VARIANT DETECTION MODEL WITH IMPROVED ROBUSTNESS AND ACCURACY FOR LOW-DEPTH TARGETED NEXT-GENERATION SEQUENCING DATA

Abstract. Massively parallel sequencing data generated by next-generation sequencing (NGS) technology is routinely used to detect single nucleotide variants (SNVs) in research samples. An emerging challenge for this technology is the identification of SNVs in heterogeneous cell populations with low read-depth data. We have developed a Bayesian statistical model is able to share information between correlated positions and call low-frequency variants in heterogeneous samples. We present a Bayesian sensitivity analysis of the model to variations in the prior function. Our model with different priors both performs a high accuracy, and a Jeffrey's prior gives a lower false discovery rate (FDR) to detect a 0.1% minor allele frequency event within minor read depth compared with the log-normal prior and an improper prior. And log-normal prior performs a higher specificity than the other two. In an analysis of a directed evolution experiment, we are able to detect the emergence of a beneficial SNV earlier than was previously shown.

1. Introduction

Massively parallel sequencing data has been generated by Next-generation sequencing (NGS) technology to benefit clinical diagnostics and sequencing based phylogenetic analyses. One primary application of NGS is variation detection among related populations and separate novel single-nucleotide variants (SNVs) candidates. Somatic SNVs are detected by comparing the tumor and corresponding normal samples.

To address the detection of SNVs at low allele frequencies, a number of algorithms from NGS platform are being under-represented. Strelka (Saunders et al., 2012), VarScan2 (Koboldt et al., 2012), JointSNVMix (Roth et al., 2012) are highly used to differentiate somatic SNVs from germline cells. Also, SAMtools (Li et al., 2009), Genome Analysis Toolkit (GATK) (McKenna et al., 2010), and MuTect (Cibulskis et al., 2013) broadly concentrate on detecting low-frequency variants. Through the comparision of these somatic mutation callers (Wang et al., 2013), VarScan2 excelled at the detection of high coverage and allele frequecy, while MuTect outperformed the other methods in detecting the low allelic fraction SNVs. However identifying the true SNVs remains challenging

because of the high false positive rate or high false negative rate which are mainly caused by the clonal heterogeneity.

Recently empirical Bayesian approaches have been made use to identify SNVs, which can automatically adjust for multiple testing and selection bias (Liao et al., 2014). A empirical Bayesian framework for somatic mutation detection from cancer genome sequencing data - EBCall, enables accurate mutations calling with low allele frequencies (less than 10%) in a minor tumour subpopulation (Shiraishi et al., 2013). Another method based on empirical Bayesian hierarchical model - RVD, was proposed for ultrasensitive rare SNV detection using beta-binomial model (Flaherty et al., 2011). RVD method is demonstrated to robustly detect mutations at 0.1% fractional representation, which means accurately call one mutant per every 1000 wild-type alleles. With the shortcoming of high read depth estimation in RVD, we originally built an improved robustness and accuracy model - RVD3 for low-depth SNVs detection. Variants calling ability is tested and analyzed both on the synthetic sequence data and the true yeast sequencing data with different read depths.

In this article, RVD3 model - a novel Bayesian structure to accurately identify SNVs with small false discovery rate is first described in detail. Secondly, Metropolis-within-Gibbs sampling is evolved for inference. And then to detect variants, a Bayesian posterior distribution is taken for hypothesis test. Furthermore, we analyze the sensitivity of the Bayesian model to different priors - Jeffrey's prior, log-normal prior, and improper prior. Finally, we choose Jeffrey's prior for the RVD3 model and demonstrate its performance on the yeast sequence data. Thus our Bayesian model achieves a enhanced robustness and accuracy when calling variants for the low read depth and minor allele frequencies.

