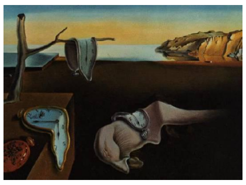

Journal of Statistical Software

November 2016, Volume 75, Issue 1. doi: 10.18637/jss.v075.i01

CLME: An R Package for Linear Mixed Effects Models under Inequality Constraints

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Abstract

In many applications researchers are typically interested in testing for inequality constraints in the context of linear fixed effects and mixed effects models. Although there exists a large body of literature for performing statistical inference under inequality constraints, user friendly statistical software implementing such methods is lacking, especially in the context of linear fixed and mixed effects models. In this article we introduce **CLME**, a package in the R language that can be used for testing a broad collection of inequality constraints. It uses residual bootstrap based methodology which is reasonably robust to non-normality as well as heteroscedasticity. The package is illustrated using two data sets. The package also contains a graphical user interface built using the **shiny** package.

Keywords: distribution free, linear inequality constraints, linear fixed effects models, linear mixed effects models, order restricted inference, residual bootstrap, R.

1. Introduction

Inequality constraints arise naturally in many applications. For example, to evaluate if a chemical is a toxin, a toxicologist may conduct a dose-response study to determine if the mean response is monotonic in dose. More precisely, suppose θ_i, $i = 1, \dots, p$ with $p \geq 2$, are the mean responses of a chemical corresponding to p dose groups. In this case the null and alternative hypotheses of interest are $H_0 : \theta_1 = \theta_2 = \dots = \theta_p$, and $H_a : \theta_1 \leq \theta_2 \leq \dots \leq \theta_p$, with at least one strict inequality (known as the *simple order* constraint), respectively. Sometimes, when the doses exceed the maximum tolerated dose (MTD), it may result in a dose-related toxicity and the monotonicity is violated causing down-turn at some (unknown) dose i (Simpson and Margolin 1986). In such cases, researchers are interested in testing for an umbrella alternative $H_{ai} : \theta_1 \leq \theta_2 \dots \leq \theta_{i-1} \leq \theta_i \leq \theta_{i+1} \geq \dots \geq \theta_p$, with at least one strict inequality.

In a multi-center rat uterotophic assay conducted by the OECD (Organization for Economic Cooperation and Development), researchers were interested in studying the effect of exposure to estrogen like compounds in the uterine weights of pre-pubertal rats. They were interested in testing if the mean uterine weights of animals exposed to estrogen like compounds increased in comparison to the uterine weights of control animals (Kanno, Onyon, Peddada, Ashby, Jacob, and Owens 2003). Thus the alternative hypothesis of interest is $H_a : \theta_1 \leq \theta_i$, $i \geq 2$, with at least one strict inequality, known as the *simple tree order*. Here θ_1 is the mean of the control group and θ_i, $i \geq 2$, are the means of the treatment groups.

In cancer trials, it is common for researchers to be interested in evaluating a cocktail of two or more experimental drugs in combination, each tried at low, medium and high doses. In such cases, the typical order restriction of interest is the *loop order* denoted by $\{\theta_{control,control} \leq \theta_{control,low} \leq \theta_{control,medium} \leq \theta_{high,high}\} \bigcup \{\theta_{control,control} \leq \theta_{low,control} \leq \theta_{medium,control} \leq \theta_{high,high}\}$, where $\theta_{a,b}$ denotes the mean response corresponding to ath dose of the first treatment and bth dose of the second treatment. The above null and alternative hypotheses can in general be expressed as $H_0 : \mathcal{C}\theta = \mathbf{c}$ and $H_a : \mathcal{C}\theta \geq \mathbf{c}$, respectively, where $\mathcal{C}$ is a suitable matrix of zeros, ones and negative ones of appropriate order, $\theta = (\theta_1, \theta_2, \ldots, \theta_p)^\top$ and $\mathbf{c}$ is a suitable vector of known scalars, for example a vector of zeros. Some examples of $\mathcal{C}$ and $\mathbf{c}$ are provided later, and an illustration of some common orders is given in Figure 1.

It is of common interest to perform statistical inference under inequality constraints, such as those described above, in a linear mixed effects model setting, especially in the context of a repeated measures design where a researcher may be interested in detecting trends. However, despite the existence of a large body of literature on constrained inference spanning over five decades and three books on testing for order restrictions (Barlow, Bartholomew, Brenner, and Brunk 1972; Robertson, Wright, and Dykstra 1988; Silvapulle and Sen 2005), it was only recently that researchers developed methods for performing constrained inference in linear mixed effects models (Rosen and Davidov 2012; Davidov and Rosen 2011; Farnan, Ivanova, and Peddada 2014). While Rosen and Davidov (2012) and Davidov and Rosen (2011) developed likelihood ratio based methods, Farnan *et al.* (2014) developed a residual bootstrap based method that is designed to be robust to non-normality as well as to heteroscedasticity. Furthermore, Farnan *et al.* (2014)'s methodology allows for modeling categorical as well as continuous covariates.

Surprisingly, not even the popular statistical analysis program SAS (SAS Institute Inc. 2011) has the capability to perform tests under general inequality constraints in a linear fixed effects model, let alone in the context of mixed effects models. As demonstrated in Farnan *et al.* (2014), statistical methods that are specifically designed for testing inequality constraints are expected to enjoy substantially higher power than the usual omnibus procedures (e.g., ANOVA) which are designed for two-sided alternatives. This observation, together with the fact that there does not exist a general software for performing statistical tests under linear inequality constraints in linear mixed effects models, motivates the current work. In this paper we introduce an R (R Core Team 2016) package, called **CLME** (for *c*onstrainted *l*inear *m*ixed *e*ffects; Jelsema and Peddada 2016) based on the distribution-free residual bootstrap methodology developed in Farnan *et al.* (2014) and available from the Comprehensive R Archive Network (CRAN) at `https://CRAN.R-project.org/package=CLME`. There are several packages in R which offer constrained fixed effects models, including **glmc** (Chaudhuri, Handcock, and Rendall 2012) and **ic.infer** (Grömping 2010), but neither of these appear to offer support for mixed models; the present work fills this void. Furthermore, since the methodology is based

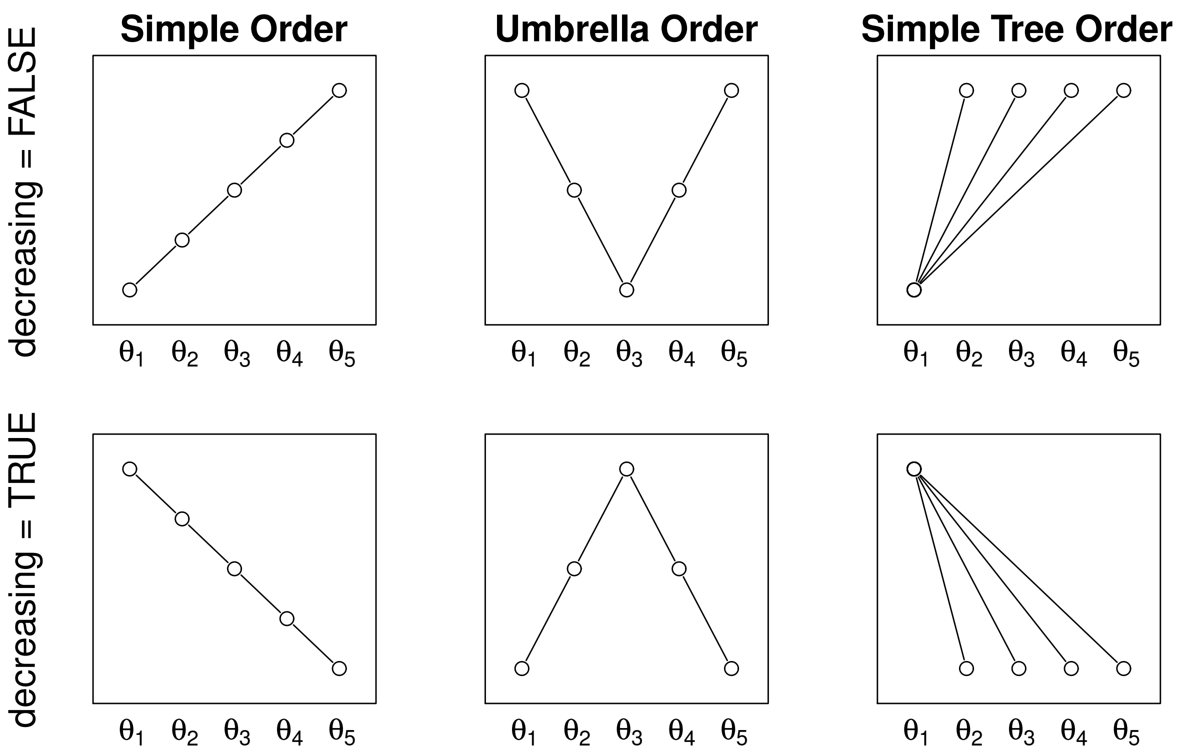


Figure 1: Illustration of order restrictions. Each circle represents a parameter of interest. Inequalities between two parameters (i.e., circles) are provided by the lines. The vertical axis denotes relative magnitude of connected parameters. No relationship (either $<$, $=$, or $>$) is known among parameters that are not connected. A nodal parameter is a parameter whose order relationship with every other parameter is known a priori or given by the hypothesis that is is being tested. For example, θ_3 is the nodal parameter in the umbrella orders.

on residual bootstrap, **CLME** does not depend on normality or homogeneity of variances for the residuals or random effects.

The rest of the paper is organized as follows: Section 2 provides a brief description of the constrained inference for linear mixed effects (LME) models presented by Farnan *et al.* (2014). Section 3 describes the contents of the package **CLME** along with implementation details. Section 4 provides some illustrative examples using the package, and Section 5 concludes the paper with a summary and some comments on planned developments of **CLME**.

2. Linear mixed effect models under inequality constraints

2.1. Definition of the model

Let

$$Y = \mathbf{X}_1\theta_1 + \mathbf{X}_2\theta_2 + \mathbf{U}\xi + \epsilon \tag{1}$$

denote a linear mixed effects (LME) model where Y is the $N \times 1$ response vector, $\mathbf{X}_1$ is a design matrix of order $N \times p_1$ and θ_1 is the corresponding $p_1 \times 1$ vector of coefficients (often treatment effects). $\mathbf{X}_2$ is an $N \times p_2$ known matrix of covariates, θ_2 is the $p_2 \times 1$ vector of regression coefficients, and $\mathbf{U}$ is an $N \times c$ matrix of known constants (random effects). For simplicity we write $\mathbf{X} = (\mathbf{X}_1 : \mathbf{X}_2)$ and $\mathbf{U} = (\mathbf{U}_1 : \mathbf{U}_2 : \ldots : \mathbf{U}_{c_q})$, where : denotes column-binding and $\mathbf{U}_i$ is an $N \times c_i$ matrix, with $\sum_{i=1}^{q} c_i = c$. We also denote $\theta = \left(\theta_1^\top, \theta_2^\top\right)^\top$ and $p = p_1 + p_2$.

