



**HAL**  
open science

# COUSIN (COdon Usage Similarity INdex): A Normalized Measure of Codon Usage Preferences

Jérôme Bourret, Samuel Alizon, Ignacio Bravo

► **To cite this version:**

Jérôme Bourret, Samuel Alizon, Ignacio Bravo. COUSIN (COdon Usage Similarity INdex): A Normalized Measure of Codon Usage Preferences. *Genome Biology and Evolution*, 2019, 11 (12), pp.3523-3528. 10.1093/gbe/evz262 . hal-02957623

**HAL Id: hal-02957623**

**<https://hal.science/hal-02957623>**

Submitted on 5 Oct 2020

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# COUSIN (COdon Usage Similarity INdex): A Normalized Measure of Codon Usage Preferences

Jérôme Bourret\*, Samuel Alizon, and Ignacio G. Bravo 

Centre National de la Recherche Scientifique, Laboratory MIVEGEC (CNRS, IRD, Uni Montpellier), Montpellier, France

\*Corresponding author: E-mail: jerome.bourret@ird.fr.

Accepted: November 25, 2019

## Abstract

Codon Usage Preferences (CUPrefs) describe the unequal usage of synonymous codons at the gene, chromosome, or genome levels. Numerous indices have been developed to evaluate CUPrefs, either in absolute terms or with respect to a reference. We introduce the normalized index COUSIN (for COdon Usage Similarity INdex), that compares the CUPrefs of a query against those of a reference and normalizes the output over a Null Hypothesis of random codon usage. The added value of COUSIN is to be easily interpreted, both quantitatively and qualitatively. An eponymous software written in Python3 is available for local or online use (<http://cousin.ird.fr>). This software allows for an easy and complete analysis of CUPrefs via COUSIN, includes seven other indices, and provides additional features such as statistical analyses, clustering, and CUPrefs optimization for gene expression. We illustrate the flexibility of COUSIN and highlight its advantages by analyzing the complete coding sequences of eight divergent genomes. Strikingly, COUSIN captures a bimodal distribution in the CUPrefs of human and chicken genes hitherto unreported with such precision. COUSIN opens new perspectives to uncover CUPrefs specificities in genomes in a practical, informative, and user-friendly way.

**Key words:** codon usage bias, mutational bias, translational selection, nucleotide composition, amino acid composition, codon adaptation index, bioinformatics, mutation–selection.

## Introduction

Translation of messenger RNAs (mRNA) into proteins is a central molecular biology process common to all forms of life. During translation, ribosomes proceed along the mRNA in steps of three nucleotides, called codons. The ribosome allows pairing of a mRNA codon against the complementary anticodon on a transfer RNA (tRNA), catalyzing the polymerization of amino acids to yield peptides and proteins (Quax et al. 2015). Sixty four nucleotide triplets are available and, in the standard genetic code, 61 codons encode for the 20 standard amino acids (Belalov and Lukashev 2013). Because of this asymmetry, certain groups of codons, known as “synonymous codons,” encode for the same amino acid (Nirenberg and Matthaei 1961; Khorana et al. 1966). Synonymous codons are not used with similar frequencies, resulting in so-called Codon Usage Preferences (CUPrefs) or Codon Usage bias. Different CUPrefs can be identified in regions within a gene, between genes within a genome and between genomes in different organisms (Grantham et al. 1980; Carbone et al. 2003).

A variety of indices have been developed since the 1980s to describe CUPrefs (Ikemura 1981; Freire-Picos et al. 1994;

Urrutia and Hurst 2001; Zhang et al. 2012). Most of them compare the CUPrefs of a query either against a reference set or against a Null Hypothesis (Shields et al. 1988; Lee et al. 2010). The “Codon Adaptation Index” (CAI; Sharp and Li 1987) and the “Effective Number of Codons” (ENC; Wright 1990) are respectively the most popular indices for each category. Numerous software packages to evaluate CUPrefs have been implemented, such as INCA (Supek and Vlahovicek 2004), JCAT (Grote et al. 2005) and CodonW (Peden and Sharp 2005). Most of them compute the CAI, sometimes the ENC, and occasionally other indices (Wan et al. 2004; Angellotti et al. 2007). Still, an important number of indices, such as the scaled  $\chi^2$  (Shields et al. 1988) or the “Maximum-likelihood Codon Bias” (MCB; Urrutia and Hurst 2001) cannot be calculated via any dedicated software.

