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AnaCoDa: Analyzing Codon Data with Bayesian mixture models

Cedric Landerer [1,2]*, Alexander Cope [3,5] Russell Zaretzki [2,4], and Michael A. Gilchrist [1,2]

[1] Department of Ecology and Evolutionary Biology, University of Tennessee, Knoxville, TN, USA. [2]National Institute for Mathematical and Biological Synthesis. [3]Genome Science and Technology, University of Tennessee, Knoxville, TN, USA. [4]Department of Statistics, Operations, and Management Science, University of Tennessee, Knoxville, TN, USA. [5]Oak Ridge National Laboratory, Oak Ridge, TN, USA.

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ABSTRACT

AnaCoDa is an R package for estimating biological parameters, such as selection against translation inefficiency, nonsense error rate, and ribosome pausing time, for sets of genes based on codon or amino acid data. **AnaCoDa** provides an adaptive Bayesian MCMC algorithm, fully implemented in C++ for high performance with an ergonomic R interface to improve usability. **AnaCoDa** employs a generic object-oriented design to allow users to extend the framework and implement their own models for analyzing biological data. Current models implemented in **AnaCoDa** can accurately estimate relevant parameters given either coding sequences or ribosome foot-printing data. Optionally, **AnaCoDa** can utilize additional data sources, such as gene expression measurements, to improve model fitting and parameter estimation. By utilizing a hierarchical object structure, some parameters can vary between sets of genes while others can be shared. Gene membership in a set can either be pre-assigned or estimated by **AnaCoDa** . This flexibility allows users to estimate the same model parameter under different biological conditions and categorize genes into different sets based on shared model properties embedded within the data. **AnaCoDa** also allows users to generate simulated data which can be used to aid model development and model analysis as well as evaluate model adequacy. Finally, **AnaCoDa** also comes with a set of visualization routines and the ability to revisit or reinitiate previous model fittings, providing researchers with a well rounded easy to use framework to analyze genome scale data.

Availability: **AnaCoDa** is freely available under the Mozilla Public License 2.0 on CRAN (`https://github.com/clandere/AnaCoDa`).

Contact: cedric.landerer@gmail.com

INTRODUCTION

The exponential increase in publicly available genomes over the past decade and the addition of novel technologies produced a vast amount of data for researchers. This influx of raw data necessitates the development of computational tools for extracting biological information. Such tools and models have to be developed and provided in easy to use software to allow researchers to analyze classical sequence data as well as novel data like ribosome foot-printing counts. Here, we describe **AnaCoDa** is an open-source software implemented in R (R Core Team, 2015) that allows researchers to analyze genome-scale data like coding sequences and ribosome foot-printing data using a Bayesian framework. Models described by Gilchrist *et al.* (2015), Wallace *et al.* (2013), and Shah and Gilchrist (2011) can be effectively fitted using **AnaCoDa** . The FONSE model analyzes ORF data for selection against of nonsense error rates. The PA and PANSE model use ribosome footprinting data to analyze for ribosome pausing time with and without nonsense error rate. **AnaCoDa** implements an adaptive Gibbs sampler within a Metropolis-Hastings Monte Carlo Markov Chain (MCMC) approach. This allows for the incorporation of prior knowledge and easy sampling from the posterior distribution to estimate parameter values and quantify the degree of uncertainty in these estimates. Currently, **AnaCoDa** provides three models to analyze codon counts obtained from coding sequences or ribosome foot-printing experiments. However, **AnaCoDa** provides a modular infrastructure such that additional genome scale or even phylogenetic models can be integrated. **AnaCoDa** provides a generic, mixture distribution option to all implemented models, allowing for estimation of condition specific parameters or the automatic categorization of data into different sets based on differences in their posterior probabilities of set membership.

The **AnaCoDa** framework works with gene specific data such as codon frequencies or position specific footprint counts. Conceptually, **AnaCoDa** uses three different types of parameters. The first type of parameters are gene specific parameters such as gene expression level or functionality. Gene-specific parameters are estimated separately for each gene and can vary between potential gene categories or sets. The second type of parameters are gene-set specific parameters, such as mutation bias terms or translation error rates. These parameters are shared across genes within a set and can be exclusive to a single set or shared with other sets. While the number of gene sets must be pre-defined by the user, set assignment

*to whom correspondence should be addressed

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of genes can be pre-defined or estimated as part of the model fitting. Estimation of the set assignment provides the probability of a gene being assigned to a set allowing the user to asses the uncertainty in each assignment. The third type of parameters are hyperparameters, such as parameters controlling the prior distribution for mutation bias or error rate. Hyperparameters can be set specific or shared across multiple sets and allow for the construction and analysis of hierarchical models, by controlling prior distributions for gene or gene-set specific parameters. In order to reduce the effect of the 'curse of dimensionality' on the sampling efficiency of the MCMC chain and allow flexibility in parallelization, **AnaCoDa** uses an adaptive Gibbs sampling approach where the MCMC sampling of one parameter type is conditioned on the other two types.