The random vector $\xi = \left(\xi_1^\top, \xi_2^\top, \ldots, \xi_q^\top\right)^\top$ is $c\times 1$, where each ξ_i is a $c_i \times 1$ vector corresponding to $\mathbf{U}_i$, for $i = 1, \ldots, q$. The elements of ξ are independently distributed with mean $\mathbf{0}$ and covariance matrix $\mathbf{T} = \text{diag}\left(\tau_1^2\mathbf{I}_{c_1}, \tau_2^2\mathbf{I}_{c_2}, \ldots, \tau_q^2\mathbf{I}_{c_q}\right)$. The residual term ϵ is similarly defined with mean $\mathbf{0}$ and covariance matrix $\boldsymbol{\Sigma} = \text{diag}\left(\sigma_1^2\mathbf{I}_{n_1}, \sigma_2^2\mathbf{I}_{n_2}, \ldots, \sigma_k^2\mathbf{I}_{n_k}\right)$, where $i = 1, \ldots, k$ and $\sum_{i=1}^{k} n_i = N$.

Although the above model description and the methodology implemented in **CLME** allows for fairly general settings, in many applications one may not require the full available flexibility. For example, in most applications it may be sufficient to assume that $T = \tau^2\mathbf{I}$, instead of the general heteroscedastic structure for T described above.

Let $\mathcal{C}$ be an $r \times p$ matrix so that $\mathcal{C}\theta$ represents the linear combinations which are subject to inequality constraints specified by the alternative hypothesis. Thus the hypotheses of interest are given by:

$$H_o : \mathcal{C}\theta = \mathbf{0} \text{ versus } H_a : \mathcal{C}\theta \geq \mathbf{0}, \tag{2}$$

such that at least one of the r inequalities is strict. Farnan *et al.* (2014) suggested the pool adjacent violators algorithm (PAVA) to implement the order constraints (isotonization). We depart from their methodology in that we use the package **isotone** (De Leeuw, Hornik, and Mair 2009) for isotonization, and in particular the function `activeSet`. In some cases using active set methodology leads to the same results as using PAVA; though using active sets is a more general approach and enables easy specification of complex order restrictions. This also enables access to a number of *solvers* for `activeSet`, including least squares, least absolute deviation, and others.

CLME is designed to implement two general classes of statistical tests. The likelihood ratio type (LRT) statistic (Rosen and Davidov 2012) is the default setting, but the user may instead choose the Williams' type test statistic (Williams 1971, 1977; Peddada, Prescott, and Conaway 2001; Farnan *et al.* 2014). In both cases, to keep the methodology robust to non-normality and potential heteroscedasticity, the p values are evaluated using the residual bootstrap methodology developed in Farnan *et al.* (2014). Thus, although our likelihood ratio type statistic is motivated by the likelihood ratio principle under the normality assumption, it does not use the normal theory based asymptotic distribution for the test statistic. Hence we use the phrase "likelihood ratio *type* test" rather than "likelihood ratio test", and results from **CLME** will not always align with those of a direct implementation of Rosen and Davidov (2012).

2.2. Performance of residual bootstrap methodology

Farnan (2011) and later Farnan *et al.* (2014) investigated the performance of the residual bootstrap based test using the above defined likelihood ratio type test (LRT) statistic and the following Williams' type statistic (W) under a wide range of distributions and variance structures. For example, Farnan (2011) generated data from a wide range of non-normal distributions including, gamma, log-normal and mixture of normals and different patterns of variances to study the Type I error and power of these tests. A subset of these results were reported in Farnan *et al.* (2014). The residual bootstrap based methodology, using the above test statistics, generally performed well in terms of Type I errors and power. The Type I errors were generally close to nominal levels.

The Williams' type statistic is defined in general as:

$$W = \max\left\{\left[\mathcal{B}\tilde{\theta}_1\right] \odot \left[\sqrt{\operatorname{diag}\left\{\mathcal{B}\mathsf{VAR}(\hat{\theta})\mathcal{B}^\top\right\}}\right]^{-1}\right\}, \tag{3}$$

where $\odot$ denotes the Schur product of vectors, i.e., $\mathbf{a} \odot \mathbf{b} = (a_1b_1, a_2b_2, \ldots, a_rb_r)^\top$, $\tilde{\theta}_1$ denotes the estimator of θ_1 under the inequality constraint of interest, and $\hat{\theta}_1$ denotes the unconstrained estimator of θ_1 (e.g., the MLE). For a given order restriction specified by $\mathcal{C}$, the contrast matrix $\mathcal{B}$ is derived from the largest hypothesized difference(s); in the simple order this is the difference between θ_1 and θ_{p_1}. The structure of $\mathcal{B}$ is similar to that of $\mathcal{C}$, and is further described in the paragraph titled "Constraints" in Section 3.1.

3. Contents of CLME

In this section we describe the functions included in **CLME** and provide some notes on their implementation. We start by describing the main function of the package, `clme`. Afterwards, we detail some of the secondary functions which users may find useful.

3.1. Main function

The main function of **CLME** is `clme`. This function implements the order restricted residual bootstrap test described in Farnan *et al.* (2014). Among the arguments listed below, only the formula and the data set are required for the model to run. A series of flowcharts are provided in Appendix A (Figures 8–10) to guide a user through the specification of the arguments for `clme`.

`formula`: a formula expression; the constrained effect(s) must come before any unconstrained effects.

`data`: data frame containing the variables in the model.

`gfix`: optional vector of group levels for residual variances. Data should be sorted by this value.

`constraints`: list containing the constraints.

`tsf`: function to calculate the test statistic.

`tsf.ind`: function to calculate the test statistic for individual contrasts.

`mySolver`: solver to use in isotonization (passed to `activeSet`).

`verbose`: logical, prints iteration step. Argument can be a vector of multiple logicals; successive elements are passed to further functions.

`levels`**:** list to manually specify labels for constrained coefficients.

`ncon`**:** the number of constrained terms in the `formula`; the first `ncon` terms are constrained.

`...`**:** space for additional arguments.

Several of the arguments to `clme` require further explanation.

Formula. The formula should be a '`formula`' object following typical specification in R. That is, it is a two-sided expression using a tilde operator to separate the dependent variable from the independent variables. For example, the formula `y ~ x1 + x2` is interpreted as modeling `y` as a linear function of `x1` and `x2`. Random effects are specified in a similar manner as in the popular **lme4** package (Bates, Mächler, Bolker, and Walker 2015), using parentheses. For example, the formula `y ~ x1 + x2 + (1 | u)` is equivalent to the previous formula, but includes `u` as a random effect.

Note that `clme` runs a model without the intercept (and removes the intercept if it was requested). This is done so that the parameters are means of interest, rather than the intercept and offsets, thereby simplifying the computations involved.

Constraints. The argument `constraints` is a list describing the order restrictions using the following elements:

`order`**:** text string specifying the type of order. Allowed values are `"simple"`, `"umbrella"`, and `"simple.tree"`.

`node`**:** numeric indicating which element of θ_1 is the node.

`decreasing`**:** logical indicating decreasing order. For simple orders, a decreasing order implies a downward trend. For umbrella or simple tree orders, a decreasing order implies a decrease from the node. See Figure 1 for an illustration.

`A`**:** matrix describing the order restrictions in $\mathcal{C}$.

`B`**:** matrix of coefficients defining the Williams' type statistic (only necessary if Williams' type test is desired). This corresponds to $\mathcal{B}$ in Equation 3.

The values of `A` and `B` use the same format as the argument `isomat` from the function `activeSet` in package **isotone**: Each is a matrix with two columns where the rows define a specific constraints. For example, `A` and `B` are shown below for the decreasing umbrella order with $p_1 = 5$ and a node at $\theta_{1,3}$ (the third element of θ_1).

$$\texttt{A} = \begin{bmatrix} 1 & 2 \\ 2 & 3 \\ 4 & 3 \\ 5 & 4 \end{bmatrix}$$

and

$$\texttt{B} = \begin{bmatrix} 1 & 3 \\ 5 & 3 \end{bmatrix}.$$

The alternative hypothesis is $H_a : \theta_1 \leq \theta_2 \leq \theta_3 \geq \theta_4 \geq \theta_5$. The first row of `A` defines the constraint $\theta_1 \leq \theta_2$, the second row defines the constraint $\theta_2 \leq \theta_3$, and so on. The entries of the rows are the coefficient indices, and the parameter indexed in the left column is hypothesized to be less than or equal to that indexed by the right column. The `B` matrix is similarly structured, but defines only the contrasts for the largest hypothesized difference(s). Under the umbrella order, this will be the node compared to the first and last values; the specific form of the Williams' type statistic from Equation 3 is:

$$W = \max \left\{ \frac{\tilde{\theta}_3 - \tilde{\theta}_1}{\sqrt{\mathsf{VAR}\left(\hat{\theta}_3 - \hat{\theta}_1\right)}}, \frac{\tilde{\theta}_3 - \tilde{\theta}_5}{\sqrt{\mathsf{VAR}\left(\hat{\theta}_3 - \hat{\theta}_5\right)}} \right\},$$

hence the `B` matrix holds the contrasts $\tilde{\theta}_3 - \tilde{\theta}_1$ and $\tilde{\theta}_3 - \tilde{\theta}_5$.

Not all of the elements of `constraints` are necessary. There are three general formats by which to pass the constraints to `clme`.

Specific defaults: One may specify only the elements `order`, `node`, and `decreasing`. In this case the program will call an internal function to generate the values of `A` and `B`. Allowed values for `order` are `"simple"`, `"umbrella"`, and `"simple.tree"`; also, the node may be omitted for simple orders. Each of the three elements may also be vector-valued (e.g., `order = c("simple", "umbrella")`) to test multiple orders.

Custom constraints: Alternatively, the list of constraints may contain `A` directly. This is particularly useful for specifying custom order restrictions such as loop orders or block orders. When a custom `A` is passed, the program will ignore any values of `order`, `node`, and `decreasing`. If the Williams' type test is selected, a custom `B` is also needed.

Unspecified: Finally, `constraints` may be left unspecified. In this case the program will search for both simple and umbrella orders with all possible nodes, both increasing and decreasing orders. As with the first case, the program will estimate the order using the maximum test statistic of all the tested orders.

