lambda2=0.752931  nlambda1=-0.009938  p1=4.960354e-01  nlambda2=-0.819858  p2=2.061486e-01
chr1:6023-16022   s1=62  s2=29  s12=0  D=9   mu=0.000266  lambda1=0.945506  lambda2=0.748086  nlambda1=-0.282582  p1=3.887486e-01  nlambda2=-0.839616  p2=2.005619e-01
chr1:7023-17022   s1=63  s2=33  s12=0  D=10  mu=0.000288  lambda1=0.861510  lambda2=0.788013  nlambda1=-0.519417  p1=3.017350e-01  nlambda2=-0.682470  p2=2.474708e-01
chr1:8023-18022   s1=59  s2=31  s12=0  D=8   mu=0.000257  lambda1=0.925393  lambda2=0.832167  nlambda1=-0.336722  p1=3.681631e-01  nlambda2=-0.522443  p2=3.006811e-01
chr1:9023-19022   s1=58  s2=33  s12=0  D=8   mu=0.000261  lambda1=0.884141  lambda2=0.876979  nlambda1=-0.452727  p1=3.253725e-01  nlambda2=-0.372932  p2=3.545994e-01
chr1:10023-20022  s1=55  s2=35  s12=0  D=7   mu=0.000251  lambda1=0.865267  lambda2=0.976694  nlambda1=-0.508188  p1=3.056607e-01  nlambda2=-0.079099  p2=4.684771e-01
chr1:11023-21022  s1=54  s2=34  s12=0  D=9   mu=0.000266  lambda1=0.777989  lambda2=0.888192  nlambda1=-0.786875  p1=2.156774e-01  nlambda2=-0.337357  p2=3.679238e-01
chr1:12023-22022  s1=57  s2=33  s12=0  D=9   mu=0.000269  lambda1=0.826614  lambda2=0.849857  nlambda1=-0.626861  p1=2.653751e-01  nlambda2=-0.461965  p2=3.220533e-01
chr1:13023-23022  s1=52  s2=35  s12=0  D=8   mu=0.000256  lambda1=0.780652  lambda2=0.958825  nlambda1=-0.777778  p1=2.183500e-01  nlambda2=-0.128256  p2=4.489731e-01
chr1:14023-24022  s1=47  s2=35  s12=0  D=8   mu=0.000247  lambda1=0.716143  lambda2=1.000819  nlambda1=-1.010356  p1=1.561624e-01  nlambda2=-0.014896  p2=4.940574e-01
```

The first row represents the estimation of global parameters.

The values of nlambda follow the standard normal distribution. The windows with p1<0.01 (corresponding to nlambda1<-2.3263) indicate species 1 has 99% confidence under positive selection. The windows with 1-p1<0.01 (corresponding to nlambda1>2.3263) indicate species 1 has 99% confidence under balancing selection.

We recommend the users calculate the p-value by the one-tailed test using the standard normal distribution to avoid numerical problems.

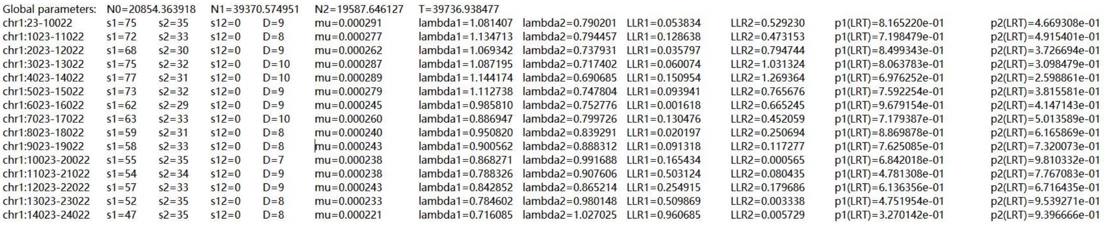
5.2 CEGA-LRT (-LRT 1)

The null hypothesis for the likelihood ratio test is: λ_1^l, $\lambda_2^l = 1$, and μ^l is free (denote the likelihood as $L(\theta_0)$). To test if species 1 is under selection, the alternative hypothesis is set to be: $\lambda_2^l = 1$, λ_1^l and μ^l are free (denote the likelihood as $L(\theta_1)$). To test if species 2 is under selection, the alternative hypothesis is: $\lambda_1^l = 1$, λ_2^l and μ^l are free (denote the likelihood as $L(\theta_2)$).

If you do the significant test by the likelihood ratio test (set the option: -LRT 1), the output file contains 12 columns organized in the following way:

<window position> <polymorphic sites within species 1> <polymorphic sites within species 2> <shared polymorphic sites of both species 1 and 2> <divergent sites> <mutation rate> <lambda1> <lambda2> <LLR1> <LLR2> <p1(LRT)> <p1(LRT)>.

