Data Analysis for the Merlin Bioassay

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1 Introduction

1.1 Assay Background

A single unit of the Merlin bioassay comprises of a 96-well microplate in which roughly 10 to 15 *Drosophila melanogaster* L1 larvae are reared in a liquid medium containing nominally known concentrations of a test compound. The eight rows of the plate allow for testing of up to eight different compounds, and each compound is tested at (generally) ten different concentrations using two-fold serial dilutions, and a set of controls are also run. After an incubation period in the treated media, the number of living larvae (a_i) and dead larvae (d_i) is determined for each well i.

Our goal is to use the results of multiple replicates of this assay to produce robust dose-response curves, calculate curve errors, determing credible intervals for data points, and find LC_{50} values and relative potenencies.

1.2 Dose-Response Curve Background

We wish to fit a logistic curve to the data, relating the logarithm of the concentration of compound as our independent variable, to the probability of survival (the dependent variable). For this, we will use the generalized logisitic curve,

$$s(x) = L + \frac{U - L}{(1 + Qe^{-Bx})^{1/\nu}},$$

where L is the lower horizontal asymptote, U is the upper horizontal asymptote, Q is related to $y(0)$, B is the growth rate, and ν is a shape asymmetry parameter. For the purposes of our bioassay, if we treat $y(x)$ as the survival propability at concentration x, then we can simplify and reparameterize as

$$s(x) = \frac{b_2}{(1 + e^{b_0 + b_1 x})^{b_3}}, \tag{1.1}$$

where b_2 is related the background control survival rate (equal to 1 if there is 100% survival, b_3 is the shape asymmetry parameter, b_1 is the growth rate, and b_0 is related to behavior at $x = 1/b_1$. Further simplifications of equation 1.1 can be made by setting one or both of b_2 or b_3 to 1, and these cases are treated *vide infra*.

2   Bootstrapping

2.1   Beginning of the Bayesian Framework

In our assay, a_i and d_i are estimated by the program I wrote that analyzes the platereader images. Because these values are probabilistic estimates, they are not confined the non-zero integers, and instead can take on any non-negative real value. While this can present an issue for a true binomial distribution, we will be using Bayesian inference methods, and this is not a problem. Instead, a_i and d_i represent the evidence in the Bayesian framework, and we will use weakly informative or uninformative priors to drive our inference.

Because a_i and d_i are representative of binomial data, we can use $\text{Beta}(1,1) = U(0,1)$ as an uninformative prior; this has the added benefit of being identical to the rule of succession method. We then have $s_i \sim \text{Beta}(a_i + 1, d_i + 1)$ as the posterior distribution, and sampling this distribution allows us to easily bootstrap thousands of replicates based on one trial to get Bayesian credible intervals.

2.1.1   Correlation between wells

Of considerable interest is the potential to have correlation between adjacent wells–this stems from the fact that the concentrations in the wells are determined by serial dilution. There are several possible ways to handle potential correlation; several of these are as follows:

1. Assume that all wells are independent, and that there is no correlation between wells of the same compound in a plate, or

2. All wells w_i and w_j have a constant correlation ρ for $i \neq j$, or

3. The correlation $\rho_{i,j}$ between wells w_i and w_j should fall as the number of dilutions between w_i and w_j increases.

The assumption of no correlation, while easiest, is likely a poor choice, as c_{i+1} is of course dependent on c_i, as any difference in the actual concentration a well and the nominal concentration are likely to propagate down the plate. If we assume that each serial dilution is performed adequately, then any differences between the nominal and actual concentrations should be due to random error, and the thus the correlation between wells w_i and w_j should fall as the number of dilutions between them increases.

2.1.2   Production of correlated Beta random variables

To produce correlated beta random variables, we first create a multivariate normal distribution $\mathcal{N}(\mathbf{0}, \boldsymbol{\Sigma})$ with dimension n and covariance matrix $\boldsymbol{\Sigma} \in \mathbb{R}^{n \times n}$ with $\text{diag}(\boldsymbol{\Sigma}) = (1, 1, \ldots, 1)$, and sample a standard normal random vector $\boldsymbol{x} = (x_1, x_2, \ldots, x_n)^T$ from this distribution N times, where N is the number of bootstraps we wish to use. We note that since $\text{diag}(\boldsymbol{\Sigma}) = (1, 1, \ldots, 1)$, the marginal of each dim of $\mathcal{N}(\mathbf{0}, \boldsymbol{\Sigma})$ is the standard normal distribution $\mathcal{N}(0, 1)$. Let $\boldsymbol{\Phi}^{-1} : \mathbb{R}^n \to \mathbb{R}^n$ be the multivariate Gaussian copula, that is, $\boldsymbol{\Phi}^{-1}(\boldsymbol{x}) = \left(\Phi^{-1}(x_1), \Phi^{-1}(x_2), \ldots, \Phi^{-1}(x_n)\right)^T \in (0, 1)^n$, where Φ^{-1} is the inverse cumulative distribution function of the standard normal distribution.

Then define function $\mathbf{F} : (0, 1)^n \times \mathbb{R}^n \times \mathbb{R}^n \to (0, 1)^n$ as the Beta quantile function such that, given shape parameter vectors $\boldsymbol{a} = (a_1, a_2, \ldots, a_n)$ and $\boldsymbol{d} = (d_1, d_2, \ldots, d_n)$, $\mathbf{F}(\boldsymbol{y}, \boldsymbol{a}, \boldsymbol{d}) = (F(y_1, a_1, d_1), F(y_2, a_2, d_2), \ldots, F(y_n, a_n, d_n))$, where $F(y_i, a_i, d_i)$ is the Beta quantile function for quantile y_i with Beta distribution shape parameters a_i and d_i. Then $\boldsymbol{F}\left(\boldsymbol{\Phi}^{-1}(\boldsymbol{x}), \boldsymbol{a}, \boldsymbol{d}\right)$ produces

random vectors of correlated beta variables, with correlations determined by $\boldsymbol{\Sigma}$. All that remains now is to construct a suitable $\boldsymbol{\Sigma}$.

Without loss of generality, number the plates a compound is tested in as $p_1, p_2, \ldots, p_k, \ldots, p_m$, where p_j has n_j wells. Additionally, order the wells by plate such that wells 1 to n_1 are arranged such that $c_1 > c_2 > \cdots > c_{n_1}$, wells n_1+1 to n_1+n_2 are arranged such that $c_{n_1+1} > c_{n_1+2} > \cdots > c_{n_1+n_2}$, and so on, up to wells $n - n_j + 1$ to n, where $c_{n-n_j+1} > c_{n-n_j+2} > \cdots > c_n$; that is, wells are ordered by plate, and within each plate, ordered by decreasing concentration. This is a logical order as this is how the plates are set up for the bioassay.