Despite this profusion of alternatives, none of the available indices evaluates CUPrefs simultaneously against a reference and against a Null Hypothesis, thus hindering direct interpretation of the results. We conceived COUSIN (for COdon Usage Similarity INdex) as a score to estimate CUPrefs of a sequence compared with those of a reference, normalized over a Null Hypothesis of equal usage of synonymous codons.

© The Author(s) 2019. Published by Oxford University Press on behalf of the Society for Molecular Biology and Evolution.

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0/>), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact journals.permissions@oup.com

The output is normalized and allows for a straightforward biological interpretation. We have implemented COUSIN together with seven other existing indices in an eponymous Python3 software available for local or online use (<http://cousin.ird.fr>). To illustrate the power of COUSIN, we compare it to the well-known CAI by analyzing eight complete Coding DNA Sequence (CDSs) data sets from a range of organisms with large differences in nucleotide composition and genome organization.

### Measuring CUPrefs with COUSIN

In COUSIN, the CUPrefs of a query are compared with those of a reference data set, and the results of this comparison are normalized over a Null Hypothesis of equal usage of synonymous codons. The notations used as well as the detailed calculation steps are given in [table 1](#).

The amino acid composition of a sequence may affect its CUPrefs (Roth et al. 2012). We therefore conceived two variants of our index: In COUSIN<sub>18</sub> each of the 18 families of synonymous codons contributes equally to the global index, whereas in COUSIN<sub>59</sub> each family contributes proportionally to the frequency of the corresponding amino acid in the query. The classical CAI score would thus correspond thus to CAI<sub>59</sub>. For the sake of comparison we have defined the equivalent CAI<sub>18</sub> as described in [supplementary data 1, Supplementary Material](#) online. By comparing the “18” and “59” scores of an index, we can estimate the impact of amino acid composition on the CUPrefs of a sequence. The COUSIN score calculation involves five steps:

1. Calculate deviation scores ( $dev_{c,a}$ ) for each codon ( $c$ ) of each amino acid ( $a$ ) in the reference data set, compared with the Null Hypothesis:

$$dev_{c,a} = f_{c,a}^{ref} - f_{c,a}^{H_0} \quad (1)$$

where  $f_{c,a}^{ref}$  is the frequency of the codon  $c$  among its synonymous in the reference data set and  $f_{c,a}^{H_0}$  the corresponding frequency under the Null Hypothesis.

2. Define a weight for each codon ( $W_{c,a}$ ), by multiplying the codon frequency in the reference by its deviation score:

$$W_{c,a}^{ref} = f_{c,a}^{ref} \times dev_{c,a} \quad (2)$$

3. Repeat step 2 for the codon frequencies in the query:

$$W_{c,a}^{que} = f_{c,a}^{que} \times dev_{c,a} \quad (3)$$

Using the same deviation score to calculate the weights allows us to compare the scores of the query and of the reference.

**Table 1**

Notations Used to Define COUSIN and CAI Indices

Symbol	Description
$c$	Codon
$a$	Amino acid
$f$	Frequency
ref	Reference
que	Query
$H_0$	Null hypothesis
$L$	Query length
$k_a$	Set of synonymous codons coding for amino acid $a$
$\mathcal{A}$	Amino acids present in both query and reference
$\mathcal{N}$	Number of amino acids present in both query and reference

4. The COUSIN<sub>18</sub> <sup>$a$</sup>  score of each amino acid is the ratio of the sum of the weights of all synonymous codons for this amino acid in the query data set over the corresponding sum of the weights in the reference data set:

$$COUSIN_{18}^a = \frac{1}{\mathcal{N}} \times \frac{\sum_{c \in k_a} W_{c,a}^{que}}{\sum_{c \in k_a} W_{c,a}^{ref}} \quad (4)$$

where  $\mathcal{N}$  is the number of amino acids present in both the query and the reference and  $k_a$  is the set of synonymous codons coding amino acid  $a$ .

