

Multivariate Methods for Detection of Rubbery Rot in Storage Apples by Monitoring Volatile Organic Compounds: An Example of Multivariate Generalised Mixed Models

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Abstract

This article is a case study illustrating the use of a multivariate statistical method for screening potential chemical markers for early detection of post-harvest disease in storage fruit. We simultaneously measure a range of volatile organic compounds (VOCs) and two measures of severity of disease infection in apples under storage: the number of apples presenting visible symptoms and the lesion area. We use multivariate generalised linear mixed models (MGLMM) for studying association patterns of those simultaneously observed responses via the covariance structure of random components. Remarkably, those MGLMMs can be used to represent patterns of association between quantities of different statistical nature. In the particular example considered in this paper, there are positive responses (concentrations of VOC, Gamma distribution based models), positive responses possibly containing observations with zero values (lesion area, Compound Poisson distribution based models) and binomially distributed responses (proportion of apples presenting infection symptoms). We represent patterns of association inferred with the MGLMMs using graphical models (a network represented by a graph), which allow us to eliminate spurious associations due to a cascade of indirect correlations between the responses.

Key-words: Multivariate-Models, Generalised-Linear-Mixed-Models, Graphical-Models, Covariance-Selection-Models

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1 Introduction

Rubbery rot is a post-harvest disease in apples caused by the fungus *Phacidiopycnis washingtonensis*, leading to significant storage losses in commercial production (Ali et al., 2018). Therefore, it is interesting to find predictors of rubbery rots onset at early stages of the infection development under fruit storage. To this purpose, a comprehensive study involving the emission of a range of specially chosen volatile organic compounds (VOCs) under apple storage conditions was performed by Holthusen et al. (2021a). In this study, experimentally induced rubbery rots infections were monitored and contrasted with the concentration of 14 VOCs along the development of the disease, aiming to find chemical predictors for rubbery rot. This article exposes details of some non-standard statistical tools used in Holthusen et al. (2021a).

The experiment we will model can be shortly described in the following way. Ten glass jars (below referred as glasses), containing nine inoculated apples were observed at three fixed observation times (6, 12 and 18 weeks post-inoculation). The following quantities were determined at each observation time: the number of apples presenting visible symptoms, the area of lesions caused by the fungal infection, and the air concentration of 14 VOCs. The details of the experiment setup and the choice of the VOCs are exposed in Holthusen et al. (2021a), see also Holthusen et al. (2021b) and Holthusen and Weber (2021).

The proper statistical modelling of the complex of experiments referred to above presents several challenges. Indeed, the simultaneously observed responses are of different statistical nature. For example, while the number of apples showing visible symptoms (used to monitor the disease development) is naturally binomially distributed, the VOC concentrations follow continuous positive non-Gaussian distributions with high skewness. Furthermore, the area of lesions (characterising disease severity) presents many zero values (absence of infection) but otherwise follows a continuously skewed distributed and therefore is not adequately described by purely continuous distributions. Thus, the first challenge we encountered was to develop methods for establishing associations between these responses of different nature. We propose to solve this problem by using suitably constructed multivariate generalised linear mixed models (MGLMMs) simultaneously describing the responses referred to above. In this way, we will model the concentrations of the VOCs using Gamma distributions, representing positive valued responses with different degrees of skewness. Moreover, the lesion area will be modelled using Gamma compound Poisson distributions with positive mass at zero and otherwise continuous with variable degrees of skewness. All these families

of distributions are particular cases of dispersion models, which are the families of distributions that form the basis of generalised linear mixed models (GLMMs).

The MGLMM we propose to use is composed of marginal GLMMs describing each of the responses studied. Each of those GLMMs will contain a random component representing the basic experimental unit (the glass), which we use to model the covariance structure of the different responses. We use the tools of graphical models to describe this covariance structure in a suitable compact form, which will allow us to draw valid general conclusions on the association between the responses, even though they are of different statistical nature. For instance, we will eliminate spurious correlations between the responses, i.e., correlations between two responses that can be explained by a cascade of correlations between those responses and the other responses in play. In this way, we will identify a minimal group of VOCs sufficient to predict the rubbery rots onset, avoiding redundancy and the pitfalls of multicollinearity.

The MGLMMs we construct allow for incorporating corrections for determining factors known to have a strong influence in the responses (e.g., the observation week) and temporal correlation due to repeated observations at the same experimental unit.