We now with to construct a suitable $\boldsymbol{\Sigma}$ in which correlations are higher for adjacent wells than for distant wells. Define a correlation matrix for plate k, $\boldsymbol{P}_k$, such that

$$
\boldsymbol{P}_{k_{i,j}} = \begin{cases} 1 & \text{if } i = j \\ \rho \cdot 2^{1-|i-j|} & \text{if } i \neq j \end{cases} = \begin{bmatrix} 1 & \rho & \frac{\rho}{2} & \frac{\rho}{4} & \frac{\rho}{8} & \cdots & \frac{\rho}{2^{2-j}} \\ \rho & 1 & \rho & \frac{\rho}{2} & \frac{\rho}{4} & \cdots & \frac{\rho}{2^{3-j}} \\ \frac{\rho}{2} & \rho & 1 & \rho & \frac{\rho}{2} & \cdots & \frac{\rho}{2^{4-j}} \\ \frac{\rho}{4} & \frac{\rho}{2} & \rho & 1 & \rho & \cdots & \frac{\rho}{2^{5-j}} \\ \frac{\rho}{8} & \frac{\rho}{4} & \frac{\rho}{2} & \rho & 1 & \cdots & \frac{\rho}{2^{6-j}} \\ \vdots & \vdots & \vdots & \vdots & \vdots & \ddots & \vdots \\ \frac{\rho}{2^{2-i}} & \frac{\rho}{2^{3-i}} & \frac{\rho}{2^{4-i}} & \frac{\rho}{2^{5-i}} & \frac{\rho}{2^{6-i}} & \cdots & 1 \end{bmatrix}
$$

for some $\rho \in (-0.25, 0.25)$.[1]

Now define the diagonal block matrix

$$
\boldsymbol{\Sigma} = \bigoplus_{k=1}^{m} \boldsymbol{P}_k = \begin{bmatrix} \boldsymbol{P}_1 & \boldsymbol{0} & \boldsymbol{0} & \cdots & \boldsymbol{0} \\ \boldsymbol{0} & \boldsymbol{P}_2 & \boldsymbol{0} & \cdots & \boldsymbol{0} \\ \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{P}_3 & \cdots & \boldsymbol{0} \\ \vdots & \vdots & \vdots & \ddots & \vdots \\ \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{0} & \cdots & \boldsymbol{P}_m \end{bmatrix}.
$$

All that remains is to show that $\boldsymbol{\Sigma}$ is symmetric positive definite, which is required for $\mathcal{N}(\boldsymbol{0}, \boldsymbol{\Sigma})$ to be a non-degenerate multivariate normal distribution.

Proof. By construction, each $\boldsymbol{P}_k$ is symmetrical and real-valued (and therefore Hermitian), and thus all eigenvalues of P_k are real. By choice of $\rho \in [-0.25, 0.25]$, we have, for any fixed i,

$$
\sum_{i \neq j} |\boldsymbol{P}_{k_{i,j}}| < 2 \sum_{i=0}^{n} |\rho| \cdot 2^{-i}
$$

$$
< 2|\rho| \sum_{i=0}^{\infty} 2^{-i}
$$

$$
= 4|\rho|
$$

$$
< \boldsymbol{P}_{k_{i,i}} = 1,
$$

---

[1]The bounds on ρ are actually substantially more generous, with $\rho \in [-1/3, 2/3]$ as $n \to \infty$. See the Appendix for details.

and so P_k is diagonally dominant. By the Gershgorin Circle theorem, all the eigenvalues of P_k are strictly positive, and thus, P_k is positive definite. Since Σ is block diagonal with off-diagonal blocks as 0, we have

$$\text{eigen}(\Sigma) = \bigcup_{k=1}^{m} \text{eigen}(P_k),$$

and thus Σ is positive definite. $\qquad\square$

2.2 General Information

Each compound of interest (herein, the compound) will eventually be tested on $m > 1$ plates for the purpose of replicates. Each plate j contains n_j wells typically 10) dosed with the compound; with m plates, we therefore have $n = \sum_{j=1}^{m} n_j$ wells to consider. Each well n_i contains the compound in a concentration of y_i, and at the end of the assay, contains a_i alive larvae and d_i dead larvae.

Our interest is in fitting a dose-response curve to this data, using the survival in a well, $s_i = \frac{a_i}{a_i+d_i}$, as a response variable to the concentration of the compound. We use the logistic curve

$$s(x) = \frac{1}{(1 + e^{b_0 + b_1 x})^{b_2}},$$

where $s(x)$ is the estimated survival at concentration $e^x = c$ (that is, $x = \log c$ for some concentration c), and $b = (b_0, b_1, b_2)$ are curve parameters. When $b_2 = 1$, we end up with the two-parameter logistic curve

$$s(x) = \frac{1}{1 + e^{b_0 + b_1 x}},$$

while we otherwise have the three parameter logistic curve.

Under traditional methods, we would use nonlinear least squares to fit the points

$$(x_1, s_1), (x_2, s_2), \ldots, (x_n, s_n)$$

to the curve $s = f(x, b)$, while minimizing the sum of squares of the residuals

$$S = \sum_{i=1}^{n} (s_i - f(x_i, b))^2.$$

If desired, we can weight the residuals based on how many larvae are in a well, on the basis that wells with more larvae ought to be considered more reliable; in this case, we would have

$$S = \sum_{i=1}^{n} W_i (s_i - f(x_i, b))^2,$$

where $W_i = (a_i + d_i)$, a weighting equal to the total number of larvae in the well, and inversely proportional to the error variance of a binomial random variable. Then we have

$$\hat{b} = \arg\min_{b} \sum_{i=1}^{n} W_i (s_i - f(x_i, b))^2,$$

which can be found using the usual optimization methods.

3 Bayesian Curve Fitting

3.1 Two-Parameter Logistic Curve

We first note that the complement to the logistic curve

$$y(x, \boldsymbol{b}) = \frac{1}{1 + \exp(b_0 + b_1 x)}$$

is

$$
\begin{aligned}
1 - y(x, \boldsymbol{b}) &= 1 - \frac{1}{1 + \exp(b_0 + b_1 x)} \\
&= \frac{\exp(b_0 + b_1 x)}{1 + \exp(b_0 + b_1 x)}.
\end{aligned}
$$

For simplicity, we set $\xi = \exp(b_0 + b_i x)$, so

$$y(x, \boldsymbol{b}) = \frac{1}{1 + \xi}; \qquad 1 - y(x, \boldsymbol{b}) = \frac{\xi}{1 + \xi}$$

3.1.1 Log-likelihood function

Using Bayes' law with evidence $\boldsymbol{\theta}$, we can fit $y(x, \boldsymbol{b})$ by finding the appropriate posterior distribution of $\boldsymbol{b}$ given $\boldsymbol{\theta}$. Specifically, we have

$$f(\boldsymbol{b}|\boldsymbol{\theta}) = kf(\boldsymbol{\theta}|\boldsymbol{b})f(\boldsymbol{b})$$

for prior distribution $f(\boldsymbol{b})$, conditional probability $f(\boldsymbol{\theta}|\boldsymbol{b})$, and regularization parameter k such that $1 = k \int_\Omega f(\boldsymbol{b}|\boldsymbol{\theta}) d\boldsymbol{\theta}$.[2] Then the conditional probability $f(\boldsymbol{b}|\boldsymbol{\theta})$ is given by

$$
\begin{aligned}
f(\boldsymbol{b}|\boldsymbol{\theta}) &= kf(\boldsymbol{b}) \cdot \prod_{i=1}^{n} \left(\frac{\exp(b_0 + b_1 x_i)}{1 + \xi_i} \right)^{\theta_i} \left(\frac{1}{1 + \xi_i} \right)^{1 - \theta_i} \\
&= kf(\boldsymbol{b}) \cdot \prod_{i=1}^{n} \left(\frac{\exp(b_0 + b_1 x_i)^{\theta_i}}{(1 + \xi_i)^{\theta_i} (1 + \xi_i)^{1 - \theta_i}} \right) \\
&= kf(\boldsymbol{b}) \cdot \frac{\exp \left(\sum_i \theta_i b_0 + \sum_i \theta_i b_1 x_i \right)}{\prod_{i=1}^{n} (1 + \xi_i)}
\end{aligned}
$$