When testing multiple orders the test statistic is taken as the maximum of all the tested orders, and the program will note the order which produced this value as the estimated order. The bootstrap null distribution of the test statistic is constructed from all the order patterns under consideration, not just the estimated order (that is, for each bootstrap sample, the test statistic is computed for all candidate orders, and the maximum is taken). For reproducibility, one may use the `seed` argument to set the seed for the pseudo-random number generator.

Test statistics: `tsf` and `tsf.ind`. The argument `tsf` is a function which computes the desired global test statistic. This defaults to `lrt.stat`, the LRT statistic. Alternatively one may select the Williams' type statistic from Equation 3 by setting `tsf = w.stat`. The related argument `tsf.ind` computes the test statistic to test the individual constraints. The Williams' type test, `w.stat.ind`, is default. These two arguments are analogous to the global F test and pairwise t tests in the context of analysis of variance. For other test statistics, the user may submit a custom function for `tsf` and/or `tsf.ind`. We refer to the documentation of `lrt.stat` for more details on the format of custom test statistic functions.

The output from any custom `tsf` should be numeric. Output with length greater than 1 corresponds to multiple global hypotheses being tested. This should not be used for testing all the individual constraints from the `A` matrix, as these are calculated separately using the `tsf.ind` argument. If desired, the test statistic function should also specify the names attribute of the test statistic, for example naming the contrast. An example of testing multiple global hypotheses is shown in Section 4.2, a reanalysis of data from the Fibroid Growth Study (Peddada *et al.* 2008).

Homogeneity of variances: `gfix`. The model described in Section 2 permits flexibility. In particular, both ξ (if random effects are included) and ϵ may be modeled under the assumption of homogeneity or heterogeneity of variances. Currently, each random effect term is modeled with a separate variance component. The argument `gfix` defines groups for the residual variance(s). By default, the data are modeled with a single residual variance. If `gfix` is supplied, then each group of `gfix` is modeled with a separate residual variance. For example if the variable `x1` contains treatment groups, then `gfix = x1` will produce a residual variance for each treatment group.

The output of `clme` is a list with elements:

- `theta`: vector of estimates of fixed-effects coefficients, θ.
- `theta.null`: vector of estimates of θ under the null hypothesis.
- `ssq`: estimate of the residual variance(s), σ_i^2, $i = 1, \ldots, k$.
- `tsq`: estimate of the random effect variance component(s), τ_i^2, $i = 1, \ldots, q$.
- `cov.theta`: the covariance matrix of the unconstrained estimates of θ.
- `ts.glb`: test statistic for the global hypothesis.
- `ts.ind`: vector of test statistics for each of the constraints (each row of `A`).
- `mySolver`: the solver used in `activeSet`.
- `constraints`: List containing the constraints (`A`) and the contrast for the global test (`B`).
- `dframe`: data frame containing the variables in the model.
- `search.grid`: matrix containing the orders to perform a search over.
- `cust.const`: logical, whether custom constraints were specified, or constraints were generated.
- `ncon`: the number of constrained effects.
- `tsf`: function to calculate the test statistic.
- `tsf.ind`: function to calculate the test statistic for individual contrasts.
- `residuals`: matrix containing residuals. For mixed models three types of residuals are given.

- `random.effects`: predicted values of the random effects.
- `gfix`: group sample sizes for residual variances.
- `gfix_group`: group membership to define residual variances.
- `gran`: group sizes for random effect variance components.
- `formula`: the formula used in the model.
- `call`: the function call.
- `P1`: the number of constrained parameters.
- `mq.phi`: initial values for the random effect variance components.
- `order`: list describing the specified constraints.

The function `clme` only fits the model. The user should run the `summary` method on the fitted '`clme`' object to obtain the bootstrap test. The `summary` method accepts arguments `object` (an object of class '`clme`', the output from function `clme`) as well as `nsim`, the number of bootstrap simulations to perform, and for reproducibility, `seed`, the seed for the random number generator (RNG). Both `nsim` and `seed` can be passed to `clme`, in which case the `summary` method will use those values. The output of the `summary` method returns the same fitted object, but appends p values.

3.2. Secondary function

Typical use of **CLME** will involve fitting a '`clme`' object, and then calling the `summary` method for '`clme`' objects to conduct inference. In the course of its evaluation, the `summary` function calls several other functions, of which the primary one is `resid_boot`. This performs the integral function of obtaining the bootstrap samples for inference. Some of the arguments for `resid_boot` are equivalent to those of `clme`, but some additional arguments are provided to allow users greater flexibility. The arguments to `resid_boot` are:

`formula`: a formula expression, the constrained effect should be the first term on the right-hand side.

`data`: data frame containing the variables in the model.

`gfix`: optional vector of group levels for residual variances.

`null.resids`: logical, whether to generate bootstrap samples under the null hypothesis. Defaults to `TRUE`.

`eps`: estimates of residuals.

`xi`: predicted values of the random effects.

`theta`: vector of fixed-effects coefficients.

`ssq`: vector of residual variance estimate(s).

`tsq`: vector of random effect variance component(s).

`cov.theta`: covariance matrix of the unconstrained fixed effects estimates.

`seed`: set the seed for the RNG.

`nsim`: number of bootstrap samples to generate (M).

`mySolver`: the solver to pass to `activeSet`.

`...`: space for additional arguments.

CLME constructs a bootstrap sample as follows:

Step 1: Obtain $\hat{\theta}_0$, the estimate of θ under the null hypothesis.

Step 2: Compute the observed values of the random effects and residuals. Denote $\hat{\boldsymbol{\Psi}} = \mathbf{U}\hat{\mathbf{T}}\mathbf{U} + \hat{\boldsymbol{\Sigma}}$, where $\hat{\mathbf{T}}$ and $\hat{\boldsymbol{\Sigma}}$ are the estimates of $\mathbf{T}$ and $\boldsymbol{\Sigma}$ described in Section 2. Then compute:

$$\hat{\boldsymbol{\epsilon}} = \left(\mathbf{I} - \mathbf{X}\left(\mathbf{X}^\top \hat{\boldsymbol{\Psi}}^{-1}\mathbf{X}\right)^{-1}\mathbf{X}^\top \hat{\boldsymbol{\Psi}}^{-1}\right)\mathbf{Y},$$

and

$$\hat{\boldsymbol{\xi}} = \hat{\mathbf{T}}\mathbf{U}^\top \hat{\boldsymbol{\Psi}}^{-1}\hat{\boldsymbol{\epsilon}}.$$

Step 3: Standardize the observed values of the random effects and residuals. Define $\hat{\boldsymbol{\delta}}_i = \frac{\hat{\boldsymbol{\xi}}_i}{sd\left(\hat{\boldsymbol{\xi}}_i\right)}$, $i = 1, \ldots, q$ and $\hat{\boldsymbol{\nu}}_i = \frac{\hat{\boldsymbol{\epsilon}}_i}{sd(\hat{\boldsymbol{\epsilon}}_i)}$, $i = 1, \ldots, k$, where $sd(\cdot)$ denotes the standard deviation of the elements in the vector.

Step 4: Obtain the bootstrap samples. Let $\hat{\boldsymbol{\nu}}^*$ denote a bootstrap sample of $\hat{\boldsymbol{\nu}}$ and let $\hat{\boldsymbol{\delta}}^*$ denote a bootstrap sample of $\hat{\boldsymbol{\delta}}$. Then define $\hat{\boldsymbol{\epsilon}}^* = \hat{\sigma}_i\hat{\boldsymbol{\nu}}_i^*$, $i = 1, \ldots, k$ and $\hat{\boldsymbol{\xi}}^* = \hat{\tau}_i\hat{\boldsymbol{\delta}}_i^*$, $i = 1, \ldots, q$. Finally, construct the final bootstrap sample as:

$$\mathbf{Y}^* = \mathbf{X}\hat{\theta}_0 + \mathbf{U}\boldsymbol{\xi}^* + \boldsymbol{\epsilon}^*.$$

In typical use `resid_boot` will follow the above procedure, and the arguments will be specified appropriately. However, for greater flexibility, the user may submit any numeric vector of the appropriate length for several of the values. In particular:

Fixed effects. The vector of fixed-effects coefficients is represented with `theta`. By default this will be $\hat{\theta}_0$, the estimate of θ under the null hypothesis. If the user wishes to center the bootstrap samples at a different location, they may submit an alternative vector for `theta`. For example, one may specify `theta` to be the fitted estimate of θ rather than the null estimate (a vector of length p). This will cause the bootstrap samples to be centered at the fitted alternative hypothesis rather than the null hypothesis (these two estimates of θ are available in a fitted '`clme`' object, elements `theta` and `theta.null`). Or the user may wish to center at an unconstrained point estimate, $\hat{\theta}$. Note that the only effect specifying `theta` will have is changing the fixed-effects estimate in Step 4 above. In particular, the observed random effects and residuals will not be affected. Also, `null.resids` must be set to `FALSE` for the specified value of `theta` to be used directly. Otherwise, `theta` will be projected onto the null hypothesis (so that $\mathcal{C}\theta = \mathbf{0}$) using `activeSet` from package **isotone**.

Variance estimates. The argument `ssq` denotes the vector of estimated residual variance terms, $\left(\hat{\sigma}_1^2, \ldots, \hat{\sigma}_k^2\right)$. If specified, then these values will be used in place of $\hat{\sigma}_i^2$, $i = 1, \ldots, k$ in Step 4 above. If `length(ssq) > 1` then the user should specify `gfix` as the residual variance groups as with the main function `clme`. The argument `tsq` is similarly defined, but containing the random-effect variance components $\left(\hat{\tau}_1^2, \ldots, \hat{\tau}_q^2\right)$.

Residuals. The observed residuals are denoted by `eps`. By default these are calculated as shown for $\hat{\epsilon}$ in Step 2 above, the residuals from an unconstrained point estimate (e.g., generalized least squares). The user may supply an $N \times 1$ vector containing residuals calculated in some other fashion. Similarly, the observed random effects are denoted with `xi`, and by default are calculated as shown for $\hat{\boldsymbol{\xi}}$ in Step 2 above. Again, the user may submit a vector with alternative values for the observed random effects.

