Chapter 1

Linear Mixed effects Models

1.1 Linear Mixed effects Models

A linear mixed effects (LME) model is a statistical model containing both fixed effects and random effects (random effects are also known as variance components). LME models are a generalization of the classical linear model, which contain fixed effects only. When the levels of factors are considered to be sampled from a population, and each level is not of particular interest, they are considered random quantities with associated variances. The effects of the levels, as described, are known as random effects. Random effects are represented by unobservable normally distributed random variables. Conversely fixed effects are considered non-random and the levels of each factor are of specific interest.

Fisher (1918) introduced variance components models for use in genetical studies. Whereas an estimate for variance must take an non-negative value, an individual variance component, i.e. a component of the overall variance, may be negative.

The methodology has developed since, including contributions from Tippett (1931), who extend the use of variance components into linear models, and Eisenhart (1947), who introduced the 'mixed model' terminology and formally distinguished between mixed and random effects models. Henderson (1950) devised a methodology for deriv-

ing estimates for both the fixed effects and the random effects, using a set of equations that would become known as 'mixed model equations' or 'Henderson's equations'. LME methodology is further enhanced by Henderson's later works (Henderson, 1953; Henderson et al., 1959, 1963, 1973, 1984). The key features of Henderson's work provide the basis for the estimation techniques.

Hartley and Rao (1967) demonstrated that unique estimates of the variance components could be obtained using maximum likelihood methods. However these estimates are known to be biased 'downwards' (i.e. underestimated) , because of the assumption that the fixed estimates are known, rather than being estimated from the data. Patterson and Thompson (1971) produced an alternative set of estimates, known as the restricted maximum likelihood (REML) estimates, that do not require the fixed effects to be known. Thusly there is a distinction the REML estimates and the original estimates, now commonly referred to as ML estimates.

Laird and Ware (1982) provides a form of notation for notation for LME models that has since become the standard form, or the basis for more complex formulations. Due to computation complexity, linear mixed effects models have not seen widespread use until many well known statistical software applications began facilitating them. SAS Institute added PROC MIXED to its software suite in 1992 (Singer, 1998). Pinheiro and Bates (1994) described how to compute LME models in the `S-plus` environment.

Using Laird-Ware form, the LME model is commonly described in matrix form,

$$y = X\beta + Zb + \epsilon \tag{1.1}$$

where y is a vector of N observable random variables, β is a vector of p fixed effects, X and Z are $N \times p$ and $N \times q$ known matrices, and b and ϵ are vectors of q and N, respectively, random effects such that $\mathrm{E}(b) = 0,\ \mathrm{E}(\epsilon) = 0$ and

$$\mathrm{var}\begin{pmatrix} b \\ \epsilon \end{pmatrix} = \begin{pmatrix} D & 0 \\ 0 & \Sigma \end{pmatrix}$$

where D and Σ are positive definite matrices parameterized by an unknown variance component parameter vector θ. The variance-covariance matrix for the vector of observations y is given by $V = ZDZ' + \Sigma$. This implies $y \sim (X\beta, V) = (X\beta, ZDZ' + \Sigma)$.

It is worth noting that V is an $n \times n$ matrix, as the dimensionality becomes relevant later on. The notation provided here is generic, and will be adapted to accord with complex formulations that will be encountered in due course.

1.1.1 Estimation

Estimation of LME models involve two complementary estimation issues'; estimating the vectors of the fixed and random effects estimates $\hat{\beta}$ and $\hat{b}$ and estimating the variance covariance matrices D and Σ. Inference about fixed effects have become known as 'estimates', while inferences about random effects have become known as 'predictions'. The most common approach to obtain estimators are Best Linear Unbiased Estimator (BLUE) and Best Linear Unbiased Predictor (BLUP). For an LME model given by (1.1), the BLUE of $\hat{\beta}$ is given by

$$\hat{\beta} = (X'V^{-1}X)^{-1}X'V^{-1}y,$$

whereas the BLUP of $\hat{b}$ is given by

$$\hat{b} = DZ'V^{-1}(y - X\hat{\beta}).$$

Henderson's equations

Because of the dimensionality of V (i.e. $n \times n$) computing the inverse of V can be difficult. As a way around the this problem Henderson (1953); Henderson et al. (1959, 1963, 1973, 1984) offered a more simpler approach of jointly estimating $\hat{\beta}$ and $\hat{b}$. Henderson (1950) made the (ad-hoc) distributional assumptions $y|b \sim \mathrm{N}(X\beta + Zb, \Sigma)$ and $b \sim \mathrm{N}(0, D)$, and proceeded to maximize the joint density of y and b

$$\left|\begin{pmatrix} D & 0 \\ 0 & \Sigma \end{pmatrix}\right|^{-\frac{1}{2}} \exp\left\{-\frac{1}{2}\begin{pmatrix} b \\ y - X\beta - Zb \end{pmatrix}'\begin{pmatrix} D & 0 \\ 0 & \Sigma \end{pmatrix}^{-1}\begin{pmatrix} b \\ y - X\beta - Zb \end{pmatrix}\right\}, \quad (1.2)$$

with respect to β and b, which ultimately requires minimizing the criterion

$$(y - X\beta - Zb)'\Sigma^{-1}(y - X\beta - Zb) + b'D^{-1}b. \quad (1.3)$$

This leads to the mixed model equations

$$\begin{pmatrix} X'\Sigma^{-1}X & X'\Sigma^{-1}Z \\ Z'\Sigma^{-1}X & X'\Sigma^{-1}X + D^{-1} \end{pmatrix} \begin{pmatrix} \beta \\ b \end{pmatrix} = \begin{pmatrix} X'\Sigma^{-1}y \\ Z'\Sigma^{-1}y \end{pmatrix}. \tag{1.4}$$

Using these equations, obtaining the estimates requires the inversion of a matrix of dimension $p+q \times p+q$, considerably smaller in size than V. **?** shows that these mixed model equations do not depend on normality and that $\hat{\beta}$ and $\hat{b}$ are the BLUE and BLUP under general conditions, provided D and Σ are known.