If we use an uncorrelated multivariate normal Gaussian centered on $(0,0)$ for $\boldsymbol{b}$ with large variance σ^2, and using likelihood function $\mathcal{L}_2(\boldsymbol{\theta}|\boldsymbol{b}) = f(\boldsymbol{b}|\boldsymbol{\theta})$ we get the log-likelihood function

$$
\begin{aligned}
\ell_2(\boldsymbol{b}|\boldsymbol{\theta}) &= \ln \mathcal{L}_2(\boldsymbol{\theta}|\boldsymbol{b}) \\
&= \ln(kf(\boldsymbol{b})f(\boldsymbol{b}|\boldsymbol{\theta})) \\
&= \ln k + \ln \left(\frac{1}{2\pi\sigma^2} \exp \left(-\frac{b_0^2 + b_1^2}{2\sigma^2} \right) \right) + \ln \left(\frac{\exp \left(\sum_i \theta_i b_0 + \sum_i \theta_i b_1 x_i \right)}{\prod_{i=1}^{n} (1 + \xi_i)} \right) \\
&= \ln k - \ln(2\pi\sigma^2) - \frac{b_0^2 + b_1^2}{2\sigma^2} + b_0 \sum_{i=1}^{n} \theta_i + b_1 \sum_{i=1}^{n} \theta_i x_i - \sum_{i=1}^{n} \ln (1 + \xi_i)
\end{aligned}
$$

---

[2]The value of k itself is not usually important at this point, since it becomes a constant factor in the log-likelihood function that is not included for our purposes.

$$\cong -\frac{b_0^2 + b_1^2}{2\sigma^2} + b_0 \sum_{i=1}^{n} \theta_i + b_1 \sum_{i=1}^{n} \theta_i x_i - \sum_{i=1}^{n} \ln\left(1 + \xi_i\right),$$

for constant hyperparameter σ after removal of constant terms.

3.1.2   Optimization of the log-likelihood function: The gradient

The gradient of the two-parameter log-likelihood function, $\nabla \ell_2(\boldsymbol{b}|\boldsymbol{\theta})$, is given by

$$\nabla \ell_2(\boldsymbol{b}|\boldsymbol{\theta}) = \begin{pmatrix} \dfrac{\partial \ell_2}{\partial b_0} \\ \dfrac{\partial \ell_2}{\partial b_1} \end{pmatrix},$$

where

$$\frac{\partial \ell_2(\boldsymbol{b}|\boldsymbol{\theta})}{\partial b_0} = -\frac{b_0}{\sigma^2} + \sum_{i=1}^{n} \theta_i - \sum_{i=1}^{n} \frac{\xi_i}{1 + \xi_i}$$

$$\frac{\partial \ell_2(\boldsymbol{b}|\boldsymbol{\theta})}{\partial b_1} = -\frac{b_1}{\sigma^2} + \sum_{i=1}^{n} \theta_i x_i - \sum_{i=1}^{n} \frac{x_i \xi_i}{1 + \xi_i}.$$

3.1.3   Optimization of the log-likelihood function: The Hessian

The Hessian matrix of $\ell_2(\boldsymbol{b}|\boldsymbol{\theta})$ is given by

$$H_{\ell_2} = \begin{bmatrix} \dfrac{\partial^2 \ell_2}{\partial b_0^2} & \dfrac{\partial^2 \ell_2}{\partial b_0 \partial b_1} \\ \dfrac{\partial^2 \ell_2}{\partial b_1 \partial b_0} & \dfrac{\partial^2 \ell_2}{\partial b_1^2} \end{bmatrix},$$

where

$$\frac{\partial^2 \ell_2(\boldsymbol{b}|\boldsymbol{\theta})}{\partial b_0^2} = -\frac{1}{\sigma^2} - \sum_{i=1}^{n} \frac{\xi_i}{(1 + \xi_i)^2}$$

$$\frac{\partial^2 \ell_2(\boldsymbol{b}|\boldsymbol{\theta})}{\partial b_0 \partial b_1} = \frac{\partial^2 \ell_2(\boldsymbol{b}|\boldsymbol{\theta})}{\partial b_1 \partial b_0} = -\sum_{i=1}^{n} \frac{x_i \xi_i}{(1 + \xi_i)^2}$$

$$\frac{\partial^2 \ell_2(\boldsymbol{b}|\boldsymbol{\theta})}{\partial b_0^2} = -\frac{1}{\sigma^2} - \sum_{i=1}^{n} \frac{x_i^2 \xi_i}{(1 + \xi_i)^2}.$$

3.2   Three-Parameter Logistic Curve (Asymmetry Parameter)

With $\xi = \exp(b_0 + b_i x)$, we first note that the complement to the three-parameter logistic curve

$$y(x, \boldsymbol{b}) = \frac{1}{(1 + \xi)^{b_2}}$$

is

$$1 - y(x, \boldsymbol{b}) = 1 - \frac{1}{(1 + \xi)^{b_2}}$$
$$= \frac{(1 + \xi)^{b_2} - 1}{(1 + \xi)^{b_2}}.$$

3.2.1 Log-likelihood function

As in Section 3.1.1, we begin with Bayes's theorem. The conditional probability $f(\boldsymbol{b}|\boldsymbol{\theta})$ is given by

$$f(\boldsymbol{b}|\boldsymbol{\theta}) = kf(\boldsymbol{\theta}|\boldsymbol{b})f(\boldsymbol{b})$$

$$= kf(\boldsymbol{b}) \cdot \prod_{i=1}^{n} \left(\frac{(1 + \xi_i)^{b_2} - 1}{(1 + \xi_i)^{b_2}} \right)^{\theta_i} \left(\frac{1}{(1 + \xi_i)^{b_2}} \right)^{1 - \theta_i}$$

$$= kf(\boldsymbol{b}) \cdot \prod_{i=1}^{n} \left(\frac{\left((1 + \xi_i)^{b_2} - 1\right)^{\theta_i}}{(1 + \xi_i)^{b_2 \theta_i} (1 + \xi_i)^{b_2(1 - \theta_i)}} \right)$$

$$= kf(\boldsymbol{b}) \cdot \frac{\prod_{i=1}^{n} \left((1 + \xi_i)^{b_2} - 1\right)^{\theta_i}}{\prod_{i=1}^{n} (1 + \xi_i)^{b_2}}.$$

