Detecting the correlated mutations based on selection pressure with `CorMut`

Zhenpeng Li

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1 Introduction

In genetics, the Ka/Ks ratio is the ratio of the number of non-synonymous substitutions per non-synonymous site (Ka) to the number of synonymous substitutions per synonymous site (Ks)[1]. Ka/Ks ratio was used as an indicator of selective pressure acting on a protein-coding gene. A Ka/Ks ratio of 1 indicates neutral selection, i.e., the observed ratio of non-synonymous mutations versus synonymous mutations exactly matches the ratio expected under a random mutation model. Thus, amino acid changes are neither being selected for nor against. A Ka/Ks value of <1 indicates negative selection pressure. That is to say most amino acid changes are deleterious and are selected against, producing an imbalance in the observed mutations that favors synonymous mutations. In the condition of Ka/Ks>1 , it indicates that amino acid changes are favored, i.e., they increase the organism's fitness. This unusual condition may reflect a change in the function of a gene or a change in environmental conditions that forces the organism to adapt. For example, highly variable viruses mutations which confer resistance to new antiviral drugs might be expected to undergo positive selection in a patient population treated with these drugs, such as HIV and HCV.

The concept of correlated mutations is one of the basic ideas in evolutionary biology. The amino acid substitution rates are expected to be limited by functional constraints. Given the functional constraints operating on gene, a mutation in one position can be compensated by an additional mutation. Then mutation patterns can be formed by correlated mutations responsible for specific conditions.

Here, we developed an R/Bioconductor package to detect the correlated mutations among positive selection sites by combining ka/ks ratio and correlated mutations analysis. The definition of Ka/Ks is based on Chen et al[2]. `CorMut` provides functions for computing kaks for individual site or specific amino acids and detecting correlated mutations among them. Two methods are provided for detecting correlated mutations,

including conditional selection pressure and mutual information, the computation consists of two steps: First, the positive selection sites are detected; Second, the mutation correlations are computed among the positive selection sites. Note that the first step is optional. Meanwhile, `CorMut` facilitates the comparison of the correlated mutations between two conditions by the means of correlated mutation network.

2 Methods

2.1 Ka/Ks calculation for individual codon position and specific amino acid substitutions

The Ka/Ks values for individual codon (specific amino acid substitutions) were determined as described by Chen et al. The Ka/Ks of individual codon (specific amino acid substitution (X2Y) for a codon) was computed as follows:

$$\frac{Ka}{Ks} = \frac{\frac{N_Y}{N_S}}{\frac{n_{Y,t}f_t + n_{Y,v}f_v}{n_{S,t}f_t + n_{S,v}f_v}}$$

where NY and NS are the count of samples with nonsynonymous mutations(X2Y mutation) at that codon and the count samples with of synonymous mutations observed at that codon respectively. Then, NY/NS is normalized by the ratio expected under a random mutation model (i.e., in the absence of any selection pressure), which was represented as the denominator of the formula. In the random mutation model, ft and fv indicate the transition and transversion frequencies respectively, and they were measured from the entire data set according to the following formulas: ft = Nt/ntS and fv = Nv/nvS, where S is the total number of samples; Nt and Nv are the numbers of observed transition and transversion mutations, respectively; nt and nv are the number of possible transitions and transversions in the focused region (simply equal to its length L and 2L respectively).LOD confidence score for a codon or mutation X2Y to be under positive selection pressure was calculated by the following formula:

$$LOD = -\log_{10} p(i \geq N_{YaXa} | N, q, \left(\frac{K_a}{K_s}\right)_{Y|Xa} = 1) = -\log_{10} \sum_{i=N_{YaXa}}^{N} \left(\begin{array}{c} N \\ i \end{array} \right) q^i (1-q)^{N-i}$$

Where N = NYaXa + NYsXa and q as defined above. If LOD > 2, the positive selection is significant.

2.2 Mutual information

MI content was adopted as a measure of the correlation between residue substitutions[3]. Accordingly, each of the N columns in the multiple sequence alignment generated for a protein of N codons is considered as a discrete random variable $Xi(1 \leq i \leq N)$. When compute Mutual information between codons, $Xi(1 \leq i \leq N)$ takes on one of the 20 amino acid types with some probability, then compute the Mutual information between two sites for specific amino acid substitutions, $Xi(1 \leq i \leq N)$ were only divided two parts (the specific amino acid mutation and the other) with some probability. Mutual information describes the mutual dependence of the two random variables Xi, Xj, The MI associated the random variables Xi and Xj corresponding to the ith and jth columns is defined as