For COUSIN<sub>59</sub>:

$$COUSIN_{59}^a = f_a^{que} \times \frac{\sum_{c \in k_a} W_{c,a}^{que}}{\sum_{c \in k_a} W_{c,a}^{ref}} \quad (5)$$

where  $f_a^{que}$  is the frequency of the amino acid  $a$  in the query.

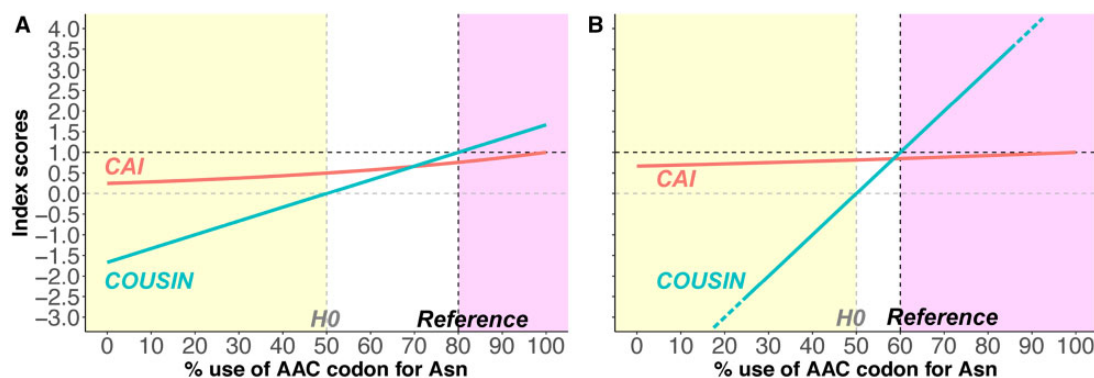
5. The final COUSIN score is obtained by adding the individual COUSIN scores of all amino acids:

$$COUSIN_{18} = \sum_{a \in \mathcal{A}} COUSIN_{18}^a \quad (6)$$

$$COUSIN_{59} = \sum_{a \in \mathcal{A}} COUSIN_{59}^a \quad (7)$$

By design, the results of COUSIN have an immediate biological interpretation and are directly suitable for hypothesis testing ([fig. 1](#)):

- a COUSIN score of 1 indicates that the CUPrefs in the query are similar to those in reference data set;
- a COUSIN score of 0 indicates that the CUPrefs in the query are similar to those in the Null Hypothesis (i.e., equal usage of synonymous codons);
- above 1, CUPrefs in the query are similar to those in the reference but of larger magnitude;



**Fig. 1.**—COUSIN (blue curve) and CAI (red curve) scores (y-axis) for a set of hypothetical queries with different frequency for the AAC and AAU codons encoding the asparagine amino acid (x-axis). Values are calculated for a reference set using (A) a strong usage bias of AAC:AAU 80:20 and (B) a slight usage bias of AAC:AAU 60:40. Vertical dashed lines indicate the composition for the Null Hypothesis of equal usage of both codons (gray line) and for the corresponding reference (black line). Horizontal dashed lines show the COUSIN key values that correspond to the Null Hypothesis (gray line) and to the reference (black line). The yellow area indicates queries with CUPrefs opposite to those in the reference, white one queries with similar but weaker CUPrefs than the reference and pink one queries with similar and stronger CUPrefs than the reference. Notice that, by design, the COUSIN values are always 0 and 1 respectively for the H0 and for the reference, independently of the CUPrefs in the reference. By definition, CAI is bounded by 0 and 1. In this example, COUSIN scores below  $-3$  and above  $4$  are omitted to facilitate results visualization and reading.