For our choice of priors on $\boldsymbol{b}$, we again select (b_0, b_1) to have a multivariate Gaussian distribution $\mathcal{N}(\boldsymbol{0}, \sigma I_2)$, where I_2 is the 2×2 identity matrix. For b_2, which should remain strictly positive, we use a gamma distribution prior

$$g(x) = \frac{\beta^\alpha}{\Gamma(\alpha)} x^{\alpha - 1} e^{-\beta x},$$

where α and β are shape and rate parameters, respectively. Using the likelihood function $\mathcal{L}_3(\boldsymbol{\theta}|\boldsymbol{b}) = f(\boldsymbol{b}|\boldsymbol{\theta})$, we get the log-likelihood function

$$\ell_3(\boldsymbol{b}|\boldsymbol{\theta}) = \ln \mathcal{L}_3(\boldsymbol{\theta}|\boldsymbol{b})$$

$$= \ln(kf(\boldsymbol{b})f(\boldsymbol{b}|\boldsymbol{\theta}))$$

$$= \ln k + \ln \left(\frac{1}{2\pi\sigma^2} \exp\left(\frac{b_0^2 + b_1^2}{2\sigma^2} \right) \right) + \ln \left(\frac{\beta^\alpha}{\Gamma(a)} b_2^{\alpha - 1} e^{-\beta b_2} \right) + \ln \prod_{i=1}^{n} \left((1 + \xi_i)^{b_2} - 1\right)^{\theta_i}$$

$$- \ln \prod_{i=1}^{n} (1 + \xi_i)^{b_2}$$

$$= \ln k - \ln(2\pi) - 2\ln\sigma + \frac{b_0^2 + b_1^2}{2\sigma^2} + \ln\left(\frac{\beta^\alpha}{\Gamma(\alpha)} \right) + (\alpha - 1)\ln b_2 - \beta b_2$$

$$+ \sum_{i=1}^{n} \theta_i \ln \left((1 + \xi_i)^{b_2} - 1\right) - b_2 \sum_{i=1}^{n} \ln(1 + \xi_i)$$

$$\cong -2\ln\sigma + \frac{b_0^2 + b_1^2}{2\sigma^2} + (\alpha - 1)\ln b_2 - \beta b_2 + \sum_{i=1}^{n} \theta_i \ln \left((1 + \xi_i)^{b_2} - 1\right) - b_2 \sum_{i=1}^{n} \ln(1 + \xi_i)$$

for constant hyperparameters σ, α, β after removal of constant terms.

3.2.2 Optimization of the log-likelihood function: The gradient

The gradient of the three-parameter log-likelihood function, $\nabla \ell_2(\boldsymbol{b}|\boldsymbol{\theta})$, is given by

$$\nabla \ell_3(\boldsymbol{b}|\boldsymbol{\theta}) = \left(\frac{\partial \ell_3}{\partial b_0}, \quad \frac{\partial \ell_3}{\partial b_1}, \quad \frac{\partial \ell_3}{\partial b_2} \right)^T,$$

where

$$\frac{\partial \ell_3(\boldsymbol{b}|\boldsymbol{\theta})}{\partial b_0} = \frac{b_0}{\sigma^2} + b_2 \sum_{i=1}^{n} \frac{\theta_i \xi_i (1 + \xi_i)^{b_2-1}}{(1 + \xi_i)^{b_2} - 1} - b_2 \sum_{i=1}^{n} \frac{\xi_i}{1 + \xi_i}$$

$$\frac{\partial \ell_3(\boldsymbol{b}|\boldsymbol{\theta})}{\partial b_1} = \frac{b_1}{\sigma^2} + b_2 \sum_{i=1}^{n} \frac{\theta_i x_i \xi_i (1 + \xi_i)^{b_2-1}}{(1 + \xi_i)^{b_2} - 1} - b_2 \sum_{i=1}^{n} \frac{x_i \xi_i}{1 + \xi_i}$$

$$\frac{\partial \ell_3(\boldsymbol{b}|\boldsymbol{\theta})}{\partial b_2} = \frac{\alpha - 1}{b_2} - \beta + \sum_{i=1}^{n} \frac{\theta_i (1 + \xi_i)^{b_2} \ln(1 + \xi_i)}{(1 + \xi_i)^{b_2} - 1} - \sum_{i=1}^{n} \ln(1 + \xi_i).$$

3.2.3 Optimization of the log-likelihood function: The Hessian

The Hessian matrix of $\ell_3(\boldsymbol{b}|\boldsymbol{\theta})$ is given by

$$H_{\ell_3} = \begin{bmatrix} \dfrac{\partial^2 \ell_3}{\partial b_0^2} & \dfrac{\partial^2 \ell_3}{\partial b_0 \partial b_1} & \dfrac{\partial^2 \ell_3}{\partial b_0 \partial b_2} \\[2mm] \dfrac{\partial^2 \ell_3}{\partial b_1 \partial b_0} & \dfrac{\partial^2 \ell_3}{\partial b_1^2} & \dfrac{\partial^2 \ell_3}{\partial b_1 \partial b_2} \\[2mm] \dfrac{\partial^2 \ell_3}{\partial b_2 \partial b_0} & \dfrac{\partial^2 \ell_3}{\partial b_2 \partial b_1} & \dfrac{\partial^2 \ell_3}{\partial b_2^2} \end{bmatrix},$$

where

$$\frac{\partial^2 \ell_3(\boldsymbol{b}|\boldsymbol{\theta})}{\partial b_0^2} = \frac{1}{\sigma^2} + b_2 \sum_{i=1}^{n} \frac{\theta_i \xi_i (1 + \xi_i)^{b_2-2} ((1 + \xi_i)^{b_2} - 1 - b_2 \xi_i)}{((1 + \xi_i)^{b_2} - 1)^2} - b_2 \sum_{i=1}^{n} \frac{\xi_i}{(1 + \xi_i)^2}$$

$$\frac{\partial^2 \ell_3(\boldsymbol{b}|\boldsymbol{\theta})}{\partial b_0 \partial b_1} = \frac{\partial^2 \ell_3(\boldsymbol{b}|\boldsymbol{\theta})}{\partial b_1 \partial b_0} = b_2 \sum_{i=1}^{n} \frac{\theta_i x_i \xi_i (1 + \xi_i)^{b_2-2} ((1 + \xi_i)^{b_2} - 1 - b_2 \xi_i)}{((1 + \xi_i)^{b_2} - 1)^2} - b_2 \sum_{i=1}^{n} \frac{x_i \xi_i}{(1 + \xi_i)^2}$$

$$\frac{\partial^2 \ell_3(\boldsymbol{b}|\boldsymbol{\theta})}{\partial b_0 \partial b_2} = \frac{\partial^2 \ell_3(\boldsymbol{b}|\boldsymbol{\theta})}{\partial b_2 \partial b_0} = \sum_{i=1}^{n} \frac{\theta_i \xi_i (1 + \xi_i)^{b_2-1} \left((1 + \xi_i)^{b_2} - b_2 \ln(1 + \xi_i) - 1\right)}{((1 + \xi_i)^{b_2} - 1)^2} - \sum_{i=1}^{n} \frac{\xi_i}{1 + \xi_i}$$

$$\frac{\partial^2 \ell_3(\boldsymbol{b}|\boldsymbol{\theta})}{\partial b_1^2} = \frac{1}{\sigma^2} + b_2 \sum_{i=1}^{n} \frac{\theta_i x_i^2 \xi_i (1 + \xi_i)^{b_2-2} ((1 + \xi_i)^{b_2} - 1 - b_2 \xi_i)}{((1 + \xi_i)^{b_2} - 1)^2} - b_2 \sum_{i=1}^{n} \frac{x_i^2 \xi_i}{(1 + \xi_i)^2}$$

$$\frac{\partial^2 \ell_3(\boldsymbol{b}|\boldsymbol{\theta})}{\partial b_1 \partial b_2} = \frac{\partial^2 \ell_3(\boldsymbol{b}|\boldsymbol{\theta})}{\partial b_2 \partial b_1} = \sum_{i=1}^{n} \frac{\theta_i x_i \xi_i (1 + \xi_i)^{b_2-1} \left((1 + \xi_i)^{b_2} - b_2 \ln(1 + \xi_i) - 1\right)}{((1 + \xi_i)^{b_2} - 1)^2} - \sum_{i=1}^{n} \frac{x_i \xi_i}{1 + \xi_i}$$

$$\frac{\partial^2 \ell_3(\boldsymbol{b}|\boldsymbol{\theta})}{\partial b_2^2} = \frac{1 - \alpha}{b_2^2} - \sum_{i=1}^{n} \frac{\theta_i (1 + \xi_i)^{b_2} \ln^2(1 + \xi_i)}{((1 + \xi_i)^{b_2} - 1)^2}$$

