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The Phylogeny Tree Reconstruction Based on the Usage Frequency of Codons and Corresponding Complementary Codons①

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Abstract: The hypothesis that a codon bias usage is identical to its complementary codon usage preference has been investigated by using the relationship analysis of codons vs their complementary ones among 70 organisms. Significantly positive usage correlations between codons and their complementary ones were found and its implication in biology was also analyzed. The codon-complementary codon tree was further built, which fairly exhibited the evolutionary relationship of these organisms. The results not only demonstrated the validity of our hypothesis, but also manifested the usefulness of correlation analysis in studying on codon usage pattern and molecular evolutionary mechanisms of organism.

Key words: codon; complementary codon; usage frequency; usage bias; correlation analysis; codon-complementary codon tree

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Genetic code is one of the most important biological languages in communications between DNA and protein. Revealing the origin and evolution mechanism of this language will bring an invaluable significance to revalue the mystery of life origin. The focus is now on what is the driving force of this selection and how does the selection work. As the logic of origination and evolution of genetic code may be staying hidden in this kind of driving force and selection mechanism, many exciting researches in the right area have been made[1～9]. Grosjean and co-worker[1,2] put forward a hypothesis namely "the optimum energy of codon-anticodon interaction" for explaining codon usage bias. Ikemura[3～6] found that the usage bias of a codon is usually determined by the abundance of its corresponding tRNAs in some unicellular organisms including *E. coli* and yeast, known as "Ikemura rule". The hypothesis of translation efficiency was used for explaining codons usage bias. As a matter of fact, we found that these two hypotheses, both being based on the interaction of codons with their corresponding anticodons, are essentially the same. If these hypotheses hold true, the optimum energy of codon-anticodon interaction should be the essential prerequisite of translation efficiency of a protein, a positive correlation of codons with their complementary codons in usage should exist. This is because that the pair-match energy of any codon with its anticodon is equal to that of this codon's complementary codon with its own anticodon. Let's take codons 'CCG' and 'CCA' as examples, 'CCG''s anticodon and complementary codon are 'CGG' and 'CGG', the same nucleotide triplet, and 'CCA''s anticodon and complementary codon 'UGG' and 'UGG', also the same kind of nucleotide triplet. It is obvious that the complexes of codon-anticodon equal to the complexes of corresponding complementary codon-codon, such as 'CCG-CGG' vs 'CGG-CCG' and 'CCA-UGG' vs 'UGG-CCA' in their

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combination energy. Therefore, if the pair-matching energy is the main selection force of a codon's usage, all corrsponding complementary codons should possess the same usage pattern as their corresponding codons. In other words, any complementary codon should have a strictly positive correlation with its codon in their usage frequency. If so, the pair-matching energy of a codon with its anticodon should probably be a selection force for the codons' usage, which just as Grosjean and Ikemura ever proposed. Hence, we put forward the hypothesis, namely when a codon is biased in usage, its complementary codon is also preferred in an organism. In order to testify the hypothesis and figure out what kind of usage relationship between codons and their complementary ones actually is, we conducted this statistical analysis by using the usage data of codons and their complementary ones from the genomes of 70 organisms including 45 Bacteria, 5 Archaea, and 20 Eukarya in this paper, and found that there in deed exists a positive usage correlation relationship between codons and their complementary ones.

1 Materials and methods

Genomic data of 70 organisms: 5 Archaea (1～5), 20 Eukaryota (6～25) and 45 Bacteria (26～70) in Table 1 were obtained from the Kazusa DNA Research Institute (http://www.kazusa.or.jp/codon/, GenBank Release 129.0, 15 April 2002). Usage correlation between all codons and their complementary ones of 70 organisms were studied using correlation analysis. The correlation coefficients between 58 codons with their corresponding complementary codons (three terminal codons and their complementary ones were excluded) in usage were calculated by using the usage frequency data of 58 codons as the independent variables and those of their corresponding complementary codons as dependent variables for all 70 organisms.

