Biostatistics

Other topics

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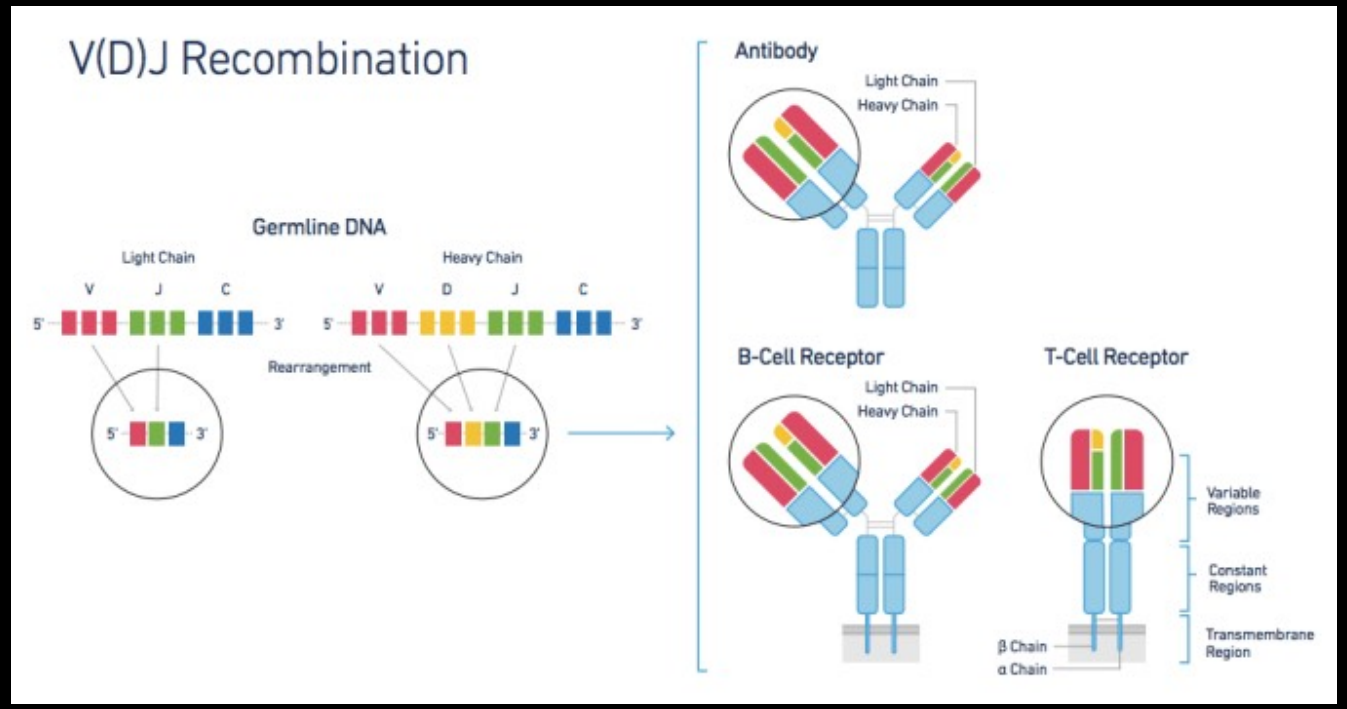
Syllabus

1. **General review**
    a. **What is Biostatistics?**
    b. **Population/Sample/Sample size**
    c. **Type of Data – quantitative and qualitative variables**
    d. **Common probability distributions**
    e. **Work example – Malaria in Tanzania**
2. **Applications in Medicine**
    a. **Construction and analysis of diagnostic tools – Binomial distribution, sensitivity, specificity, ROC curve,Rogal-Gladen estimator**
    b. **Estimation of treatment effects - generalized linear models**
    c. **Survival analysis - Kaplan-Meier curve, log-rank test, Cox's proportional hazards model**
3. **Applications in Genetics, Genomics, and other 'omics data**
    a. Genetic association studies – Hardy-Weinberg test, homozygosity, minor allele frequencies, additive model, multiple testing correction
    b. **Methylation association studies – M versus beta values, estimation of biological age**
    c. **Gene expression studies based on RNA-seq experiments – Tests based on Poisson and Negative-Binomial**
4. **Other Topics**
    a. **Estimation of Species diversity – Diversity indexes, Poisson mixture models**
    b. **Serological analysis – Gaussian (skew-normal) mixture models**
    c. **Advanced sample size and power calculations**

Species Biodiversity

Microbial diversity

B/T-cell diversity



Species richness

The number of different species present in a given population

Species diversity

The number of different species and their abundance present in a given population

Main research question

How many species exist in the population according to the information from a sample?