3.3   Three-Parameter Logistic Curve (Scale Parameter)

We now explore a three-parameter logistic curve wherein the third parameter is scale parameter, $b_2 \in (0, 1)$, such that our curve is given by the formula

$$y(x, \boldsymbol{b}) = \frac{b_2}{1 + \xi}.$$

This curve is useful when we have high control mortality (with an average mortality rate of $1 - b_2$), and thus an upper asymptote of 1 is no longer appropriate. We first note that the complement to this scaled three-parameter logistic curve is

$$
\begin{aligned}
1 - y(x, \boldsymbol{b}) &= 1 - \frac{b_2}{1 + \xi} \\
&= \frac{1 + \xi - b_2}{1 + \xi}.
\end{aligned}
$$

3.3.1   Log-likelihood function

Beginning with Bayes's theorem, the conditional probability $f(\boldsymbol{b}|\boldsymbol{\theta})$ is given by

$$
\begin{aligned}
f(\boldsymbol{b}|\boldsymbol{\theta}) &= k f(\boldsymbol{\theta}|\boldsymbol{b}) f(\boldsymbol{b}) \\
&= k f(\boldsymbol{b}) \cdot \prod_{i=1}^{n} \left(\frac{1 + \xi_i - b_2}{1 + \xi_i} \right)^{\theta_i} \left(\frac{b_2}{1 + \xi_i} \right)^{1-\theta_i} \\
&= k f(\boldsymbol{b}) \cdot \prod_{i=1}^{n} \frac{(1 + \xi_i - b_2)^{\theta_i} b_2^{1-\theta_i}}{1 + \xi_i}.
\end{aligned}
$$

For our choice of priors on $\boldsymbol{b}$, we again select (b_0, b_1) to have multivariate Gaussian distribution $\mathcal{N}(\boldsymbol{0}, \sigma I_2)$, where I_2 is the 2×2 identity matrix. For $b_2 \in (0, 1)$, we use a beta distribution prior

$$g(x) = \frac{\Gamma(\alpha + \beta)}{\Gamma(\alpha)\Gamma(\beta)} x^{\alpha-1}(1 - x)^{\beta-1},$$

where α and β are shape parameters. Using the likelihood function $\mathcal{L}_{3'}(\boldsymbol{\theta}|\boldsymbol{b}) = f(\boldsymbol{b}|\boldsymbol{\theta})$, we get the log-likelihood function

$$
\begin{aligned}
\ell_{3'}(\boldsymbol{b}|\boldsymbol{\theta}) &= \ln \mathcal{L}_{3'}(\boldsymbol{\theta}|\boldsymbol{b}) \\
&= \ln \left(k \cdot \frac{1}{2\pi\sigma^2} \exp\left(-\frac{b_0^2 + b_1^2}{2\sigma^2} \right) \cdot \frac{\Gamma(\alpha + \beta)}{\Gamma(\alpha)\Gamma(\beta)} b_2^{\alpha-1} (1 - b_2)^{\beta-1} \cdot \prod_{i=1}^{n} \frac{(1 + \xi_i - b_2)^{\theta_i} b_2^{1-\theta_i}}{1 + \xi_i} \right) \\
&= \ln k - \ln 2\pi - 2 \ln \sigma - \frac{b_0^2 + b_1^2}{2\sigma^2} + \ln \Gamma(\alpha + \beta) - \ln \Gamma(\alpha) - \ln \Gamma(\beta) + (\alpha - 1) \ln b_2 \\
&\quad + (\beta - 1) \ln(1 - b_2) + \sum_{i=1}^{n} \theta_i \ln(1 + \xi_i - b_2) + \sum_{i=1}^{n} (1 - \theta_i) b_2 - \sum_{i=1}^{n} \ln(1 + \xi_i) \\
&\cong -\frac{b_0^2 + b_1^2}{2\sigma^2} + (\alpha - 1) \ln b_2 + (\beta - 1) \ln(1 - b_2) + \sum_{i=1}^{n} \theta_i \ln(1 + \xi_i - b_2) + \sum_{i=1}^{n} (1 - \theta_i) \ln b_2 \\
&\quad - \sum_{i=1}^{n} \ln(1 + \xi_i)
\end{aligned}
$$

for constant hyperparameters σ, α, β after removal of constant terms.

3.4 Four-Parameter Logistic Curve (Scale and Shape Parameters)

We now explore a four-parameter logistic curve given by the formula

$$y(x, \boldsymbol{b}) = \frac{b_3}{(1 + \xi)^{b_2}}.$$

This curve is useful when we have high control mortality (with an average mortality rate of $1 - b_3$), and thus an upper asymptote of 1 is no longer appropriate, coupled with an asymmetric dose response. We first note that the complement to this scaled three-parameter logistic curve is

$$1 - y(x, \boldsymbol{b}) = 1 - \frac{b_3}{(1 + \xi)^{b_2}}$$

$$= \frac{(1 + \xi)^{b_2} - b_3}{(1 + \xi)^{b_2}}.$$

3.4.1 Log-likelihood function

Beginning with Bayes's theorem, the conditional probability $f(\boldsymbol{b}|\boldsymbol{\theta})$ is given by

$$f(\boldsymbol{b}|\boldsymbol{\theta}) = k f(\boldsymbol{\theta}|\boldsymbol{b}) f(\boldsymbol{b})$$

$$= k f(\boldsymbol{b}) \cdot \prod_{i=1}^{n} \left(\frac{(1 + \xi_i)^{b_2} - b_3}{(1 + \xi_i)^{b_2}} \right)^{\theta_i} \left(\frac{b_3}{(1 + \xi_i)^{b_2}} \right)^{1 - \theta_i}$$

$$= k f(\boldsymbol{b}) \cdot \prod_{i=1}^{n} \frac{\left((1 + \xi_i)^{b_2} - b_3 \right)^{\theta_i} b_3^{1 - \theta_i}}{(1 + \xi_i)^{b_2}}.$$

For our choice of priors on $\boldsymbol{b}$, we again select (b_0, b_1) to have multivariate Gaussian distribution $\mathcal{N}(\boldsymbol{0}, \sigma I_2)$, where I_2 is the 2×2 identity matrix. For shape parameter $b_2 \in \mathbb{R}^+$, we use a gamma distribution prior

$$g(b_2) = \frac{\beta^{\alpha}}{\Gamma(\alpha)} b_2^{\alpha - 1} e^{-\beta b_2},$$

where α and β are shape and rate hyperparameters, respectively. We would really prefer parameter $b_3 \in (0, 1)$, but for numerical optimizatino p

$$g(b_3) = \frac{\Gamma(\kappa + \tau)}{\Gamma(\kappa)\Gamma(\tau)} b_3^{\kappa - 1} (1 - b_3)^{\tau - 1},$$

where κ and τ are shape hyperparameters. Using the likelihood function $\mathcal{L}_4(\boldsymbol{\theta}|\boldsymbol{b}) = f(\boldsymbol{b}|\boldsymbol{\theta})$, we get the log-likelihood function