Robinson (1991) points out that although Henderson (1950) initially referred to the estimates $\hat{\beta}$ and $\hat{b}$ from (1.4) as "joint maximum likelihood estimates", Henderson (1973) later advised that these estimates should not be referred to as "maximum likelihood" as the function being maximized in (1.3) is a joint density rather than a likelihood function. Lee et al. (2006) remarks that it is clear that Henderson used joint estimation for computational purposes, without recognizing the theoretical implications.

Estimation of the fixed parameters

The vector y has marginal density $y \sim \mathrm{N}(X\beta, V)$, where $V = \Sigma + ZDZ'$ is specified through the variance component parameters θ. The log-likelihood of the fixed parameters (β, θ) is

$$\ell(\beta, \theta|y) = -\frac{1}{2}\log|V| - \frac{1}{2}(y - X\beta)'V^{-1}(y - X\beta), \tag{1.5}$$

and for fixed θ the estimate $\hat{\beta}$ of β is obtained as the solution of

$$(X'V^{-1}X)\beta = X'V^{-1}y. \tag{1.6}$$

Substituting $\hat{\beta}$ from (1.6) into $\ell(\beta, \theta|y)$ from (1.5) returns the *profile* log-likelihood

$$\begin{aligned} \ell_P(\theta \mid y) &= \ell(\hat{\beta}, \theta \mid y) \\ &= -\frac{1}{2}\log|V| - \frac{1}{2}(y - X\hat{\beta})'V^{-1}(y - X\hat{\beta}) \end{aligned}$$

of the variance parameter θ. Estimates of the parameters θ specifying V can be found by maximizing $\ell_P(\theta \mid y)$ over θ. These are the ML estimates.

For REML estimation the *restricted* log-likelihood is defined as

$$\ell_R(\theta \mid y) = \ell_P(\theta \mid y) - \frac{1}{2}\log|X'VX|.$$

The REML approach does not base estimates on a maximum likelihood fit of all the information, but instead uses a likelihood function derived from a data set, transformed to remove the irrelevant influences (Dodge, 2003). Restricted maximum likelihood is often preferred to maximum likelihood because REML estimation reduces the bias in the variance component by taking into account the loss of degrees of freedom that results from estimating the fixed effects in $\boldsymbol{\beta}$. Restricted maximum likelihood also handles high correlations more effectively, and is less sensitive to outliers than maximum likelihood. The problem with REML for model building is that the likelihoods obtained for different fixed effects are not comparable. Hence it is not valid to compare models with different fixed effects using a likelihood ratio test or AIC when REML is used to estimate the model. Therefore models derived using ML must be used instead.

Estimation of the random effects

The established approach for estimating the random effects is to use the best linear predictor of b from y, which for a given β equals $DZ'V^{-1}(y-X\beta)$. In practice β is replaced by an estimator such as $\hat{\beta}$ from (1.6) so that $\hat{b} = DZ'V^{-1}(y - X\hat{\beta})$. Pre-multiplying by the appropriate matrices it is straightforward to show that these estimates $\hat{\beta}$ and $\hat{b}$ satisfy the equations in (1.4).

Algorithms for likelihood function optimization

Iterative numerical techniques are used to optimize the log-likelihood function and estimate the covariance parameters θ. The procedure is subject to the constraint that R and D are both positive definite. The most common iterative algorithms for optimizing the likelihood function are the Newton-Raphson method, which is the preferred method, the expectation maximization (EM) algorithm and the Fisher scoring methods.

The EM algorithm, introduced by Dempster et al. (1977), is an iterative technique for maximizing complicated likelihood functions. The algorithm alternates between performing an expectation (E) step and the maximization (M) step. The 'E' step computes the expectation of the log-likelihood evaluated using the current estimate for the variables. In the 'M' step, parameters that maximize the expected log-likelihood, found on the previous 'E' step, are computed. These parameter estimates are then used to determine the distribution of the variables in the next 'E' step. The algorithm alternatives between these two steps until convergence is reached.

The main drawback of the EM algorithm is its slow rate of convergence. Consequently the EM algorithm is rarely used entirely in LME estimation, instead providing an initial set of values that can be passed to other optimization techniques.

The Newton Raphson (NR) method is the most common, and recommended technique for ML and REML estimation. The NR algorithm minimizes an objective function defines as -2 times the log likelihood for the covariance parameters θ. At every iteration the NR algorithm requires the calculation of a vector of partial derivatives, known as the gradient, and the second derivative matrix with respect to the covariance parameters. This is known as the observed Hessian matrix. Due to the Hessian matrix, the NR algorithm is more time-consuming, but convergence is reached with fewer iterations compared to the EM algorithm. The Fisher scoring algorithm is an variant of the NR algorithm that is more numerically stable and likely to converge, but not recommended to obtain final estimates.