Formulation of the problem

Data

Species	Abundance
Species_1	n_1
Species_2	n_2
Species_3	n_3
….	
Species_k	n_k

$n =$ sample size $\qquad n = \sum_{i=1}^{k} n_i$

n is the hypothetical maximum species richness due to sampling

$n_i =$ frequency of individuals (abundance) from species i

$k =$ number of different species represented in the sample

$D =$ unknown number of different species in the population

$\hat{D} = ?$

Under the assumption of a large population

Formulation of the problem

Summarising the Data

Abundance	Number of Species
1	m_1
2	m_2
3	m_3
….	
l	m_l

Species-abundance distribution

$n =$ sample size

$$n = \sum_{i=1}^{l} i \times m_i$$

$m_i =$ frequency of species with abundance i

$l =$ maximum abundance recorded in the sample

Example

TABLE 1

DISTRIBUTION OF LEPIDOPTERA CAUGHT IN A LIGHT TRAP AT ROTHAMSTED IN 1934

Individuals per species (r)	Number of species (n_r)	Expected		
		Logarithmic	Grouped lognormal	Poisson lognormal
1	34	39.0	32.7	31.2
2	19	19.3	20.5	20.8
3	15	12.7	14.6	15.0
4	10	9.4	11.2	11.5
5	10	7.4	8.9	9.1
6-7	9	11.3	13.4	13.7
8-10	17	12.0	13.7	13.9
11-14	9	11.0	12.0	12.1
15-20	14	11.2	11.3	11.3
21-28	10	9.8	9.1	9.0
29-39	6	8.7	7.4	7.3
40-55	7	7.8	6.1	6.0
56-77	3	6.2	4.6	4.5
78-108	5	4.6	3.4	3.4
109-151	4	2.8	2.4	2.4
152-	4	2.9	4.5	4.7
Total	176	176.1	175.8	175.9
χ^2		9.2	6.9	7.4
Degrees of freedom		14	13	13

Simpson's Diversity index

$$D_s = 1 - \underbrace{\frac{\sum_{i=1}^{k} n_i(n_i - 1)}{n(n-1)}} = 1 - \frac{\sum_{i=1}^{l} m_i i(i-1)}{n(n-1)}$$

The probability that two individuals taken at random from the sample (with replacement) are from the same species

$D_s = 0 \Rightarrow$ Individuals are from the same species $(k = 1, n_1 = n)$

$D_s = 1 \Rightarrow$ Every individual is from a different species $(n_i = 1, \forall_i)$

Shannon's Diversity index

$$H = -\sum_{i=1}^{k} p_i \log p_i = \sum_{i=1}^{l} m_i \times \frac{i}{n} \log \frac{i}{n}$$

where $p_i = n_i/n$

It quantifies the uncertainty associated with the species prediction when one takes an individual from the sample randomly

Maximal entropy $n_i = 1, \forall_i \Rightarrow H_{\max} = \log n$

Exercise: Data_lecture_13_TCR_diversity.csv

Calculate the Simpson's and Shannon's diversity indexes for the species abundance distribution of DP CD3low

	Thymus			Lymph nodes	
i	DP CD3low	SP $CD4^+$	SP $CD8^+$	LN $CD4^+$	LN $CD8^+$
1	79	33	16	34	17
2	17	6	3	8	8
3	6	2	3	2	1
4	5	2	5	1	2
5	1	0	3	0	1
6	1	0	1	0	0
7	1	0	1	0	0
8		0	1	1	0
10		1	0	1	0
11		0	1	0	0
16		1		0	0
20		0		1	0
21		0			1
28		1			0
52					1

How to estimate species richness?

Abundance	Number of Species
0	D-k
1	m_1
2	m_2
3	m_3
….	
l	m_l
> l	0

Augmented Species-abundance distribution

If this is a contingency table, what is a possible sampling model?

How to estimate species richness?