$$\ell_4(\boldsymbol{b}|\boldsymbol{\theta}) = \ln \mathcal{L}_4(\boldsymbol{\theta}|\boldsymbol{b})$$

$$= \ln \left(k \cdot \frac{1}{2\pi\sigma^2} \exp\left(-\frac{b_0^2 + b_1^2}{2\sigma^2} \right) \cdot \frac{\beta^{\alpha}}{\Gamma(\alpha)} b_2^{\alpha - 1} e^{-\beta b_2} \cdot \frac{\Gamma(\kappa + \tau)}{\Gamma(\kappa)\Gamma(\tau)} b_3^{\kappa - 1} (1 - b_3)^{\tau - 1} \right.$$

$$\left. \cdot \prod_{i=1}^{n} \frac{(1 + \xi_i - b_2)^{\theta_i} b_2^{1 - \theta_i}}{1 + \xi_i} \right)$$

$$= \ln k - \ln 2\pi - 2 \ln \sigma - \frac{b_0^2 + b_1^2}{2\sigma^2} + \ln \frac{\beta^{\alpha}}{\Gamma(\alpha)} + (\alpha - 1) \ln b_2 - \beta b_2 + \ln \frac{\Gamma(\kappa + \tau)}{\Gamma(\kappa)\Gamma(\tau)}$$

$$+ (\kappa - 1) \ln b_3 + (\tau - 1) \ln(1 - b_3) + \sum_{i=1}^{n} \theta_i \ln \left((1 + \xi_i)^{b_2} - b_3 \right) + \sum_{i=1}^{n} (1 - \theta_i) \ln b_3$$

$$- b_2 \sum_{i=1}^{n} \ln(1 + \xi_i)$$

$$\cong -\frac{b_0^2 + b_1^2}{2\sigma^2} + (\alpha - 1) \ln b_2 - \beta b_2 + (\kappa - 1) \ln b_3 + (\tau - 1) \ln(1 - b_3)$$

$$+ \sum_{i=1}^{n} \theta_i \ln \left((1 + \xi_i)^{b_2} - b_3 \right) + \sum_{i=1}^{n} (1 - \theta_i) \ln b_3 - b_2 \sum_{i=1}^{n} \ln(1 + \xi_i)$$

for constant hyperparameters $\sigma, \alpha, \beta, \kappa, \tau$ after removal of constant terms that do not depend on $\boldsymbol{b}$.

3.4.2 Optimization of the log-likelihood function: The gradient

The gradient of the four-parameter log-likelihood function, $\nabla \ell_4(\boldsymbol{b}|\boldsymbol{\theta})$, is given by

$$\nabla \ell_4(\boldsymbol{b}|\boldsymbol{\theta}) = \left(\frac{\partial \ell_4}{\partial b_0}, \quad \frac{\partial \ell_4}{\partial b_1}, \quad \frac{\partial \ell_4}{\partial b_2}, \quad \frac{\partial \ell_4}{\partial b_3} \right)^T,$$

where

$$\frac{\partial \ell_4(\boldsymbol{b}|\boldsymbol{\theta})}{\partial b_0} = \frac{b_0}{\sigma^2} + b_2 \sum_{i=1}^{n} \frac{\theta_i \xi_i (1 + \xi_i)^{b_2 - 1}}{(1 + \xi_i)^{b_2} - b_3} - b_2 \sum_{i=1}^{n} \frac{\xi_i}{1 + \xi_i}$$

$$\frac{\partial \ell_4(\boldsymbol{b}|\boldsymbol{\theta})}{\partial b_1} = \frac{b_0}{\sigma^2} + b_2 \sum_{i=1}^{n} \frac{x_i \theta_i \xi_i (1 + \xi_i)^{b_2 - 1}}{(1 + \xi_i)^{b_2} - b_3} - b_2 \sum_{i=1}^{n} \frac{x_i \xi_i}{1 + \xi_i}$$

$$\frac{\partial \ell_4(\boldsymbol{b}|\boldsymbol{\theta})}{\partial b_2} = \frac{\alpha - 1}{b_2} - \beta + \sum_{i=1}^{n} \frac{\theta_i (1 + \xi_i)^{b_2} \ln(1 + \xi_i)}{(1 + \xi_i)^{b_2} - b_3} - \sum_{i=1}^{n} \ln(1 + \xi_i)$$

$$\frac{\partial \ell_4(\boldsymbol{b}|\boldsymbol{\theta})}{\partial b_3} = \frac{\kappa - 1}{b_3} - \frac{\tau - 1}{1 - b_3} - \sum_{i=1}^{n} \frac{\theta_i}{(1 + \xi_i)^{b_2} - b_3} + \sum_{i=1}^{n} \frac{1 - \theta_i}{b_3}$$

3.4.3 Optimization of the log-likelihood function: The Hessian

The Hessian matrix of $\ell_4(\boldsymbol{b}|\boldsymbol{\theta})$ is given by

$$H_{\ell_4} = \begin{bmatrix} \dfrac{\partial^2 \ell_4}{\partial b_0^2} & \dfrac{\partial^2 \ell_4}{\partial b_0 \partial b_1} & \dfrac{\partial^2 \ell_4}{\partial b_0 \partial b_2} & \dfrac{\partial^2 \ell_4}{\partial b_0 \partial b_3} \\[2ex] \dfrac{\partial^2 \ell_4}{\partial b_1 \partial b_0} & \dfrac{\partial^2 \ell_4}{\partial b_1^2} & \dfrac{\partial^2 \ell_4}{\partial b_1 \partial b_2} & \dfrac{\partial^2 \ell_4}{\partial b_1 \partial b_3} \\[2ex] \dfrac{\partial^2 \ell_4}{\partial b_2 \partial b_0} & \dfrac{\partial^2 \ell_4}{\partial b_2 \partial b_1} & \dfrac{\partial^2 \ell_4}{\partial b_2^2} & \dfrac{\partial^2 \ell_4}{\partial b_2 \partial b_3} \\[2ex] \dfrac{\partial^2 \ell_4}{\partial b_3 \partial b_0} & \dfrac{\partial^2 \ell_4}{\partial b_3 \partial b_1} & \dfrac{\partial^2 \ell_4}{\partial b_3 \partial b_2} & \dfrac{\partial^2 \ell_4}{\partial b_3^2} \end{bmatrix},$$

where

$$\frac{\partial^2 \ell_4(\boldsymbol{b}|\boldsymbol{\theta})}{\partial b_0^2} = \frac{1}{\sigma^2} + b_2 \sum_{i=1}^{n} \frac{\theta_i \xi_i (1 + \xi_i)^{b_2 - 2} ((1 + \xi_i)^{b_2} - b_2 b_3 \xi_i - b_3)}{((1 + \xi_i)^{b_2} - b_3)^2} - b_2 \sum_{i=1}^{n} \frac{\xi_i}{(1 + \xi_i)^2}$$

$$\frac{\partial^2 \ell_4(\boldsymbol{b}|\boldsymbol{\theta})}{\partial b_0 \partial b_1} = \frac{\partial^2 \ell_4(\boldsymbol{b}|\boldsymbol{\theta})}{\partial b_1 \partial b_0} = b_2 \sum_{i=1}^{n} \frac{\theta_i x_i \xi_i (1+\xi_i)^{b_2-2}((1+\xi_i)^{b_2} - b_2 b_3 \xi_i - b_3)}{((1+\xi_i)^{b_2} - b_3)^2} - b_2 \sum_{i=1}^{n} \frac{x_i \xi_i}{(1+\xi_i)^2}$$