For example:



```
Global parameters:  N0=20854.363918  N1=39370.574951  N2=19587.646127  T=39736.938477
chr1:23-10022       s1=75   s2=35   s12=0   D=9    mu=0.000291   lambda1=1.081407  lambda2=0.790201  LLR1=0.053834    LLR2=0.529230    p1(LRT)=8.165220e-01    p2(LRT)=4.669308e-01
chr1:1023-11022     s1=72   s2=33   s12=0   D=8    mu=0.000277   lambda1=1.134713  lambda2=0.794457  LLR1=0.128638    LLR2=0.473153    p1(LRT)=7.198479e-01    p2(LRT)=4.915401e-01
chr1:2023-12022     s1=68   s2=30   s12=0   D=9    mu=0.000262   lambda1=1.069342  lambda2=0.737931  LLR1=0.035797    LLR2=0.794744    p1(LRT)=8.499343e-01    p2(LRT)=3.726694e-01
chr1:3023-13022     s1=75   s2=32   s12=0   D=10   mu=0.000287   lambda1=1.087195  lambda2=0.717402  LLR1=0.060074    LLR2=1.031324    p1(LRT)=8.063783e-01    p2(LRT)=3.098479e-01
chr1:4023-14022     s1=77   s2=31   s12=0   D=10   mu=0.000289   lambda1=1.144174  lambda2=0.690685  LLR1=0.150954    LLR2=1.269364    p1(LRT)=6.976252e-01    p2(LRT)=2.598861e-01
chr1:5023-15022     s1=73   s2=32   s12=0   D=9    mu=0.000279   lambda1=1.112738  lambda2=0.747804  LLR1=0.093941    LLR2=0.765676    p1(LRT)=7.592254e-01    p2(LRT)=3.815581e-01
chr1:6023-16022     s1=62   s2=29   s12=0   D=9    mu=0.000245   lambda1=0.985810  lambda2=0.752776  LLR1=0.001618    LLR2=0.665245    p1(LRT)=9.679154e-01    p2(LRT)=4.147143e-01
chr1:7023-17022     s1=63   s2=33   s12=0   D=10   mu=0.000260   lambda1=0.886947  lambda2=0.799726  LLR1=0.130476    LLR2=0.452059    p1(LRT)=7.179387e-01    p2(LRT)=5.013589e-01
chr1:8023-18022     s1=59   s2=31   s12=0   D=8    mu=0.000240   lambda1=0.950820  lambda2=0.839291  LLR1=0.020197    LLR2=0.250694    p1(LRT)=8.869878e-01    p2(LRT)=6.165869e-01
chr1:9023-19022     s1=58   s2=33   s12=0   D=8    mu=0.000243   lambda1=0.900562  lambda2=0.888312  LLR1=0.091318    LLR2=0.117277    p1(LRT)=7.625085e-01    p2(LRT)=7.320073e-01
chr1:10023-20022    s1=55   s2=35   s12=0   D=7    mu=0.000238   lambda1=0.868271  lambda2=0.991688  LLR1=0.165434    LLR2=0.000565    p1(LRT)=6.842018e-01    p2(LRT)=9.810332e-01
chr1:11023-21022    s1=54   s2=34   s12=0   D=9    mu=0.000238   lambda1=0.788326  lambda2=0.907606  LLR1=0.503124    LLR2=0.080435    p1(LRT)=4.781308e-01    p2(LRT)=7.767083e-01
chr1:12023-22022    s1=57   s2=33   s12=0   D=9    mu=0.000243   lambda1=0.842852  lambda2=0.865214  LLR1=0.254915    LLR2=0.179686    p1(LRT)=6.136356e-01    p2(LRT)=6.716435e-01
chr1:13023-23022    s1=52   s2=35   s12=0   D=8    mu=0.000233   lambda1=0.784602  lambda2=0.980148  LLR1=0.509869    LLR2=0.003338    p1(LRT)=4.751954e-01    p2(LRT)=9.539271e-01
chr1:14023-24022    s1=47   s2=35   s12=0   D=8    mu=0.000221   lambda1=0.716085  lambda2=1.027025  LLR1=0.960685    LLR2=0.005729    p1(LRT)=3.270142e-01    p2(LRT)=9.396666e-01
```

In the output file, LLR1=2[$ln(L(\theta_1))$-$ln(L(\theta_0))$], and LLR2=2[$ln(L(\theta_2))$-$ln(L(\theta_0))$]. After adjustment, LLR follows the Chi-squared distribution with 1 degree of freedom (see details in the published paper). The windows with p1(LRT)<0.01 and lambda1<1 indicate species 1 has 99% confidence under positive selection. The windows with p1(LRT)<0.01 and lambda1>1 indicate species 1 has 99% confidence under balancing selection.