Abundance	Number of Species
0	D-k
1	m_1
2	m_2
3	m_3
….	
l	m_l
> l	0

Augmented Species-Abundance distribution

Multinomial distribution

$$f(\{m_i\} \mid D, \{\theta_i\}) = \frac{D!}{(D-k)!m_1!\cdots m_l!}\theta_0^{D-M}\prod_{i=1}^{k}\theta_i^{m_i}$$

$\theta_i =$ probability of sampling i individuals from a given species

Unknown parameters $D, \theta_i, i = 0,1,\cdots,l$

Is it possible to estimate this model?

How to estimate species richness?

Abundance	Number of Species
0	D-k
1	m_1
2	m_2
3	m_3
….	
l	m_l
> l	0

Augmented Species-Abundance distribution

Modelling θ_i

$$\theta_i = P[X = i \mid \lambda]$$

$$X \mid \lambda \rightsquigarrow \text{Poisson}(\lambda)$$

$$\theta_i = \frac{e^{-\lambda}\lambda^i}{i!}$$

$$f(\{m_i\} \mid D, \{\theta_i\}) = \frac{D!}{(D-k)!m_1!\cdots m_l!} e^{-\lambda(D-M)} \prod_{i=1}^{k} \frac{e^{-\lambda m_i}\lambda^{i m_i}}{i!}$$

How can we estimate this model?

D is an integer parameter while λ is a positive continuous parameter

How to estimate species richness?

Abundance	Number of Species
0	D-k
1	m_1
2	m_2
3	m_3
….	
l	m_l

Augmented Species-Abundance distribution

First solution (truncated Poisson)

$$k \mid D, \theta_0 \rightsquigarrow \text{Binomial}(D, 1-\theta_0)$$

$$f(\{m_i\} \mid k, \{\theta_i\}) = \frac{k!}{m_1! \cdots m_l!} \prod_{i=1}^{k} \left(\frac{\theta_i}{1-\theta_0} \right)^{m_i}$$

1. Estimate a Poisson truncated at zero using raw data only
2. Estimate D from the binomial distribution by $\hat{D} = \frac{k}{1-\hat{\theta}_0}$

$$\hat{\theta}_0 = e^{-\hat{\lambda}}$$

Exercise: Data_lecture_13_TCR_diversity.csv

Estimate the species richness D for the DP CD3low cells using the first solution. Use vglm from package VGAM to estimate the truncated Poisson model.

	Thymus			Lymph nodes	
i	DP CD3low	SP $CD4^+$	SP $CD8^+$	LN $CD4^+$	LN $CD8^+$
1	79	33	16	34	17
2	17	6	3	8	8
3	6	2	3	2	1
4	5	2	5	1	2
5	1	0	3	0	1
6	1	0	1	0	0
7	1	0	1	0	0
8		0	1	1	0
10		1	0	1	0
11		0	1	0	0
16		1		0	0
20		0		1	0
21		0			1
28		1			0
52					1

How to estimate species richness?

