

Article

# Comparative Multivariate Analysis of Codon and Amino Acid Usage in Three *Leishmania* Genomes

Nutan Chauhan, Ambarish Sharan Vidyarthi, and Raju Poddar\*

*Department of Biotechnology, Birla Institute of Technology, Mesra, Ranchi-835215, India*

Genomics Proteomics Bioinformatics 2011 Dec; 9(6): 218-228 DOI: 10.1016/S1672-0229(11)60025-9

Received: May 02, 2011; Accepted: Oct 31, 2011

## Abstract

Multivariate analysis of codon and amino acid usage was performed for three *Leishmania* species, including *L. donovani*, *L. infantum* and *L. major*. It was revealed that all three species are under mutational bias and translational selection. Lower GC<sub>12</sub> and higher GC<sub>3S</sub> in all three parasites suggests that the ancestral highly expressed genes (HEGs), compared to lowly expressed genes (LEGs), might have been rich in AT-content. This also suggests that there must have been a faster rate of evolution under GC-bias in LEGs. It was observed from the estimation of synonymous/non-synonymous substitutions in HEGs that the HEG dataset of *L. donovani* is much closer to *L. major* evolutionarily. This is also supported by the higher  $d_N$  value as compared to  $d_S$  between *L. donovani* and *L. major*, suggesting the conservation of synonymous codon positions between these two species and the role of translational selection in shaping the composition of protein-coding genes.

**Key words:** *Leishmania*, relative synonymous codon usage, multivariate analysis, hydrophathy, aromaticity

## Introduction

Leishmaniasis, an infectious protozoal disease caused by parasites belonging to the genus *Leishmania*, is still one of the world's most neglected diseases, affecting mainly developing countries (1). *L. major* causes the most common form of infection, cutaneous leishmaniasis, while *L. donovani* and *L. infantum* are associated with visceral leishmaniasis (2, 3), also known as Kala-azar, in the Indian subcontinent, East Africa, and Mediterranean regions (4). Despite the continuous ongoing efforts in antileishmanial drug discovery and development, there is no effective medicine available so far. The results from current

chemotherapeutic drugs available for the treatment of *Leishmania* infection are not satisfactory (5). The toxic nature of available drugs and the tendency of *Leishmania* to become resistant reflect the need for discovery of more effective antileishmanial agents (5). Therefore, there is an urgent need to understand the biology of these three *Leishmania* pathogens. The published genomic details of *L. infantum* (6) and *L. major* (7) show that the average GC content was around 59% for both of them. The whole genome of *L. donovani* has yet not been sequenced, but sequences of some genes and proteins are available online.

Many genes demonstrate a non-random selection of codons in their protein-coding regions. For any given protein we can distinguish at least two sources of bias in codon usage. The first, "amino acid preference", is the uneven amino acid composition of typical proteins,

\*Corresponding author.

E-mail: [rpoddar@bitmesra.ac.in](mailto:rpoddar@bitmesra.ac.in)

© 2011 Beijing Institute of Genomics. All rights reserved.

*i.e.*, some amino acids are used far more frequently than others (8). The second is that once an amino acid has been chosen, there are generally preferences for the use of certain codons. Relative synonymous codon usage (RSCU) and relative amino acid usage (RAAU) are used to measure the non-random usage of specific amino acid. Genes with strong codon bias appear to be expressed at a higher level compared to other genes. Biased codon usage may result from a combination of several factors, namely, biases in the pattern of mutation (9), or translational selection (10) among synonymous codons. Within-species heterogeneity in codon usage has been most clearly elucidated in *E. coli* (11). The major trend includes a strong bias towards a particular subset of codons in highly expressed genes (HEGs) and more even codon usage in lowly expressed genes (LEGs) (12-14). Our comparative multivariate analysis of codon and amino acid usage patterns in *Leishmania* species will provide an insight into the divergence and compositional similarities within and across their genomes and may lead to a better understanding of the biology of the parasites and the development of more effective drug treatments.

## Results

### Major sources of RSCU variation in the three *Leishmania* species

Correspondence analysis (COA) was used to explore the variation of RSCU values in the genes from *L. donovani*, *L. infantum* and *L. major*. After plotting

genes in 59-dimensional hyperspace, according to the usage of the 59 synonymous sense codons (stop codons and codons with one-to-one mapping to amino acids, *i.e.*, Met, Trp were excluded), COA identifies a series of new orthogonal axes accounting for the greatest variation among genes. The coordinate of each gene on each new axis and the fraction of the total variation accounted for by each axis is generated by COA. Axis 1 and Axis 2 indicate the major trends of variations among genes. Axis 1 accounts for 31.5%, 15.7% and 17.2% of the total variations for RSCU in *L. donovani*, *L. infantum* and *L. major*, respectively (Table 1). In all cases, GC<sub>3S</sub> (GC content at synonymous codon sites excluding ATG for Met and TGG for Trp) and N<sub>C</sub> (effective number of codons) exhibited strong correlation with Axis 1. The correlation between GC<sub>3S</sub> and Axis 1 is negative in *L. donovani* and *L. major* but positive in *L. infantum*. Conversely, the correlation between N<sub>C</sub> and Axis 1 is positive in *L. donovani* and *L. major* but negative in *L. infantum*. The correlations between GC<sub>3S</sub> and Axis 1 suggests that highly biased genes, those with G/C-ending codons, are clustered on the negative side in *L. donovani* and *L. major* but on the positive side of Axis 1 in *L. infantum* (Table 1). Also, the high degree of correlation between GC<sub>3S</sub> and Axis 1 suggests that directional mutational pressure plays a major role in governing the synonymous codon usage. In addition, the low value of N<sub>C</sub> (Table 1) indicates that HEGs are under translational selection. In *L. donovani*, GT<sub>3S</sub>, gravity and aromaticity all significantly contributed to the variation on Axis 2. In *L. infantum*, both GT<sub>3S</sub> and gravity significantly correlated with Axis 2 in *L. donovani*,

**Table 1** Major trends in synonymous codon usage in *L. donovani*, *L. infantum* and *L. major* as revealed by COA on RSCU of genes on Axis 1 and 2

Organism	Axis 1			Axis 2		
	Total variability	Source of variation	Correlation coefficient (r) <sup>a</sup>	Total variability	Source of variation	Correlation coefficient (r) <sup>a</sup>
<i>L. donovani</i>	31.5%	N <sub>C</sub>	0.693	7.4%	GT <sub>3S</sub>	-0.371
		GC <sub>3S</sub>	-0.983		Aromaticity	-0.358
<i>L. infantum</i>	15.7%	N <sub>C</sub>	-0.940	4.7%	Gravy	-0.265
		GC <sub>3S</sub>	0.951		GT <sub>3S</sub>	0.621
					Gravy	-0.131
<i>L. major</i>	17.2%	N <sub>C</sub>	0.957	4.7%	Aromaticity	-0.117
		GC <sub>3S</sub>	-0.953			

Note: <sup>a</sup>All correlations are significant at  $P < 0.01$ .

while in *L. major*, aromaticity was found to be the only major source of variation on Axis 2.