$$I(X_i, X_j) = S(X_i) + S(X_j) - S(X_i, X_j) = S(X_i) - S(X_i|X_j)$$

Where

$$S(X_i) = -\sum_{allx_i} P(X_i = x_i) \log P(X_i = x_i)$$

is the entropy of Xi,

$$S(X_i|X_j) = -\sum_{allx_i} \sum_{allx_j} P(X_i = x_i, X_j = x_j) \log P(X_i = x_i | X_j = x_j)$$

is the conditional entropy of Xi given Xj, S(Xi, Xj) is the joint entropy of Xi and Xj. Here P(Xi = xi, Xj = xj) is the joint probability of occurrence of amino acid types xi and xj at the ith and jth positions, respectively, P(Xi = xi) and P(Xj = xj) are the corresponding singlet probabilities.
I(Xi, Xj) is the ijth element of the $N \times N$ MI matrix I corresponding to the examined multiple sequence alignment.
2.3 Jaccard index
Jaccard index measures similarity between two variables, and it has been widely used to measure the correlated mutations. For a pair of mutations X and Y, the Jaccard index is calculated as Nxy/(Nxy+Nx0+Ny0), where Nxy represents the number of sequences where X and Y are jointly mutated, Nx0 represents the number of sequences with only X mutation, but not Y, and Ny0 represents the sequences with only Y mutation, but not X. The computation was deemed to effectively avoid the exaggeration of rare mutation pairs where the number of sequences without either X or Y is very large.

3 Implementation

3.1 Process the multiple sequence alignment files *seqFormat* replace the raw bases with common bases and delete the gaps according to the reference sequence. As one raw base has several common bases, then a base causing amino acid mutation will be randomly chosen. Here, HIV protease sequences of treatment-naive and treatment will be used as examples.

```
> library(CorMut)
> examplefile=system.file("extdata","PI_treatment.aln",package="CorMut")
> examplefile02=system.file("extdata","PI_treatment_naive.aln",package="CorMut"
> example=seqFormat(examplefile)
> example02= seqFormat(examplefile02)
```

3.2 Compute kaks for individual codon

```
> result=kaksCodon(example)
> fresult=filterSites(result)
> head(fresult)
```

```
  mutation       kaks       lod   freq
1        3 150.500000 52.827378 1.0000
2       10   8.500000 24.207139 0.7367
3       12  15.000000  5.106647 0.0967
4       19   2.539578  3.209634 0.1167
5       24   3.234973  3.134242 0.1000
6       35   4.301692  4.602898 0.2633
```

3.3 Compute kaks for individual amino acid mutation

```
> result=kaksAA(example)
> fresult=filterSites(result)
> head(fresult)
```

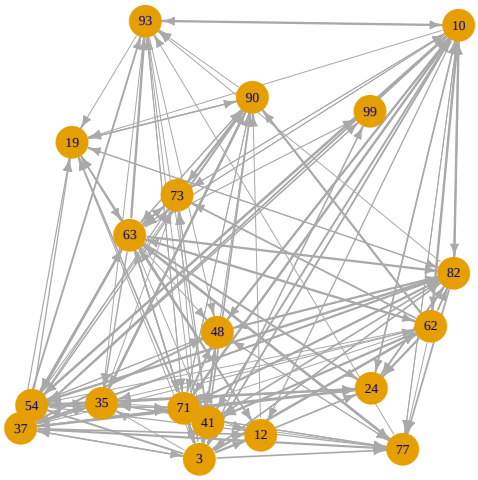
```
  position mutation             kaks              lod   freq
1        3      V3I 404.727477477477 179.620396535997 0.9967
```

```
2        10     L10I 106.595786379226 194.844486413216 0.6033
3        12     T12N 6.45071770334928              Inf   0.01
4        12     T12P 61.2484076433121 4.49662674477974 0.0267
5        12     T12E 15.0516746411483              Inf 0.0233
6        12     T12S 38.2802547770701 2.04746382429526 0.0167
```

3.4 Compute the conditional kaks(conditional selection pressure) among codons

```
> result=ckaksCodon(example)

> plot(result)
```



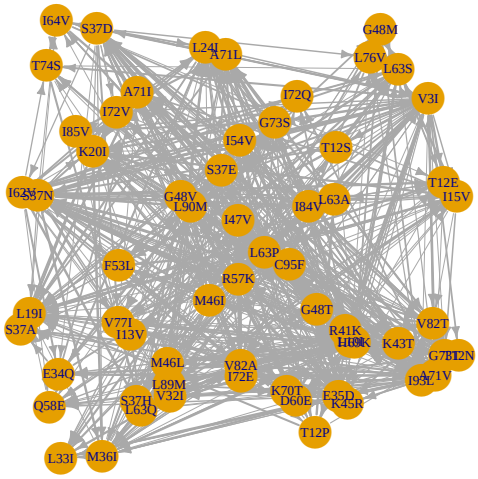

```
> fresult=filterSites(result)
> head(fresult)

  position1 position2             ckaks              lod
1         3        10                17 24.2071393392468
2         3        12                30 5.10664651261475
3         3        19 1.84210526315789 3.20963382207299
4         3        24 3.33333333333333 3.13424219813808
5         3        35 13.1666666666667  4.6028980810425
6         3        37               142 31.4661779679137
```