Abundance	Number of Species
0	D-k
1	m_1
2	m_2
3	m_3
….	
l	m_l

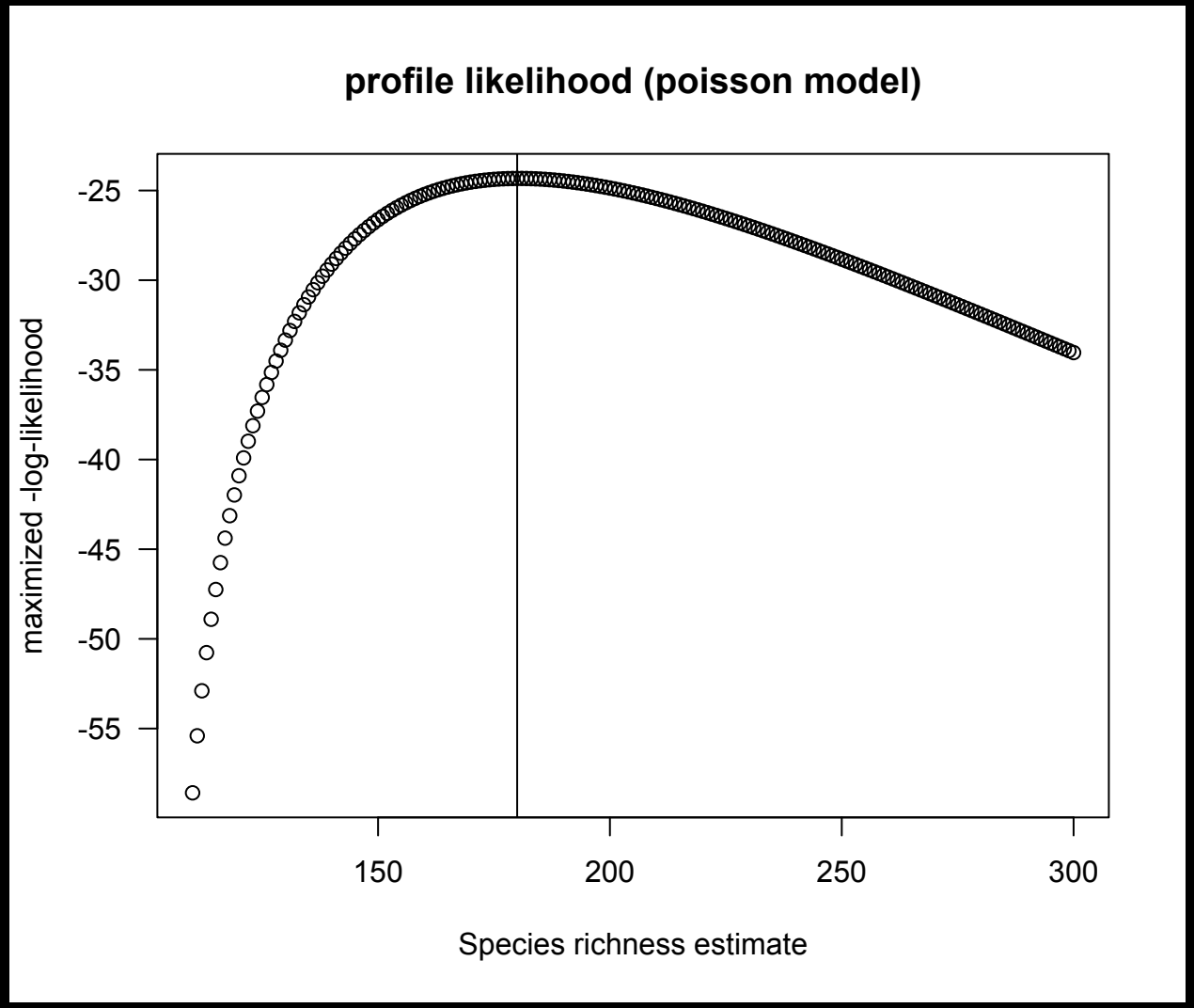
Augmented Species-Abundance distribution

Second solution (profile likelihood)

$$f(\{m_i\} \mid D, \{\theta_i\}) = \frac{D!}{(D-k)!m_1!\cdots m_l!}\theta_0^{D-M}\prod_{i=1}^{k}\theta_i^{m_i}$$

1. Fix D=k
2. Estimate the parameter of the Poisson distribution via maximum likelihood and calculate the respective maximized log-likelihood. (What is the MLE of λ?)
3. Do $D+1$ and repeat previous step
4. Keep incrementing if the maximised log-likelihood is increasing
5. $\hat{D}_{MLE}$ is the value immediately before when the maximized log-likelihood starts decreasing

How to estimate species richness?



Exercise: Data_lecture_13_TCR_diversity.csv

Estimate the species richness D for the DP CD3low cells using the second solution.

	Thymus			Lymph nodes	
i	DP CD3low	SP $CD4^+$	SP $CD8^+$	LN $CD4^+$	LN $CD8^+$
1	79	33	16	34	17
2	17	6	3	8	8
3	6	2	3	2	1
4	5	2	5	1	2
5	1	0	3	0	1
6	1	0	1	0	0
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21		0			1
28		1			0
52					1

How to estimate species richness?

Abundance	Number of Species
0	D-k
1	m_1
2	m_2
3	m_3
….	
l	m_l

Augmented Species-Abundance distribution

Calculation of a 95% confidence interval using the profile likelihood

Use the critical value of the Wilks' ratio test

$$H_0 : D = D_0 \text{ versus } H_1 : D \neq D_0$$

$$\Lambda = -2(\log L_{D_0} - \log L_{\hat{D}}) \,|\, H_0 \rightsquigarrow \chi^2_{(1)}$$

$$\text{critical value} = q_{95\%,\chi^2_{(1)}}$$

accept H_0 if $\Lambda < q_{95\%,\chi^2_{(1)}}$ reject H_0, otherwise

How to estimate species richness?