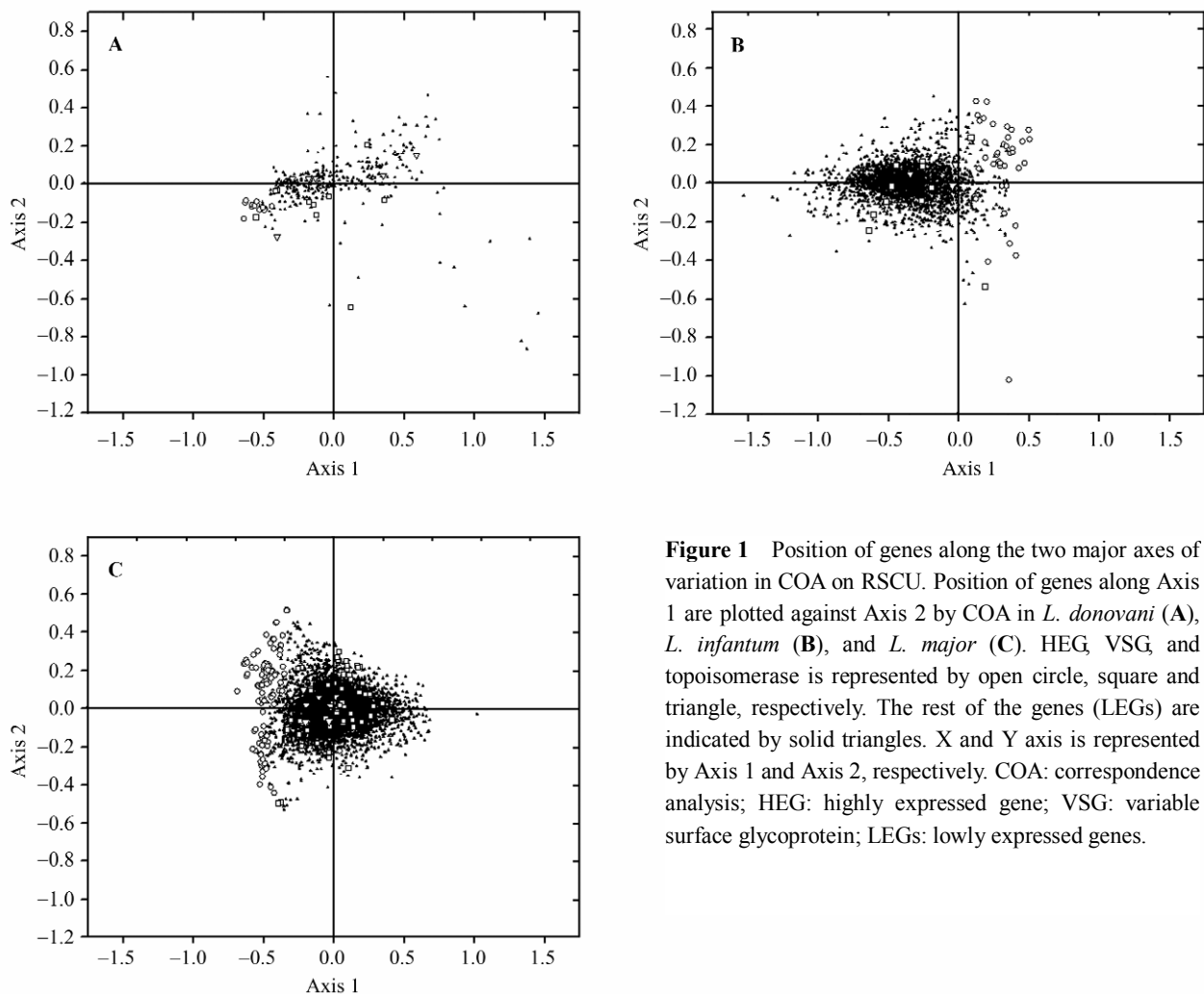
A plot of Axis 1–Axis 2 of each genome under study including *L. donovani*, *L. major*, and *L. infantum* was drawn, showing that HEGs are clustered at one end of Axis 1 (**Figure 1**, circle), indicating that these genes follow a distinct pattern of synonymous codon usage.

A comparison of RSCU values of the HEGs with those of the LEGs shows that in all three parasites examined, a similar subset of synonymous codons, mostly G/C-ending, are preferred by the HEGs (Table S1, codons with bold values). The LEGs exhibit relatively higher usage of A/U-ending codons. But in all three species, even the LEGs prefer to use G/C-ending codons for most of the amino acids, though the frequencies of such codons are low. This is in agreement with the high GC content in the genes

from *L. donovani* (58.8%), *L. infantum* (59.3%) and *L. major* (59.7%). As seen in Table S1, high extent of bias in the synonymous codon usage suggests that the influence of translational selection is strong in all the three *Leishmania* species.

### Codon usage in variant surface glycoproteins, HEGs and the topoisomerase gene

Variable surface glycoproteins (VSGs) have been identified as parasite virulence factors that make possible the survival of *Leishmania* inside the macrophages (15). DNA topoisomerases are a family of DNA-processing enzymes involved in catalysis of the breakage and rejoining of DNA strands (16). DNA topoisomerase of *L. donovani* is distinct from other eukaryotic counterparts with respect to its biological properties and preferential sensitivity to many



**Figure 1** Position of genes along the two major axes of variation in COA on RSCU. Position of genes along Axis 1 are plotted against Axis 2 by COA in *L. donovani* (**A**), *L. infantum* (**B**), and *L. major* (**C**). HEG, VSG, and topoisomerase is represented by open circle, square and triangle, respectively. The rest of the genes (LEGs) are indicated by solid triangles. X and Y axis is represented by Axis 1 and Axis 2, respectively. COA: correspondence analysis; HEG: highly expressed gene; VSG: variable surface glycoprotein; LEGs: lowly expressed genes.

therapeutic agents (17). Due to the therapeutical importance of VSGs and topoisomerases, we have included them separately for analysis of codon and amino acid usage.