2. Data Sets

2.1. **Synthetic DNA Sequence Data.** Two 400bp DNA sequences(control/case) were synthesized with only 14 different single nucleotide positions. Sample of the case and control DNA were mixed to yield 0.1%, 0.3%, 1%, 10%, and 100% defined minor allele frequencies (MAFs). The details of the experimental protocol are available from the original publication (Flaherty et al., 2011). We used BWA v0.7.5a to align the short sequencing reads to the reference sequence. The -C50 option of BWA was taken to remove the reads of low mapping quality. BAM files were sampled by

$10\times$, $100\times$, $1,000\times$, and $10,000\times$ using Picard v1.104 (http://picard.sourceforge.net). The final data set contains read pairs for $N = 6$ replicates for the control at different MAF levels.

2.2. **Yeast Data.** We first mapped the wild-tpye strain GSY1135 (Kvitek and Sherlock, 2011) to Chromosome 10 in S288c reference genome (SGD; http://www.yeastgenome.org/) by BWA v0.7.5a (Li and Durbin, 2009). Then called SNPs by GATK v2.5 UnifiedGenotyper (McKenna et al., 2010; DePristo et al., 2011) and created a FASTA GSY1135 reference using GATK FastaAlternative. Secondly, we downloaded generation 7 as control and generation 133 as case in experiment 1 from (Kvitek and Sherlock, 2013), and removed WT population using FASTX Barcode Splitter and cut down the pair ends accordingly. The FASTQ files of case and control were mapped to the corresponding reference genome created before. Then wen used SAMtools v0.1.19 (Li et al., 2009) to convert the alignment files to the binary alignment map (BAM) format. Next, pileup files were generated by SAMtools and depth chart file were derived for further SNVs detection.

3. RVD3 Model

3.1. **Model Structure.** RVD is based on a two-stage hierarchical Bayesian model for variant detection (Flaherty et al., 2011). Through hypothesis test on case and control samples by RVD, we can call the variants successfully. Now RVD3 has three-stage model including priors built on the former RVD model. The definitions for sample data are given: r_{ji} is the number of reads with a non-reference base at position j in replicate i, and n_{ji} is the total number of reads at position j in replicate i. Three parameters of the model are: μ_0, a global error rate; M_0, the global position which estimates the variation in the error rate across the positions; to choose a priori distribution for M_j, log-normal prior and Jeffrey's prior (Jeffreys, 1946) are employed, which enhances the former RVD model (Flaherty et al., 2011). Here M_j is the local precision measures the alteration in the error rate across replicates at position j. The graphical chart for RVD3 is shown in Figure 1.

RVD3 hierarchically includes three levels of samplings: $r_{ji}|n_{ji} \sim \text{Binomial}(\theta_{ji}, n_{ji})$ models the variation due to sampling the pool of DNA molecules on the sequencer. $\theta_{ji} \sim \text{Beta}(\mu_j, M_j)$ models the variation caused by experimental repeatability. The variation in error rate due to sequence context is modeled by $\mu_j \sim \text{Beta}(\mu_0, M_0)$. And the local precision is modeled by $M_j \sim \text{log-normal}(\mu, \sigma)$ (log-normal prior), and Jeffrey's prior for M_j.

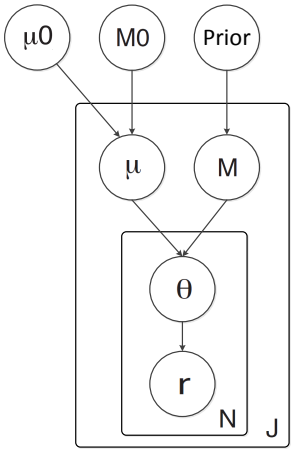

FIGURE 1. RVD3 Graphical Model

3.2. **Inference and Hypothesis Testing.**

3.3. **Priors for precision parameter.** The prior distribution characterizes the knowledge of the parameters in the statistical model. Including prior information in the Bayesian approach is difficult but meaningful. A way to choose the prior function is to use the Schwarz criterion or Bayesian information criterion (BIC) (Weakliem, 1999). Besides, the Bayes factor does tend to be more sensitive to access the prior distributions on the model parameters (Kass and Raftery, 1995). To consider the Bayesian sensitivity analysis, a more practical way is to consider the range of posterior quantities of interest- the posterior mean or posterior probability (Bayarri et al., 1998; Harrison et al., 2013). Based on this theory, we analyze the posterior and prior probability of the precision hyper-parameter. For this purpose, a bunch of different priors should be chosen for analysis (Gelman, 2006). In a polygenic modeling with Bayesian sparse linear mixed model research, the results don't change dramatically by changing the prior measurement which reveals that the prior specification for the hyper-parameters are fine (Zhou et al., 2013). So we performed three different plausible prior distributions: improper prior, informative prior (log-normal), and non-informative prior (Jeffrey's).