$$\frac{\partial^2 \ell_4(\boldsymbol{b}|\boldsymbol{\theta})}{\partial b_0 \partial b_2} = \frac{\partial^2 \ell_4(\boldsymbol{b}|\boldsymbol{\theta})}{\partial b_2 \partial b_0} = \sum_{i=1}^{n} \frac{\theta_i \xi_i (1+\xi_i)^{b_2-1} \left((1+\xi_i)^{b_2} - b_2 b_3 \ln(1+\xi_i) - b_3\right)}{((1+\xi_i)^{b_2} - b_3)^2} - \sum_{i=1}^{n} \frac{\xi_i}{1-\xi_i}$$

$$\frac{\partial^2 \ell_4(\boldsymbol{b}|\boldsymbol{\theta})}{\partial b_0 \partial b_3} = \frac{\partial^2 \ell_4(\boldsymbol{b}|\boldsymbol{\theta})}{\partial b_3 \partial b_0} = b_2 \sum_{i=1}^{n} \frac{\theta_i \xi_i (1+\xi_i)^{b_2+1}}{((1+\xi_i)^{b_2} - b_3)^2}$$

$$\frac{\partial^2 \ell_4(\boldsymbol{b}|\boldsymbol{\theta})}{\partial b_1^2} = \frac{1}{\sigma^2} + b_2 \sum_{i=1}^{n} \frac{\theta_i x_i^2 \xi_i (1+\xi_i)^{b_2-2}(1 + b_2 \xi_i - (1+\xi_i)^{b_2})}{((1+\xi_i)^{b_2} - 1)^2} - b_2 \sum_{i=1}^{n} \frac{x_i^2 \xi_i}{(1+\xi_i)^2}$$

$$\frac{\partial^2 \ell_4(\boldsymbol{b}|\boldsymbol{\theta})}{\partial b_1 \partial b_2} = \frac{\partial^2 \ell_4(\boldsymbol{b}|\boldsymbol{\theta})}{\partial b_2 \partial b_1} = \sum_{i=1}^{n} \frac{\theta_i x_i \xi_i (1+\xi_i)^{b_2-1} \left((1+\xi_i)^{b_2} - b_2 \ln(1+\xi_i) - 1\right)}{((1+\xi_i)^{b_2} - 1)^2}$$

$$\frac{\partial^2 \ell_4(\boldsymbol{b}|\boldsymbol{\theta})}{\partial b_1 \partial b_3} = \frac{\partial^2 \ell_4(\boldsymbol{b}|\boldsymbol{\theta})}{\partial b_3 \partial b_1} =$$

$$\frac{\partial^2 \ell_4(\boldsymbol{b}|\boldsymbol{\theta})}{\partial b_2^2} = \frac{1-\alpha}{b_2^2} - \sum_{i=1}^{n} \frac{\theta_i (1+\xi_i)^{b_2} \ln^2(1+\xi_i)}{((1+\xi_i)^{b_2} - 1)^2}$$

$$\frac{\partial^2 \ell_4(\boldsymbol{b}|\boldsymbol{\theta})}{\partial b_2 \partial b_3} = \frac{\partial^2 \ell_4(\boldsymbol{b}|\boldsymbol{\theta})}{\partial b_3 \partial b_2} =$$

$$\frac{\partial^2 \ell_4(\boldsymbol{b}|\boldsymbol{\theta})}{\partial b_3^2} =$$

4   Appendix

4.1   Larger permissible values of ρ in covariance matrix

In section 2.1.2, we proved that in the covariance matrix

$$\boldsymbol{\Sigma} = \bigoplus_{k=1}^{m} \boldsymbol{P}_k$$

with

$$\boldsymbol{P}_{k_{i,j}} = \begin{cases} 1 & \text{if } i = j \le n \\ \rho \cdot 2^{1-|i-j|} & \text{if } i \ne j, i \le n \end{cases} \tag{4.1}$$

for $\boldsymbol{P}_k \in \mathbb{R}^{n \times n}$ and some $\rho \in (0.25, 0.25)$, and alluded to the fact that larger bounds on ρ were permissible. In particular, we claim the following.

Theorem 1. *As $n \to \infty$, the matrix defined by equation 4.1 is positive-definite for $\rho \in [-0.25, 0.75]$.*

Proof. First, we design matrix $A_n \in \mathbb{R}^{n \times n}$ such that

$$A_{k_{i,j}} = \begin{cases} b & \text{if } i = j \\ x^{|i-j|} & \text{if } i \ne j \end{cases}$$

We note that if $b = \rho^{-1}$, then

$$\boldsymbol{P}_k = \rho x^{-1} A_n, \tag{4.2}$$

and thus for fixed dilution ratio x, we can find the full range of b that gives positive definite A_n, and thence the values of ρ that give positive definite $\boldsymbol{P}_k$.[3]

First, we find the recursive formula for D_n, the determinant of A_n.

$$D_n = \det A_n = \begin{vmatrix} b & x & x^2 & x^3 & \cdots & x^n \\ x & b & x & x^2 & \cdots & x^{n-1} \\ x^2 & x & b & x & \cdots & x^{n-2} \\ x^3 & x^2 & x & b & \cdots & x^{n-3} \\ \vdots & \vdots & \vdots & \vdots & \ddots & \vdots \\ x^n & x^{n-1} & x^{n-2} & x^{n-3} & \cdots & b \end{vmatrix}$$

$$= \begin{vmatrix} b - x^2 & x & x^2 & x^3 & \cdots & x^n \\ x - bx & b & x & x^2 & \cdots & x^{n-1} \\ 0 & x & b & x & \cdots & x^{n-2} \\ 0 & x^2 & x & b & \cdots & x^{n-3} \\ \vdots & \vdots & \vdots & \vdots & \ddots & \vdots \\ 0 & x^{n-1} & x^{n-2} & x^{n-3} & \cdots & b \end{vmatrix}$$

$$= \begin{vmatrix} b - 2x^2 + bx^2 & x - bx & 0 & 0 & \cdots & 0 \\ x - bx & b & x & x^2 & \cdots & x^{n-1} \\ 0 & x & b & x & \cdots & x^{n-2} \\ 0 & x^2 & x & b & \cdots & x^{n-3} \\ \vdots & & \vdots & \vdots & \vdots & \ddots & \vdots \\ 0 & x^{n-1} & x^{n-2} & x^{n-3} & \cdots & b \end{vmatrix}$$

$$= \left(\big(b + (b-2)x^2\big) \det(A_{n-1}) \right) - \big((1-b)x\big) \begin{vmatrix} (1-b)x & x & x^2 & \cdots & x^{n-1} \\ 0 & b & x & \cdots & x^{n-2} \\ 0 & x & b & \cdots & x^{n-3} \\ \vdots & \vdots & \vdots & \ddots & \vdots \\ 0 & x^{n-2} & x^{n-3} & \cdots & b \end{vmatrix}$$

$$= \left(\big(b + (b-2)x^2\big) \det(A_{n-1}) \right) - \big((1-b)x\big)^2 \begin{vmatrix} b & x & \cdots & x^{n-2} \\ x & b & \cdots & x^{n-3} \\ \vdots & \vdots & \ddots & \vdots \\ x^{n-2} & x^{n-3} & \cdots & b \end{vmatrix}$$

$$= \left(\big(b + (b-2)x^2\big) \det(A_{n-1}) \right) - \big((1-b)x\big)^2 \det(A_{n-2})$$