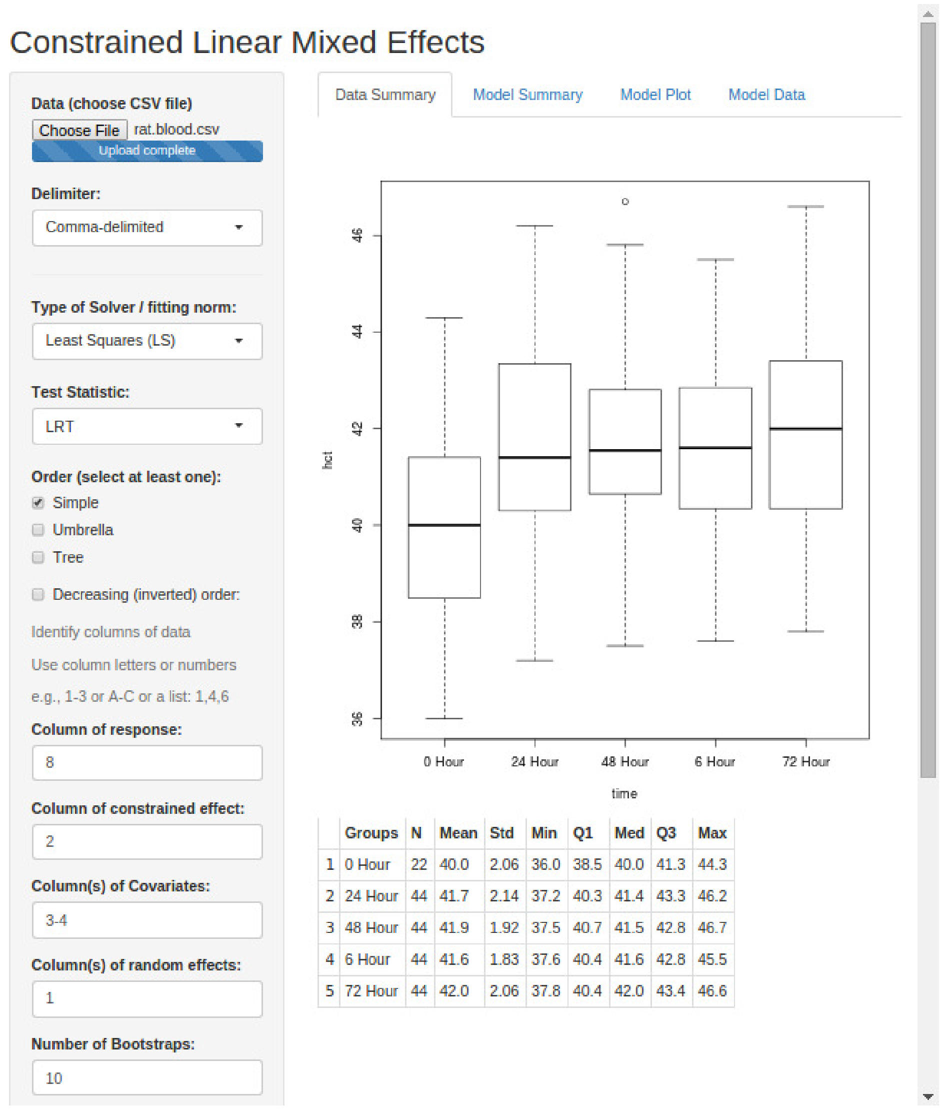
The only necessary arguments for `resid_boot` are `formula` and `data`, these will be used to obtain the model matrices. If provided, the values of `theta`, `ssq`, `tsq`, `eps` and `xi` will be used for bootstrapping. Any of these values that are left unspecified will be computed as described in Steps 1– 4 above.

3.3. Other package contents

shiny application. The **shiny** package (RStudio Inc. 2016) offers the ability to develop a graphical user interface (GUI) which implements **CLME**. A GUI developed in **shiny** can be run locally or deployed online. This is particularly beneficial to researchers who are not as familiar with R, or programming in general, but wish to use the methods described here. The package **CLME** includes a **shiny** application to run `clme`. After installing the package, a user may run the command `shiny_clme()` to call the GUI and begin using **CLME** without any need for further programming.

Figure 2 shows the GUI for `clme` with the arguments filled in. This example uses the `rat.blood` data set (which was printed to a comma-delimited file). The column headers are: id, time, temp, sex, wbc, rbc, hgb, hct, spun, mcv, mch, mchc, plts, and grp_ord. The final column was added to the data set, it contains the values '0 Hour', '6 Hour', '24 Hour', '48 Hour', and '72 Hour'. This column was added so that the proper order of groups for the constrained effect can be defined. A screenshot of the first 15 rows of this file can be seen in Figure 3.

First the user should browse to the data set (which should be a CSV file with the first row being a header), and then select the desired function arguments. Some arguments are provided by default (e.g., the global test statistic and the type of solver for isotonization). There are several check boxes to define the order, and then the user must tell the application which variables to use. The variables are identified using either their column number or column letter (e.g., 1 or A). Multiple variables may be separated by a comma (e.g., '1,2,4' or 'A,B,D'), a range of variables may be defined with a dash (e.g., '1–4' or 'A–D'), or a combination of the two can be used. These values should be set to 'None' to indicate no covariates or random effects. Group levels for the constrained effect may not be read into R with the correct order; an extra column may contain the ordered group levels (it may therefore have different length than the rest of the data set). The user is recommended to inspect the bottom of the summary output to verify the ordering of the group levels.



Constrained Linear Mixed Effects

Data (choose CSV file): rat.blood.csv

Delimiter: Comma-delimited

Type of Solver / fitting norm: Least Squares (LS)

Test Statistic: LRT

Order (select at least one):
[✓ Simple]
Umbrella
Tree

Decreasing (inverted) order:

Identify columns of data

Use column letters or numbers

e.g., 1-3 or A-C or a list: 1,4,6

Column of response: 8

Column of constrained effect: 2

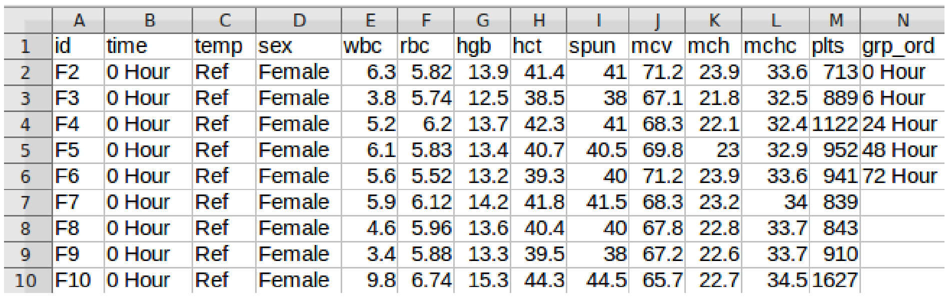
Column(s) of Covariates: 3-4

Column(s) of random effects: 1

Number of Bootstraps: 10

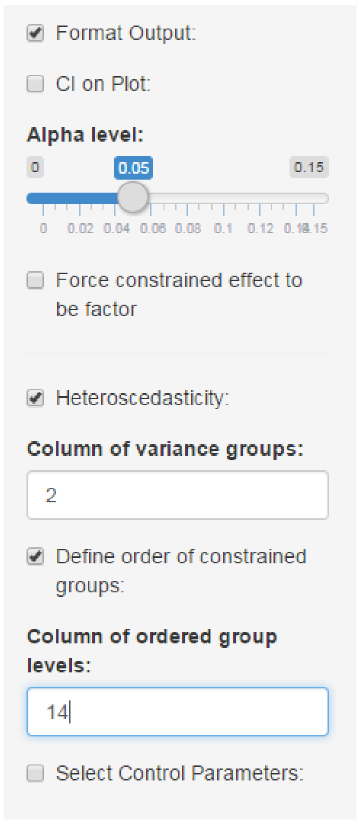
	Groups	N	Mean	Std	Min	Q1	Med	Q3	Max
1	0 Hour	22	40.0	2.06	36.0	38.5	40.0	41.3	44.3
2	24 Hour	44	41.7	2.14	37.2	40.3	41.4	43.3	46.2
3	48 Hour	44	41.9	1.92	37.5	40.7	41.5	42.8	46.7
4	6 Hour	44	41.6	1.83	37.6	40.4	41.6	42.8	45.5
5	72 Hour	44	42.0	2.06	37.8	40.4	42.0	43.4	46.6

Figure 2: Screenshot of the GUI for `clme`, built in **shiny**.



	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	id	time	temp	sex	wbc	rbc	hgb	hct	spun	mcv	mch	mchc	plts	grp_ord
2	F2	0 Hour	Ref	Female	6.3	5.82	13.9	41.4	41	71.2	23.9	33.6	713	0 Hour
3	F3	0 Hour	Ref	Female	3.8	5.74	12.5	38.5	38	67.1	21.8	32.5	889	6 Hour
4	F4	0 Hour	Ref	Female	5.2	6.2	13.7	42.3	41	68.3	22.1	32.4	1122	24 Hour
5	F5	0 Hour	Ref	Female	6.1	5.83	13.4	40.7	40.5	69.8	23	32.9	952	48 Hour
6	F6	0 Hour	Ref	Female	5.6	5.52	13.2	39.3	40	71.2	23.9	33.6	941	72 Hour
7	F7	0 Hour	Ref	Female	5.9	6.12	14.2	41.8	41.5	68.3	23.2	34	839	
8	F8	0 Hour	Ref	Female	4.6	5.96	13.6	40.4	40	67.8	22.8	33.7	843	
9	F9	0 Hour	Ref	Female	3.4	5.88	13.3	39.5	38	67.2	22.6	33.7	910	
10	F10	0 Hour	Ref	Female	9.8	6.74	15.3	44.3	44.5	65.7	22.7	34.5	1627	

Figure 3: Screenshot of the top several rows of the data set used in Figure 2.



☑ Format Output:

☐ CI on Plot:

Alpha level:

0.05

☐ Force constrained effect to be factor

☑ Heteroscedasticity:

Column of variance groups:

2

☑ Define order of constrained groups:

Column of ordered group levels:

14

☐ Select Control Parameters:

Figure 4: Some additional control parameters available in the GUI for `clme`.

If we wish to model `hct`, we specify column 8 for the response variable. Then the constrained effect is `time`, which is in column 2. The other covariates, `temp` and `sex`, are in columns 3 and 4, so we type '3–4' (alternatively, we could have typed '3,4', or 'C,D'), and finally the random effect, `id`, is in column 1. Since this is just for illustration, we specified only 10 bootstrap samples. Figure 2 shows the first panel of output, which contains summary information on the data set: a box plot of the response variable for each level of the constrained effect (if the constrained effect is a factor), and a table of descriptive statistics. The other three panels are: 'Model Summary', which provides the summary printout of the fitted model (several examples are shown in Section 4); 'Model Plot', which provides a plot of the fitted means with indication of significance (e.g., Figure 5); and 'Model Data' prints the data used for the analysis.