This paper is structured as follows. Section 2 introduces the marginal GLMMs for each of the responses considered. Those models are used to construct a MGLMM in Section 3. The details of the construction are given in Section 3.1, and the graphical model representing the covariance structure of the random components is discussed in section 3.2. Section 4 briefly discusses the results obtained.

2 Models for Several Responses with Different Nature

We introduce below a range of GLMMs describing each of the observed responses. Those models contain a random component representing the glass (which is viewed as the basic experimental unit) and a fixed effect representing the observation time (week). We used a GLMM defined with the binomial distribution and logistic link function for describing the number of apples presenting visible symptoms. The concentrations of VOCs were modelled using GLMMs defined with a Gamma distribution and the logarithmic link function. Finally, we used a GLMM defined with the family of Gamma compound Poisson distributions and a logarithmic link to describe the lesion area. We give the full details of those models below using a notation suitable for defining the multivariate model for simultaneously describing the 16 responses in play.

2.1 Concentrations of Volatiles Organic Compounds - Positive Responses

We describe below the GLMM used for modelling the concentration of each of the 14 VOCs. We label those VOCs by the index j ($j = 1, \dots, 14$), which is kept fixed along this section (referring to a choice of one of the VOCs). Denote by $X_{tg}^{[j]}$ the random variable representing the concentration of the value of the j^{th} VOC measured at the t^{th} week ($t = 6, 12, 18$) in the g^{th} glass ($g = 1, \dots, 10$). According the GLMM we are defining, there exist 10 unobservable random variables, denoted by $U_1^{[j]}, \dots, U_{10}^{[j]}$, such that for $t = 6, 12, 18$ and $g = 1, \dots, 10$ the response $X_{tg}^{[j]}$ is conditional Gamma distributed given $U_g^{[j]}$ with conditional expectation given by

$$\log(\mathbb{E}[X_{tg}^{[j]} | U_g^{[j]} = u]) = \theta_t^{[j]} + u \quad \text{for all } u \in \mathbb{R}.$$

Moreover, according to the GLMM the responses, $X_{gt}^{[j]}$ for $t = 6, 12, 18$ and $g = 1, \dots, 10$, are conditionally independent given $U_1^{[j]}, \dots, U_{10}^{[j]}$. The specification of the GLMM is completed by stating that $U_1^{[j]}, \dots, U_{10}^{[j]}$ are independent and identically normally distributed with expectation 0. Here $\theta_6^{[j]}, \theta_{12}^{[j]}$ and $\theta_{18}^{[j]}$ are fixed effects describing the variation of the concentration of the j^{th} VOC at different observation times.

2.2 Number of apples Presenting Symptoms - Binomial Counts

Let Y_{tg} be a random variable representing the number of apples presenting symptoms in the g^{th} glass ($g = 1, \dots, 10$), at the at the t^{th} week ($t = 6, 12, 18$) out of the nine apples contained in each glass. We assume that there exist 10 unobservable random variables, denoted by V_1, \dots, V_{10} such that, for $t = 6, 12, 18$ and $g = 1, \dots, 10$, Y_{tg} is conditionally binomially distributed given V_g with size 9 and conditional expectation given by

$$\text{logit}(\mathbb{E}[Y_{tg} | V_g = v]) = \alpha_t + v \quad \text{for all } v \in \mathbb{R}.$$

According to the model the responses, Y_{gt} for $t = 6, 12, 18$ and $g = 1, \dots, 10$, are conditionally independent given V_1, \dots, V_{10} . Moreover, the random components V_1, \dots, V_{10} are assumed to be independent and identically normally distributed with expectation zero.

2.3 Lesion Area of Infection - Positive Responses with Zero Values

Denote by Z_{tg} the random variable describing the observed lesion area in the g^{th} glass ($g = 1, \dots, 10$), at the t^{th} week ($t = 6, 12, 18$). We assume that there exist 10 independent and normally distributed random variables with expectation zero, denoted by W_1, \dots, W_{10} , such that, for $t = 6, 12, 18$ and $g = 1, \dots, 10$, the response Z_{tg} is distributed according to a Gamma-compound Poisson distribution with conditional expectation given by

$$\log(\mathbb{E}[Z_{tg}|W_g = w]) = \beta_t + w, \quad \text{for all } w \in \mathbb{R}.$$

Note that the Gamma-compound Poisson family of distributions is an exponential dispersion model (see Jørgensen,1987); therefore, the model we are defining is a genuine GLMM. Furthermore, the distributions in the Gamma-compound Poisson family have the peculiarity of attributing positive probability to the value zero and otherwise being a continuous distribution taking positive values, making them suitable for describing the lesion area.