We let $D_0 = 1$, and $\det A_1 = |b| = b$. Let $D_i = \det A_i$. (Note that a quick sanity check of the recurrence relation for $\det A_2 = \begin{vmatrix} b & x \\ x & b \end{vmatrix}$ gives $b^2 - x^2$ as expected.) The first several determinants are:

$$D_0 = 1$$

---

[3]If $\rho < 0$, then we are looking for a negative-definite A_n, to produce a positive definite $\boldsymbol{P}_k$, since all eigenvalues are multiplied by ρx^{-1}.

$$D_1 = b$$
$$D_2 = b^2 - x^2$$
$$D_3 = b^3 - bx^4 - 2bx^2 + 2x^4$$
$$D_4 = b^4 - 3b^2x^2 + x^4 + 4bx^4 - 2b^2x^4 - 4x^6 + 4bx^6 - b^2x^6$$

If we constrain $x = 1/2$ for our two-fold dilutions, we get

$$D_0 = 1$$
$$D_1 = b$$
$$D_2 = b^2 - \frac{1}{4}$$
$$D_3 = b^3 - \frac{9b}{16} + \frac{1}{8}$$
$$D_4 = b^4 - \frac{57b^2}{64} + \frac{5b}{16}$$
$$D_5 = b^5 - \frac{313b^3}{256} + \frac{67b^2}{128} + \frac{3b}{64} - \frac{1}{32}$$
$$D_6 = b^6 - \frac{1593b^4}{1024} + \frac{95b^3}{128} + \frac{45b^2}{256} - \frac{9b}{64} + \frac{1}{64}$$
$$D_7 = b^7 - \frac{7737b^5}{4096} + \frac{1973b^4}{2048} + \frac{207b^3}{512} - \frac{93b^2}{256} + \frac{b}{16}$$
$$D_8 = b^8 - \frac{36409b^6}{16384} + \frac{4855b^5}{4096} + \frac{1515b^4}{2048} - \frac{23b^3}{32} + \frac{145b^2}{1024} + \frac{3b}{256} - \frac{1}{256}$$

The constant-recurrence sequence

$$D_n = \left(b + (b-2)x^2\right) D_{n-1} - \left((1-b)x\right)^2 D_{n-2}$$

has characteristic equation

$$0 = d^2 - \left(b + (b-2)x^2\right) d + \left((1-b)x\right)^2$$
$$= d^2 - Pd + Q,$$

where $P = \left(b + (b-2)x^2\right)$ and $Q = (1-b)^2x^2$. By simple use of the quadratic formula, we get

$$A = \frac{P + \sqrt{D}}{2}, \qquad B = \frac{P - \sqrt{D}}{2}, \qquad D = P^2 - 4Q.$$

Then, using the initial conditions $D_0 = 1$ and $D_1 = b$, we must solve the system of equations

$$\begin{cases} D_0 = 1 = kA^0 + lB^0 \\ D_1 = b = kA^1 + lB^1. \end{cases}$$

Trivially, we have $l = 1 - k$, and then, by noting that $A - B = \sqrt{D}$, it is easy to verify

$$k = \frac{b - B}{\sqrt{D}}, \qquad l = \frac{A - b}{\sqrt{D}},$$

and we thus have

$$D_n = \frac{b - B}{\sqrt{D}} A^n + \frac{A - b}{\sqrt{D}} B^n$$

$$= \frac{b - \frac{P - \sqrt{D}}{2}}{\sqrt{D}} \left(\frac{P + \sqrt{D}}{2} \right)^n + \frac{\frac{P + \sqrt{D}}{2} - b}{\sqrt{D}} \left(\frac{P - \sqrt{D}}{2} \right)^n$$

$$= \frac{b - \frac{(b + (b-2)x^2) - \sqrt{D}}{2}}{\sqrt{D}} \left(\frac{(b + (b-2)x^2) + \sqrt{D}}{2} \right)^n$$

$$+ \frac{\frac{(b + (b-2)x^2) + \sqrt{D}}{2} - b}{\sqrt{D}} \left(\frac{(b + (b-2)x^2) - \sqrt{D}}{2} \right)^n$$

$$= \frac{b - (b-2)x^2 + \sqrt{D}}{2\sqrt{D}} \left(\frac{b + (b-2)x^2 + \sqrt{D}}{2} \right)^n$$

$$+ \frac{-b + (b-2)x^2 + \sqrt{D}}{2\sqrt{D}} \left(\frac{b + (b-2)x^2 - \sqrt{D}}{2} \right)^n.$$

When $x = 1/2$, we then have

$$D_n = \frac{\frac{3b+2}{4} + \frac{1}{4}D'}{\frac{2}{4}D'} \left(\frac{\frac{5b-2}{4} + \frac{1}{4}D'}{2} \right)^n + \frac{-\frac{3b+2}{4} + \frac{1}{4}D'}{\frac{2}{4}D'} \left(\frac{\frac{5b-2}{4} - \frac{1}{4}D'}{2} \right)^n$$

$$= \frac{1}{2D'} \left[(D' + 3b + 2) \left(\frac{5b - 2 + D'}{8} \right)^n + (D' - 3b - 2) \left(\frac{5b - 2 - D'}{8} \right)^n \right]$$

$$= \frac{1}{2^{3n+1}D'} \left[(D' + 3b + 2) (5b - 2 + D')^n + (D' - 3b - 2) (5b - 2 - D')^n \right], \qquad (4.3)$$

where

$$D' = \sqrt{9b^2 + 12b - 12}.$$

If we are interested in the limiting case as $n \to \infty$, we require $D_n = 0$, so we must solve

$$0 = \lim_{n \to \infty} \left[(D' + 3b + 2) (5b - 2 + D')^n + (D' - 3b - 2) (5b - 2 - D')^n \right].$$

In particular, we note that the limit is equal to 0 in the following cases:

$$0 = D' + 3b + 2 = D' - 3b - 2 \qquad (4.4)$$
$$0 = 5b - 2 + D' = 5b - 2 - D' \qquad (4.5)$$
$$0 = D' + 3b + 2 = 5b - 2 - D' \qquad (4.6)$$
$$0 = D' - 3b - 2 = 5b - 2 + D' \qquad (4.7)$$

We note that in equations 4.4 and 4.5, we end up with the solution $D' = -D'$, which requires

$$0 = D'$$
$$= 3b^2 + 4b - 4,$$

so $b = -2$ or $b = 2/3$. For equations 4.6 and 4.7, it is easy to show that there is no solution to $D' + 3b + 2 = 0$, $5b - 2 - D' = 0$, or $D' - 3b - 2 = 0$, while $5b - 2 + D' = 0$ has the solution $b = 1$, and so there are no values of b that meet the dule requirements of equations 4.6 and 4.7.

Next we note that $D' = \sqrt{9b^2 + 12b - 12}$ has real solutions in the domain $(-\infty, -2] \cup [2/3, \infty)$. Additionally, equation 4.2 specifies the relation between matrix A_n, for which equation 4.3 was

derived, to the covariance matrix $\boldsymbol{P}_k$. Specifically, we note that the eigenvalues of A_n are exactly equal to $x\rho^{-1}$ times the eigenvalues of $\boldsymbol{P}_k$, and as $n \to \infty$, the permissible values of ρ are equal to x times the inverse values of $(-\infty, -2] \cup [2/3, \infty)$. In particular, when $x = 1/2$, we get $\rho \in [-1/4, 3/4]$.

$\square$