An improper prior is the prior distribution integrates to infinity, and may cause an improper posterior which results in an invalid inferences (Lesaffre and Lawson, 2012). Furthermore when the Markov chain Monte Carlo method is taken to derive the posterior, it is possibly hard to sniff out the improper posterior. Even though no problems happened in estimation by improper prior, other

troubles could be caused in the Bayesian inference and analysis by it (Stein, 1965). Playing no prior on M_j is exactly an implicit improper prior. Therefore we considered about non-informative prior and informative prior for sensitivity analysis.

Informative prior distribution is the most specific type of prior. A good informative prior is imperative to promote accurate posterior estimates. In our study, log-normal prior is a proper prior for the beta density's parameters, $\theta_{ji} \sim \text{Beta}(\mu_j, M_j)$. The parameters of it denoted μ (mean) and σ (standard deviation) respectively.

Non-informative prior seems to be more unbiased and objective. Various non-informative prior distributions have been suggested for parameters in hierarchical models. Jeffrey's prior, as a typical and influential one, is proposed to establish a least informative prior that is automatically invariant to transformations by Harold Jeffreys (Jeffreys, 1946). It is defined in terms of the Fisher information and works well with a single parameter. In our research Jeffrey's prior for M_j is the square root of Fisher information of M_j.

4. Results

4.1. **Sensitivity analysis.** The Bayesian posterior distributions and the priors distributions are shown in Figure 2. These plots indicate the probability distribution over different M values when dilution is 10% at 100× read depth rate. The positions are chosen in the middle of the base length-position 185 and 265 are mutant, and position 200 and 300 are non-mutant. The posterior probability distributions for M_j are estimated by Gaussian Kernel Density (Silverman, 1986). Generally the distribution curves display normal and stable. Jeffrey's posterior distribution changes a lot which shows Jeffrey's piror is more sensitive than the log-normal prior between the mutant and non-mutant positions.

Figure 3 shows that across all priors the non-mutant positions indicate that the replicate variance is small. At the known mutant positions, the replicate parameter M_j is less variable compared to the reference position with the log-normal and improper prior compared with Jeffrey's prior, which corresponds to the information in Figure 2 However, at the begining and the end of the sequence which are the low read-depth positions, the improper and log-normal prior are more variable compared to Jeffrey's prior. Additionally, the error rate across positions is captured by the M_0 parameter shown as a horizontal dotted line in the plots. M_j is greater than M_0 shows