Abundance	Number of Species
0	D-k
1	m_1
2	m_2
3	m_3
….	
l	m_l

Augmented Species-Abundance distribution

Calculation of a 95% confidence interval using the profile likelihood

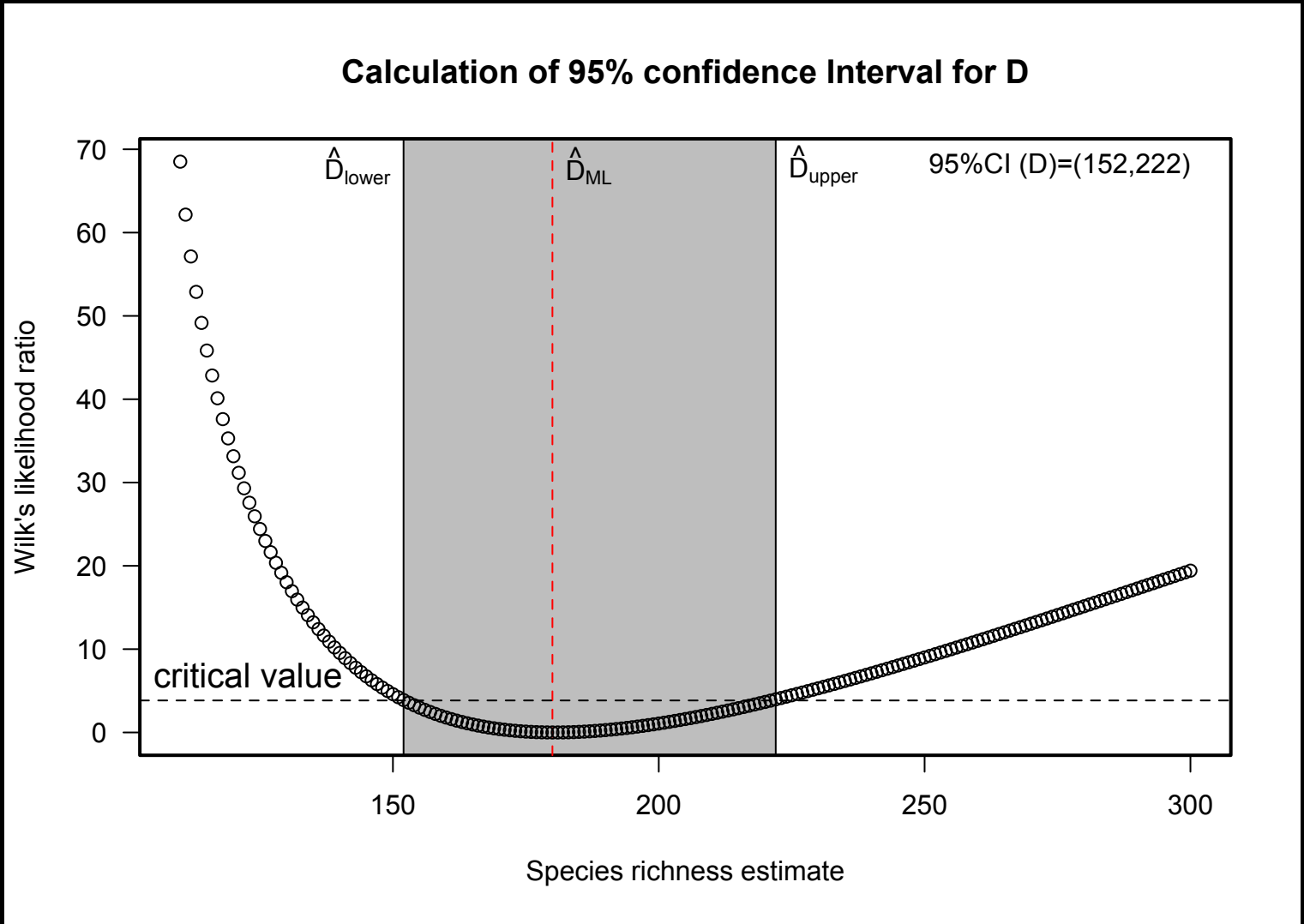
Use the critical value of the Wilks's ratio test

$$q_{95\%,\chi^2_{(1)}} = -2(\log L_{D_0} - \log L_{\hat{D}})$$

$$(\hat{D}_{lower}; \hat{D}_{upper})$$

$\hat{D}_{lower}$ and $\hat{D}_{upper}$ are the solutions of the above question

How to estimate species richness?