The extended likelihood

The desire to have an entirely likelihood-based justification for estimates of random effects, in contrast to Henderson's equation, has motivated Pawitan (2001, page 429) to define the *extended likelihood*. He remarks "In mixed effects modelling the extended likelihood has been called *h-likelihood* (for hierarchical likelihood) by Lee and Nelder (1996), while in smoothing literature it is known as the *penalized likelihood* (e.g. Green and Silverman 1994)." The extended likelihood can be written $L(\beta, \theta, b|y) =$

$p(y|b;\beta,\theta)p(b;\theta)$ and adopting the same distributional assumptions used by Henderson (1950) yields the log-likelihood function

$$\begin{aligned}\ell_h(\beta,\theta,b|y) \;&= -\frac{1}{2}\left\{\log|\Sigma| + (y-X\beta-Zb)'\Sigma^{-1}(y-X\beta-Zb)\right.\\ &\qquad\left.+\log|D| + b'D^{-1}b\right\}.\end{aligned}$$

Given θ, differentiating with respect to β and b returns Henderson's equations in (1.4).

The LME model as a general linear model

Henderson's equations in (1.4) can be rewritten $(T'W^{-1}T)\delta = T'W^{-1}y_a$ using

$$\delta = \begin{pmatrix}\beta\\ b\end{pmatrix},\; y_a = \begin{pmatrix}y\\ \psi\end{pmatrix},\; T = \begin{pmatrix}X & Z\\ 0 & I\end{pmatrix},\; \text{and } W = \begin{pmatrix}\Sigma & 0\\ 0 & D\end{pmatrix},$$

where Lee et al. (2006) describe $\psi = 0$ as quasi-data with mean $\mathrm{E}(\psi) = b$. Their formulation suggests that the joint estimation of the coefficients β and b of the linear mixed effects model can be derived via a classical augmented general linear model $y_a = T\delta + \varepsilon$ where $\mathrm{E}(\varepsilon) = 0$ and $\mathrm{var}(\varepsilon) = W$, with *both* β and b appearing as fixed parameters. The usefulness of this reformulation of an LME as a general linear model will be revisited.

1.2 Repeated measurements in LME models

In many statistical analyzes, the need to determine parameter estimates where multiple measurements are available on each of a set of variables often arises. Further to Lam et al. (1999), Hamlett et al. (2004) performs an analysis of the correlation of replicate measurements, for two variables of interest, using LME models.

Let y_{Aij} and y_{Bij} be the jth repeated observations of the variables of interest A and B taken on the ith subject. The number of repeated measurements for each variable may differ for each individual. Both variables are measured on each time points. Let n_i be the number of observations for each variable, hence $2 \times n_i$ observations in total.

It is assumed that the pair y_{Aij} and y_{Bij} follow a bivariate normal distribution.

$$\begin{pmatrix} y_{Aij} \\ y_{Bij} \end{pmatrix} \sim \mathcal{N}(\boldsymbol{\mu}, \boldsymbol{\Sigma}) \text{ where } \boldsymbol{\mu} = \begin{pmatrix} \mu_A \\ \mu_B \end{pmatrix}$$

The matrix Σ represents the variance component matrix between response variables at a given time point j.

$$\boldsymbol{\Sigma} = \begin{pmatrix} \sigma_A^2 & \sigma_{AB} \\ \sigma_{AB} & \sigma_B^2 \end{pmatrix}$$

σ_A^2 is the variance of variable A, σ_B^2 is the variance of variable B and σ_{AB} is the covariance of the two variable. It is assumed that $\boldsymbol{\Sigma}$ does not depend on a particular time point, and is the same over all time points.

1.2.1 Formulation of the response vector

Information of individual i is recorded in a response vector $\boldsymbol{y}_i$. The response vector is constructed by stacking the response of the 2 responses at the first instance, then the 2 responses at the second instance, and so on. Therefore the response vector is a $2n_i \times 1$ column vector. The covariance matrix of $\boldsymbol{y_i}$ is a $2n_i \times 2n_i$ positive definite matrix $\boldsymbol{\Omega}_i$.

Consider the case where three measurements are taken by both methods A and B,

$\boldsymbol{y}_i$ is a 6×1 random vector describing the ith subject.

$$\boldsymbol{y}_i = (y_i^{A1}, y_i^{B1}, y_i^{A2}, y_i^{B2}, y_i^{A3}, y_i^{B3})\boldsymbol{\prime}$$

The response vector $\boldsymbol{y_i}$ can be formulated as an LME model according to Laird-Ware form.

$$\begin{aligned} \boldsymbol{y_i} &= \boldsymbol{X_i\beta} + \boldsymbol{Z_i b_i} + \boldsymbol{\epsilon_i} \\ \boldsymbol{b_i} &\sim \mathcal{N}(\boldsymbol{0}, \boldsymbol{D}) \\ \boldsymbol{\epsilon_i} &\sim \mathcal{N}(\boldsymbol{0}, \boldsymbol{R_i}) \end{aligned}$$

Information on the fixed effects are contained in a three dimensional vector $\boldsymbol{\beta} = (\beta_0, \beta_1, \beta_2)\boldsymbol{\prime}$. For computational purposes β_2 is conventionally set to zero. Consequently $\boldsymbol{\beta}$ is the solutions of the means of the two methods, i.e. $E(\boldsymbol{y}_i) = \boldsymbol{X}_i\boldsymbol{\beta}$. The variance covariance matrix $\boldsymbol{D}$ is a general 2×2 matrix, while $\boldsymbol{R}_i$ is a $2n_i \times 2n_i$ matrix.