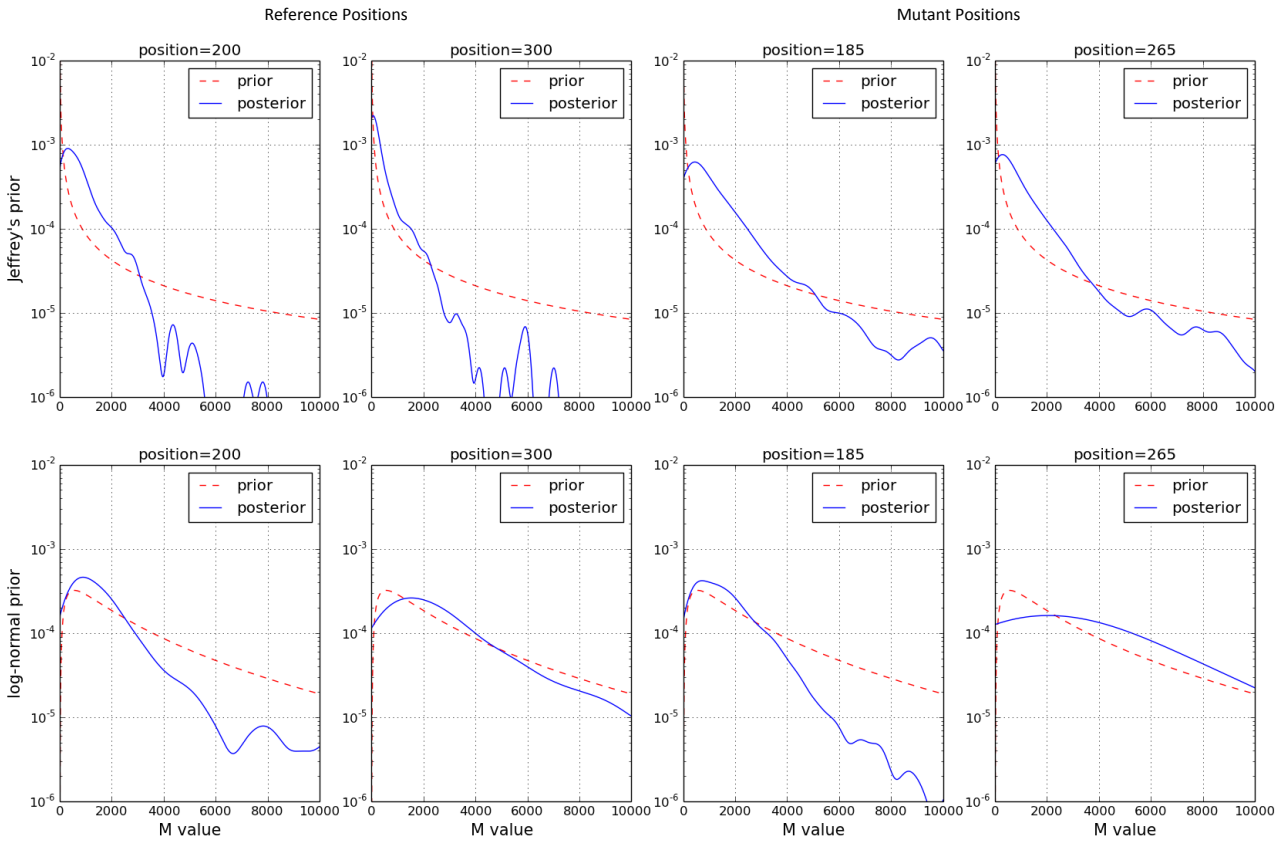

FIGURE 2. Distribution of priors and posteriors when MAF is 10%

that the precision between replicates is higher than the precision between positions. Here $\widetilde{E}[M|D]$ represents the expectation of empirical posterior approximation.

4.2. **Results of priors on synthetic data.** The RVD3 model is analyzed by the selecting two reasonable prior distributions, and the corresponding results are compared from the aspects of performance with different read depths, sensitivity and specificity, and FDR.

4.2.1. *Performance with read depth.* To evaluate the performance of RVD3 model with priors, we generated receiver-operating characteristic curves (ROCs) for median read depth and minor allele frequencies (MAFs). Here the Bayesian test is used without the χ^2 test. Figure 4 and Figure 5 shows ROC curves with a fixed $\alpha = 0.05$. The performance improves when the read depth goes up. (ROC shows that the model with priors performs better than improper prior situation especially on the small read depth). Noticed at the lowest depth (22) with 10.0% mutant mixture, the sensitivity