The Gamma-compound Poisson family has been known for a long time (see Tweedie, 1984; Jørgensen,1987, and Cordeiro et al.,2021), however, these distributions are not routinely used in applications of generalised linear models (or GLMMs). Therefore, we shortly describe the inference procedure we used (for a detailed study see Labouriau ,2021). The Gamma-compound Poisson family is characterised by having a power variance function (*i.e.*, a function expressing the variance as a function of the expectation) of the form $V(\mu) = k\mu^p$ for p in the open interval $(1, 2)$ (see Cordeiro et al.,2021), where different power indices p yield different Gamma-compound Poisson families. The probability of observing a zero value can be calculated as a function of the power index p and the expectation (see Jørgensen,1987). In the analysis described above, we calculated the probability of each observation taking the value zero using a grid of values of the power index p . We estimated the expected number of zeroes for each observation week by summing the probability of observing a zero for each observation made in this week. This process was repeated for each value of the power index p in a grid of possible values. We used in the analysis the value of the power index p that minimised the Euclidean distance between the vector containing the observed proportions of zeroes for each week and the vector of expected number of zeroes for each week.

3 Multivariate Simultaneous Models for Responses of Different Statistical Nature

3.1 A Multivariate Construction

We use now the marginal GLMMs described above to formulate a MGLMM that simultaneously describe the 16 responses observed in this experiment. The idea explored here is to combine the marginal models into a MGLMM by constructing a 16-dimensional multivariate Gaussian random component (corresponding to the 16 responses) for each glass. More precisely, define, for $g = 1, \dots, 10$,

$$\mathbf{B}_g = (U_g^{[1]}, \dots, U_g^{[14]}, V_g, W_g).$$

According to the MGLMM we define here, the random components $\mathbf{B}_1, \dots, \mathbf{B}_{10}$ are independent and multivariate normally distributed with distribution given by

$$\mathbf{B}_g \sim N_{16}(\mathbf{0}, \Sigma), \text{ for } g = 1, \dots, 10.$$

Moreover, we assume that the multivariate vectors of responses, $(X_{tg}^{[1]}, \dots, X_{tg}^{[14]}, Y_{tg}, Z_{tg})$, for $t = 6, 12, 18$ and $g = 1, \dots, 10$, are conditionally independent given the random components $\mathbf{B}_1, \dots, \mathbf{B}_{10}$. Moreover, according to the MGLMM, for $t = 6, 12, 18$ and $g = 1, \dots, 10$, the vector of responses $(X_{tg}^{[1]}, \dots, X_{tg}^{[14]}, Y_{tg}, Z_{tg})$ is conditionally distributed given \mathbf{B}_g as specified below

$$\left\{ \begin{array}{l} X_{tg}^{[1]} | U_g^{[1]} = u_1 \sim \text{Ga} \left(\exp \left\{ \theta_t^{[1]} + u_1 \right\}, \lambda_1 \right), \forall u_1 \in \mathbb{R} \\ \vdots \\ X_{tg}^{[14]} | U_g^{[14]} = u_{14} \sim \text{Ga} \left(\exp \left\{ \theta_t^{[14]} + u_{14} \right\}, \lambda_{14} \right), \forall u_{14} \in \mathbb{R} \\ Y_{tg} | V_g = v \sim \text{Bi} \left(9, \text{logit}^{-1} \left\{ \alpha_t + v \right\} \right), \forall v \in \mathbb{R} \\ Z_{tg} | W_g = w \sim \text{ComPo} \left(\exp \left\{ \beta_t + w \right\}, \lambda_Z \right), \forall w \in \mathbb{R}. \end{array} \right. \quad (1)$$

Here the notation $X \sim \text{Ga}(\mu, \lambda)$ and $Z \sim \text{ComPo}(\mu, \lambda)$ indicates that X and Z are distributed according to the Gamma and Gamma-compound Poisson distributions with mean μ and dispersion parameter λ , respectively. $Y \sim \text{Bi}(n, p)$ denotes that Y is binomially distributed with size n and probability parameter p .