Table 1 The usage correlation of codons vs complementary codons of the genomes in 70 organisms

Organisms	Domains		No. of codons	GC content	GC3s content	r_{58}
01. *M. jannaschii*	Archaea	Thermphile	504 594	31.85	24.73	0.3254*
02. *M. thermautotrophicus*		Thermphile	595 693	50.44	56.54	0.3903**
03. *P. horikoshii*		Thermphile	486 103	42.45	43.37	0.3728**
04. *T. acidophilum*		Thermphile	471 498	47.38	55.04	0.2914*
05. *T. volcanium*		Thermphile	454 874	40.99	40.71	0.4027**
06. *Homo sapiens*	Eukaryota	Vertebrate	19 894 411	52.65	59.31	0.4940**
07. *Sus scrofa*		Vertebrate	406 321	54.07	64.80	0.4804**
08. *O. cuniculus*		Vertebrate	443 600	54.55	67.27	0.4503**
09. *Mus musculus*		Vertebrate	9 549 215	52.41	59.45	0.4981**
10. *Rattus norvegicus*		Vertebrate	3 435 705	52.79	61.26	0.4635**
11. *X. laevis*		Vertebrate	973 234	47.35	48.81	0.3145*
12. *C. elegans*		Protostome	9 684 274	42.76	39.89	0.4766**
13. *S. cerevisiae*		Fungi	5 664 727	39.70	37.94	0.4790**
14. *K. lactis*		Fungi	114 708	39.09	35.88	0.4485**
15. *C. albicans*		Fungi	330 853	36.90	28.96	0.4592**
16. *S. pombe*		Fungi	2 840 951	39.80	33.12	0.3693**
17. *N. crassa*		Fungi	939 373	56.21	65.58	0.5062**
18. *E. nidulans*		Fungi	204 431	53.17	58.86	0.5057**
19. *A. niger*		Fungi	82 096	56.22	67.84	0.4781**
20. *A. thaliana*		Plant	19 602 801	44.44	42.19	0.3982**
21. *O. sativa*		Plant	1 701 592	54.60	61.26	0.4974**
22. *N. tabacum*		Plant	347 019	43.55	39.49	0.2965*
23. *Zea mays*		Plant	556 901	54.88	63.94	0.4613**
24. *H. vulgare subsp. vulgare*		Plant	151 278	58.06	71.60	0.5089**
25. *L. esculentum*		Plant	391 001	42.62	37.61	0.3344*
26. *E. coli* K12	Bacteria	γ subdivision	1 363 716	51.83	55.89	0.4913**