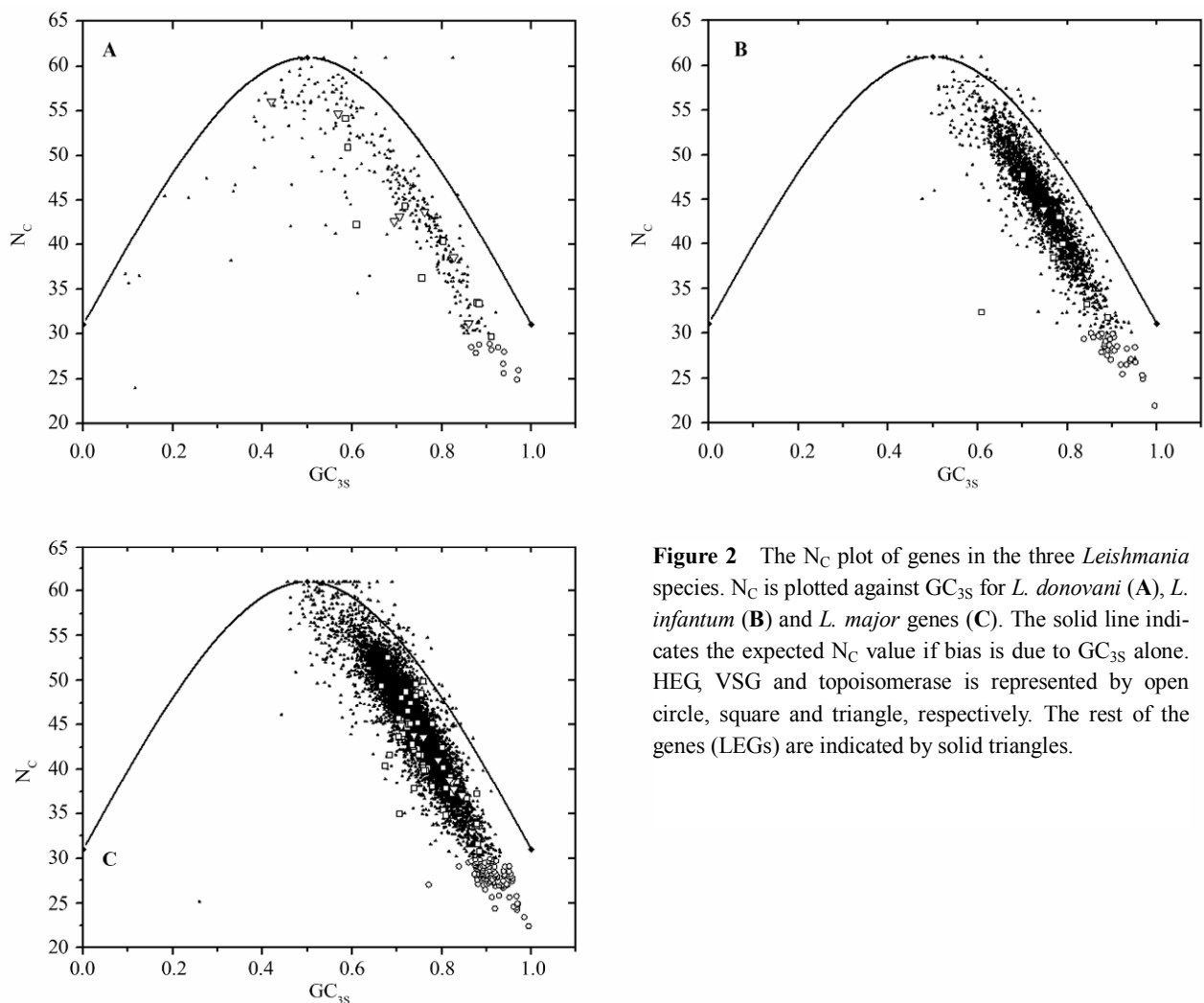
In all the three species of *Leishmania* examined, genes other than HEGs constitute a single cluster (Figure 1). But this is not the case for some genes, i.e., VSG and topoisomerase genes. Their highly scattered nature on Axis 1–Axis 2 plot suggests that these genes have different codon usage due to mutational pressure or different translational selection. As indicated in Figure 1 and Figure 2, all these genes are also characterized by high GC<sub>3S</sub> and high N<sub>C</sub> values.

### Major sources of variation in amino acid usages

To identify the major trends of intra-proteomic variations in amino acid composition in the three

*Leishmania* species, COA on amino acid usage was performed. The first axis generated by COA accounts for 32%, 24% and 30% of the total variations in *L. donovani*, *L. infantum*, and *L. major*, respectively (Table 2).

In all three species, codon adaptation index (CAI) and GC<sub>12</sub> were common primary sources of intra-proteomic variations in amino acid usage (Table 2). It was also observed that GC<sub>3S</sub> and N<sub>C</sub> provide additional trends of variability in all three *Leishmania* species. GT<sub>3S</sub> accounted for the variation on Axis 1 only in *L. donovani*. Variation on Axis 2 was determined by gravity and aromaticity for all three species. Observations from Axis 1–Axis 2 plots of COA on amino acid usage (Figure 3) showed that distribution of the HEGs in *L. donovani* (Figure 3A) overlapped with that of other genes. In *L. infantum* most of these genes lie on the left side of Axis 1 (Figure 3B). In the case of *L. major*,