3.5 Compute the conditional kaks(conditional selection pressure) among amino acid mutations

```
> result=ckaksAA(example)
```

```
> plot(result)
```




```
> fresult=filterSites(result)
> head(fresult)
```

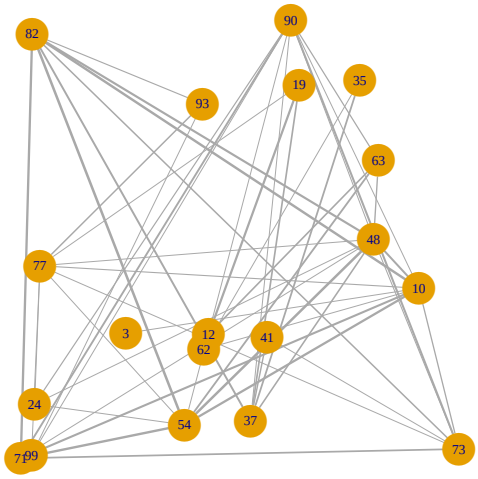
```
  position1 position2 ckaks    lod
1       V3I       L10I 13.92 226.85
2       V3I       T12N     4    Inf
3       V3I       T12P     9  10.89
4       V3I       T12E     8    Inf
5       V3I       T12S     6   6.81
6       V3I       I13V    33  18.67
```

3.6 Compute the mutual information among codons

An instance with two matrixes will be returned, they are mutation information matrix and p matrix, The ijth element of the $N \times N$ MI matrix indicates the mutation information or p value between position i and position j.

```
> result=miCodon(example)
```

```
> plot(result)
```




```
> fresult=filterSites(result)
> head(fresult)
```

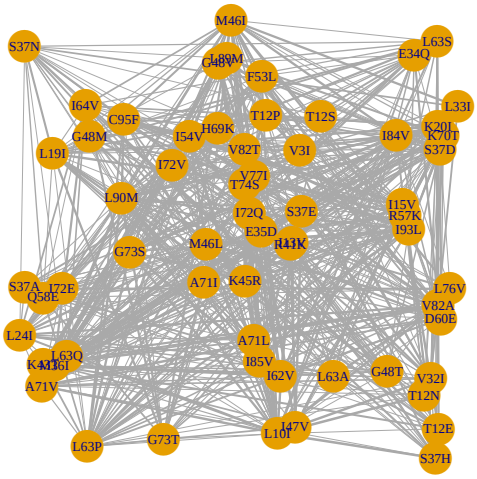
```
  position1 position2                 mi             p.value
1        10         3 0.0223403798434836  0.0195330905975584
2        19        12  0.128809592522988 0.00427286356821589
3        37        19 0.0747160966532552  0.0298106760573202
4        37        35  0.075806272221832 0.00427286356821589
5        41        37   0.06512044936218 0.00427286356821589
6        48        10 0.0897750853050767 0.00427286356821589
```

3.7 Compute the mutual information among individual amino acid mutations

An instance with two matrixes will be returned, they are mutation information matrix and p matrix, The ijth element of the $N \times N$ MI matrix indicates the mutation information or p value between two amino acid mutations.

```
> result=miAA(example)
```

```
> plot(result)
```




```
> fresult=filterSites(result)
> head(fresult)
```

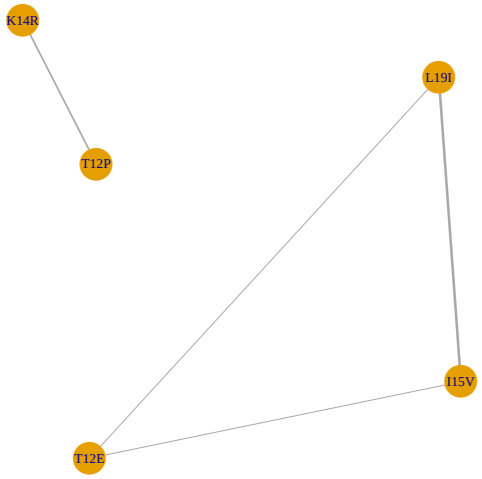
	position1	position2	mi	p.value
1	L10I	V3I	0.00309067986573841	7.90929048032942e-40
2	T12N	L10I	8.64708687707827e-05	0.040207170030247
3	T12E	L10I	0.000640519978094889	1.63583633828262e-09
4	I13V	V3I	0.000389134320368023	3.1062662483976e-05
5	I13V	T12S	0.00478659729991238	6.31583852904427e-63
6	I15V	V3I	0.00051661973913858	7.31371985853487e-07