1.2.2 Decomposition of the response covariance matrix

The variance covariance structure can be re-expressed in the following form,

$$\mathrm{Cov}(\mathrm{y}_i) = \boldsymbol{\Omega_i} = \boldsymbol{Z}_i\boldsymbol{D}\boldsymbol{Z}_i' + \boldsymbol{R_i}.$$

$\boldsymbol{R_i}$ can be shown to be the Kronecker product of a correlation matrix $\boldsymbol{V}$ and $\boldsymbol{\Lambda}$. The correlation matrix $\boldsymbol{V}$ of the repeated measures on a given response variable is assumed to be the same for all response variables. Both Hamlett et al. (2004) and Lam et al. (1999) use the identity matrix, with dimensions $n_i \times n_i$ as the formulation for $\boldsymbol{V}$. Roy (2009) remarks that, with repeated measures, the response for each subject is correlated for each variable, and that such correlation must be taken into account in order to produce a valid inference on correlation estimates. Roy (2006) proposes various correlation structures may be assumed for repeated measure correlations, such as the compound symmetry and autoregressive structures, as alternative to the identity matrix.

However, for the purposes of method comparison studies, the necessary estimates are currently only determinable when the identity matrix is specified, and the results in Roy (2009) indicate its use.

For the response vector described, Hamlett et al. (2004) presents a detailed covariance matrix. A brief summary shall be presented here only. The overall variance matrix is a 6×6 matrix composed of two types of 2×2 blocks. Each block represents one separate time of measurement.

$$\boldsymbol{\Omega}_i = \begin{pmatrix} \boldsymbol{\Sigma} & \boldsymbol{D} & \boldsymbol{D} \\ \boldsymbol{D} & \boldsymbol{\Sigma} & \boldsymbol{D} \\ \boldsymbol{D} & \boldsymbol{D} & \boldsymbol{\Sigma} \end{pmatrix}$$

The diagonal blocks are Σ, as described previously. The 2×2 block diagonal matrix in $\boldsymbol{\Omega}$ gives $\boldsymbol{\Sigma}$. $\boldsymbol{\Sigma}$ is the sum of the between-subject variability $\boldsymbol{D}$ and the within subject variability $\boldsymbol{\Lambda}$.

$\boldsymbol{\Omega_i}$ can be expressed as

$$\boldsymbol{\Omega_i} = \boldsymbol{Z}_i \boldsymbol{D} \boldsymbol{Z}_i' + (\boldsymbol{I}_{\boldsymbol{n_i}} \otimes \boldsymbol{\Lambda}).$$

The notation $\dim_{n_i}$ means an $n_i \times n_i$ diagonal block.

1.2.3 Correlation terms

Hamlett et al. (2004) demonstrated how the between-subject and within subject variabilities can be expressed in terms of correlation terms.

$$\boldsymbol{D} = \begin{pmatrix} \sigma_A^2 \rho_A & \sigma_A \sigma_b \rho_{AB} \delta \\ \sigma_A \sigma_b \rho_{AB} \delta & \sigma_B^2 \rho_B \end{pmatrix}$$

$$\boldsymbol{\Lambda} = \begin{pmatrix} \sigma_A^2 (1 - \rho_A) & \sigma_{AB} (1 - \delta) \\ \sigma_{AB} (1 - \delta) & \sigma_B^2 (1 - \rho_B) \end{pmatrix}.$$

ρ_A describe the correlations of measurements made by the method A at different times. Similarly ρ_B describe the correlation of measurements made by the method B

at different times. Correlations among repeated measures within the same method are known as intra-class correlation coefficients. ρ_{AB} describes the correlation of measurements taken at the same same time by both methods. The coefficient δ is added for when the measurements are taken at different times, and is a constant of less than 1 for linked replicates. This is based on the assumption that linked replicates measurements taken at the same time would have greater correlation than those taken at different times. For unlinked replicates δ is simply 1. Hamlett et al. (2004) provides a useful graphical depiction of the role of each correlation coefficients.

1.3 Using LME for method comparison

Due to the prevalence of modern statistical software, Carstensen et al. (2008) advocates the adoption of computer based approaches, such as LME models, to method comparison studies. Carstensen et al. (2008) remarks upon 'by-hand' approaches advocated in Bland and Altman (1999) discouragingly, describing them as tedious, unnecessary and 'outdated'. Rather than using the 'by hand' methods, estimates for required LME parameters can be read directly from program output. Furthermore, using computer approaches removes constraints associated with 'by-hand' approaches, such as the need for the design to be perfectly balanced.

1.3.1 Roy's methodology

For the purposes of comparing two methods of measurement, Roy (2009) presents a methodology utilizing linear mixed effects model. This methodology provides for the formal testing of inter-method bias, between-subject variability and within-subject variability of two methods. The formulation contains a Kronecker product covariance structure in a doubly multivariate setup. By doubly multivariate set up, Roy means that the information on each patient or item is multivariate at two levels, the number of methods and number of replicated measurements. Further to Lam et al. (1999), it is assumed that the replicates are linked over time. However it is easy to modify to the unlinked case.

Roy (2009) sets out three criteria for two methods to be considered in agreement. Firstly that there be no significant bias. Second that there is no difference in the between-subject variabilities, and lastly that there is no significant difference in the within-subject variabilities. Roy further proposes examination of the the overall variability by considering the second and third criteria be examined jointly. Should both the second and third criteria be fulfilled, then the overall variabilities of both methods would be equal.