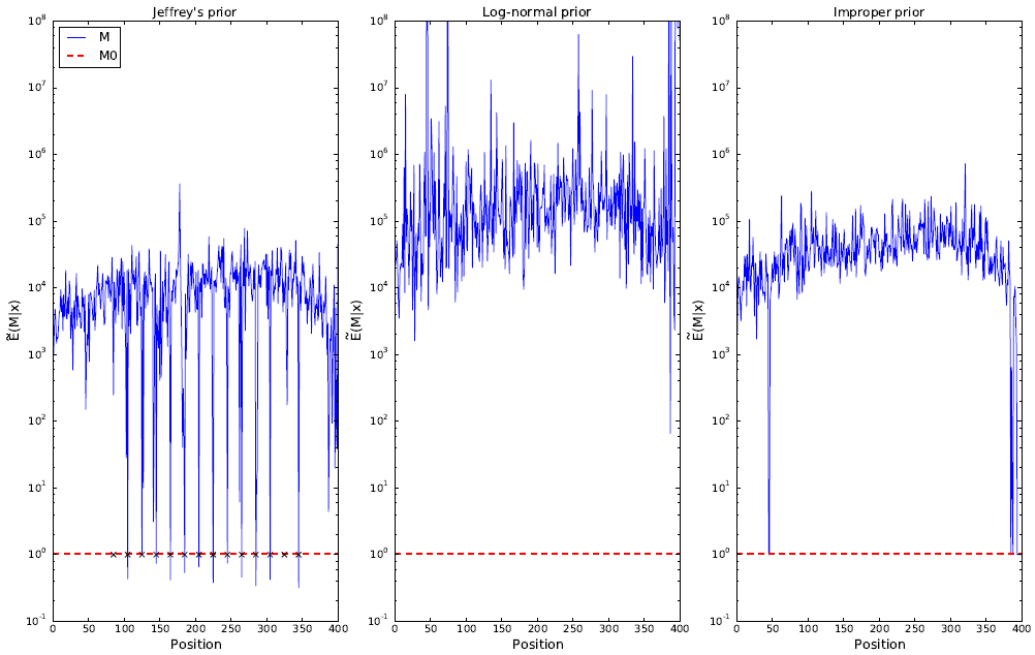

FIGURE 3. Precision parameter for RVD3 model with different priors. M_0 is the global precision. X indicates mutant position.

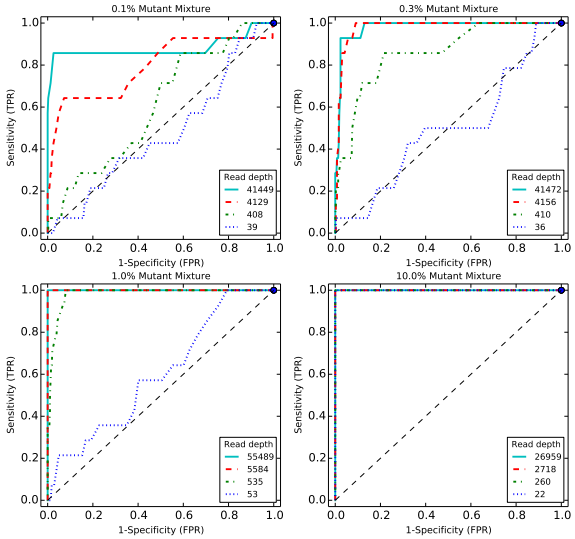

FIGURE 4. ROC curve for variants detection performance by Jeffrey's prior.

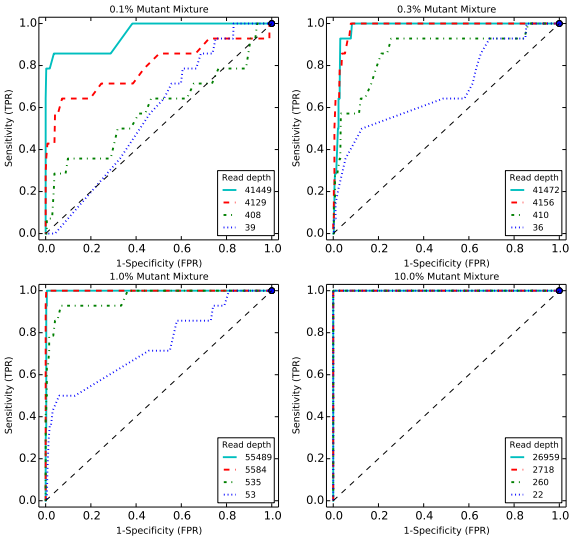

FIGURE 5. ROC curve for variants detection performance by log-normal prior.

and specificity value are 1 and much better than the model with improper priors for M_j, which definitely demonstrates the advantage of priors.