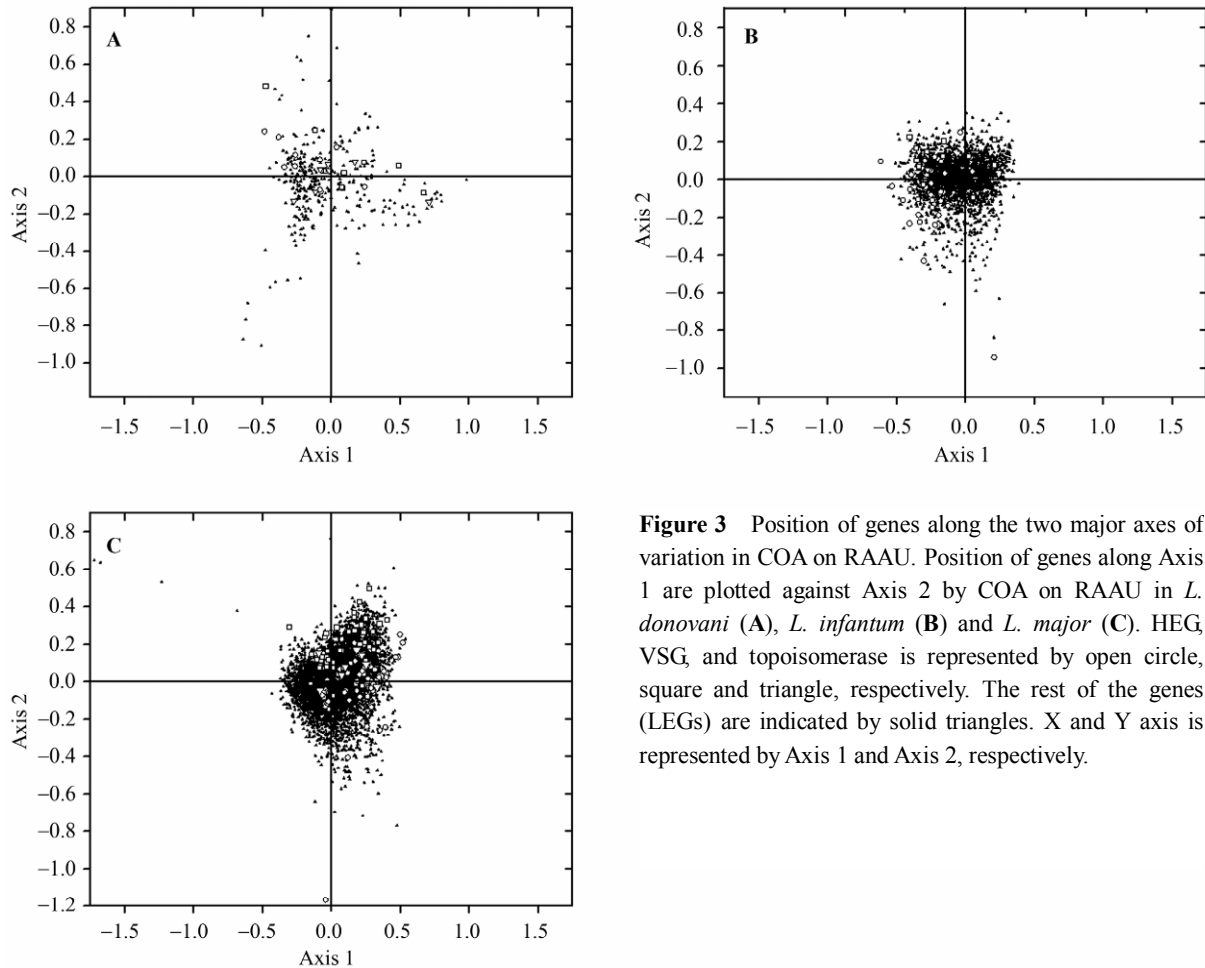


**Figure 2** The N<sub>C</sub> plot of genes in the three *Leishmania* species. N<sub>C</sub> is plotted against GC<sub>3S</sub> for *L. donovani* (A), *L. infantum* (B) and *L. major* genes (C). The solid line indicates the expected N<sub>C</sub> value if bias is due to GC<sub>3S</sub> alone. HEG, VSG and topoisomerase is represented by open circle, square and triangle, respectively. The rest of the genes (LEGs) are indicated by solid triangles.

**Table 2 Major trends in synonymous codon usage in *L. donovani*, *L. infantum* and *L. major* as revealed by COA on amino acid usage of encoded proteins on Axis 1 and Axis 2**

Organism	Axis 1			Axis 2		
	Total variability	Source of variation	Correlation coefficient (r) <sup>a</sup>	Total variability	Source of variation	Correlation coefficient (r) <sup>a</sup>
<i>L. donovani</i>	32%	CAI	-0.599	20%	Aromaticity	-0.792
		N <sub>C</sub>	0.540		Gravy	-0.717
		GT <sub>3S</sub>	-0.541			
		GC <sub>12</sub>	0.767			
<i>L. infantum</i>	24.2%	CAI	-0.645	14.3%	Aromaticity	0.397
		N <sub>C</sub>	0.430		Gravy	0.334
		GC <sub>3S</sub>	-0.424			
		GC <sub>12</sub>	0.946			
<i>L. major</i>	30%	CAI	0.529	14.3%	Aromaticity	0.669
		GC <sub>3S</sub>	0.400		Gravy	0.531
		GC <sub>12</sub>	-0.862			

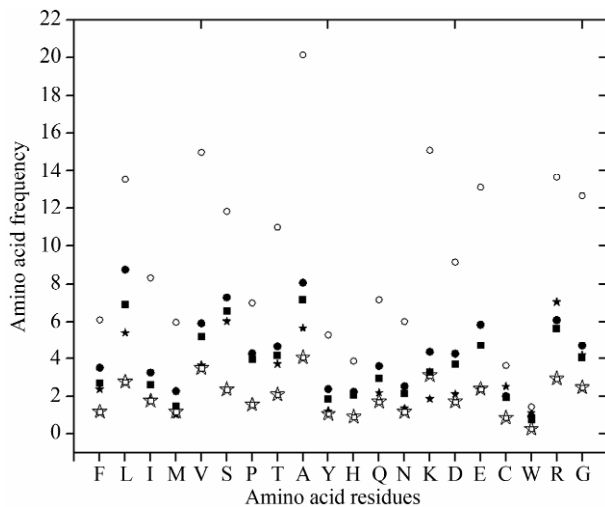
Note: <sup>a</sup>All correlations are significant at  $P=0.01$ . GC<sub>12</sub>: G/C content at first and second codon sites; CAI, codon adaptation index.