FEATURES

AnaCoDa provides an interface written in R, a freely available programming language noted for its ease of use for even inexperienced programmers. As a result, **AnaCoDa** is accessible to researchers with minimal computational experience.

The **AnaCoDa** interface is designed for quick and efficient data analysis. Generally, the only input needed for fitting a model to the data are protein-coding nucleotide sequences in the form of a FASTA file or a flat-file containing codon counts obtained from ribosome foot-printing experiments. If available, users may also provide additional types of data such as estimates of gene expression. **AnaCoDa** can simultaneously utilize the information embedded in these additional data types and/or estimate the error associated with it. **AnaCoDa** also provides visualization functionality, including plotting functions to compare parameter estimates for different mixture distributions and display codon usage patterns. In addition, diagnostic functions such as those for calculating and visualizing the degree of autocorrelation in MCMC samples are provided.

Robust and efficient model fitting **AnaCoDa** has built-in features designed to improve the robustness and performance of the implemented MCMC approach. For example, the implemented MCMC approach automatically adapts the proposal width for sampled parameters so that a user defined acceptance rate is met, improving sampling efficiency of the MCMC and computational performance. Even though **AnaCoDa** is written in C++, analysis of large datasets and/or complex models can be very computationally intensive. In order to protect users from computer failures or aid in the collection of additional MCMC samples, **AnaCoDa** periodically produces output files which can be used to restart an MCMC chain from a previous time point. In addition, **AnaCoDa** is capable of thinning the MCMC chain, meaning only every k^{th} sample is kept. Thinning increases the effective number of samples by reducing the auto-correlation between samples and reduces the amount of memory required by the underlying data structures. **AnaCoDa** is also able to create file R compatible representations of its parameter and MCMC objects. These objects can be loaded into the R environment for model analysis and visualization.

Although **AnaCoDa** is provided as an R package, the main computational work is implemented in C++. Because R does not provide native C++ support, we used the R package Rcpp which allows for the exposure of whole C++ classes as modules to R (Edelbuettel and Francois, 2011). Using Rcpp eliminates time consuming data transfer between the R environment and the C++ core during runs, resulting in improved computational performance and allowed for a fully object-oriented code design (Booch, 1993). As expected, the runtime of **AnaCoDa** scales linearly with genome size and number of iterations, and polynomial with the number of mixture distributions in the data set. The polynomial increase in the number of mixture distributions is explained by the necessity to estimate the protein production rate for each gene in each mixture distribution, as it is a gene specific parameter and the probability of a gene being assigned to a mixture has to be conditioned on it.

Data Simulation In addition to model fitting to actual datasets, **AnaCoDa** can be used to generate simulated data sets as well. On their own, simulated datasets are useful for model development and analysis. Simulating data under different conditions allows the user to explore model behavior. Different conditions can include the addition or elimination of parameters, or simply allowing a set of parameter values to vary. Fitting models to simulated data can provide users insight into potential pitfalls or shortcomings when fitting observational data and can serve as the basis for evaluating model adequacy of a model fit to observational data (Mi *et al.*, 2015). Significant differences between simulated and observational data suggests the current set of parameters or the model as a whole fail to include or adequately represent biological mechanisms underlying the observational data.

Available models **AnaCoDa** currently provides codon models for analyzing genome scale data. The ROC model extends the codon usage bias (CUB) model developed by Gilchrist *et al.* (2015); Wallace *et al.* (2013); Shah and Gilchrist (2011), which can reliably estimate the strength of selection on ribosome overhead cost, mutation bias and allows for the inference of protein synthesis rates. This model allows for the separation of effects of mutation and selection based on gene ordering by protein synthesis rate, and the added mixture distribution allows for gene clustering based on these effects. In addition to identifying the most efficient codons, ROC provides information on the direction of mutation bias, which can be used to approximate mutation rates between codons (see Gilchrist *et al.* (2015); Wallace *et al.* (2013)). The ability to estimate protein synthesis rates in the absence of empirical data is useful for investigating CUB of non-model organisms for which such data is lacking. These estimates may also be used for other models which require gene expression information. Use of the mixture model allows for the investigation of CUB heterogeneity at the genome or gene level. Additional models included in **AnaCoDa** provide estimates of codon-specific nonsense errors rates (FONSE) and ribosome pausing times (PA and PANSE).

Furthermore, **AnaCoDa** implements a ribosome Pausing (PA) model to estimate codon specific ribosome pausing times from ribosome foot-printing data.

Parameters estimated with these models can serve as expected results for empirical work. Assuming no major errors in the experimental procedure, significant deviations from the estimated parameters might suggest an underlying assumption of the model is violated or the selective forces involved are particularly weak. For example, if the estimated protein syntheis rates from ROC do not correlate with empirically measured protein synthesis rates for an organism, then selection for translation efficiency might be

relatively weak in this organism. These types of conflicts indicate the need for further empirical and computational studies. **AnaCoDa** allows for users to modify the current models to suit their needs, as well as implement new models for genomic data anaylsis.

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