6 How to use CEGA

6.1 Basic usage

CEGA is a command-line tool. All supported command-line flags are provided in section 3. The basic execution examples under different formatted genetic variants input files are:

(1) .vcf format

```
./CEGA -i1 ./testdata/testdata_species1.vcf -i2 ./testdata/testdata_species2.vcf -ws 10000 1000 -t 10 -o result.out
```

(2) .tped format

```
./CEGA -i1 ./testdata/testdata_species1.tped -i2 ./testdata/testdata_species2.tped -ws 10000 1000 -t 10 -o result.out
```

(3) .hap format

```
./CEGA -i1 ./testdata/testdata_species1.hap -i2 ./testdata/testdata_species2.hap -p1 ./testdata/testdata_species1.pos -p2 ./testdata/testdata_species2.pos -ws 10000 1000 -t 10 -o result.out
```

Note: When .hap format input files are used, position files must also be provided. We also provided the test files of “wf_10kb_1kb.txt” (for -wf) and “wf_g.txt” (for -wf_g).

6.2 Analyzing population genomic data of humans and chimpanzees

We applied CEGA to whole-genome sequencing data of nine Homo sapiens and nine Pan troglodytes [1, 2]. Considering the running time, we cut out the 40Mb genome segments (Chr6: 10,000,001-50,000,000) as an example. The command line is:

```
./CEGA -i1 ./testdata/MHC_human.vcf.gz -i2 ./testdata/MHC_chimpanzee.vcf.gz -N0 30000.0 24000.0 42000.0 -mu 2.5e-8 -t 30 -d 50 -wf ./testdata/wf_MHC.txt -wf_g ./testdata/wf_g_MHC.txt -o result_MHC.out
```

Here, human and chimpanzee genomes are aligned to the same reference genome. Genome segments with tandem repeats, segmental duplication, genomic gaps, and structural variants may cause false positive selection signals. A strict filtering strategy can help to avoid artifact bias in analyzing genomic data [2]. To do this, we first removed the SNPs on the filtering regions from .vcf data files of both species. Then, we prepared the file “wf_MHC.txt” to specify local windows and their effective size (length of the window after excluding the filtering regions). The whole genome was divided into 39,991 windows with a window size of 10 kb and a step size of 1 kb. We excluded the windows with an effective size <2kb from the window file. The selection signals will be detected among the remaining 37,814 windows.

We prepared the file “wf_g_MHC.txt” to specify regions for estimating the global parameters. We excluded the following genome segments from estimating global parameters: (1) regions prone to under selection (such as gene regions and their flanking regions of 10kb); (2) CpG islands with more shared polymorphic sites that are recurrent mutations from identical by state processes rather than identical by descent processes; (3) tandem repeats, segmental duplication, genomic gaps, and structural variants, etc.

For two species with long-term divergence time (such as T>10Ne generations), the estimation of N0 and T may not be accurate as a result of less information from shared polymorphic sites. The additional information on N0 or T can help to estimate the global parameters more precisely. Here, we used the arguments “-N0 30000.0 24000.0 42000.0” to restrict N0 from 24000.0 to 42000.0 according to the previous research [3]. **Warning:** Avoid setting narrow bounds for both N0 and T at the same time, or it may affect the optimization.

We also filtered 568 windows with s1+s2+s12+D<50 by setting “-d 50” considering less information. After completing the running, the results of 37,246 windows will be recorded on the file “result_MHC.out”. Figure 1 and Figure 2 show the balancing selection signals in the MHC region (between the bash lines) from the “result_MHC.out” file.