**Figure 3** Position of genes along the two major axes of variation in COA on RAAU. Position of genes along Axis 1 are plotted against Axis 2 by COA on RAAU in *L. donovani* (A), *L. infantum* (B) and *L. major* (C). HEG, VSG, and topoisomerase is represented by open circle, square and triangle, respectively. The rest of the genes (LEGs) are indicated by solid triangles. X and Y axis is represented by Axis 1 and Axis 2, respectively.

HEGs clustered at the right side of the Axis 1 (Figure 3C). Figure 2 (A and B) and Table 2 together suggest that the HEGs of *L. donovani* and *L. infantum* are characterized by relatively high GC<sub>12</sub>. However, GC<sub>12</sub> was low in *L. major*, which was not expected because of the high GC content in *L. major*. This may be due to the effect of mutational pressure on *L. major*.

GC<sub>1</sub> (G/C content at first codon sites) and GC<sub>2</sub> (G/C content at first codon sites) of HEGs are similar in all three species (Table S2). GC<sub>1</sub> and GC<sub>2</sub> of HEGs in *L. donovani* are lower than those in LEGs in all species, which could be due to mutational bias in *L. donovani*, suggesting the higher AT content in LEGs in *L. donovani*. **Figure 4** shows the average amino acid frequencies in proteins encoded by the HEGs and LEGs in the three parasites under study. The frequency of many amino acids differs in these two sets of genes in *L. major* and is distributed widely, whereas GC-rich codons are dominant in HEGs as compared to LEGs (Figure 4, open and solid circles). But this distribution is restricted to one extreme end in the case of *L. donovani* (Figure 4, open and solid stars) and *L. infantum* (Figure 4, open and solid squares). These data suggest that there is a major variation in selecting the codons for amino acids usage.



**Figure 4** Frequency of amino acid residues in proteins encoded by HEGs and LEGs in three *Leishmania* species. Open stars, squares and circles represent HEGs in *L. donovani*, *L. infantum* and *L. major*, respectively and solid stars, squares and circles indicate LEGs in *L. donovani*, *L. infantum* and *L. major*, respectively. The amino acid (single letter code) residues are showed on X axis and the frequency of amino acid occurring in proteins encoded by LEGs and HEGs is indicated on Y axis.

### Conservation of HEGs

Estimation of  $d_s$  (number of synonymous substitutions per synonymous sites) and  $d_N$  (non-synonymous substitutions per non-synonymous site) on the orthologs of HEGs in *L. donovani* with *L. infantum* and *L. major* was performed to investigate the evolution of amino acid substitution. Pairwise alignment was done between the orthologs of HEGs of *L. donovani*–*L. infantum* and *L. donovani*–*L. major*, and the total numbers of synonymous substitutions and non-synonymous substitutions are calculated. **Table 3** shows that  $d_N$  is higher than  $d_s$  in both the groups. It is noteworthy that the  $d_s$  and  $d_N$  values of *L. donovani*–*L. major* are lower, while the  $d_N/d_s$  ratio is higher than those of *L. donovani*–*L. infantum*. This means that *L. infantum* has deviated at the synonymous and non-synonymous codon positions at a much faster rate than *L. major*.

### Codon and amino acid usage analysis for homologous genes

According to COA on RSCU, Axis 1 accounts for 30.54%, 26.47% and 32.53% of the total variations due to GC<sub>3S</sub> and N<sub>C</sub> in three species (**Table 4**). On Axis 2, GT<sub>3S</sub> and aromaticity account for the major trends of variation. N<sub>C</sub> is correlated with Axis 1 positively in *L. infantum* but negatively in *L. donovani* and *L. major*, while an opposite trend was observed for the correlation between GC<sub>3S</sub> and Axis 1 in these three species, suggesting that the genes with G/C-ending codons are clustered on the right side but on the negative side in *L. infantum* due to negative correlation (**Figure 5**, Axis 1–Axis 2 plot of homologous genes). It has also been noted (Table 4) that N<sub>C</sub> is negatively correlated with Axis 1 in *L. donovani* and *L. major*, which may be due to the decrease in codon bias among the genes lying towards the right side of Axis 1. This high correlation suggests that directional mutational pressure is dominating for governing synonymous codon usage.

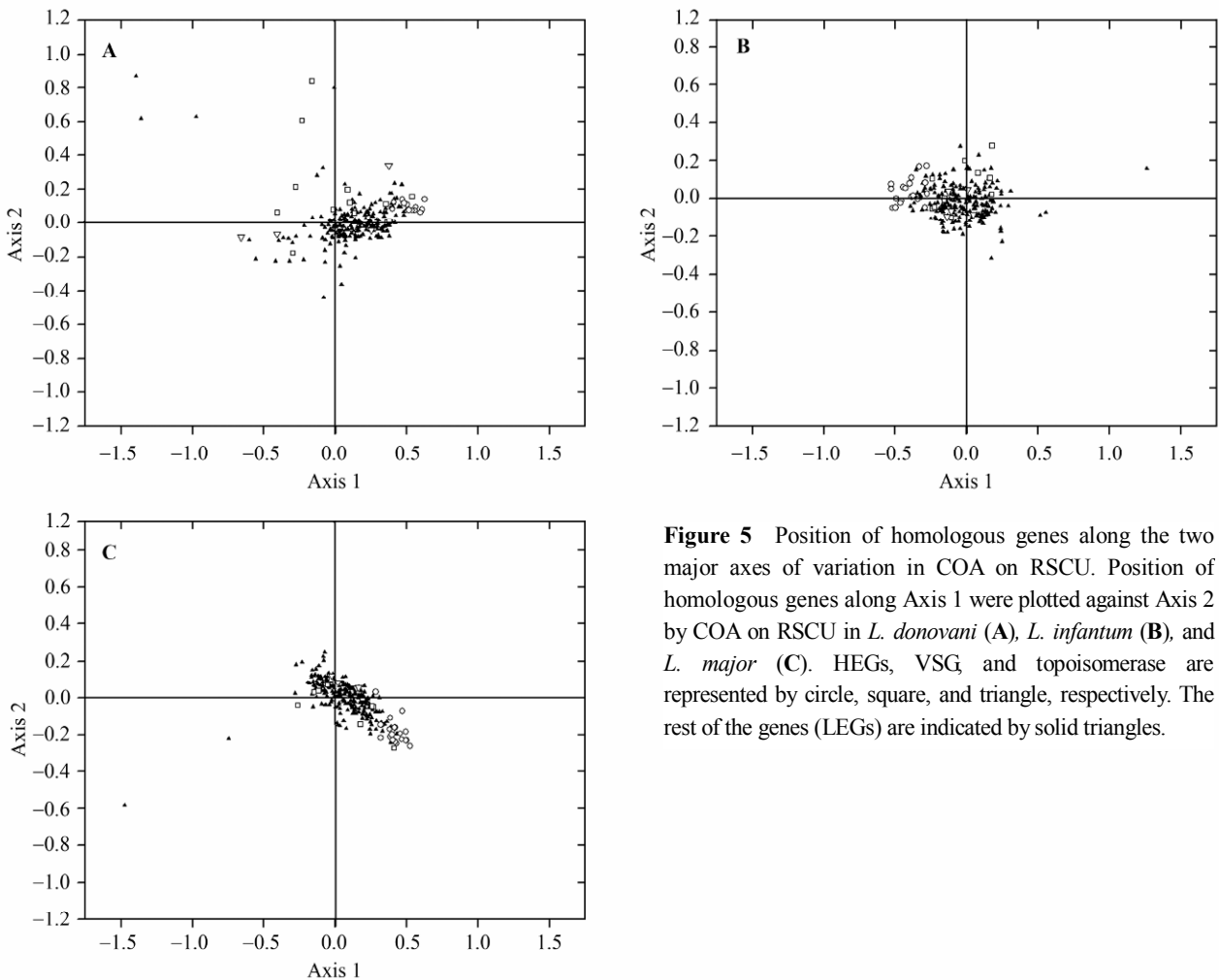
**Table 3**  $d_s$  and  $d_N$  in orthologs of HEGs