Table 1 continued

27. *E. coli*	(Gram-negative	γ subdivision	3 662 594	50.58	53.36	0.4716**
28. *S. typhimurium*	proteobacteria)	γ subdivision	360 681	52.82	58.70	0.5345**
29. *S. typhimurium* LT2		γ subdivision	1 477 278	53.36	59.53	0.5465**
30. *K. pneumoniae*		γ subdivision	178 416	55.77	64.84	0.6530**
31. *Y. enterocolitica*		γ subdivision	123 436	47.17	46.45	0.4056**
32. *Y. pestis*		γ subdivision	1 471 174	48.97	50.17	0.4963**
33. *V. cholerae*		γ subdivision	1 399 331	47.35	47.69	0.4346**
34. *H. influenzae* Rd		γ subdivision	523 322	38.76	29.08	0.2800*
35. *C. burnetii*		γ subdivision	86 514	42.51	39.68	0.3975**
36. *B. aphidicola*		γ subdivision	90 279	26.99	13.66	0.5578**
37. *Buchnera sp.* APS		γ subdivision	188 858	27.43	14.29	0.5831**
38. *P. aeruginosa*		γ subdivision	2 313 442	66.44	86.27	0.6446**
39. *P. putida*		γ subdivision	345 718	60.25	73.64	0.6373**
40. *A. vinelandii*		γ subdivision	78 438	65.24	85.93	0.6167**
41. *B. pertussis*		β subdivision	91 147	67.87	85.13	0.7175**
42. *N. gonorrhoeae*		β subdivision	140 675	52.56	59.92	0.5963**
43. *N. meningitidis*		β subdivision	303 391	51.12	56.80	0.5768**
44. *N. meningitidis* MC58		β subdivision	589 048	53.06	61.39	0.5735**
45. *N. meningitidis* Z2491		β subdivision	583 889	53.32	62.08	0.5660**
46. *A. tumefaciens*		α subdivision	294 124	56.72	64.20	0.7771**
47. *A. tumefaciens* str. C58 (U.Washington)		α subdivision	1 668 270	59.77	71.48	0.7658**
48. *A. tumefaciens* str. C58 (Cereon)		α subdivision	1 697 312	59.74	71.45	0.7581**
49. *R. leguminosarum*		α subdivision	86 387	60.13	72.71	0.7650**
50. *R. rhizogenes*		α subdivision	96 351	57.60	66.18	0.8180**
51. *Rhizobium sp.* NGR234		α subdivision	129 817	58.92	68.24	0.7993**
52. *R. capsulatus*		α subdivision	163 774	66.38	83.81	0.5871**
53. *R. sphaeroides*		α subdivision	143 497	68.18	88.07	0.6839**
54. *R. prowazekii*		α subdivision	312 081	30.63	18.43	0.5281**
55. *R. conorii*		α subdivision	347 826	32.92	23.58	0.5168**
56. *P. denitrificans*		α subdivision	57 421	66.31	86.91	0.5322**
57. *B. japonicum*		α subdivision	232 339	62.30	75.90	0.7422**
58. *M. xanthus*		δ subdivision	137 602	69.24	89.05	0.6032**
59. *H. pylori*		ε subdivision	265 678	39.60	39.80	0.5601**
60. *H. pylori* 26695		ε subdivision	498 249	39.56	41.95	0.6047**
61. *H. pylori* J99		ε subdivision	495 471	39.90	42.66	0.5967**
62. *B. subtilis*	Bacteria	Low G+C	2 783 908	44.31	44.60	0.3171*
63. *S. aureus*		Gram-positive	431 609	32.88	22.93	0.3711**
64. *S. aureus subsp. aureus* Mu50			811 148	33.54	22.69	0.3435*
65. *S. aureus subsp. aureus* N315			790 215	33.51	22.53	0.3402*
66. *S. pneumoniae*			370 868	39.16	33.51	0.2967*
67. *L. lactis*			233 617	35.49	26.41	0.3739**
68. *E. faecalis*			145 562	37.81	31.65	0.2875*
69. *M. genitalium*		Mycoplasma	181 435	31.74	23.24	0.6631**
70. *M. pulmonis*		Mycoplasma	302 999	27.14	14.72	0.5914**

GC, percentage of guanine + cytosine; GC3s, frequency of guanine + cytosine at the synonymous third positions of codons; *r* is correlation coefficient of 58 codons with their complementary codons (three terminal codons and their corresponding complementary codons are not included); *, ** Significance at the 5% and 1% probability levels respectively.