3.2 The Covariance Structure of the Random Components

The covariance structure of the random components (given by the covariance matrix Σ) will be characterised using the tools of graphical models. Before embracing this task, we give a short account of the basic theory of graphical models required for the exposition (see Whittaker, 1990 and Lauritzen, 1996 for details).

Let $\mathcal{G} = (\mathcal{V}, \mathcal{E})$ denote an undirected graph with vertices composed of random variables. A pair of vertices belong to the set of edges $\mathcal{E} \subseteq \mathcal{V} \times \mathcal{V}$ if, and only if, the corresponding variables are conditionally dependent given the remaining variables in the set of vertices \mathcal{V} . Usually, we represent the graph $\mathcal{G} = (\mathcal{V}, \mathcal{E})$ by a set of points in the plane corresponding to the vertices in \mathcal{V} ; an edge connecting two vertices is represented by a line connecting the two points corresponding to the vertices. The following basic definitions of graph theory will be necessary to characterise the covariance structure of the MGLMM we work with. We say that there is a path connecting two vertices, say v_1 and v_n , if there exists a sequence of vertices v_1, \dots, v_n such that, for $i = 1, \dots, n-1$, the pair (v_i, v_{i+1}) is in \mathcal{E} . A set of vertices S , separates two disjoint sets of vertices A and B in the graph $\mathcal{G} = (\mathcal{V}, \mathcal{E})$ when every path connecting a vertex in A to a vertex in B necessarily contains a vertex in S . According to the theory of graphical models (see Lauritzen, 1996 and Perl, 2009), the graph defined above satisfies the *separation principle*, which states that if a set of vertices S , separates two disjoint subsets of vertices A and B in the graph $\mathcal{G} = (\mathcal{V}, \mathcal{E})$, then all variables in A are independent of all variables in B given S . Moreover, if the subsets A and B are isolated (*i.e.*, there are no paths connecting a vertex in A to a vertex in B), then the variables in A are independent of the variables in B .

We characterise the covariance structure of the MGLMM defined in Section 3.1 by defining a graphical model constructed with its random components. In this way, for each g in $\{1, \dots, 10\}$, we might construct the graph $\mathcal{G}_g = (\mathcal{V}_g, \mathcal{E}_g)$ with the set of vertices $\mathcal{V}_g = \{U_g^{[1]}, \dots, U_g^{[14]}, V_g, W_g\}$ using the conventions defined above. Since the random vectors, $(U_g^{[1]}, \dots, U_g^{[14]}, V_g, W_g)$ for $g = 1, \dots, 10$, are per construction independent and identically distributed, the graphs $\mathcal{G}_1, \dots, \mathcal{G}_{10}$ are identical; therefore, we suppress the subindex g in the discussion below and use the notation $\mathcal{G} = (\mathcal{V}, \mathcal{E})$ to refer to a generic graph representing the (common) covariance structure of the random components.

Suppose that the multivariate random components of the MGLMM have a covariance structure encoded by the graph $\mathcal{G} = (\mathcal{V}, \mathcal{E})$. This covariance structure allows us to draw conclusions on the dependence of the unobservable random components, which is not our primary interest. In order to extend those conclusions to the observed re-

sponses, we should use the *induced separation principle*, defined in Pelck and Labouriau (2021B), which states that if two disjoint sets of random components, for example $A = \{U^{[5]}, \dots, U^{[14]}\}$ and $B = \{V, W\}$, are conditional independent given a separating set of random components $S = \{U^{[1]}, \dots, U^{[4]}\}$, then the corresponding set of conditional responses $\tilde{A} = \{X^{[5]}, \dots, X^{[14]}\}$ and $\tilde{B} = \{Y, Z\}$ are conditional independent given the set of random components S . This result implies that the knowledge of the random components in S renders the VOC's in \tilde{A} uninformative with respect to the lesion area and the proportion of apples presenting visible symptoms.

In the analysis of the experiment described above, we adjusted the GLMMs introduced in Section 2 using the Laplace approximation method proposed by Breslow and Clayton (1993). We modelled the predicted values of the random components of those models by finding the graph which minimises the BIC (Bayesian Information Criterium) as exposed in Abreu et al. (2010) (see also Edwards et al., 2010).