Ortholog pairs	$d_s$	$d_N$	$d_N/d_s$
<i>L. donovani</i> – <i>L. infantum</i>	0.094	0.12	1.27
<i>L. donovani</i> – <i>L. major</i>	0.056	0.074	1.32

**Table 4 Major trends in synonymous codon usage of homologous genes in three *Leishmania* species as revealed by COA on codon usage of encoded proteins on Axis 1 and 2**

Organism	Axis 1			Axis 2		
	Total variability	Source of variation	Correlation coefficient (r) <sup>a</sup>	Total variability	Source of variation	Correlation coefficient (r) <sup>a</sup>
<i>L. donovani</i>	30.54%	N <sub>C</sub>	-0.797	7.74%	GT <sub>3S</sub>	0.338
		GC <sub>3S</sub>	0.982		Aromaticity	0.125
<i>L. infantum</i>	26.47%	N <sub>C</sub>	0.891	8.13%	GT <sub>3S</sub>	0.237
		GC <sub>3S</sub>	-0.958		Aromaticity	-0.309
					Gravy	-0.408
<i>L. major</i>	32.53%	N <sub>C</sub>	-0.761	6.95%	GT <sub>3S</sub>	-0.223
		GC <sub>3S</sub>	0.976		Aromaticity	-0.288
					Gravy	-0.221

Note: <sup>a</sup>All correlations are significant at  $P < 0.01$ .



**Figure 5** Position of homologous genes along the two major axes of variation in COA on RSCU. Position of homologous genes along Axis 1 were plotted against Axis 2 by COA on RSCU in *L. donovani* (A), *L. infantum* (B), and *L. major* (C). HEGs, VSG and topoisomerase are represented by circle, square, and triangle, respectively. The rest of the genes (LEGs) are indicated by solid triangles.

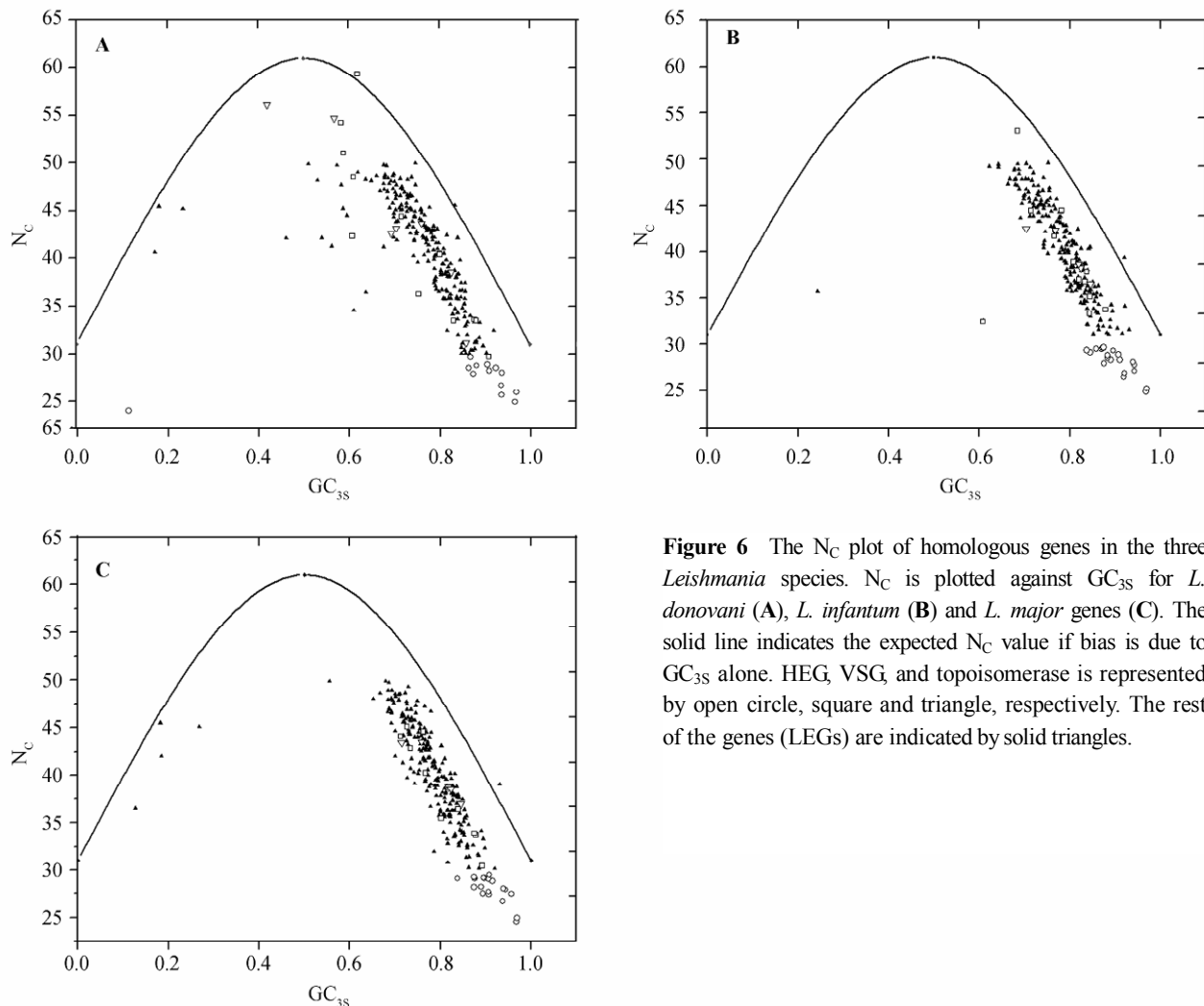
$N_C$ – $GC_{3S}$  plot (**Figure 6**) indicates that HEGs constitute a single cluster, but VSG and topoisomerase genes demonstrate different codon usage pattern and are characterized by high  $N_C$  and  $GC_{3S}$  for *L. infantum* and *L. major*, while topoisomerase genes in *L. donovani* are distributed randomly (range 0.42– 0.86). COA on amino acid usage has been performed for proteomic variability. Axis 1 accounts for 33.51%, 39.3% and 31.59% of total variation in the three species of *Leishmania* (**Table 5**). CAI is the common source of intra-proteomic variation in all species.  $GC_{12}$  accounts for the additional variation in *L. donovani* and *L. infantum*, while for *L. major*, gravity and aromaticity contribute to variation besides CAI. Variation on Axis 2 is determined by gravity and aromaticity in *L. donovani* and *L. infantum*, while for *L. major*,  $GC_{12}$  and  $GT_{3S}$  were the main contributors for the in-