3.8 Compute the Jaccard index among individual amino acid mutations

An instance with two matrixes will be returned, they are Jaccard index matrix and p matrix, The ijth element of the $N \times N$ MI matrix indicates the Jaccard index or p value between two amino acid mutations.

```
> result=jiAA(example)

> plot(result)
```




```
> fresult=filterSites(result)
> head(fresult)
```

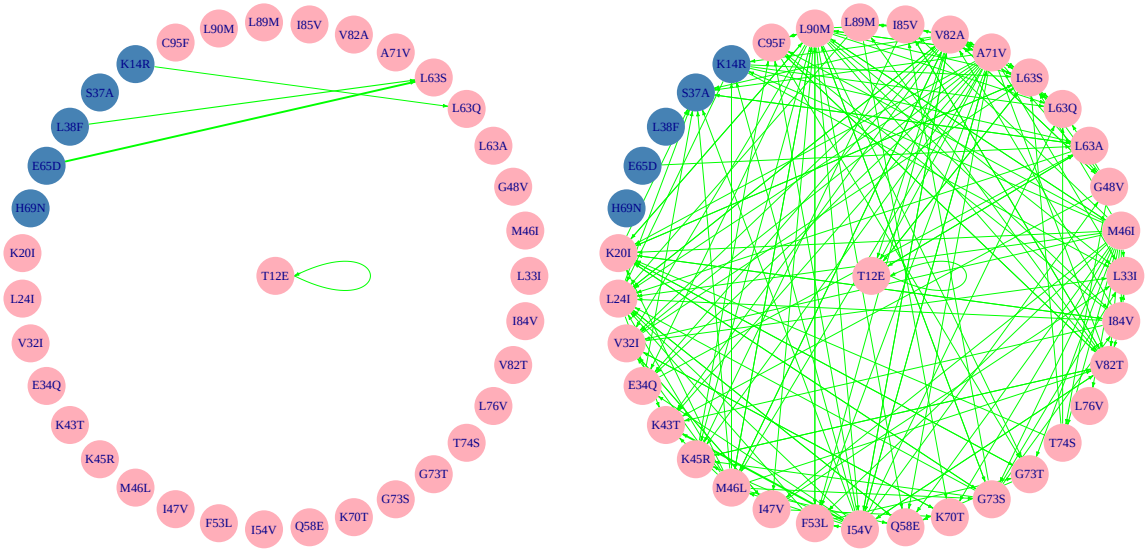
```
  position1 position2                JI              p.value
1      T12P      K14R 0.192307692307692   0.0203598174209213
2      L19I      I15V 0.228070175438596  0.00322947408828938
3      T12E      I15V 0.162790697674419 0.000394448679750347
4      T12E      L19I 0.172413793103448   0.0203598174209213
```

3.9 Comparison of the correlated mutations between two conditions by the means of correlated mutation network

biCompare compare the correlated mutation relationship between two conditions, such as HIV treatment-naive and treatment. The result of *biCompare* can be visualized by *plot* method. Only positive selection codons or amino acid mutations were displayed on the plot, blue nodes indicate the distinct positive codons or amino acid mutations of the first condition, that is to say these nodes will be non-positive selection in second condition. While red nodes indicate the distinct positive codons or amino acid mutations appeared in the second condition. *plot* also has an option for displaying the unchanged positive selection nodes in both conditions. If *plotUnchanged* is FALSE, the unchanged positive selection nodes in both conditions will not be displayed.

```
> biexample=biCkaksAA(example02,example)
> result=biCompare(biexample)

> plot(result)
```



References

[1] Hurst, L. D. *The Ka/Ks ratio: diagnosing the form of sequence evolution.* Trends in genetics: TIG 18, 486 (2002).

[2] Chen, L., Perlina, A., Lee, C. J. *Positive selection detection in 40,000 human immunodeficiency virus (HIV) type 1 sequences automatically identifies drug resistance and positive fitness mutations in HIV protease and reverse transcriptase.* Journal of virology 78, 3722-3732 (2004).

[3] Cover, T. M., Thomas, J. A., Wiley, J. *Elements of information theory.* 6, (Wiley Online Library: 1991).