4 Results

We give below a brief description of the results obtained, see Holthusen et al. (2021a) for a full discussion. Figure 1 displays the representation of the estimated graph describing the covariance structure of the random components of the MGLMM we adjusted. It is remarkable that a group of only four random components related to VOCs (composed by anisole, 3-pentanone, 2-methyl-1-propanol and 2-phenylethanol) separates the random components associated to the two infection responses (*i.e.*, the infection proportion and the lesion area) from the random components connected with the other VOCs. Therefore, by the extended separation principle, the knowledge of the random components corresponding to the concentration of anisole, 3-pentanone, 2-methyl-1-propanol and 2-phenylethanol renders the concentrations of the other VOCs independent of the infection proportion and the lesion area.

We verified the adequacy of the MGLMM described in the following way. First, we checked the marginal GLMMs by plotting the Pearson residuals against the fitted values (not shown). No anomalies were encountered. Moreover, we applied the cumulative distribution function of the putative distribution to each observation (with the estimated mean and dispersion) and verified whether the resulted transformed observations adhered to the uniform distribution in the interval between 0 and 1. All the p-values found were larger than 0.10.

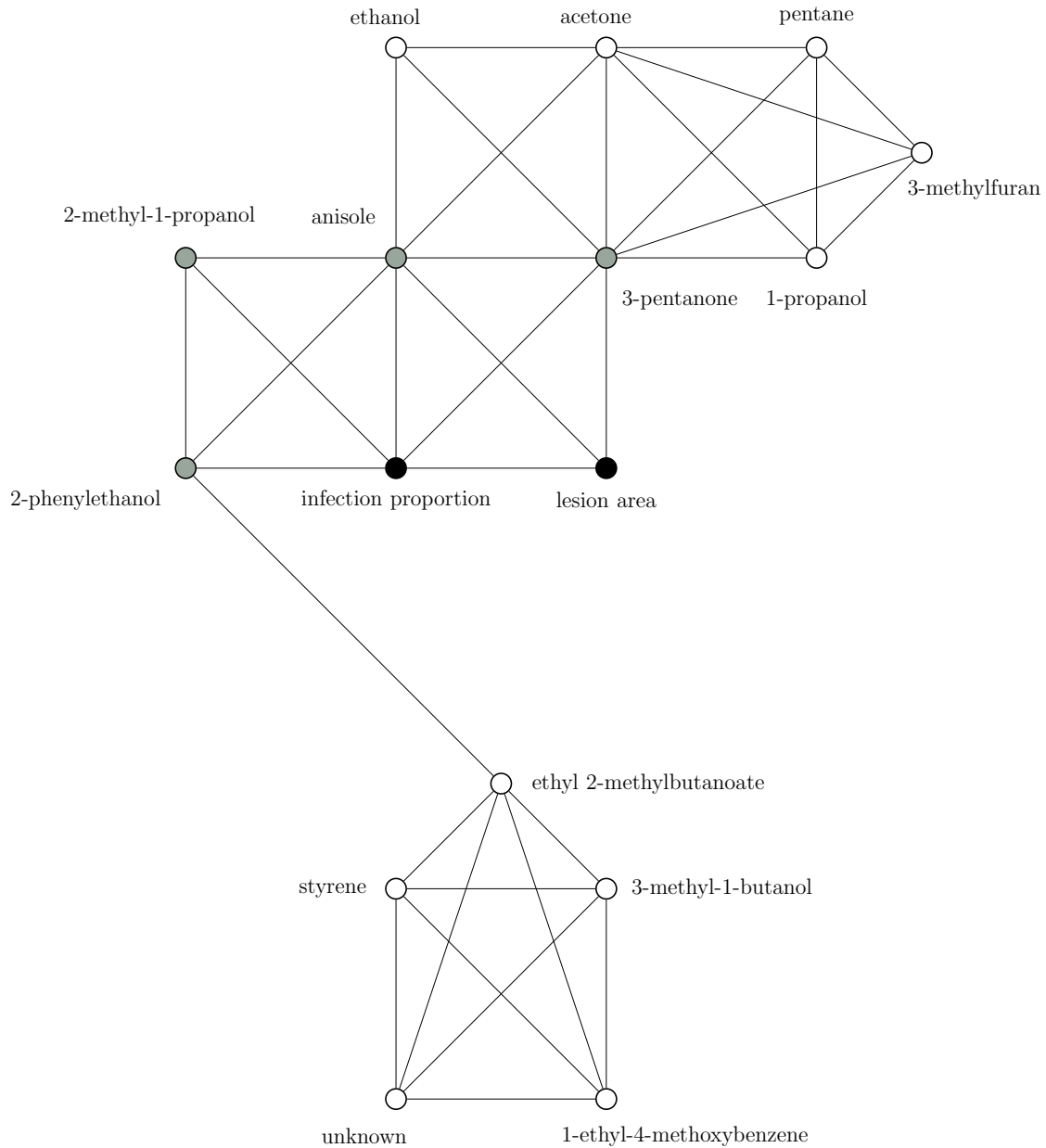


Figure 1: Graphical model representing the covariance structure of the random components related to the 14 VOCs, the lesion area and the proportion of infection. The vertices related to the infection responses (lesion area and proportion of infection) are represented as black circles. The vertices directly connected to the infection responses (depicted as grey circles) separate the vertices related to the infection responses from the vertices related to the other VOCs (represented as white circles).

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References

- Abreu, C. G., Edwards, D. and Labouriau, R. (2010). High-Dimensional Graphical Model Search with the gRapHD R Package. *Journal of Statistical Software*, Vol. **37**, issue 1.
- Ali, E.Md., Pandit, L.K., Mulvaney, K.A., Amiri, A. (2018). Sensitivity of *Phacidiopycnis spp.* isolates from pome fruit to six pre- and postharvest fungicides. *Plant Disease*, Vol. **102**, 533-539.
- Breslow, N. E. and Clayton, D. G (1993). Approximate inference in generalized linear mixed models. *Journal of the American statistical Association*, Vol. **88**, No 421, 9–25.