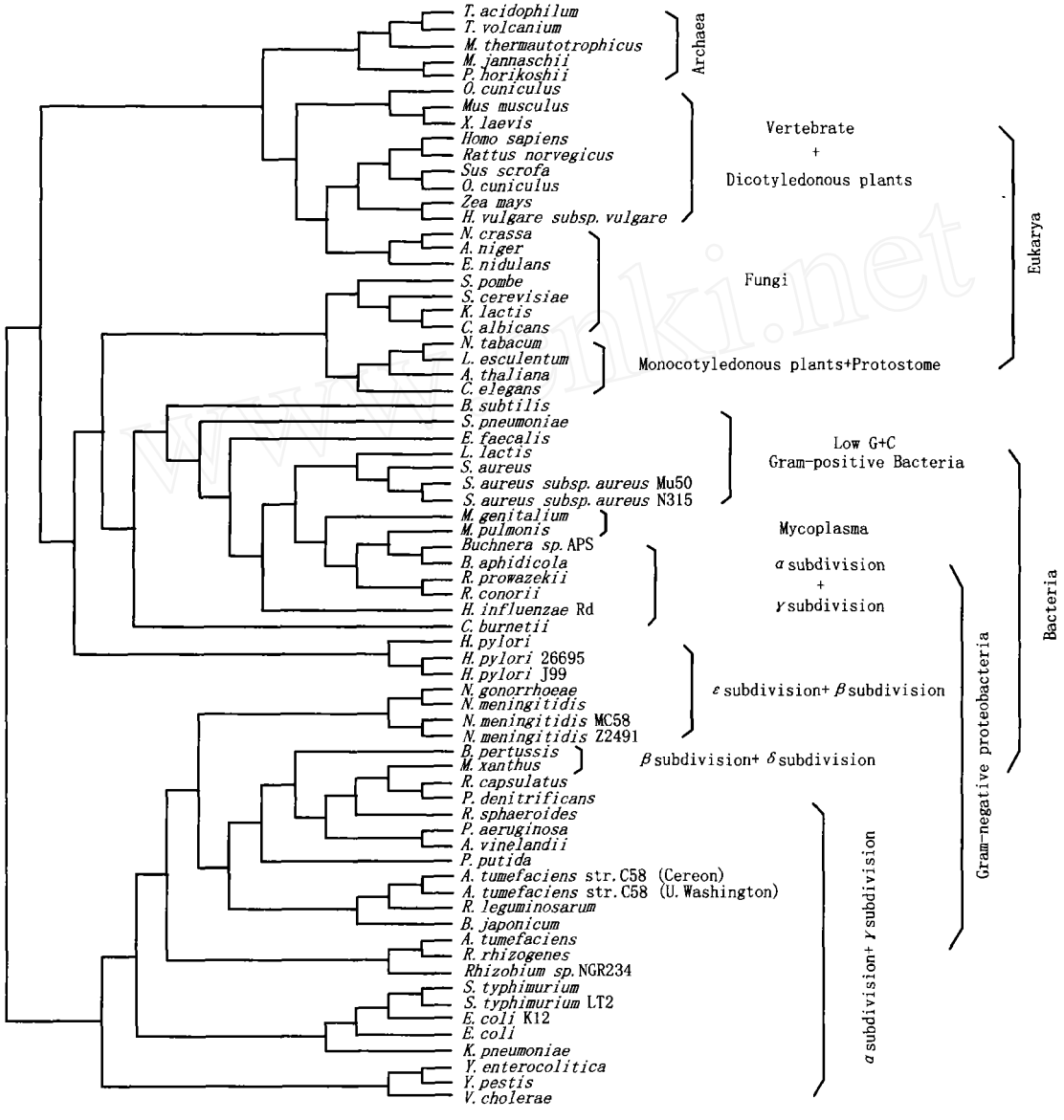
2 Results and discussion

The analysis results of codons usage with their corresponding complimentary ones of genomes from 70 organisms were summarized in Table 1. From Table 1, one could easily see that codons usage has a highly significant or significant positive correlation with their corresponding complementary codons in 70 organisms. The high average correlation coefficients of codons vs complementary codons of genomes from 70 organisms, 0.5101 with the standard deviation 0.1389, indicates that the positive usage correlation relationship of codons with their corresponding complementary ones do objectively exist.

