

Cell-Signalling in Streptococcus Pneumoniae (SPN)

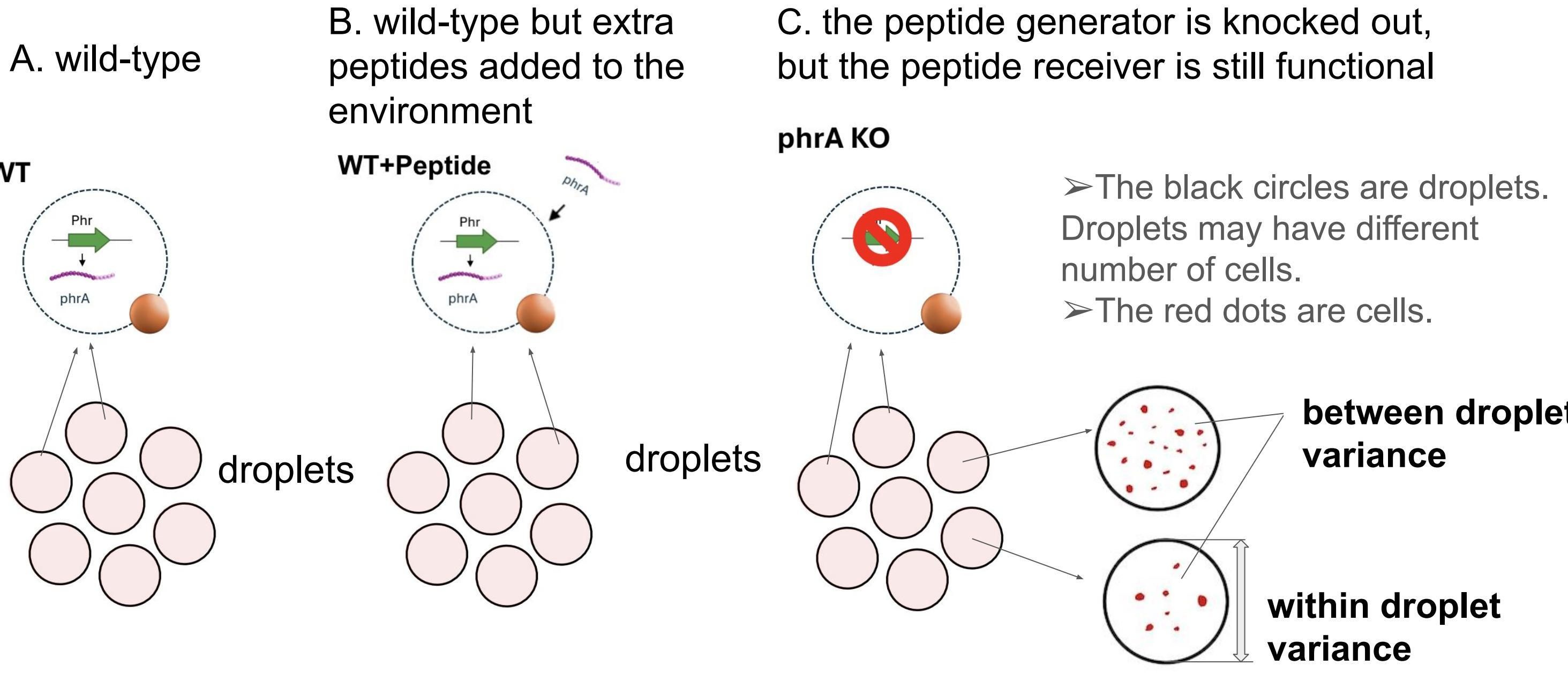
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Background

- Spn* is the leading cause of pneumonia mortality globally, killing more than 300,000 children under 5 years old worldwide annually
- Spn* cells are inserted in microdroplets
- How cells communicate → use the **peptide** produced by PhrA induction
- How we measure communication → **fluorescence intensity (FL)**
 - > more peptides → more communication → greater FL
- Research Objective
 - > **Investigate cell communication under 3 experiment conditions**