- between 0 and 1, CUPrefs in the query are similar to those in the reference but of smaller magnitude;
- below 0, CUPrefs in the query are opposite to those in the reference;

Upper and lower boundaries to COUSIN values depend on the CUPrefs of the reference: The closer the CUPrefs of the reference are to the null hypothesis, the largest the range of the possible COUSIN scores. As an example, in the case of *Homo sapiens*, with a light global bias in CUPrefs, the range of possible COUSIN values is  $[-4.48; 6.13]$ . On the other hand, for *Plasmodium falciparum*, with a strong global bias in CUPrefs, the boundaries for COUSIN values are  $[0.15; 1.35]$ . To facilitate interpretation of CUPrefs, artificial boundaries can be given when calculating a COUSIN score. The COUSIN software, described below, proposes such solution.

## COUSIN Software

We designed a Python3 software package to implement COUSIN along with other seven existing indices to facilitate CUPrefs analysis and comparisons between methods. The COUSIN software and its documentation are accessible online at <http://cousin.ird.fr>. A local version can be downloaded from the same website to be used on a UNIX-like Operating System via command lines. For most tasks, the COUSIN software requires query sequences in a FASTA format and a reference data set in a kazusa-like format (Nakamura et al. 2000). The global architecture of the COUSIN software is described in [supplementary data 2, Supplementary Material](#) online.

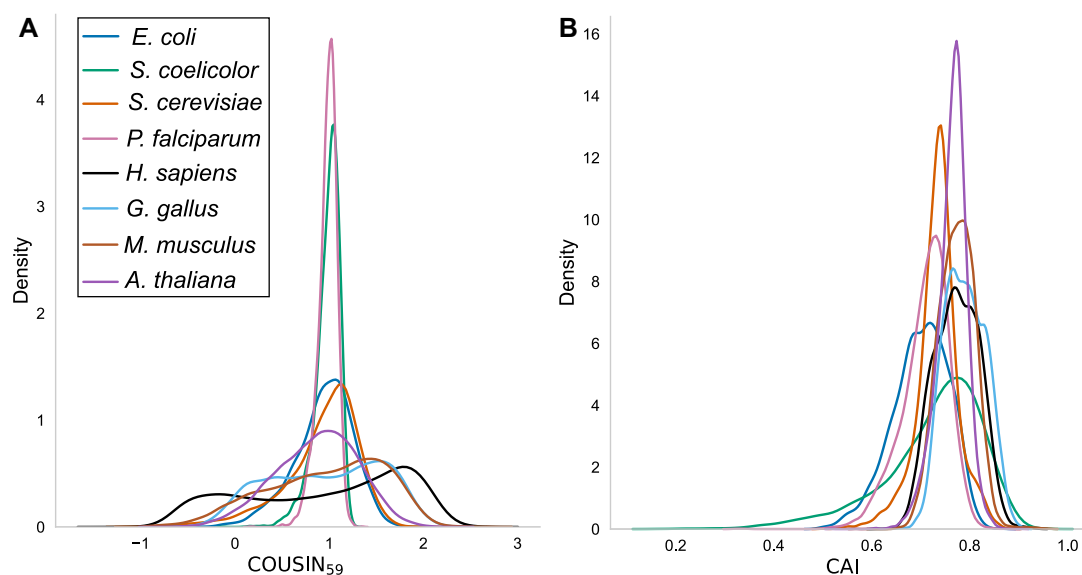
For any entry, the COUSIN software initially calculates basic nucleotide and amino acid composition statistics

and estimates CUPrefs. The COUSIN software currently features eight indices that evaluate CUPrefs: COUSIN, CAI (Sharp and Li 1987), ENC (Wright 1990), FOP (Ikemura 1981), SCUO (Angellotti et al. 2007), ICDI (Freire-Picos et al. 1994), CBI (Bennetzen and Hall 1982), and scaled  $\chi^2$  (Shields et al. 1988).