Figure 4 shows several of the optional parameters in the GUI. By clicking the 'Format Output' checkbox, one can request a confidence interval to show on the plot, adjust the Type I error rate, and force the constrained effect to be a factor (not all file imports will bring in text variables as a factor). The 'Heteroscedasticity' box bring up another input field to select a variable, which allows one to set the `gfix` parameter of `clme`. In this case, we have entered '2', which corresponds to the variable `time`. This has the effect of assuming each time group has a unique variance.

The checkbox 'Define order of constrained groups' will bring up another input field. Here we enter 14, to select the 'grp_ord' variable which was discussed above. Note on the box plot in

Generic name	Description
`AIC`	Computes Akaike information criterion.
`as`	Coerces an object to class '`clme`'.
`BIC`	Computes Bayesian information criterion.
`confint`	Computes individual confidence intervals for fixed effects parameters. Intervals are centered at the constrained estimates, but use standard errors of the unconstrained estimates.
`fixef`	Extracts estimates fixed-effects coefficients, θ (also, `fixed.effects` and `coef`).
`formula`	Extracts the formula for the model.
`is`	Tests if an object is of class '`clme`'.
`logLik`	Computes the log-likelihood under the assumption of normality.
`model.frame`	Data frame with the variables in the model.
`model.matrix`	The fixed-effects design matrix.
`nobs`	Number of observations.
`plot`	Produces a plot of the constrained coefficients and denotes statistical significance.
`print`	A basic printout of the model results.
`ranef`	Extracts predictions of the random effects (also, `random.effects`).
`residuals`	Extract various types of residuals.
`sigma`	The residual variance(s).
`summary`	Obtains inference (e.g., p values) for objects of class '`clme`'.
`VarCorr`	Estimates of variance components.
`vcov`	The variance-covariance matrix of the fixed-effects estimates.

Table 1: List of methods currently defined for objects of class '`clme`'. All methods extracting some value (e.g., `fixef`) will also work on the result of summary results of an object of class '`clme`'.

Figure 2 that the groups are out of order: Without this column, the time periods would be out of order: the '6 Hour' group would be placed between the '48 Hour' and '72 Hour' groups. By specifying this input, we correct the order (output not shown). The checkbox 'Select Control Parameters' allows one to set the seed for the RNG, as well as the maximum number of iterations and the convergence threshold for both the EM algorithm and the MINQUE algorithm.

Methods. The function `clme` outputs an object of the S3 class '`clme`'. The methods available for this class are briefly described in Table 1.

4. Sample implementation

In this section we demonstrate the use of **CLME** by applying it to two real-world data sets. Some of the analyses mimic those performed in the original papers but in the context of order-restricted inference. Other analyses are intended to exhibit certain features of the package or compare the available options. We emphasize that these analyses are intended not as a scientific reanalysis, but as an illustration. Consequently some modeling choices, the assumption of homogeneity of variances in particular, are not thoroughly investigated. The data analyzed are included in the package as the data sets `rat.blood` and `fibroid`.

4.1. Hematologic parameters from Sprague-Dawley rats

In a recent study on the effect the amount of time a sample is stored has on various hematological parameters, Cora, King, Betz, Wilson, and Travlos (2012) conducted a time course study using blood samples drawn from Sprague-Dawley rats. Blood samples from 11 female and 11 male rats were kept at either room temperature 21 °C (the control group) or refrigerated at 3 °C for 6, 24, 48 or 72 hours (see Cora *et al.* 2012 for more details). Although the authors obtained data on a variety of hematological variables in this repeated measure time course study, we shall focus on hematocrit (HCT) and the white blood cell (WBC) count over time. In the case of HCT we shall illustrate some of the options of **CLME** while testing for simple order with an increasing trend in time. In the case of WBC we test for simple tree order the mean WBC count in the freezer group was at least as high as that of the 0 hour.

First, we load the package and the data.

```
R> library("CLME")
R> data("rat.blood", package = "CLME")
```

Hematocrit. We illustrate **CLME** using three different settings. In the first case (Case A) we test the following hypotheses:

$$H_0 : \theta_1 = \theta_2 = \theta_3 = \theta_4 = \theta_5$$

vs.

$$H_{aA} : \theta_1 \leq \theta_2 \leq \theta_3 \leq \theta_4 \leq \theta_5, \tag{A}$$

with at least one strict inequality, here θ_i is the mean corresponding to either 0, 6, 24, 48 or 72 hours. In the second case (Case B), we test for a union of umbrella alternatives. If the null hypothesis is rejected then the algorithm selects the pattern that has largest value of test statistic:

$$H_0 : \theta_1 = \theta_2 = \theta_3 = \theta_4 = \theta_5$$

vs.

$$H_{aB} : \left\{ \bigcup_{i=1}^{5} \theta_1 \leq \theta_2 \leq \ldots \leq \theta_i \geq \ldots \geq \theta_5 \ \cup \ \bigcup_{i=1}^{5} \theta_1 \geq \theta_2 \geq \ldots \geq \theta_i \leq \ldots \leq \theta_5. \right\}. \tag{B}$$

Thus in (B) the order is unspecified but limited to either umbrella or inverted umbrella orders. Note that simple orders (increasing or decreasing) are a special case of umbrella orders, where the peak is the first or last parameter. The peak or the trough of each umbrella is specified using the specification of `node`. Case (C) is a repeat of case (A), but there we will assume heteroscedasticity of variances between the time groups.

We initially use the default arguments as far as possible. We use the gender of the rat and the storage temperature of the sample as covariates. The R code to test case (A) is provided below along with the results.

```
R> const <- list(order = "simple", node = 1, decreasing = FALSE)
R> hct1 <- clme(hct ~ time + temp + sex + (1 | id), data = rat.blood,
+    constraints = const, levels = list(2, levels(rat.blood$time)))
R> hct1b <- summary(hct1, seed = 42)
R> hct1b

Linear mixed model subject to order restrictions
Formula: hct ~ time + temp + sex + (1 | id) - 1

Order specified: increasing simple order

log-likelihood: -296.6
AIC:             611.3
BIC:             624.3
(log-likelihood, AIC, BIC computed under normality)

Global test:
 Contrast       Statistic  p-value
 Bootstrap LRT       0.17   0.0020

Individual Tests (Williams' type tests):
 Contrast           Estimate  Statistic  p-value
 6 Hour - 0 Hour       1.342      4.862   0.0000
 24 Hour - 6 Hour      0.086      0.399   0.1480
 48 Hour - 24 Hour     0.180      0.829   0.0550
 72 Hour - 48 Hour     0.150      0.693   0.1070

Variance components:
         Std. Error
id         1.483449
Residual   1.015556

Fixed effect coefficients (theta):
          Estimate  Std. Err  95% lower  95% upper
 0 Hour    40.9121    0.5226     39.888     41.936
 6 Hour    42.2542    0.5055     41.263     43.245
 24 Hour   42.3405    0.5055     41.350     43.331
 48 Hour   42.5201    0.5055     41.529     43.511
 72 Hour   42.6701    0.5055     41.679     43.661
 tempRT     0.5023    0.1531      0.202      0.802
 sexMale   -1.8333    0.6804     -3.167     -0.500
Std. Errors and confidence limits based on unconstrained covariance matrix

Parameters are ordered according to the following factor levels:
0 Hour, 6 Hour, 24 Hour, 48 Hour, 72 Hour

Model based on 1000 bootstrap samples
```

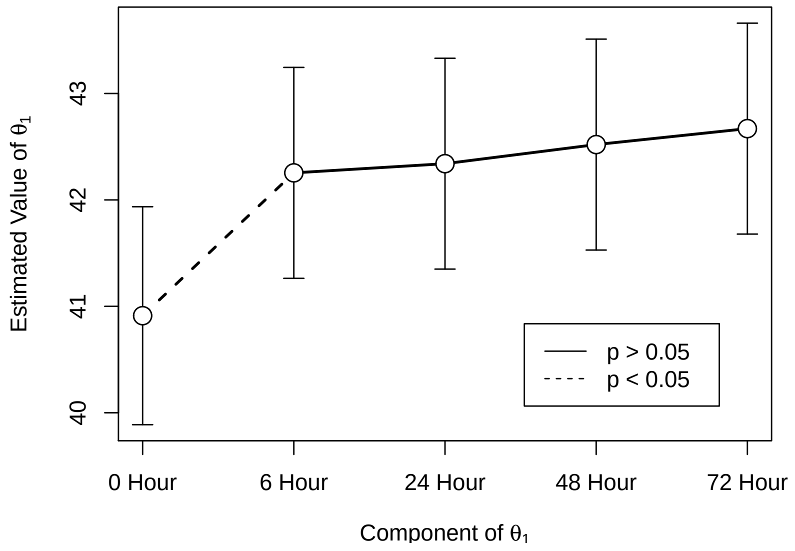


Figure 5: Plot of estimated coefficients of mean hematocrit (HCT) from Case (A). The model assumed an increasing simple order and homogeneity of variances across treatment groups. Solid lines denote no significant difference, while dashed lines denote statistical significance.

```
R> plot(hct1b, ci = TRUE, legendx = "bottomright", inset = 0.08)
```

We find strong evidence ($p = 0.002$) of an increasing pattern in mean HCT. The coefficients are plotted in Figure 5 with indications of significance for the individual contrasts.

To test case (B) we simply need to omit the constraints from the call to `clme`. The code and results are given below.

```
R> hct2 <- clme(hct ~ time + temp + sex + (1 | id), data = rat.blood,
+    levels = list(2, levels(rat.blood$time)))
R> summary(hct2, seed = 42)

Linear mixed model subject to order restrictions
Formula: hct ~ time + temp + sex + (1 | id) - 1

Order estimated: increasing simple order

log-likelihood: -296.6
AIC:             611.3
BIC:             624.3
(log-likelihood, AIC, BIC computed under normality)

Global test:
 Contrast       Statistic  p-value
 Bootstrap LRT       0.17   0.0070

Individual Tests (Williams' type tests):
 Contrast           Estimate  Statistic  p-value
 6 Hour - 0 Hour       1.342      4.862   0.0000
 24 Hour - 6 Hour      0.086      0.399   0.1480
 48 Hour - 24 Hour     0.180      0.829   0.0550
 72 Hour - 48 Hour     0.150      0.693   0.1070

Variance components:
         Std. Error
id         1.483449
Residual   1.015556

Fixed effect coefficients (theta):
          Estimate  Std. Err  95% lower  95% upper
 0 Hour    40.9121    0.5226     39.888     41.936
 6 Hour    42.2542    0.5055     41.263     43.245
 24 Hour   42.3405    0.5055     41.350     43.331
 48 Hour   42.5201    0.5055     41.529     43.511
 72 Hour   42.6701    0.5055     41.679     43.661
 tempRT     0.5023    0.1531      0.202      0.802
 sexMale   -1.8333    0.6804     -3.167     -0.500
Std. Errors and confidence limits based on unconstrained covariance matrix

Parameters are ordered according to the following factor levels:
0 Hour, 6 Hour, 24 Hour, 48 Hour, 72 Hour

Model based on 1000 bootstrap samples
```

Observe that the alternative hypothesis in (B) is much larger than the alternative hypothesis in (A). Thus, while the conclusions of tests for (A) and (B) are the same, that the parameters satisfy an increasing simple order, the *p* value associated with (B) is larger because the alternative hypothesis in (B) is larger than the alternative in (A).