However, there was also strong heterogeneity of correlation coefficients among 70 organisms (Table 1). For Archaea, the range of correlation coefficient between all codons vs complimentary ones usage is from 0.2914 to 0.4027 with a mean of 0.3565 and standard deviation of 0.0468; while in Eukaryota, from 0.2965 to 0.5089 with a mean of 0.4460 and standard deviation of 0.0665; and in Bacteria, from 0.2800 to 0.8180 with a mean of 0.5556 and standard deviation of 0.1466. The mean of correlation coefficient from 45 Bacteria is rather higher than that from Archaea and Eukaryota. It was further found that there exists general descending tendency of their means of correlation coefficient: α subdivision (0.6895) > *Mycoplasma* Bacteria (0.6272) > β subdivision (0.6060) > δ subdivision (0.6032) > ε subdivision (0.5872) > γ subdivision (0.5167) > Low (G+C) Gram-positive Bacteria (0.3329) in Bacteria dataset, while in Eukaryota dataset: Fungi (0.4637) > Vertebrates (0.4501) > Plants (0.4161). The results suggest that the correlation coefficient of codons vs complimentary ones usage to some extent not only reflect the evolutionary history of these organisms, but also reveal the co-evolution processing of codons vs complimentary codons in organisms. Specially, for thermophiles from Archaea, *M. jannaschii, M. thermautotrophicus, P. horikoshii, T. acidophilum* and *T. volcanium* grow at environment of relative high temperature from 60℃ to over 100℃[10]. Withstanding higher temperatures, they show that their bio-molecules composition, especially proteins, must have been changed for normal biological function. Therefore, in order to maintain the kind of function stability, these thermophiles should have endured a much stronger selective pressure during their evolution. This kind of selection pressure may have intensively twisted the correlation between codons and their complementary codons usage, and result in a lower correlation coefficient. Hence, the correlation extent of codons vs their complementary codons usage may be used as a fair index for measuring the degree of selection force during organism evolution.

In order to determine the effect of GC content on correlation of all codons with complimentary ones usage, the relation analysis was carried out between G+C content and correlation coefficient of all codons vs complimentary ones usage among the 70 genomes. The results show a highly significant positive correlation between GC content and correlation coefficient ($r = 0.5230$, $P < 0.0001$) as well as GC3s content and correlation coefficient ($r = 0.5307$, $P < 0.0001$). The higher correlation coefficient is with higher GC content of the complete genome of organism. Therefore GC and GC3s content also reveal strong effects upon the correlation between all codons vs complementary ones usage. Recently it was suggested that compositional constraints are the main factors of deciding the codon usage changes among the genes and organisms[11,12], and that the combination of translational selection and compositional constraints acts for dictating the codon usage variation among genes[13,14]. However, among prokaryotes, it appears that the influences of natural selection and mutational biases are different if the genome is skewed towards AT or GC, and the analyses of the completed genomes of Rickettsia prowazekii and Borrelia burgdorferi with a genomic GC level of 29% show that the mutational bias is the dominant factor shaping codon usage, while in Mycobacterium tuberculosis (GC=65%) translational selection on codon choices has been displayed[11,15~19]. Therefore, the present results suggested that the correlation degree of codons with complementary ones may provide a useful mark for distinguishing the pattern of codon usage among different organisms. For example, Romero, *et al* [13] found that compositional pressure and translational selection determine codon usage in the extremely GC-poor unicellular eukaryote Entamoeba histolytica. Compositional pressure and translational

selection may also be detected by correlation analysis of codons with complementary ones based on the composition of both codons and their complimentary ones, for both are virtually the same either in GC or AU content. If the usage of codons tends to be selected by the composition of the genome, the usage of the complementary codons should accordingly be selected by the same factor. Such usage relationship of codons and complementary ones under compositional pressure and translational selection could then be reflected by their correlation coefficient.



The codon-complementary codon tree was built by the difference values between every single pair usage frequency of codon vs complementary codon, and five frequency combination datasets, namely double low frequency numbers (the frequency of codon and its complementary codon < 10‰), double high frequency numbers (the frequency of codon and its complementary codon > 40‰), single low frequency numbers (one of the frequency of codon and its complementary codon < 10‰), single high frequency numbers (one of the frequency of codon and its complementary codon > 40‰), and the others (the frequency of codon and its complementary codon between 10‰ and 40‰) in the genomes of 70 organisms. Distance between two species were estimated on the 37-dimensional vectors (32 the difference values between every single pair usage frequency of codon vs complementary codon and 5 frequency combination numbers), with each axis representing a pair. With the parsed data, a distance matrix was then calculated by using Minkowski distance. The codon-complementary codon tree was subsequently constructed by using the Pairwise Distance program with the Neighbor-Joining method in the Mega2 Software[23].