If instructed by the user, the COUSIN software performs simulations to assess whether the score of a query is statistically close to that of a standard CDS encoded by the reference ([supplementary data 2, Supplementary Material](#) online). The COUSIN software offers additional features to further analyze CUPrefs, such as a clustering analysis to group sequences according to their CUPrefs, or an optimization step to modify the CUPrefs of a sequence to adhere to those in the reference. The COUSIN software can also create a Codon Usage Table in a kazusa-like style from a set of sequences.

## COUSIN Analysis

We illustrate the potential of the COUSIN and compare it to the widely used CAI by performing an analysis on the complete CDSs of eight unrelated organisms with contrasted GC content: Two prokaryotes (*Escherichia coli*, *Streptomyces coelicolor*), a plant (*Arabidopsis thaliana*), a yeast (*Saccharomyces cerevisiae*), a protist (*P. falciparum*), a bird (*Gallus gallus*), and two mammals (*H. sapiens*, *Mus musculus*). We extracted the complete nuclear CDSs from these genomes using the Emboss extractfeat function (Rice et al. 2000). To avoid redundancy, when there were alternative spliced forms of a gene, only the first isoform was kept. Only CDSs  $>300$  nucleotides were kept for the analyses. Indeed, most CUPrefs methods show strong biases when analyzing sequences  $<100$  amino acids (Comeron and Aguadé 1998;



**Fig. 2.**—Density curves for COUSIN<sub>59</sub> (A) and CAI (B) indices for the complete CDSs of the eight organisms studied (see color legend). For each CDS the values for COUSIN<sub>59</sub> and CAI were calculated against the average codon usage reference table of the corresponding genome. The COUSIN<sub>59</sub> normalization renders curves centered around 1 allowing for rapid identification of differential dispersion in the leptokurtic curves for organisms with strong nucleotide compositional biases (e.g., *Streptomyces coelicolor*, in green) compared with those more platykurtic for organisms with weaker compositional biases (e.g., *Escherichia coli* in blue). Notice the bimodal distributions for *Homo sapiens* (black) and *Gallus gallus* (light blue) in panel A.

Roth et al. 2012). Further details about the selected sequences are in [supplementary data 3, Supplementary Material](#) online.

For each genome data set, we constructed a codon usage reference table using the corresponding the COUSIN software utility, and calculated the CUPrefs scores of each CDS against this reference. The resulting density curves for COUSIN<sub>59</sub> and CAI are presented in [figure 2](#). Details about this analysis are given in [supplementary data 4–6, Supplementary Material](#) online.

Analysing CDSs with COUSIN highlights shared patterns as well as differences between organisms. All COUSIN frequency curves ([fig. 2](#), panel A) are centered around 1 (i.e., with similar CUPrefs to those of the reference), but differ strongly in terms of dispersion and of the global shape of data distribution (unimodal, bimodal, or nearly flat). For *S. coelicolor* and *P. falciparum* COUSIN distributions are unimodal and display little variance, consistent with the strong nucleotide composition bias in these genomes (respectively 92.4% GC3 and 17.8% GC3). For other organisms with unimodal distribution but less biased nucleotide composition (e.g., *E. coli*, with 54.9% GC3), the distributions display a larger variance. For larger genomes with strong local differences in nucleotide composition (e.g., chromosome isochores in *H. sapiens*, and microchromosomes in *G. gallus*), the COUSIN frequency curves capture a hitherto not described bimodal shape of CUPrefs ([supplementary data 4, Supplementary Material](#) online). For the CAI results obtained with the same data sets ([fig. 2](#), panel B), all frequency curves display unimodal shapes while

exhibiting differences in their central value and dispersion, preventing direct contrast with one another.