Accounting for heteroscedasticity is simple in **CLME**. For example, suppose we wish to model each of the time points with a different residual variance. To do this we pass the time groups as the argument `gfix`, as shown below. We will call this case (C).

```
R> hct3 <- clme(hct ~ time + temp + sex + (1 | id), data = rat.blood,
+    gfix = rat.blood$time, constraints = const,
+    levels = list(2, levels(rat.blood$time)))
R> summary(hct3, seed = 42)

Linear mixed model subject to order restrictions
Formula: hct ~ time + temp + sex + (1 | id) - 1

Order specified: increasing simple order

log-likelihood: -291.4
AIC:             608.9
```

```
BIC:              627.7
(log-likelihood, AIC, BIC computed under normality)

Global test:
 Contrast       Statistic  p-value
 Bootstrap LRT      0.266   0.0010

Individual Tests (Williams' type tests):
 Contrast           Estimate  Statistic  p-value
 6 Hour - 0 Hour       1.326      4.937   0.0000
 24 Hour - 6 Hour      0.086      0.465   0.1250
 48 Hour - 24 Hour     0.180      0.902   0.0430
 72 Hour - 48 Hour     0.150      0.823   0.0890

Variance components:
        Std. Error
id       1.4597664
0 Hour   1.1073252
6 Hour   0.7380298
24 Hour  0.9852592
48 Hour  0.8780926
72 Hour  0.8315274

Fixed effect coefficients (theta):
          Estimate  Std. Err  95% lower  95% upper
 0 Hour    40.9258    0.5032     39.940     41.912
 6 Hour    42.2521    0.4625     41.346     43.159
 24 Hour   42.3385    0.4729     41.412     43.265
 48 Hour   42.5180    0.4681     41.601     43.435
 72 Hour   42.6680    0.4661     41.754     43.582
 tempRT     0.5341    0.1273      0.285      0.784
 sexMale   -1.8511    0.6345     -3.095     -0.608
Std. Errors and confidence limits based on unconstrained covariance matrix

Parameters are ordered according to the following factor levels:
0 Hour, 6 Hour, 24 Hour, 48 Hour, 72 Hour

Model based on 1000 bootstrap samples
```

White blood cell count. Using the white blood cell count data of Cora *et al.* (2012), we now illustrate our package for testing a simple tree order. Here the nodal parameter is taken to be the population mean corresponding to the 0 hour group. Since box plots of the residuals (not shown) suggested the variances were potentially equal across the groups, we assume homogeneity of variances. For illustration, in this example we use the Williams' type test statistic. The code and results are below, and the coefficients are plotted in Figure 6.

```
R> const <- list(order = "simple.tree", node = 1, decreasing = FALSE)
R> wbc <- summary(clme(wbc ~ time + temp + sex + (1 | id), data = rat.blood,
+    constraints = const, levels = list(2, levels(rat.blood$time)),
+    tsf = w.stat), seed = 42)
R> wbc

Linear mixed model subject to order restrictions
Formula: wbc ~ time + temp + sex + (1 | id) - 1

Order specified: increasing tree order with node at 1

log-likelihood: -236.3
AIC:            490.5
BIC:            503.6
(log-likelihood, AIC, BIC computed under normality)

Global test:
 Contrast          Statistic  p-value
 72 Hour - 0 Hour      5.207   0.0000

Individual Tests (Williams' type tests):
 Contrast          Estimate  Statistic  p-value
 6 Hour - 0 Hour      0.000      0.000   1.0000
 24 Hour - 0 Hour     0.409      2.240   0.0120
 48 Hour - 0 Hour     0.574      3.150   0.0000
 72 Hour - 0 Hour     0.949      5.207   0.0000

Variance components:
         Std. Error
id        1.2325597
Residual  0.6709482

Fixed effect coefficients (theta):
          Estimate  Std. Err  95% lower  95% upper
 0 Hour     5.4262    0.4007      4.641      6.212
 6 Hour     5.4262    0.3910      4.660      6.193
 24 Hour    5.8348    0.3910      5.068      6.601
 48 Hour    6.0007    0.3910      5.234      6.767
 72 Hour    6.3757    0.3910      5.609      7.142
 tempRT    -0.1947    0.1011     -0.393      0.004
 sexMale    1.8229    0.5336      0.777      2.869
Std. Errors and confidence limits based on unconstrained covariance matrix

Parameters are ordered according to the following factor levels:
0 Hour, 6 Hour, 24 Hour, 48 Hour, 72 Hour

Model based on 1000 bootstrap samples
```

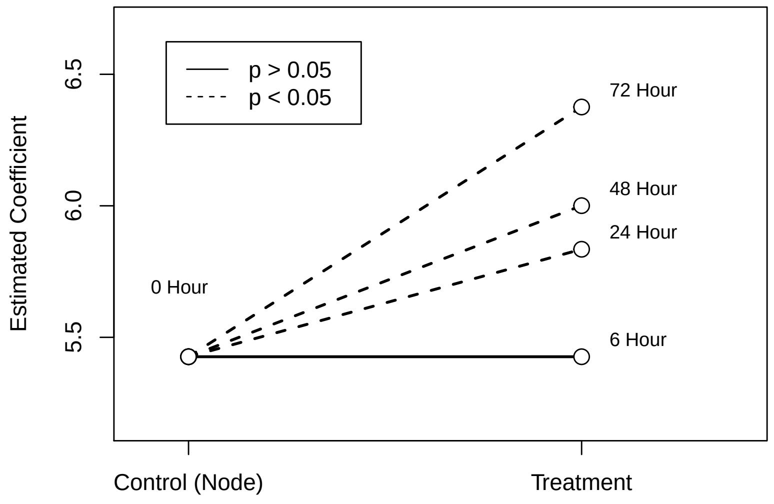


Figure 6: Plot of estimated coefficients of white blood cell (WBC) count. Solid lines denote no significant difference, while dashed lines denote statistical significance.

```
R> plot(wbc, legend = "topleft", inset = 0.08)
```

Our results are consistent with those of Cora *et al.* (2012), but we have in addition detected the 0 hour − 48 hour and 0 hour − 24 hour contrasts as being significant, which were not identified by Cora *et al.* (2012). There does appear to be an increasing pattern over time, but the differences from control are not statistically significant until sufficient time has passed.

As an alternative to Williams' type test, we repeated the analysis using the LRT (results not provided) and discovered that the LRT did not reject the null hypothesis at the 5% level of significance ($p = 0.252$). This discrepancy between Williams' type and LRT is not surprising in view of the simulation study reported in Farnan *et al.* (2014), which indicated that Williams' type test can be more powerful than LRT in some cases.

4.2. Fibroid growth rates

Peddada *et al.* (2008) investigated growth rate of uterine leiomyomata (fibroids) in black and white women. Since fibroids are hormonally mediated and there is a drop in estrogen levels as women age, it may be reasonable to hypothesize a reduction in fibroid growth rates. Interestingly, Peddada *et al.* (2008) reported that for white women the rate of growth of fibroids decreased with age (i.e., simple order with decreasing pattern), whereas they did not find any reduction in the average growth rate of fibroids with age for black women. They defined the three age groups as follows: Young (< 35), Middle (35–44), and Old (≥ 45). We shall now re-analyze their data using the methodology available in our package **CLME** where the alternative hypothesis for women of each race group is a decreasing simple order. Note that for confidentiality, we use a subset of the data from the Fibroid Growth Study, excluding cases which may be personally identifiable, particularly those with only one fibroid analyzed in the study. This subset of the data represents 240 fibroids on 54 women. The original analysis in Peddada *et al.* (2008) represented 262 fibroids on 72 women.

The interest in this case is to test for a simple order for *each* race using a linear mixed effects model. This analysis serves as a useful illustration of customizing the order restrictions, because it cannot be performed with the default settings of **CLME**. First we load the data and perform some manipulations to get a factor that we can use. We define the variable `race.age` to encode the interaction of the race and age variables; with six levels ordered as: young black, middle-age black, older black, young white, middle-age white, and older white.

```
R> data("fibroid", package = "CLME")
R> race.age <- factor(paste0(fibroid$race, ".", fibroid$age),
+    levels = c("Black.Yng", "Black.Mid", "Black.Old", "White.Yng",
+    "White.Mid", "White.Old"))
R> fibroid$race.age <- race.age
```

We performed our analysis adjusting for the initial fibroid volume as a covariate, which was grouped into three categories: < 14 cm^3, 14–65 cm^3, ≥ 65 cm^3 with the < 14 category taken to be the baseline. To deal with repeated measurements, we took subject ID as the random effect.

```
R> fibroid$initVol <- cut(fibroid$vol, c(0, 14000, 65000, Inf),
+    c("small", "medium", "large"), right = FALSE)
```

For the interaction between the age and race terms, we require constraints which define a decreasing simple order over age for both blacks and whites, but do not impose any order restriction *between* blacks and whites. We do this as follows:

```
R> const <- list(A = cbind(2:6, 1:5)[-3, ], B = rbind(c(3, 1), c(6, 4)))
R> const

$A
     [,1] [,2]
[1,]    2    1
[2,]    3    2
[3,]    5    4
[4,]    6    5

$B
     [,1] [,2]
[1,]    3    1
[2,]    6    4
```

To understand the construction of these matrices, recall the parameter vector θ_1 is ordered as: young black, middle-age black, older black, young white, middle-age white, and older white. The groups of the constrained effect are transformed into column indicators, meaning there will be six parameters: $(\theta_{YB}, \theta_{MB}, \theta_{OB}, \theta_{YW}, \theta_{MW}, \theta_{OW})$, where YB denotes 'young black', MB denotes 'middle-aged black', and so on. Hence, the first three elements of θ correspond to the blacks, and the last three elements correspond to the whites.