How to estimate species richness?

Abundance	Number of Species
1	m_1
2	m_2
3	m_3
….	
l	m_l
>l	0

Augmented Species-Abundance distribution

Pearson's goodness of fit test to check whether the model fits the data well

Use only the observed data

$$f(\{m_i\} \mid k, \{\theta_i\}) = \frac{k!}{m_1! \cdots m_l!} \prod_{i=1}^{k} \left(\frac{\theta_i}{1-\theta_0} \right)^{m_i}$$

$$\hat{\theta}_i = \frac{e^{-\hat{\lambda}} \hat{\lambda}^i}{i!}$$

Exercise: Data_lecture_13_TCR_diversity.csv

Calculate the confidence interval for the species richness D for the DP CD3low cells using the profile likelihood plot. Check whether the Poisson model fits the data well using the Pearson's goodness of fit test.

	Thymus			Lymph nodes	
i	DP CD3low	SP $CD4^+$	SP $CD8^+$	LN $CD4^+$	LN $CD8^+$
1	79	33	16	34	17
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16		1		0	0
20		0		1	0
21		0			1
28		1			0
52					1

Poisson-Gamma mixture model for estimating diversity richness

Abundance	Number of Species
0	D-k
1	m_1
2	m_2
3	m_3
….	
l	m_l

Augmented Species-Abundance distribution

Modelling θ_i

$$\theta_i = P[X = i \mid \lambda]$$

$X \mid \lambda \rightsquigarrow \text{Poisson}(\lambda)$ $\qquad$ $\lambda \mid \alpha, \beta \rightsquigarrow \text{Gamma}(\alpha, \beta)$

$$P[X = x] = \int_0^\infty P[X = x \mid \lambda] P[\lambda] d\lambda = \int_0^\infty \frac{e^\lambda \lambda^x}{x!} \times \frac{\beta^\alpha \lambda^{\alpha-1} e^{\beta\lambda}}{\Gamma(\alpha)} d\lambda$$

$$= \frac{\Gamma(i+\alpha)}{\Gamma(i+1)\Gamma(\alpha)} \left(\frac{\beta}{\beta+1}\right)^\alpha \left(\frac{1}{\beta+1}\right)^i$$

Negative Binomial

How to estimate species richness?

Abundance	Number of Species
0	D-k
1	m_1
2	m_2
3	m_3
….	
l	m_l

Augmented Species-Abundance distribution

First solution (truncated Negative Binomial)

$$k \mid D, \theta_0 \rightsquigarrow \text{Binomial}(D, 1-\theta_0)$$

$$f(\{m_i\} \mid k, \{\theta_i\}) = \frac{k!}{m_1! \cdots m_l!} \prod_{i=1}^{k} \left(\frac{\theta_i}{1-\theta_0} \right)^{m_i}$$

1. Estimate the Negative-Binomial truncated at zero using the raw data only
2. Estimate D from the binomial distribution by $\hat{D} = \frac{k}{1-\hat{\theta}_0}$

$$\hat{\theta}_0 = \left(\frac{\hat{\beta}}{\hat{\beta}+1} \right)^{\hat{\alpha}}$$

How to estimate species richness?

Abundance	Number of Species
0	D-k
1	m_1
2	m_2
3	m_3
….	
l	m_l

Augmented Species-Abundance distribution

Second solution (profile likelihood)

$$f(\{m_i\} \mid D, \{\theta_i\}) = \frac{D!}{(D-k)!m_1!\cdots m_l!}\theta_0^{D-M}\prod_{i=1}^{k}\theta_i^{m_i}$$

1. Fix $\hat{D}$=k
2. Estimate the parameters of the Negative distribution via maximum likelihood and calculate the respective maximized log-likelihood.
3. Do $\hat{D}+1$ in one unit and repeat previous step
4. Keep incrementing if the maximised log-likelihood is increasing
5. $\hat{D}_{MLE}$ is the value immediately before when the maximized log-likelihood starts decreasing

Exercise: Data_lecture_13_TCR_diversity.csv

Estimate the species richness D for the DP CD3low cells using the Negative Binomial distribution. Estimate via the second solution.

	Thymus			Lymph nodes	
i	DP CD3low	SP $CD4^+$	SP $CD8^+$	LN $CD4^+$	LN $CD8^+$
1	79	33	16	34	17
2	17	6	3	8	8
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52					1