tra-proteomic variation on Axis 2. In all three species, HEGs, when plotted on Axis 1–Axis 2 (Figure S1), were scattered, which was not expected because the average GC content of these species is high. This discrepancy may be due to the influence of mutational pressure.

## Discussion

The present study reveals the major trends involved in the selection of gene/protein composition of the three *Leishmania* species examined. The analysis of synonymous codon usage and amino acid variations shows that genomes of all the three *Leishmania* species are under mutational bias and translational selection.

In all three species, the lower  $GC_{12}$  and higher



**Figure 6** The  $N_C$  plot of homologous genes in the three *Leishmania* species.  $N_C$  is plotted against  $GC_{3S}$  for *L. donovani* (A), *L. infantum* (B) and *L. major* genes (C). The solid line indicates the expected  $N_C$  value if bias is due to  $GC_{3S}$  alone. HEG, VSG, and topoisomerase is represented by open circle, square and triangle, respectively. The rest of the genes (LEGs) are indicated by solid triangles.

**Table 5 Major trends in synonymous codon usage of homologous genes in 3 *Leishmania* species as revealed by COA on amino acid usage of encoded proteins on Axis 1 and 2**

Organism	Axis 1			Axis 2		
	Total variability	Source of variation	Correlation coefficient (r) <sup>a</sup>	Total variability	Source of variation	Correlation coefficient (r) <sup>a</sup>
<i>L. donovani</i>	33.51%	CAI	0.569	19.51%	Gravy	0.629
		GC <sub>12</sub>	-0.761		Aromaticity	0.751
		GT <sub>3S</sub>	0.519			
<i>L. infantum</i>	39.3%	CAI	0.478	8.13%	Aromaticity	0.667
		GC <sub>12</sub>	-0.557		Gravy	0.550
<i>L. major</i>	31.59%	CAI	0.443	19.5%	GT <sub>3S</sub>	-0.460
		Gravy	0.567		Aromaticity	-0.242
		Aromaticity	0.700		GC <sub>12</sub>	0.715

Note: <sup>a</sup>All correlations are significant at  $P \leq 0.01$ .

GC<sub>3S</sub> in HEGs as compared to LEGs suggest that the ancestor of the HEGs might have been relatively rich in AT-content. Previous studies have suggested a universal AT mutational bias, because many types of spontaneous mutations (e.g., the deamination of cytosine) cause GC to AT changes (18). This also suggests that the LEGs have evolved at a faster rate and become GC-rich. The lower  $d_S$  and  $d_N$  values in *L. donovani*–*L. major* than those in *L. donovani*–*L. infantum* suggests that the HEG dataset of *L. donovani* is evolutionarily much closer to *L. major*. The higher value of  $d_N$  as compared to  $d_S$  shows that synonymous positions are more conserved between *L. donovani* and *L. major*, and mutational bias plays a major role in shaping the composition of protein-coding genes. Additionally, optimal codons in all three *Leishmania* species are G/C-ending in HEGs but A/T-ending in LEGs. This supports the fact that translational selection works more strongly on synonymous sites of HEGs (19-21). As a result, the HEGs of these three species are characterized by low GC<sub>12</sub> and high GC<sub>3S</sub> in comparison to the LEGs. The HEGs may further be explored to identify the essential genes, for example, by applying *in silico* subtracting genomic approach, and could be helpful in searching potential therapeutic drug targets for curing leishmaniasis.

## Materials and Methods

### Sequence dataset

Complete protein-coding gene sequences of *L. infantum* and *L. major* were extracted from the Sanger database (<http://www.sanger.ac.uk/>) while protein-coding sequences of *L. donovani* were obtained from NCBI (<http://www.ncbi.nlm.nih.gov/>), which contain 2,655, 9,159 and 368 (till April 30, 2011) protein-coding genes, respectively. To minimize sampling error, genes with less than 100 codons, internal stop codons, not-translatable codons, incomplete start and stop codons, and pseudogenes were excluded from the analysis. Therefore, finally 2,559 and 8,132 genes were included for analysis for *L. infantum* and *L. major*, respectively. No such filter was applied for *L. donovani* due to fewer gene sequences.

Homologs for *L. donovani* were searched using BLAST. For this purpose the E-value cut-off was set to e-100 and genes with E-value less than e-100 were considered as homologs. According to this criterion, a total of 341 genes from *L. infantum* and 340 genes from *L. major* were found as homologs for 347 genes from *L. donovani*.