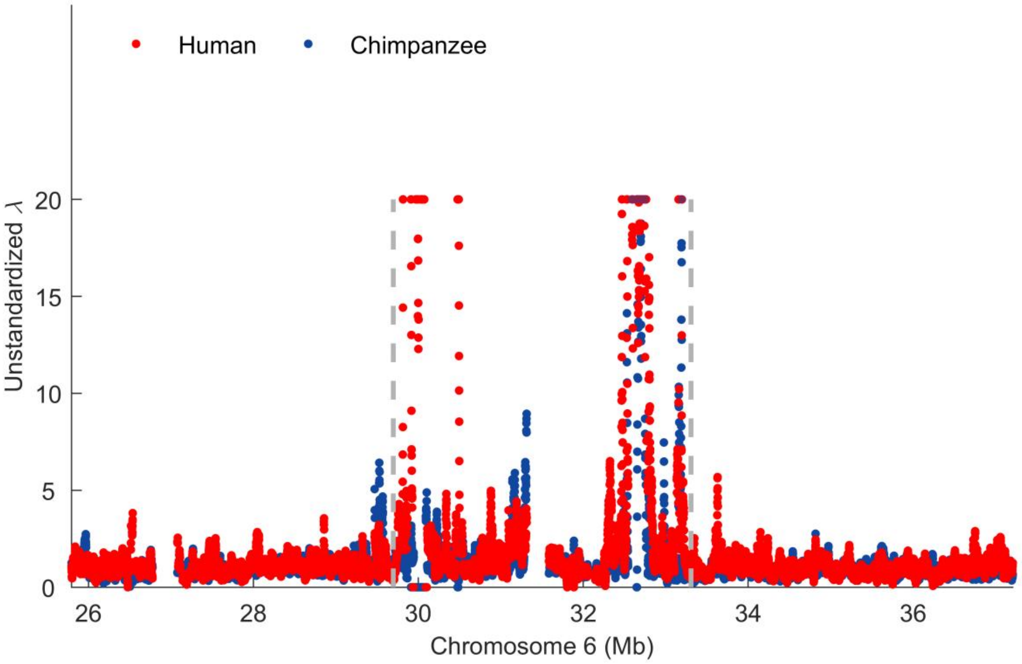


Figure 1. The unstandardized λ values. Values larger than 20 were set to 20 for better illustration.

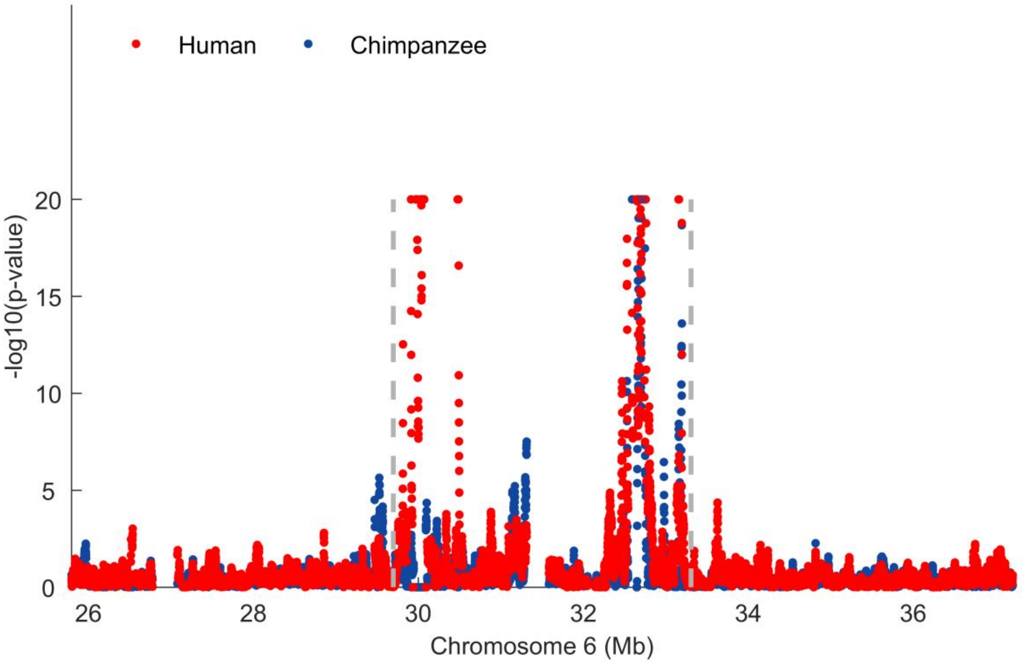


Figure 2. The significant test by CEGA-λ. Here p-values equal to 1-p1 and 1-p2, since we aim to show balancing selection signals. Values larger than 20 were set to 20 for better illustration.

7 Main changes compared with CEGA v1.1

(1) CEGA v1.2 adds the option -LRT for implementing the likelihood ratio test as an alternative to the significant test (set -LRT 1 for CEGA-LRT).

(2) The running speed of CEGA v1.2 is improved.

(3) The lower bound of λ is changed from 10^{-6} to 10^{-4}. The upper bound of divergence time T is changed from 10^{6} to 10^{7}.

(4) The normalization of λ is changed.

Reference

1. Prado-Martinez J, Sudmant PH, Kidd JM, Li H, Kelley JL, Lorente-Galdos B, Veeramah KR, Woerner AE, O'Connor TD, Santpere G, et al: **Great ape genetic diversity and population history.** *Nature* 2013, **499:**471-475.
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