Data

Experimental Conditions – approximately 35-40 droplets @4 hr

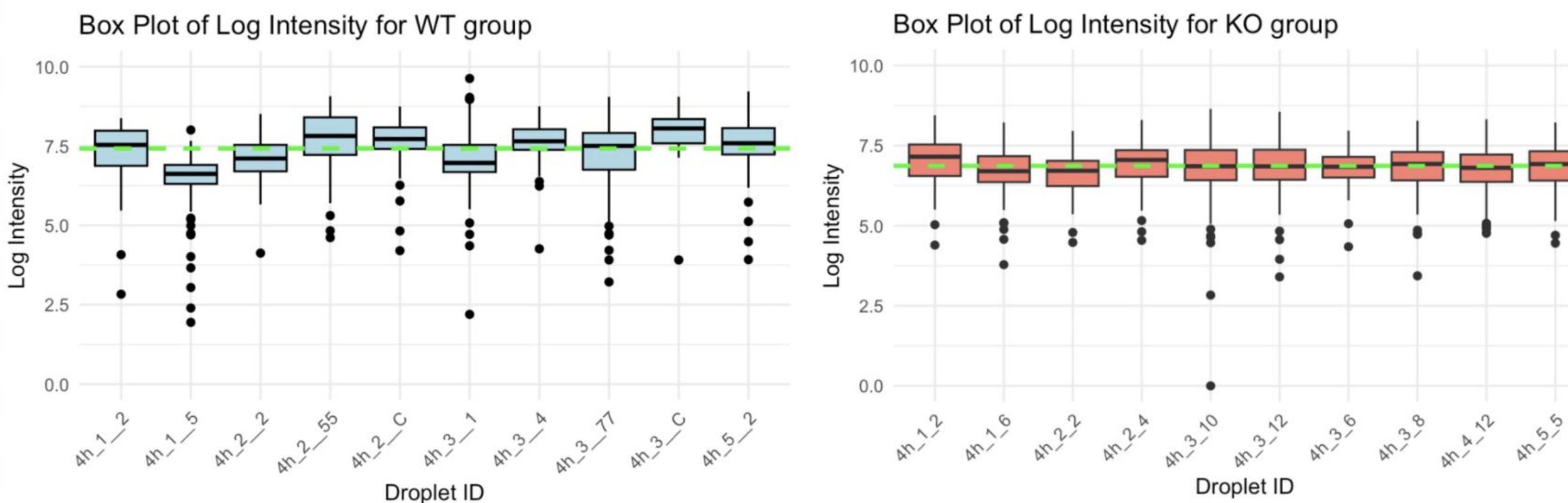


Method

Mixed effect model

- Model that accounts for both fixed effects (systematic, population-level variability) and random effects (individual-level variability)
- model = lmer(response ~ fixed effect + (1 | random effect), data = data)
- Intraclass Correlation Coefficient (ICC) measures the proportion of total variance in the response variable that is attributable to between unit (droplet) variation
- ICC =
$$\frac{\sigma_{\text{between}}^2}{\sigma_{\text{between}}^2 + \sigma_{\text{within}}^2}$$