The `A` matrix must define the proper order restriction on these elements. The first row defines the constraint $\theta_{MB} \leq \theta_{YB}$, the first row defines the constraint $\theta_{OB} \leq \theta_{MB}$. The second two rows define similar constraints for the white women. None of the rows define a restriction between any of the first three elements (blacks) and any of the last three elements (whites); hence there is no order restriction imposed between the two races.

To test for a decreasing simple order for both blacks and whites, we must also define a function to compute the Williams' type test statistic of Farnan *et al.* (2014) for both blacks and whites separately. While the matrix of contrasts is provided above, by default the Williams' type test will take the maximum and report a single test statistic. We require a test statistic for each of these contrasts. This is similar to the function `w.stat.ind` which calculates the test statistics for the individual constraints. However, submitting `tsf = w.stat.ind` will test all of the constraints in the matrix `constA` instead of the contrasts in `constB`. To correct this, we make a small modification to `w.stat.ind`.

```
R> w.blk.wht <- function (theta, cov.theta, B, A, ...) {
+    stats <- vector("numeric", length = nrow(B))
+    ctd <- diag(cov.theta)
+    stats <- apply(B, 1, FUN = function(a, theta, cov, ctd) {
+      std <- sqrt(ctd[a[1]] + ctd[a[2]] - 2 * cov.theta[a[1], a[2]])
+      (theta[a[2]] - theta[a[1]]) / std
+    }, theta = theta, cov = cov.theta, ctd = ctd)
+    names(stats) <- c("Black.Yng - Black.Old", "White.Yng - White.Old" )
+    return(stats)
+  }
```

All we have done is replace calls to `A` with calls to `B`; this will accomplish our goal of producing a global test for both blacks and whites individually.

We are then ready to run the analysis. For simplicity, we assume homogeneity of variances. Results of the analysis are shown in Figure 7. The code for this figure is given below, since it cannot be produced through **CLME**.

```
R> fib <- summary(clme(lfgr ~ race.age + initVol + (1 | id), data = fibroid,
+    constraints = const, tsf = w.blk.wht,
+    levels = list(10, levels(race.age))), seed = 42)
R> fib

Linear mixed model subject to order restrictions
Formula: lfgr ~ race.age + initVol + (1 | id) - 1

Custom order constraints were provided

log-likelihood: -1085
AIC:             2189
BIC:             2206
(log-likelihood, AIC, BIC computed under normality)

Global tests:
 Contrast               Statistic  p-value
 Black.Yng - Black.Old      1.116   0.1880
 White.Yng - White.Old      2.270   0.0150

Individual Tests (Williams' type tests):
 Contrast              Estimate  Statistic  p-value
 Black.Yng - Black.Mid    8.7520      1.454   0.0580
 Black.Mid - Black.Old    0.0000      0.000   1.0000
 White.Yng - White.Mid    10.391      1.217   0.0640
 White.Mid - White.Old    7.7030      1.059   0.0830

Variance components:
         Std. Error
id         10.37229
Residual   20.47316

Fixed effect coefficients (theta):
                Estimate  Std. Err  95% lower  95% upper
 Black.Yng        21.471     5.087     11.501     31.441
 Black.Mid        12.719     3.910      5.055     20.383
 Black.Old        12.719     6.277      0.416     25.023
 White.Yng        21.795     6.746      8.573     35.016
 White.Mid        11.403     5.751      0.132     22.674
 White.Old         3.700     4.872     -5.848     13.249
 initVolmedium    -4.720     3.201    -10.995      1.554
 initVollarge     -3.641     3.854    -11.194      3.912
Std. Errors and confidence limits based on unconstrained covariance matrix

Parameters are ordered according to the following factor levels:
Black.Yng, Black.Mid, Black.Old, White.Yng, White.Mid, White.Old

Model based on 1000 bootstrap samples
```

```r
R> plot(x = 1, y = 0, col = 0, ylim = c(-6, 22), xlim = c(0.9, 3.1),
+    xlab = "", ylab = "Estimated Coefficient", xaxt = "n")
R> axis(side = 1, at = 1:3,
+    labels = c("Young (<35)", "Middle aged (35-44)", "Older (>45)"))
R> for (y1 in seq(-15, 25, 5)) {
+    lines( x = c(0, 7), y = c(y1, y1), col = "gray", lty = 2)
+  }
R> lty1 <- 1 + (fib$p.value.ind[1] < 0.05)
R> lty2 <- 1 + (fib$p.value.ind[2] < 0.05)
R> lty3 <- 1 + (fib$p.value.ind[3] < 0.05)
R> lty4 <- 1 + (fib$p.value.ind[4] < 0.05)
R> points(c(1, 2), fib$theta[1:2], col = 1, type = "l", lwd = 2 , lty = lty1)
R> points(c(2, 3), fib$theta[2:3], col = 1, type = "l", lwd = 2 , lty = lty2)
R> points(c(1, 2), fib$theta[4:5], col = 3, type = "l", lwd = 2 , lty = lty3)
R> points(c(2, 3), fib$theta[5:6], col = 3, type = "l", lwd = 2 , lty = lty4)
R> points(1:3, fib$theta[1:3], col = 1, cex = 1.5, pch = 21, bg = "white")
```

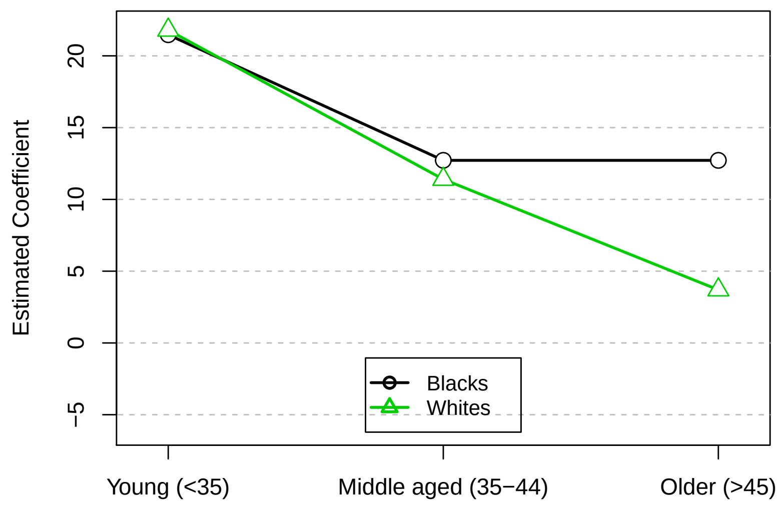


Figure 7: Plot of estimated coefficients of 6-month mean fibroid growth by race and age group. Black lines with circles correspond to Blacks, green lines with triangles correspond to Whites. Growth rates for each fibroid were averaged over the 2–4 time points. None of the individual constraints were significant. The global tests found a significant decreasing trend for white women, but not for black women.

```
R> points(1:3, fib$theta[4:6], col = 3, cex = 1.5, pch = 24, bg = "white")
R> legend("bottom", lty = c(1, 1), pch = c(21, 24), col = c(1, 3), pt.bg = 0,
+    pt.cex = 1.1, lwd = 2, inset = 0.03, cex = 0.9,
+    legend = c("Blacks    ", "Whites"))
```

The global tests found significant evidence of a decreasing simple order for white women ($p = 0.0150$) but not for black women ($p = 0.1880$). In particular, fibroid growth in older white women was found to be less than that of younger women. Neither of the individual contrasts for the white women (Young-Middle and Middle-Old) were significant at the $\alpha = 0.05$ level. The significant decreasing trend confirms the conclusions of Peddada *et al.* (2008).

4.3. Comparison with other packages

In this section we provide some comparisons of **CLME** to **lme4**, which is a popular package for the analysis of linear mixed models. We will use the `rat.blood` data, and in particular the response variable `hct`. We chose this variable because the observed means at each time point happen to be ordered according to a simple order. Hence, the fitted means will not be changed by `clme`, so the results between `clme` and `lmer` will be more comparable.

```
R> library("lme4")
R> cons  <- list(order = "simple", decreasing = FALSE, node = 1)
R> clme1 <- clme(hct ~ time + temp + sex + (1 | id), data = rat.blood,
+    constraints = cons)
R> lmer1 <- lmer(hct ~ time + temp + sex + (1 | id) - 1, data = rat.blood)
R> lmer2 <- lmer(hct ~ time + temp + sex + (1 | id) - 1, data = rat.blood,
+    REML = FALSE)
```

```
R> rbind(fixef(clme1), fixef(lmer1), fixef(lmer2))

     time0 Hour time6 Hour time24 Hour time48 Hour time72 Hour    tempRT
[1,]   40.91212   42.25417    42.34053    42.52008    42.67008 0.5022727
[2,]   40.91212   42.25417    42.34053    42.52008    42.67008 0.5022727
[3,]   40.91212   42.25417    42.34053    42.52008    42.67008 0.5022727
       sexMale
[1,] -1.833333
[2,] -1.833333
[3,] -1.833333

R> VarCorr(clme1)

         Std. Error
id         1.483449
Residual   1.015556

R> VarCorr(lmer1)

 Groups   Name        Std.Dev.
 id       (Intercept) 1.55933
 Residual             0.90356

R> VarCorr(lmer2)

 Groups   Name        Std.Dev.
 id       (Intercept) 1.48486
 Residual             0.89063

R> c(AIC(clme1), AIC(lmer1), AIC(lmer2))

[1] 611.2926 613.7119 605.7245
```

These results indicate that, when the observed means do not violate the assumed order, `clme` produces results that are similar to those of `lmer`. The variance estimates (and hence the log-likelihood and AIC) differ somewhat but, as shown, this is also true when using `lmer` by specifying a different optimization criterion (`REML = FALSE`).