A formal test for inter-method bias can be implemented by examining the fixed ef-

fects of the model. This is common to well known classical linear model methodologies. The null hypotheses, that both methods have the same mean, which is tested against the alternative hypothesis, that both methods have different means. The inter-method bias and necessary $t-$value and $p-$value are presented in computer output. A decision on whether the first of Roy's criteria is fulfilled can be based on these values.

Importantly Roy (2009) further proposes a series of three tests on the variance components of an LME model, which allow decisions on the second and third of Roy's criteria. For these tests, four candidate LME models are constructed. The differences in the models are specifically in how the the D and Λ matrices are constructed, using either an unstructured form or a compound symmetry form. To illustrate these differences, consider a generic matrix A,

$$\boldsymbol{A} = \begin{pmatrix} a_{11} & a_{12} \\ a_{21} & a_{22} \end{pmatrix}.$$

A symmetric matrix allows the diagonal terms a_{11} and a_{22} to differ. The compound symmetry structure requires that both of these terms be equal, i.e $a_{11} = a_{22}$.

The first model acts as an alternative hypothesis to be compared against each of three other models, acting as null hypothesis models, successively. The models are compared using the likelihood ratio test. Likelihood ratio tests are a class of tests based on the comparison of the values of the likelihood functions of two candidate models. LRTs can be used to test hypotheses about covariance parameters or fixed effects parameters in the context of LMEs. The test statistic for the likelihood ratio test is the difference of the log-likelihood functions, multiplied by -2. The probability distribution of the test statistic is approximated by the χ^2 distribution with $(\nu_1 - \nu_2)$ degrees of freedom, where ν_1 and ν_2 are the degrees of freedom of models 1 and 2 respectively. Each of these three test shall be examined in more detail shortly.

1.3.2 Correlation

In addition to the variability tests, Roy advises that it is preferable that a correlation of greater than 0.82 exist for two methods to be considered interchangeable. However if two methods fulfil all the other conditions for agreement, failure to comply with this one can be overlooked. Indeed Roy demonstrates that placing undue importance to it can lead to incorrect conclusions. Roy (2009) remarks that current computer implementations only gives overall correlation coefficients, but not their variances. Consequently it is not possible to carry out inferences based on all overall correlation coefficients.

1.3.3 Variability test 1

The first test determines whether or not both methods A and B have the same between-subject variability, further to the second of Roy's criteria.

$$H_0 : \ d_A = d_B$$
$$H_A : \ d_A \neq d_B$$

This test is facilitated by constructing a model specifying a symmetric form for D (i.e. the alternative model) and comparing it with a model that has compound symmetric form for D (i.e. the null model). For this test $\hat{\mathbf{\Lambda}}$ has a symmetric form for both models, and will be the same for both.

1.3.4 Variability test 2

This test determines whether or not both methods A and B have the same within-subject variability, thus enabling a decision on the third of Roy's criteria.

$$H_0 : \ \lambda_A = \lambda_B$$
$$H_A : \ \lambda_A = \lambda_B$$

This model is performed in the same manner as the first test, only reversing the roles of $\hat{\boldsymbol{D}}$ and $\hat{\boldsymbol{\Lambda}}$. The null model is constructed a symmetric form for $\hat{\boldsymbol{\Lambda}}$ while the alternative model uses a compound symmetry form. This time $\hat{\boldsymbol{D}}$ has a symmetric form for both models, and will be the same for both.

As the within-subject variabilities are fundamental to the coefficient of repeatability, this variability test likelihood ratio test is equivalent to testing the equality of two coefficients of repeatability of two methods. In presenting the results of this test, Roy (2009) includes the coefficients of repeatability for both methods.

1.3.5 Variability test 3

The last of the variability test examines whether or not methods A and B have the same overall variability. This enables the joint consideration of second and third criteria.

$$H_0 : \ \sigma_A = \sigma_B$$
$$H_A : \ \sigma_A = \sigma_B$$

The null model is constructed a symmetric form for both $\hat{\boldsymbol{D}}$ and $\hat{\boldsymbol{\Lambda}}$ while the alternative model uses a compound symmetry form for both.

1.3.6 Demonstration of Roy's testing

Roy provides three case studies, using data sets well known in method comparison studies, to demonstrate how the methodology should be used. The first two examples used are from the 'blood pressure' data set introduced by Bland and Altman (1999). The data set is a tabulation of simultaneous measurements of systolic blood pressure were made by each of two experienced observers (denoted 'J' and 'R') using a sphygmomanometer and by a semi-automatic blood pressure monitor (denoted 'S'). Three sets of readings were made in quick succession. Roy compares the 'J' and 'S' methods in the first of her examples.

The inter-method bias between the two method is found to be 15.62 , with a $t-$value of -7.64, with a $p-$value of less than 0.0001. Consequently there is a significant inter-method bias present between methods J and S, and the first of the Roy's three agreement criteria is unfulfilled.

Next, the first variability test is carried out, yielding maximum likelihood estimates of the between-subject variance covariance matrix, for both the null model, in compound symmetry (CS) form, and the alternative model in symmetric (symm) form. These matrices are determined to be as follows;

$$\hat{\boldsymbol{D}}_{CS} = \begin{pmatrix} 946.50 & 784.32 \\ 784.32 & 946.50 \end{pmatrix}, \qquad \hat{\boldsymbol{D}}_{Symm} = \begin{pmatrix} 923.98 & 785.24 \\ 785.24 & 971.30 \end{pmatrix}.$$

A likelihood ratio test is perform to compare both candidate models. The log-likelihood of the null model is -2030.7, and for the alternative model -2030.8. The test statistic, presented with greater precision than the log-likelihoods, is 0.1592. The $p-$value is 0.6958. Consequently we fail to reject the null model, and by extension, conclude that the hypothesis that methods J and S have the same between-subject variability. Thus the second of the criteria is fulfilled.