Figure 1　The codon-complementary codon tree

Theoretically, any genetic codons with their corresponding complementary ones in usage should be complete-

ly positive correlated (namely their correlation coefficients were close to 1). In fact, it has proved that the first base of many anticodons is usually modified and its pairing with the third base of corresponding codon usually wobbled[20,21]. In these cases, the equality of the 'codon-anticodon' vs 'complementary codon-anticodon' in pairing energy can not be preserved, which results in a biased correlation relationship between them. That is why the calculated correlation coefficients of codons with complementary ones in usage are always much less than 1. The wobbling and the modification in anticodons may directly result in the result of lower correlation coefficients as indicated in our results, and the stronger the wobbling as well as the modification are, the smaller the correlation coefficients will become. This implies correlation coefficients of codons with complementary ones could also be used as the index of anticodons' modification and wobbling, and may provide much useful information about all anticodons' modification and wobbling-pairing with corresponding codons within this organism.

As it is true for the pair usage information of codons vs complementary codons in an organism may imply the evolutionary history of the organism, the neighbouring species in evolution may have similar pair usage patterns of codons vs complementary codons. In order to test the validity of our hypothesis, we built a codon-complementary codon tree based on 32 single pair usage of codon vs complementary codon (Fig 1). As expected, the five Archae bacteria, twenty Eukarya and forty five Bacteria are grouped together respectively, and *M. pulmonis* is nearly grouped with *M. genitalium*, besides the placement of dicotyledonous plants and monocotyledonous plants as well as Fungi was slightly dispatched, the codon-complementary codon tree exhibited very high fidelity to the phylogenetic lineage of the organisms as shown in[22] Figure 1 and Table 1. This suggests that the usage relationship of codons vs complementary codon in organism may strongly reflect their co-evolution history.

In summary, the significantly positive correlation of all codons with their complementary ones in usage among 70 organisms not only proves the validity of our assumption that when a codon is biased in usage, its complementary codon is also biased, but also demonstrates the rationality of Henri Grosjean's 'optimal combination of codon-anticodon complex'[2] and Ikemura's 'translation efficiency'[3,6] hypotheses in a new point of view. This manifests the usefulness of correlation analysis between codon and complementary codon in studying pattern of codon usage bias, especially for elucidating molecular evolutionary mechanisms.

References

[1] Grosjean H, Sankoff D and Fiers W. Bacteriophage MS2 RNA: A correlation between the stability of the codon: anticodon interaction and the choice of code words[J]. J Mol Evol, 1978, 12: 113～119

[2] Grosjean H and Fiers W. Preferential codon usage in prokaryotic genes: the optimal codon-anticodon interaction energy and the selective codon usage in efficiently expressed genes[J]. Gene, 1982, 18: 199～209

[3] Ikemura T. Correlation between the abundance of *Escherichia coli* transfer RNAs and the occurrence of the respective codons in its protein genes: a proposal for a synonymous codon choice that is optimal for the *E. coli* translational system[J]. J Mol Biol, 1981, 151: 389～409

[4] Ikemura T. Correlation between the abundance of yeast transfer RNAs and the occurrence of the respective codons in proteins genes. Differences in synonymous codon choice patterns of yeast and *Escherichia coli* with reference to the abundance of isoaccepting transfer RNAs[J]. J Mol Biol, 1982, 158: 573～587

[5] Ikemura T. Codon usage and tRNA content in unicellular and multicellular organisms[J]. Mol Biol Evol, 1985, 2: 13～34

[6] Ikemura T. Correlation between codon usage and tRNA content in microorganisms[A]. In: Transfer RNA in Protein Synthesis[M]. d D L Hatfield, B J Lee and R M Pirtle. eds. Boca Raton, FL, CRC Press, 1992. 87～111