- Cordeiro, G. M., Labouriau, R. and Botter, D. (2021). An introduction to Bent Jørgensen’s ideas. *Brazilian Journal of Probability and Statistics*, Vol. **35**, No 1, 2–20.
- Edwards, D. (1995). Introduction to Graphical Modelling. *Springer-Verlag*, New York.
- Edwards, D., Abreu, C. G. and Labouriau, R. (2010). Selecting high-dimensional mixed graphical models using minimal AIC or BIC forests. *BMC Bioinformatics*, Vol. **11**, issue 18. doi:10.1186/1471-2105-11-18.
- Holthusen, H.H.F., Luca A., Pelck, J.S., Labouriau, R., and Edelenbos, M. (2021). Detection of rubbery rot caused by *Phacidiopycnis washingtonensis* by use of volatile monitoring in apple storage. *In preparation*.
- Holthusen, H.H.F., Luca A. , Weber, R.W.S and Edelenbos, M. (2021). Volatile markers emitted by *Phacidiopycnis washingtonensis* from agar culture and naturally infected apple fruit. *In preparation*.
- Holthusen, H.H.F. and Weber, R.W.S. (2021). Infection conditions for *Neofabraea perennans* and *Phacidiopycnis washingtonensis* on developing apple fruit in the orchard. *In preparation*.

Jørgensen, B. (1987). Exponential dispersion models. *Journal of the Royal Statistical Society. Series B (Methodological)*. JSTOR

Jørgensen, B. and Labouriau, R. (2012). Exponential Families and Theoretical Inference. *Monografias de Matematica 52*, 196p. Instituto de Matematica Pura e Aplicada (IMPA), Rio de Janeiro, Brazil. <https://pure.au.dk/portal/files/51499534/Mon52.pdf>

Labouriau, R. (2021). Inference and Characterisation of the Gamma-compound Poisson Family. *In preparation*.

Lauritzen, S. L. (1969). Graphical models. *Clarendon Press*.

Pelck, J. S. and Labouriau, R. (2021). Conditional inference for multivariate generalised linear mixed models. *In preparation*.

Pelck, J. S. and Labouriau, R. (2021). Multivariate generalised linear mixed models with graphical latent covariance structure. *In preparation*.

Perl, J. (2009). Causality: models, reasoning and inference. Second Edition. Cambridge University Press.

Tweedie, M.C.K. (1984). An index with distinguishes between some important exponential families. In *Statistics: Applications and New Directions. Proceedings of the Indian Golden Jubilee International Conference* (Eds. J.K. Ghosh and J. Roy), pp 579–604. Calcuta: Indian Statistical Institute.

Whittaker, J (1990). Graphical models in applied multivariate analysis. *Chichester New York et al: John Wiley & Sons*.