The second variability test determines maximum likelihood estimates of the within-subject variance covariance matrix, for both the null model, in CS form, and the alternative model in symmetric form.

$$\hat{\boldsymbol{\Lambda}}_{\boldsymbol{CS}} = \begin{pmatrix} 60.27 & 16.06 \\ 16.06 & 60.27 \end{pmatrix}, \qquad \hat{\boldsymbol{\Lambda}}_{Symm} = \begin{pmatrix} 37.40 & 16.06 \\ 16.06 & 83.14 \end{pmatrix}.$$

Again, A likelihood ratio test is perform to compare both candidate models. The log-likelihood of the alternative model model is -2045.0. As before, the null model has a log-likelihood of -2030.7. The test statistic is computed as 28.617, again presented with greater precision. The $p-$value is less than 0.0001. In this case we reject the null hypothesis of equal within-subject variability. Consequently the third of Roy's criteria is unfulfilled. The coefficient of repeatability for methods J and S are found to be 16.95 mmHg and 25.28 mmHg respectively.

The last of the three variability tests is carried out to compare the overall variabilities of both methods. With the null model the MLE of the within-subject variance covariance matrix is given below. The overall variabilities for the null and alternative models, respectively, are determined to be as follows;

$$\hat{\boldsymbol{\Sigma}}_{CS} = \begin{pmatrix} 1007.92 & 801.65 \\ 801.65 & 1007.92 \end{pmatrix}, \qquad \hat{\boldsymbol{\Sigma}}_{Symm} = \begin{pmatrix} 961.38 & 801.40 \\ 801.40 & 1054.43 \end{pmatrix},$$

The log-likelihood of the alternative model model is -2045.2, and again, the null model has a log-likelihood of -2030.7. The test statistic is 28.884, and the $p-$value is less than 0.0001. The null hypothesis, that both methods have equal overall variability, is rejected. Further to the second variability test, it is known that this difference is specifically due to the difference of within-subject variabilities.

Lastly, Roy considers the overall correlation coefficient. The diagonal blocks $\hat{\boldsymbol{r}}_{\boldsymbol{\Omega}ii}$ of the correlation matrix indicate an overall coefficient of 0.7959. This is less than the threshold of 0.82 that Roy recommends.

$$\hat{\boldsymbol{r}}_{\boldsymbol{\Omega}ii} = \begin{pmatrix} 1 & 0.7959 \\ 0.7959 & 1 \end{pmatrix}$$

The off-diagonal blocks of the overall correlation matrix $\hat{\boldsymbol{r}}_{\boldsymbol{\Omega}ii'}$ present the correlation coefficients further to Hamlett et al. (2004).

$$\hat{\boldsymbol{r}}_{\boldsymbol{\Omega}ii'} = \begin{pmatrix} 0.9611 & 0.7799 \\ 0.7799 & 0.9212 \end{pmatrix}.$$

The overall conclusion of the procedure is that method J and S are not in agreement, specifically due to the within-subject variability, and the inter-method bias. The repeatability coefficients are substantially different, with the coefficient for method S being 49% larger than for method J. Additionally the overall correlation coefficient did not exceed the recommended threshold of 0.82.

1.4 Limits of agreement in LME models

Limits of agreement are used extensively for assessing agreement, because they are intuitive and easy to use. Necessarily their prevalence in literature has meant that they are now the best known measurement for agreement, and therefore any newer methodology would benefit by making reference to them.

Carstensen et al. (2008) uses LME models to determine the limits of agreement. Between-subject variation for method m is given by d_m^2 and within-subject variation is given by λ_m^2. Carstensen et al. (2008) remarks that for two methods A and B, separate values of d_A^2 and d_B^2 cannot be estimated, only their average. Hence the assumption that $d_x = d_y = d$ is necessary. The between-subject variability $\boldsymbol{D}$ and within-subject variability $\boldsymbol{\Lambda}$ can be presented in matrix form,

$$\boldsymbol{D} = \begin{pmatrix} d_A^2 & 0 \\ 0 & d_B^2 \end{pmatrix} = \begin{pmatrix} d^2 & 0 \\ 0 & d^2 \end{pmatrix}, \qquad \boldsymbol{\Lambda} = \begin{pmatrix} \lambda_A^2 & 0 \\ 0 & \lambda_B^2 \end{pmatrix}.$$

The variance for method m is $d_m^2 + \lambda_m^2$. Limits of agreement are determined using the standard deviation of the case-wise differences between the sets of measurements by two methods A and B, given by

$$\text{var}(y_A - y_B) = 2d^2 + \lambda_A^2 + \lambda_B^2. \tag{1.7}$$

Importantly the covariance terms in both variability matrices are zero, and no covariance component is present.

Carstensen et al. (2008) presents a data set 'fat', which is a comparison of measurements of subcutaneous fat by two observers at the Steno Diabetes Center, Copenhagen. Measurements are in millimeters (mm). Each person is measured three times by each observer. The observations are considered to be 'true' replicates.