The key difference between COUSIN and CAI resides in the direct interpretation of the COUSIN results. Indeed, the correlation between CAI and COUSIN scores for each CDS is strong and positive, ranging from 0.661 in *A. thaliana* to 0.978 in *S. coelicolor* (see complete comparisons in [supplementary data 6, Supplementary Material](#) online). However, for COUSIN we compare here the CUPrefs of individuals CDSs to a reference representing the average CUPrefs of the organism, therefore expecting—and obtaining—an average score close to 1. For CAI, the central value of the obtained distribution depends on the precise CUPrefs of the reference, and are therefore not comparable between organisms. This lack of normalization hampers any direct comparison of CAI values for genes against different reference sets. Furthermore, the COUSIN score seems to better capture the impact of the query's GC3 content on CUPrefs with, for instance, a Pearson correlation score of 0.91 between GC3 and COUSIN<sub>59</sub> and of 0.86 between GC3 and CAI for *H. sapiens* CDSs ([supplementary data 4, Supplementary Material](#) online).

## Discussion

A large number of indices have been conceived to evaluate CUPrefs. Nevertheless, in most cases they do not allow for a straightforward interpretation. As an example, the CAI is often considered as a direct measure of CUPrefs against a reference. However, the CAI value of the reference against itself

is different for each reference, preventing comparisons between genomes. Further, the CAI value of 1 is virtually never reached by any CDS in a given genome. Similarly, the different flavors of ENC (Wright 1990; Novembre 2002; Satapathy et al. 2017) allow to evaluate the presence and extent of CUPrefs against a Null Hypothesis of equal codon usage, but cannot inform on the precise trends of the detected CUPrefs.

We introduced here COUSIN, a new index to measure the CUPrefs of a sequence with respect to both a reference and a Null Hypothesis of equal usage of synonymous codons. The COUSIN value has a straightforward quantitative and qualitative meaning: It allows for an easy comparison 1) between the CUPrefs of the query CDS and those of both the reference and random CUPrefs, and 2) between queries and/or between data sets. We implemented the calculation of the COUSIN index, as well as of a number of additional features and existing indices to evaluate CUPrefs, into an eponymous bioinformatic software, available in a stand-alone as well as in an online version (COUSIN, at <http://cousin.ird.fr>).

We briefly illustrated the novelty and potential of the COUSIN by analyzing all CDSs in the genomes of eight divergent organisms. Taking the average genomic CUPrefs as a reference, we showed that COUSIN brings to light strong differences between CDSs within organisms, as well as between organisms. Such differences are far less obvious when using the CAI. Importantly, using the average genomic CUPrefs as a reference may or not be relevant when analyzing tissue or condition-dependent CUPrefs based on gene expression data. It remains the responsibility of the user to choose the appropriate reference and to interpret the results accordingly. Our results on differences in COUSIN values distribution and variance (exemplified by the bimodality in *H. sapiens* and *G. gallus*) demonstrate the power and utility of this novel index to identify differential heterogeneity between and within genomic data sets.

## Supplementary Material

Supplementary data are available at *Genome Biology and Evolution* online.

## Acknowledgments

J.B. is funded by a PhD fellowship from the French Ministry of Education and Research. This study was supported by the European Union's Horizon 2020 research and innovation program under the grant agreement CODOVIREVOL (ERC-2014-CoG-647916) to I.G.B. The authors acknowledge the CNRS and the IRD for additional, intramural support. The computational results presented have been achieved in part using the IRD Bioinformatic Cluster *itrop*, which also hosts the COUSIN

online server (<http://cousin.ird.fr>). We thank Frédéric Delsuc for driving our attention onto the composition particularities of the *G. gallus* genome.