Results & Analysis

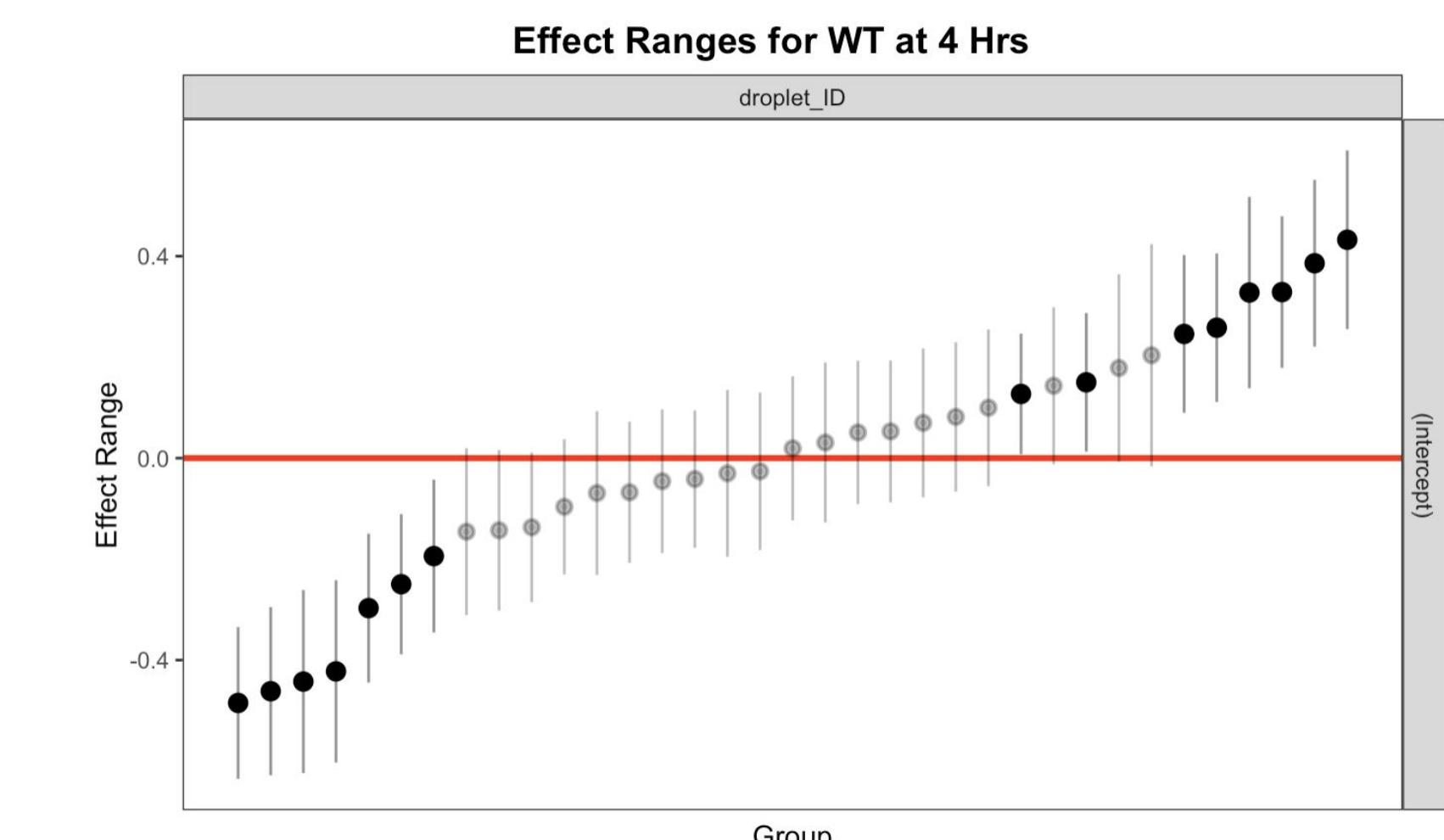


- Random selection** of 10 droplets
- Mean of WT (greenline) higher than KO
- WT group has more variation **both between and within droplet** and has more extreme outliers

Table 1: Comparison of KO vs WT @ 4 hrs

Exp	Measure	Mixed Model		Fixed Effects	
		KO	WT	KO	WT
Exp #1	Mean (KO)	6.752	6.764		
	Group Effect (WT)	0.582	0.552		
	t-value (group)	8.554	29.61		
	ICC	10.35%	NA		
Exp #2	Mean (KO)	7.10398	6.82107		
	Group Effect (WT)	0.43544	0.52545		
	t-value (group)	5.401	32.27		
	ICC	9.55%	NA		
Exp #3	Mean (KO)	6.722	6.873		
	Group Effect (WT)	0.498	0.546		
	t-value (group)	22.04	32.74		
	ICC	12.06%	NA		

- More active communication in WT than KO**
 - > mean FL WT > mean FL KO
- Between-droplet variability is significant**
 - > ICC significantly > 0
- t-values differ** significantly between two models
- Fixed-effect** models yield much **larger** t-values
- Mixed model accounts for droplet-level variability
 - > more conservative significance



- Figure shows each droplet's 95% CI for the deviation in mean FL from the overall WT average
- There is **heterogeneity** among WT droplets in their mean FL under the 4-hour condition

Table 2: Comparison of WT vs WT+Peptide @ 4 hrs

Exp	Measure	Mixed Model		Fixed Effects	
		Intercept (WT)	Group Effect (+ peptide)	Intercept (WT)	Group Effect (+ peptide)
Exp #1	Intercept (WT)	7.006	6.964		
	Group Effect (+ peptide)	0.391	0.426		
	t-value (group)	14.30	22.86		
	ICC	10.90%	NA		
Exp #2	Intercept (WT)	6.900	6.821		
	Group Effect (+ peptide)	0.423	0.525		
	t-value (group)	16.99	32.27		
	ICC	7.05%	NA		
Exp #3	Intercept (WT)	7.191	7.092		
	Group Effect (+ peptide)	0.244	0.383		
	t-value (group)	8.41	18.31		
	ICC	12.38%	NA		

- t-values of group effect range from 8.41 to 32.27, all significant with $p < .001$ → **strong statistical support** for group effect
- More active communication in WT+Peptide**
 - > mean FL WT+Peptide > mean FL WT
- ICC shows **significant variability across droplets**

Conclusion

Biological insights

- WT cells show **stronger fluorescence** than KO
- WT+peptide further increases fluorescence Intensity
- Between-droplet variability** is consistently significant across experiments

Modeling Insights

- Fixed-effects models lead to inflated t-values and potentially **overstated** significance by ignoring between-droplet variability
- Mixed effect model is more suitable for this experiment**

References

- Clark, M. (2020). *Random Intercepts — Mixed Models with R*. Retrieved from m-clark.github.io
- Mudge, L. (n.d.). *Mixed Effects Models*. Retrieved from lmudge13.github.io
- Aggarwal, S. D., Yesilkaya, H., Dawid, S., & Hiller, N. L. (2020). *The pneumococcal social network*. PLOS Pathogens, 16(10), e1008931. <https://doi.org/10.1371/journal.ppat.1008931>