[7] Wright F. The effective number of codons used by a gene[J]. Gene, 1990, 87: 23～29

[8] Karlin S and Mrázek J. What drives codon choices in human genes? [J]. J Mol Biol, 1996, 262: 459～472

[9] Kim C H, Oh Y and Lee T H. Codon optimization for high-level expression of human erythropoietin (EPO) in mammalian cells[J]. Gene, 1997, 199: 293～301

[10] de Farias S and Bonato M. Preferred codons and amino acid couples in hyperthermophiles[J]. Genome Biology, 2002, 3: 1～18, preprint

[11] Andersson S, Zomorodipour Andersson J, Sicheritz-Ponten T, *et al* The genome sequence of *Rickettsia prowazekii* and the ori-

gin of mitochondria[J]. Nature, 1998, 396: 133～140

[12] Musto H, Romero H, Zavala A, *et al* Synonymous codon choices in the extremely GC-poor genome of plasmodium falciparum: compositional constraints and translational selection[J]. J Mol Evol, 1999, 49: 27～35

[13] Romero H, Zavala A and Musto H. Compositional pressure and translational selection determine codon usage in the extremely GC-poor unicellular eukaryote *Entamoeba histolytica* [J]. Gene, 2000, 242: 307～311

[14] Ghosh T C, Gupta S K and Majumdar S. Studies on codon usage in *Entamoeba histolytica* [J]. Int J Parasitol, 2000, 30: 725～722

[15] Naya H, Romero H, Carels N, *et al* Translational selection shapes codon usage in the GC-rich genome of *Chlamydomonas reinhardtii* [J]. FEBS Lett, 2000, 501: 127～130

[16] Andersson S and Sharp P. Codon usage and base composition in *Rickettsia prowazekii* [J]. J Mol Evol, 1996, 42: 525～536

[17] Lafay B, Lloyd A, McLean M, *et al* Protostome composition and codon usage in spirochaetes: species-specific and DNA strand-specific mutational biases[J]. Nucleic Acids Res, 1999, 27: 1642～1649

[18] McInerney J O. Replicational and transcriptional selection on codon usage in *Borrelia burgdorferi* [J]. Proc Natl Acad Sci USA, 1998, 95: 10698～10703

[19] De Miranda A, Alvarez-Valin F, Jabbari K, *et al* Gene expression, amino acid conservation and hydrophobicity are the main factors shaping codon preferences in *Mycobacterium tuberculosis* and *Mycobacterium leprae* [J]. J Mol Evol, 2000, 50: 45～55

[20] Crick F H C. The origin of the genetic code[J]. J Mol Biol, 1968, 38: 367～79

[21] Yoshiki A, Fumiaki Y, Akira M, *et al* Codon recognition patterns as deduced from sequences of the complete set of transfer RNA species in *Mycoplasma capricolum* [J]. Resemblance to mitochondria. J Mol Biol, 1989, 209: 37～54

[22] Kirlin S, Campbell A M and Mrázek J. Comparative DNA analysis across diverse genomes[J]. Annu Rev Genet, 1998, 32: 185～225

[23] Kumar S, Tamura K, Jakobsen I B, *et al* MEGA2: molecular evolutionary genetics analysis software[J]. Bioinformatics, 2001, 17: 1244～1245

基于密码子与互补密码子使用的进化树的重构

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摘　要:为了更深入地了解密码子与互补密码子使用相关的内涵,70个单细胞和多细胞生物被更进一步的分析。结果得到 70种细胞生物具有较高的相关系数平均值 (0.5101,标准差是 0.1389),表明密码子与其互补密码子使用间确实存在正的使用相关联系,进一步地支持了以前的结果。此外,通过对 70个单细胞和多细胞生物在基于密码子与互补密码子使用频率差异,以及配对频率的高低信息的分析基础上,绘制进化树,得到的进化树与传统的进化树非常接近。

关键词:密码子;互补密码子;使用频率;使用偏爱;相关分析;密码子 - 互补密码子树