## Literature Cited

- Angellotti MC, Bhuiyan SB, Chen G, Wan XF. 2007. CodonO: codon usage bias analysis within and across genomes. *Nucleic Acids Res.* 35(Web Server):W132–W136.
- Belalov IS, Lukashev AN. 2013. Causes and implications of codon usage bias in RNA viruses. *PLoS One* 8(2):e56642.
- Bennetzen JL, Hall BD. 1982. Codon selection in yeast. *J Biol Chem.* 257(6):3026–3031.
- Carbone A, Zinovyev A, Képès F. 2003. Codon adaptation index as a measure of dominating codon bias. *Bioinformatics* 19(16):2005–2015.
- Cameron JM, Aguadé M. 1998. An evaluation of measures of synonymous codon usage bias. *J Mol Evol.* 47(3):268–274.
- Freire-Picos MA, et al. 1994. Codon usage in *Kluyveromyces lactis* and in yeast cytochrome c-encoding genes. *Gene* 139(1):43–49.
- Grantham R, Gautier C, Gouy M, Mercier R, Pavé A. 1980. Codon catalog usage and the genome hypothesis. *Nucleic Acids Res.* 8(1):r49–r62.
- Grote A, et al. 2005. JCat: a novel tool to adapt codon usage of a target gene to its potential expression host. *Nucleic Acids Res.* 33(Web Server):W526–W531.
- Ikemura T. 1981. 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 Mol Biol.* 151(3):389–409.
- Khorana HG, et al. 1966. Polynucleotide synthesis and the genetic code. *Cold Spring Harb Symp Quant Biol.* 31:39–49.
- Lee S, Weon S, Lee S, Kang C. 2010. Relative codon adaptation index, a sensitive measure of codon usage bias. *Evol Bioinform Online.* 6:47–55.
- Nakamura Y, Gojobori T, Ikemura T. 2000. Codon usage tabulated from international DNA sequence databases: status for the year 2000. *Nucleic Acids Res.* 28(1):292.
- Nirenberg MW, Matthaei JH. 1961. The dependence of cell-free protein synthesis in *E. coli* upon naturally occurring or synthetic polyribonucleotides. *Proc Natl Acad Sci U S A.* 47(10):1588–1602.
- Novembre JA. 2002. Accounting for background nucleotide composition when measuring codon usage bias. *Mol Biol Evol.* 19(8):1390–1394.
- Peden J, Sharp P. 2005. CodonW: Correspondence analysis of Codon Usage. <http://codonw.sourceforge.net/>; last accessed November 2019.
- Quax TEF, Claessens NJ, Söll D, vanderOost J. 2015. Codon bias as a means to fine-tune gene expression. *Mol Cell.* 59(2):149–161.
- Rice P, Longden I, Bleasby A. 2000. EMBOSS: the European molecular biology open software suite. *Trends Genet.* 16(6):276–277.
- Roth A, Anisimova M, Cannarozzi G. 2012. Measuring codon usage bias. In *Codon Evolution Mechanisms and Models*, Chapter: 13. Oxford University Press.
- Satapathy SS, Sahoo AK, Ray SK, Ghosh TC. 2017. Codon degeneracy and amino acid abundance influence the measures of codon usage bias: improved Nc (Nc) and ENCprime (N'c) measures. *Genes Cells* 22(3):277–283.
- Sharp PM, Li WH. 1987. The codon Adaptation Index—a measure of directional synonymous codon usage bias, and its potential applications. *Nucleic Acids Res.* 15(3):1281–1295.
- Shields DC, Sharp PM, Higgins DG, Wright F. 1988. “Silent” sites in *Drosophila* genes are not neutral: evidence of selection among synonymous codons. *Mol Biol Evol.* 5(6):704–716.
- Supek F, Vlahovicek K. 2004. INCA: synonymous codon usage analysis and clustering by means of self-organizing map. *Bioinformatics* 20(14):2329–2330.

- Urrutia AO, Hurst LD. 2001. Codon usage bias covaries with expression breadth and the rate of synonymous evolution in humans, but this is not evidence for selection. *Genetics* 159(3):1191–1199.
- Wan XF, Xu D, Kleinhofs A, Zhou J. 2004. Quantitative relationship between synonymous codon usage bias and GC composition across unicellular genomes. *BMC Evol Biol.* 4(1):19.
- Wright F. 1990. The 'effective number of codons' used in a gene. *Gene* 87(1):23–29.
- Zhang Z, et al. 2012. Codon Deviation Coefficient: a novel measure for estimating codon usage bias and its statistical significance. *BMC Bioinformatics* 13(1):43.

**Associate editor:** Gwenael Piganeau