4.2.2. *Sensitivity/Specificity/FDR.* Figure 6 shows that the sensitivity and specificity of the RVD3 of different priors. Log-normal prior performs a higher sensitivity and specificity value, and the specificity of log-normal prior is a little better than the improper prior and much better than the performance of Jeffrey's prior. It shows that log-normal prior performs a smaller false positive rate than the other two priors.

Figure **??** shows the false discovery rate of the RVD3 with different priors. Jeffrey's prior shows a smaller false discovery rate than others over all the MAF levels at various read depths. It is obvious no matter Jeffrey's or log-normal prior, the variant detection performance acquires lower FDR to a known 0.1% minor allele frequency event, compared with improper priors, which seems desirable of our model for extending for a broad application. Based on the various advantages for Jeffrey's and log-normal prior, RVD3 can afford a more appropriate choice for the precision parameter to accommodate to the target. When we do the variants calling research, we should choose Jeffrey's prior model because it's a non-informative prior, and can produce a smaller false discovery rate and high accuracy; when we do the clinical experiment or diagnosis, we prefer to choose log-normal prior which cares more on true positive rate and true negative rate.

MAF	Median Depth	Log-normal Prior	Jeffreys Prior	Improper Prior
0.10%	39	0.00/1.00	0.00/1.00	0.00/1.00
	408	0.00/1.00	0.00/1.00	0.00/1.00
	4129	0.07/1.00	0.00/1.00	0.14/1.00
	41449	0.79/0.98	0.36/1.00	0.86/0.96
0.30%	36	0.00/1.00	0.00/1.00	0.00/1.00
	410	0.00/1.00	0.00/1.00	0.00/1.00
	4156	1.00/0.99	0.86/0.99	1.00/0.99
	41472	1.00/0.88	0.93/0.92	1.00/0.86
1.00%	53	0.00/1.00	0.00/1.00	0.00/1.00
	535	0.21/1.00	0.14/1.00	0.21/1.00
	5584	1.00/0.99	1.00/0.99	1.00/0.98
	55489	1.00/0.88	1.00/0.91	1.00/0.87
10.00%	22	0.00/1.00	0.00/1.00	0.00/1.00
	260	1.00/1.00	1.00/1.00	1.00/1.00
	2718	1.00/1.00	1.00/1.00	1.00/1.00
	26959	1.00/1.00	1.00/1.00	1.00/1.00
100.00%	27	1.00/1.00	1.00/1.00	1.00/1.00
	298	1.00/1.00	1.00/1.00	1.00/1.00
	3089	1.00/1.00	1.00/1.00	1.00/1.00
	30590	1.00/1.00	1.00/1.00	1.00/1.00

FIGURE 6. Sensitivity/Specificity comparison of RVD3 with different priors.

Model with different priors				
	Median	Jeffrey's	Log-normal	Improper
MAF	Depth	FDR	FDR	FDR
0.10%	39			
	408			
	4129		0	0
	41449	0	0.39	0.54
0.30%	36			
	410			
	4156	0.2	0.26	0.26
	41472	0.7	0.77	0.8
1.00%	53			
	535	0	0	0
	5584	0.18	0.26	0.33
	55489	0.71	0.77	0.78
10.00%	22			
	260	0	0	0
	2718	0	0	0
	26959	0	0	0
100.00%	27	0	0	0
	298	0	0	0
	3089	0	0	0
	30590	0	0	0

FIGURE 7. False Discovery Rate comparison with different priors on RVD3.

4.3. **Results of Jeffrey's prior on yeast data.** We demonstrated our RVD3 model with Jeffrey's prior on yeast data to identify the variants (Kvitek and Sherlock, 2013).