A linear mixed effects model is formulated, and implementation through several software packages is demonstrated. All of the necessary terms are presented in the computer output. The limits of agreement are therefore,

$$0.0449 \pm 1.96 \times \sqrt{2 \times 0.0596^2 + 0.0772^2 + 0.0724^2} = (-0.220, 0.309). \tag{1.8}$$

Roy (2009) has demonstrated a methodology whereby d_A^2 and d_B^2 can be estimated separately. Also covariance terms are present in both $\boldsymbol{D}$ and $\boldsymbol{\Lambda}$. Using Roy's methodology, the variance of the differences is

$$\text{var}(y_{iA} - y_{iB}) = d_A^2 + \lambda_B^2 + d_A^2 + \lambda_B^2 - 2(d_{AB} + \lambda_{AB}) \tag{1.9}$$

All of these terms are given or determinable in computer output. The limits of agreement can therefore be evaluated using

$$\bar{y_A} - \bar{y_B} \pm 1.96 \times \sqrt{\sigma_A^2 + \sigma_B^2 - 2(\sigma_{AB})}. \tag{1.10}$$

For Carstensen's 'fat' data, the limits of agreement computed using Roy's method are consistent with the estimates given by Carstensen et al. (2008); $0.044884 \pm 1.96 \times 0.1373979 = (-0.224, 0.314)$.

1.4.1 Linked replicates

Carstensen et al. (2008) proposes the addition of an random effects term to their model when the replicates are linked. This term is used to describe the 'item by replicate' interaction, which is independent of the methods. This interaction is a source of variability independent of the methods. Therefore failure to account for it will result in variability being wrongly attributed to the methods.

Carstensen et al. (2008) introduces a second data set; the oximetry study. This study done at the Royal Childrens Hospital in Melbourne to assess the agreement between co-oximetry and pulse oximetry in small babies.

In most cases, measurements were taken by both method at three different times. In some cases there are either one or two pairs of measurements, hence the data is unbalanced. Carstensen et al. (2008) describes many of the children as being very sick, and with very low oxygen saturations levels. Therefore it must be assumed that a biological change can occur in interim periods, and measurements are not true replicates.

Carstensen et al. (2008) demonstrate the necessity of accounting for linked replicated by comparing the limits of agreement from the 'oximetry' data set using a model

with the additional term, and one without. When the interaction is accounted for the limits of agreement are (-9.62,14.56). When the interaction is not accounted for, the limits of agreement are (-11.88,16.83). It is shown that the failure to include this additional term results in an over-estimation of the standard deviations of differences.

Limits of agreement are determined using Roy's methodology, without adding any additional terms, are found to be consistent with the 'interaction' model; $(-9.562, 14.504)$. Roy's methodology assumes that replicates are linked. However, following Carstensen's example, an addition interaction term is added to the implementation of Roy's model to assess the effect, the limits of agreement estimates do not change. However there is a conspicuous difference in within-subject matrices of Roy's model and the modified model (denoted 1 and 2 respectively);

$$\hat{\mathbf{\Lambda}}_1 = \begin{pmatrix} 16.61 & 11.67 \\ 11.67 & 27.65 \end{pmatrix} \qquad \hat{\mathbf{\Lambda}}_2 = \begin{pmatrix} 7.55 & 2.60 \\ 2.60 & 18.59 \end{pmatrix}. \tag{1.11}$$

(The variance of the additional random effect in model 2 is 3.01.)

Akaike (1974) introduces the Akaike information criterion (AIC), a model selection tool based on the likelihood function. Given a data set, candidate models are ranked according to their AIC values, with the model having the lowest AIC being considered the best fit.Two candidate models can said to be equally good if there is a difference of less than 2 in their AIC values.

The Akaike information criterion (AIC) for both models are $AIC_1 = 2304.226$ and $AIC_2 = 2306.226$, indicating little difference in models. The AIC values for the Carstensen 'unlinked' and 'linked' models are 1994.66 and 1955.48 respectively, indicating an improvement by adding the interaction term.

The $\hat{\mathbf{\Lambda}}$ matrices are informative as to the difference between Carstensen's unlinked and linked models. For the oximetry data, the covariance terms (given above as 11.67 and 2.6 respectively) are of similar magnitudes to the variance terms. Conversely for the 'fat' data the covariance term (-0.00032) is negligible. When the interaction term is added to the model, the covariance term remains negligible. (For the 'fat' data, the difference in AIC values is also approximately 2).

To conclude, Carstensen's models provided a rigorous way to determine limits of agreement, but don't provide for the computation of $\hat{\boldsymbol{D}}$ and $\hat{\boldsymbol{\Lambda}}$. Therefore the test's proposed by Roy (2009) can not be implemented. Conversely, accurate limits of agreement as determined by Carstensen's model may also be found using Roy's method. Addition of the interaction term erodes the capability of Roy's methodology to compare candidate models, and therefore shall not be adopted.

Finally, to complement the blood pressure (i.e.'J vs S') method comparison from the previous section (i.e.'J vs S'), the limits of agreement are $15.62 \pm 1.96 \times 20.33 = (-24.22, 55.46)$.)

1.5 Implementation in R

To implement an LME model in `R`, the `nlme` package is used. This package is loaded into the `R` environment using the library command, (i.e. `library(nlme)`). The `lme` command is used to fit LME models. The first two arguments to the `lme` function specify the fixed effect component of the model, and the data set to which the model is to be fitted. The first candidate model ('MCS1') fits an LME model on the data set 'dat'. The variable 'method' is assigned as the fixed effect, with the response variable 'BP' (i.e. blood pressure).