## Parameters used for identifying trends of variations

For each protein-coding gene under study, the following parameters were calculated, which include RSCU, RAAU, CAI, GC<sub>12</sub>, GC<sub>3S</sub> at synonymous codon sites excluding ATG for Met, TGG for Trp and stop codons, average hydrophathy (22) and aromaticity (23) of the gene products.

## Datasets of HEGs and LEGs

Datasets of putative HEGs and LEGs were obtained by taking genes from the two extreme ends of Axis 1 of COA on RSCU in all three parasites.

## Statistical analyses

The program CodonW 1.1.4 (Peden, J., 1999. available at <http://sourceforge.net/projects/codonw/>) was used to analyze codon usage, COA (24), GC<sub>3S</sub>, RSCU (22), and CAI (14, 18). A 2×2 contingency table  $\chi^2$  was used to detect the significant differences in codon and amino acid usage.

## Estimation of non-synonymous and synonymous substitutions in HEGs

Orthologs for HEGs (genes lying at the one extreme end of Axis 1 of COA) of *L. donovani* were extracted using BLAST. The cut-off E-value for searching orthologs was set to e-50 so the homologs with E-value less than e-50 were considered as orthologs. Pairwise alignments between the orthologs and estimation of  $d_S$  and  $d_N$  were carried out using MEGA4 program (25).

## Acknowledgements

The authors are thankful to the Sub-Distributed Information Center (BTISnet SubDIC) and Department of Biotechnology, BIT, Mesra, Ranchi for their kind support.

## Authors' contributions

NC and RP were involved in this study on all aspects,

contributed to the design of the project and wrote the manuscript. ASV performed synonymous/non-synonymous substitutions analysis. All authors read and approved the final manuscript.

## Competing interests

The authors have declared that no competing interests exist.

## References

- 1 World Health Organization. 2010. Control of the leishmaniases: report of a meeting of the WHO Expert Committee on the Control of Leishmaniases. WHO technical report series (no. 949). WHO Press, Geneva, Switzerland.
- 2 Minodier, P. and Parola, P. 2007. Cutaneous leishmaniasis treatment. *Travel Med. Infect. Dis.* 5: 150-158.
- 3 Gibson, M.E. 1983. The identification of kala-azar and the discovery of *Leishmania donovani*. *Med. Hist.* 27: 203-213.
- 4 Desjeux, P. 2004. Leishmaniasis: current situation and new perspectives. *Comp. Immunol. Microbiol. Infect. Dis.* 27: 305-318.
- 5 Singh, S. 2006. New developments in diagnosis of leishmaniasis. *Indian J. Med. Res.* 123: 311-330.
- 6 Peacock, C.S., et al. 2007. Comparative genomic analysis of three *Leishmania* species that cause diverse human disease. *Nature Genet.* 39: 839-847.
- 7 Ivens, A.C., et al. 2005. The genome of the kinetoplastid parasite, *Leishmania major*. *Science* 309: 436-442.
- 8 Dayhoff, M.O. 1978. *Atlas of Protein Sequence and Structure*. Vol 5 Supplement 3 (eds. Hunt, L.T., et al.), National Biomedical Research Foundation, Washington D.C, USA.
- 9 Levin, D.B. and Whittome, B. 2000. Codon usage in nucleopolyhedroviruses. *J. Gen. Virol.* 81: 2313-2325.
- 10 Grantham, R., et al. 1981. Codon catalog usage is a genome strategy modulated for gene expressivity. *Nucleic Acids Res.* 9: 43-74.
- 11 Elton, B., et al. 1976. Doublet frequencies and codon weighting in the DNA of *Escherichia coli*. *J. Mol. Evol.* 8: 117-135.
- 12 Gouy, M. and Gautier, C. 1982. Codon usage in bacteria: correlation with gene expressivity. *Nucleic Acids Res.* 10: 7055-7074.
- 13 Ikemura, T. 1985. Codon usage and tRNA content in unicellular and multicellular organisms. *Mol. Biol. Evol.* 2: 13-34.
- 14 Sharp, P.M. and Li, W.H. 1986. An evolutionary perspec-

- tive on synonymous codon usage in unicellular organisms. *J. Mol. Evol.* 24: 28-38.
- 15 Chaudhuri, G., et al. 1989. Surface acid proteinase (gp63) of *Leishmania mexicana*. A metalloenzyme capable of protecting of liposome-encapsulated proteins from phagolysosomal degradation by macrophages. *J. Biol. Chem.* 264: 7483-7489.
  - 16 Wang, J.C. 2002. Cellular roles of DNA topoisomerases: a molecular perspective. *Nat. Rev. Mol. Cell Biol.* 6:430-440.
  - 17 Cheesman, S.J. 2000. The topoisomerases of protozoan parasites. *Parasitol. Today* 7: 277-281.
  - 18 Sharp, P.M. and Li, W.H. 1987. The codon adaptation index—a measure of directional synonymous codon usage bias, and its potential applications. *Nucleic Acids Res.* 15: 1281-1295.
  - 19 Birdsell, J.A. 2002. Integrating genomics, bioinformatics, and classical genetics to study the effects of recombination on genome evolution. *Mol. Biol. Evol.* 19: 1181-1197.
  - 20 Iida, K and Akashi, H. 2000. A test of translational selection at ‘silent’ sites in the human genome: base composition comparisons in alternatively spliced genes. *Gene* 261: 93-105.
  - 21 Lafay, B., et al. 2000. Absence of translationally selected synonymous codon usage bias in *Helicobacter pylori*. *Microbiology* 146: 851-860.
  - 22 Kyte, J. and Doolittle, R.F. 1982. A simple method for displaying the hydropathic character of a protein. *J. Mol. Biol.* 157: 105-132.
  - 23 Lobry, J.R. and Gautier, C. 1994. Hydrophobicity, expressivity and aromaticity are the major trends of amino acid usage in 999 *Escherichia coli* chromosome-encoded genes. *Nucleic Acids Res.* 22: 3174-3180.
  - 24 Greenacre, M.J. 1984. *Theory and Applications of Correspondence Analysis*. Academic Press, New York, USA.
  - 25 Tamura, K., et al. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) Software Version 4.0. *Mol. Biol. Evol.* 24: 1596-1599.

**Supplementary Material**

Tables S1 and S2; Figure S1  
 DOI: 10.1016/S1672-0229(11)60025-9