Users should be aware that `clme` is less computationally efficient than `lmer`. This should not be entirely surprising, as **lme4** includes functions written in C++, while **CLME** is written entirely in R. While computation times depend on computer specifications, we provide some time relative time benchmarks between the packages using **rbenchmark** (Kusnierczyk 2012). First we compare the relative time for model fitting.

```
R> library("rbenchmark")
R> set.seed(42)
R> BMclme <- function(x = 0) {
```

```
+    clme(hct ~ time + temp + sex + (1 | id), data = rat.blood,
+      constraints = cons)
+  }
R> BMlmer <- function(x = 0) {
+    lmer(hct ~ time + temp + sex + (1 | id), data = rat.blood)
+  }
R> benchmark(BMlmer(), BMclme(), replications = 100)[, 1:4]

      test replications elapsed relative
2 BMclme()          100  33.333   16.559
1 BMlmer()          100   2.013    1.000
```

As one can see, `clme` is significantly slower relative to `lmer`, though note that the elapsed time is over 100 runs of each functions; in this test an average run of `clme` evaluated in less than 1 second. Additionally, we tested the speed of the bootstrap functions of **CLME** and **lme4**. Note that for simplicity we used 100 bootstrap samples for each, and only performed 10 replications for time benchmarks.

```
R> set.seed(42)
R> beta_sum <- function(.) {
+    beta = fixef(.)
+  }
R> BMclme <- function(x = 0) {
+    summary(clme1, nsim = 100, seed = 42)
+  }
R> BMlmer1 <- function(x = 0) {
+    bootMer(lmer1, FUN = beta_sum, nsim = 100, seed = 42,
+      type = "parametric")
+  }
R> BMlmer2 <- function(x = 0) {
+    bootMer(lmer1, FUN = beta_sum, nsim = 100, seed = 42,
+      use.u = TRUE, type = "semiparametric")
+  }
R> benchmark(BMclme(), BMlmer1(), BMlmer2(), replications = 10)[, 1:4]

       test replications elapsed relative
1  BMclme()           10  33.849    2.955
2 BMlmer1()           10  11.465    1.001
3 BMlmer2()           10  11.453    1.000
```

While `lmer` still evaluates much faster than `clme`, the relative difference is not as extreme as for the model fitting.

5. Summary

In this paper we have introduced the R package **CLME** for performing statistical tests under linear inequality constraints. It allows the user to choose either the likelihood ratio type statistic or Williams' type statistic. Since it is based on the residual bootstrap methodology it is not dependent on any normality assumption. As demonstrated in the paper, the package is simple to implement with default settings (Section 4.1), and more complex hypotheses (Section 4.2) can be accommodated with relatively little effort.

Due to the flexibility and distribution-free nature of the model, as well as the ease of use, we anticipate that many researchers may benefit from using the order-restricted model implemented in **CLME** instead of standard ANOVA models. Other than this package, there does not appear to be any software which offers constrained inference for linear mixed effects models.

While the current release is stable, the authors have an interest in further developing the functionality of **CLME**. There are many potential improvements that we foresee. On the methodological side these include: adding more models, such as logistic models; implementing an automated choice of the number of bootstrap samples (see Jiang and Salzman 2012); allowing for correlated random effects; and adding the ability to perform power or sample size calculations. Furthermore, the software does not currently allow for complex covariance structures for the variance components, such as the AR(1) process, although it may be extended to accommodate such structures. Other projected developments include enabling the program to take advantage of parallel processing to speed up the repetitive calculations for each bootstrap sample. Finally, as noted, **shiny** offers the ability to create apps, making complex models easily available to researchers without the need to write R code. The included app can be run locally, but **shiny** apps can be hosted on a server and deployed online. A well-designed and web-based application could put the power and flexibility of **CLME** at a researcher's fingertips. Future developments include improving the app and deploying it online.

Acknowledgments

The first author was a Research Fellow at National Institute of Environmental Health Sciences (NIEHS) when this work was conducted. The **CLME** package will be maintained, and updated versions may be available on the Comprehensive R Archive Network (CRAN), located at `https://CRAN.R-project.org/package=CLME`. Developmental versions may be available at `https://github.com/jelsema/CLME`; issues/bug reports are also directed to this site.

This research is supported (in part) by the Intramural Research Program of the NIH, NIEHS (Z01 ES101744). The authors thank the following individuals: Drs. Katie O'Brien, Keith Shockley, and Bahjat Qaqish for carefully reading the manuscript and making numerous suggestions which substantially improved the presentation; Dr. Michelle Cora for providing the data on Sprague-Dawley rats analyzed in Section 4.1; and the editors of the Journal of Statistical Software and the two anonymous reviewers for providing suggestions which improved this work.

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A. Flowcharts to determine arguments

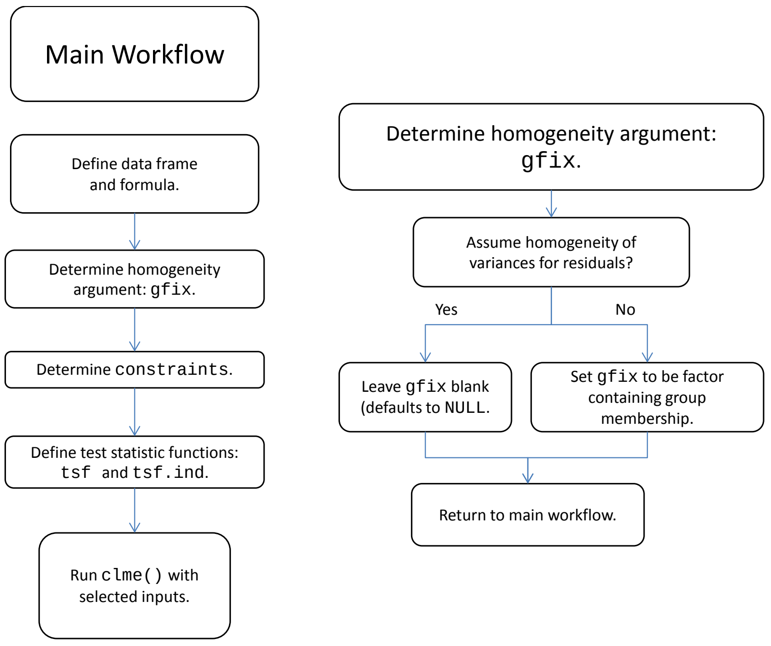
Main Workflow
Define data frame and formula.
Determine homogeneity argument: gfix.
Determine constraints.
Define test statistic functions: tsf and tsf.ind.
Run clme() with selected inputs.
Determine homogeneity argument: gfix.
Assume homogeneity of variances for residuals?
Yes
No
Leave gfix blank (defaults to NULL.
Set gfix to be factor containing group membership.
Return to main workflow.

Figure 8: Main flowchart to determine arguments (left) and flowchart to determine groups for residual variance (right).

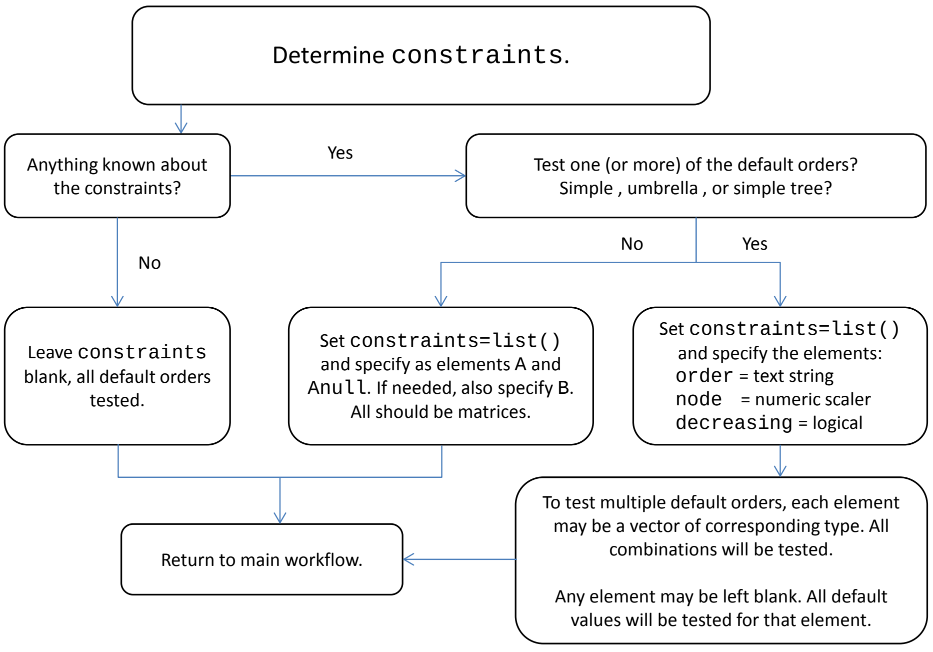
Determine constraints.
Anything known about the constraints?
Yes
Test one (or more) of the default orders? Simple , umbrella , or simple tree?
No
No
Yes
Leave constraints blank, all default orders tested.
Set constraints=list() and specify as elements A and Anull. If needed, also specify B. All should be matrices.
Set constraints=list() and specify the elements:
order = text string
node = numeric scaler
decreasing = logical
Return to main workflow.
To test multiple default orders, each element may be a vector of corresponding type. All combinations will be tested.
Any element may be left blank. All default values will be tested for that element.

Figure 9: Flowchart to determine constraints.

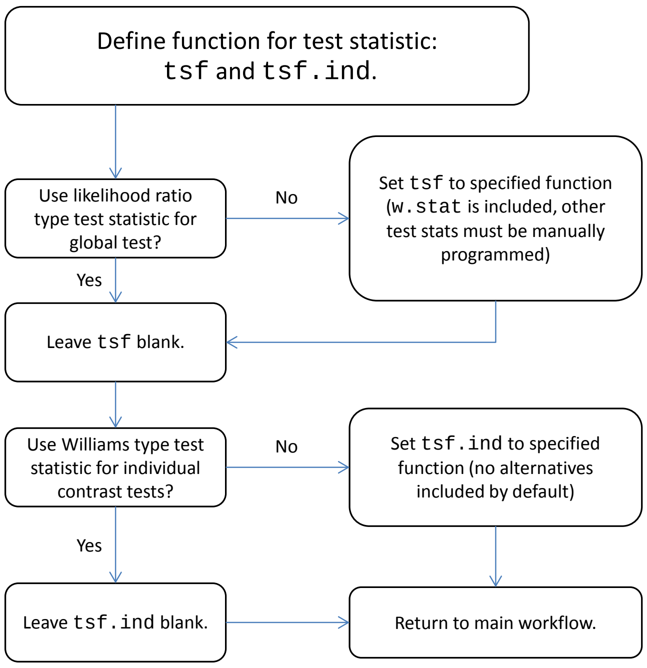
Define function for test statistic: tsf and tsf.ind.
Use likelihood ratio type test statistic for global test?
No
Set tsf to specified function (w.stat is included, other test stats must be manually programmed)
Yes
Leave tsf blank.
Use Williams type test statistic for individual contrast tests?
No
Set tsf.ind to specified function (no alternatives included by default)
Yes
Leave tsf.ind blank.
Return to main workflow.

Figure 10: Flowcharts to determine arguments defining the test statistic.

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Journal of Statistical Software http://www.jstatsoft.org/
published by the Foundation for Open Access Statistics http://www.foastat.org/
November 2016, Volume 75, Issue 1 *Submitted:* 2014-10-24
doi:10.18637/jss.v075.i01 *Accepted:* 2015-09-29