The third argument contain the random effects component of the formulation, describing the random effects, and their grouping structure. The `nlme` package provides a set of positive-definite matrices , the `pdMat` class, that can be used to specify a structure for the between-subject variance-covariance matrix for the random effects. For Roy's methodology, we will use the `pdSymm` and `pdCompSymm` to specify a symmetric structure and a compound symmetry structure respectively. A full discussion of these structures can be found in Pinheiro and Bates (1994, pg. 158).

Similarly a variety of structures for the with-subject variance-covariance matrix can be implemented using `nlme`. To implement a particular matrix structure, one must specify both a variance function and correlation structure accordingly. Variance functions are used to model the variance structure of the within-subject errors. `varIdent` is a variance function object used to allow different variances according to the levels of a classification factor in the data. A compound symmetry structure is implemented using the `corCompSymm` class, while the symmetric form is specified by `corSymm` class. Finally, the estimation methods is specified as "ML" or "REML".

The first of Roy's candidate model can be implemented using the following code;

```
MCS1 = lme(BP ~ method-1, data = dat,
random =  list(subject=pdSymm(~ method-1)),
weights=varIdent(form=~1|method),
correlation = corSymm(form=~1 | subject/obs), method="ML")
```

For the blood pressure data used in Roy (2009), all four candidate models are implemented by slight variations of this piece of code, specifying either `pdSymm` or `pdCompSymm` in the second line, and either `corSymm` or `corCompSymm` in the fourth line. For example, the second candidate model 'MCS2' is implemented with the same code as MCS1, except for the term `pdCompSymm` in the second line, rather than `pdSymm`.

```
MCS2 = lme(BP ~ method-1, data = dat,
random = list(subject=pdCompSymm(~ method-1)),
weights = varIdent(form=~1|method),
correlation = corSymm(form=~1 | subject/obs), method="ML")
```

Using this `R` implementation for other data sets requires that the data set is structured appropriately (i.e. each case of observation records the index, response, method and replicate). Once formatted properly, implementation is simply a case of re-writing the first line of code, and computing the four candidate models accordingly.

To perform a likelihood ratio test for two candidate models, simply use the `anova` command with the names of the candidate models as arguments. The following piece of code implement the first of Roy's variability tests.

```
> anova(MCS1,MCS2)
     Model df    AIC    BIC  logLik   Test L.Ratio p-value
MCS1     1  8 4077.5 4111.3 -2030.7
MCS2     2  7 4075.6 4105.3 -2030.8 1 vs 2 0.15291  0.6958
>
```

The fixed effects estimates are the same for all four candidate models. The inter-method bias can be easily determined by inspecting a summary of any model. The summary presents estimates for all of the important parameters, but not the complete variance-covariance matrices (although some simple `R` functions can be written to overcome this). The variance estimates for the random effects for MCS2 is presented below.

```
Random effects:
Formula: ~method - 1 | subject
Structure: Compound Symmetry
          StdDev Corr
methodJ  30.765
methodS  30.765 0.829
Residual  6.115
```

Similarly, for computing the limits of agreement the standard deviation of the differences is not explicitly given. Again, A simple `R` function can be written to calculate the limits of agreement directly.

1.6 Extension of Roy's methodology

Roy's methodology is constructed to compare two methods in the presence of replicate measurements. Necessarily it is worth examining whether this methodology can be adapted for different circumstances.

An implementation of Roy's methodology, whereby three or more methods are used, is not feasible due to computational restrictions. Specifically there is a failure to reach convergence before the iteration limit is reached. This may be due to the presence of additional variables, causing the problem of non-identifiability. In the case of two variables, it is required to estimate two variance terms and four correlation terms, six in all. For the case of three variabilities, three variance terms must be estimated as well as nine correlation terms, twelve in all. In general for n methods has $2 \times T_n$ variance terms, where T_n is the triangular number for n, i.e. the addition analogue of the factorial. Hence the computational complexity quite increases substantially for every increase in n.

Should an implementation be feasible, further difficulty arises when interpreting the results. The fundamental question is whether two methods have close agreement so as to be interchangeable. When three methods are present in the model, the null hypothesis is that all three methods have the same variability relevant to the respective tests. The outcome of the analysis will either be that all three are interchangeable or that all three are not interchangeable.

The tests would not be informative as to whether any two of those three were interchangeable, or equivalently if one method in particular disagreed with the other two. Indeed it is easier to perform three pair-wise comparisons separately and then to combine the results.

Roy's methodology is not suitable for the case of single measurements because it follows from the decomposition for the covariance matrix of the response vector y_i, as presented in Hamlett et al. (2004). The decomposition depends on the estimation of correlation terms, which would be absent in the single measurement case. Indeed

there can be no within-subject variability if there are no repeated terms for it to describe. There would only be the covariance matrix of the measurements by both methods, which doesn't require the use of LME models. To conclude, simpler existing methodologies, such as Deming regression, would be the correct approach where there only one measurements by each method.

1.7 Conclusion

Carstensen et al. (2008) and Roy (2009) highlight the need for method comparison methodologies suitable for use in the presence of replicate measurements. Roy (2009) presents a comprehensive methodology for assessing the agreement of two methods, for replicate measurements. This methodology has the added benefit of overcoming the problems of unbalanced data and unequal numbers of replicates. Implementation of the methodology, and interpretation of the results, is relatively easy for practitioners who have only basic statistical training. Furthermore, it can be shown that widely used existing methodologies, such as the limits of agreement, can be incorporated into Roy's methodology.

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