5. Discussion

6. Conclusion

Appendix A. Parameter Initialization

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Since $r_{ji} \sim \text{Binomial}(n_{ji}, \theta_{ji})$, the first population moment is $E[r_{ji}] = \theta_{ji} n_{ji}$ and the first sample moment is simply $m_1 = r_{ji}$. Therefore the MoM estimator is

$$\tilde{\theta}_{ji} = \frac{r_{ji}}{n_{ji}} \tag{1}$$

We take the MoM estimate, $\tilde{\theta}_{ji}$, as data for the next conditional distribution in the hierarchical model. The distribution is $\theta_{ji} \sim \text{Beta}(\mu_j M_j, (1-\mu_j) M_j)$. The first and second population moments are

$$E[\theta_{ji}] = \quad \mu_j, \tag{2}$$

$$\text{Var}[\theta_{ji}] = \quad \tfrac{\mu_j(1-\mu_j)}{M_j+1}. \tag{3}$$

The first and second sample moments are $m_1 = \frac{1}{N}\sum_{i=1}^{N} \theta_{ji}$ and $m_2 = \frac{1}{N}\sum_{i=1}^{N} \theta_{ji}^2$. Setting the population moments equal to the sample moments and solving for μ_j and M_j gives

$$\tilde{\mu}_j = \quad \tfrac{1}{N}\textstyle\sum_{i=1}^{N} \theta_{ji}, \tag{4}$$

$$\tilde{M}_j = \quad \tfrac{\tilde{\mu}_j(1-\tilde{\mu}_j)}{\frac{1}{N}\sum_{i=1}^{N} \theta_{ji}^2} - 1. \tag{5}$$

Following the same procedure for the parameters of $\mu_j \sim \text{Beta}(\mu_0, M_0)$ gives the following MoM estimates

$$\tilde{\mu}_0 = \quad \tfrac{1}{J}\textstyle\sum_{j=1}^{J} \mu_j \tag{6}$$

$$\tilde{M}_0 = \quad \tfrac{\tilde{\mu}_0(1-\tilde{\mu}_0)}{\frac{1}{J}\sum_{j=1}^{J} \mu_j^2} - 1. \tag{7}$$

Appendix B. Inference of Jeffrey's prior

We assume there is only one replicate (i=1),

$$p\left(\theta_j\right) = \frac{\Gamma\left(M_j\right)}{\Gamma\left(\mu_j M_j\right)\Gamma\left((1-\mu_j)M_j\right)}{\theta_j}^{\mu_j M_j - 1}(1-\theta)_j^{(1-\mu_j)M_j - 1} \tag{8}$$

$$\begin{aligned}\log p\left(\theta_j|\mu_j, M_j\right) &= \log\Gamma\left(M_j\right) - \log\Gamma\left(\mu_j, M_j\right) \\ &- \log\Gamma\left(1-\mu_j, M_j\right) + \left(\mu_j M_j - 1\right)\log\theta_j \\ &+ \left((1-\ u_j)M_j - 1\right)\log(1-\theta_j)\end{aligned} \tag{9}$$

$$\begin{aligned}\frac{\delta \log p(\theta_j)}{\delta M_j} &= \Psi(M_j) - \Psi(\mu_j M_j)\mu_j \\ &- \Psi((1-\mu_j)M_j)(1-\mu_j) + \mu_j \log\theta_j + (1-\mu_j)\log(1-\theta_j)\end{aligned} \tag{10}$$

$$\frac{\delta^2 \log p(\theta_j)}{\delta M_j^2} = \Psi_1(M_j) - \Psi_1(\mu_j M_j)\mu_j^2 - \Psi_1((1-\mu_j)M_j)(1-\mu_j)^2 \tag{11}$$

Now we have the Jeffreys' prior $\pi\left(M_j\right)$ for M_j:

$$[-\left(\Psi_1(M_j) - \Psi_1(\mu_j M_j)\mu_j^2 - \Psi_1((1-\mu_j)M_j)(1-\mu_j)^2\right)]^{\frac{1}{2}} \tag{12}$$

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