

# *Osmia* Apple Orchard Nesting Structure Survival Rate

Batoule Hyjazie

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## 1. Introduction

### 1.1 Background

Like many species around the world, pollinators are heavily affected by habitat loss, often driven by agricultural intensification, among other factors like pesticide use, disease and climate change (Potts et al. 2010). Natural habitats provide floral and nesting resources to pollinators which are both essential to survival and growth (Roulston and Goodell 2011). Ironically, while, most pollinator population declines are often attributed to agriculture, pollinators have been shown to not only enhance crop production but also, to be uniquely essential to the production of certain crops like macadamia, cantaloupe and watermelon (Klein et al. 2006). Several studies have used artificial nesting structures, also known as trap nests or bee hotels (Geslin et al. 2020), to not only study wild bees, but also to promote their conservation in urban and agricultural settings (MacIvor and Packer 2015; N. K. Boyle and Pitts-Singer 2017). Understanding how bees respond to their environment, and in particular, to changes in resource availability like with floral and nesting resources, can allow us to better predict their abundance and by extension their survival, as well as to develop better conservation strategies in both natural and agricultural environments.

### 1.2 Study System

While nesting structures are widely used in the study of bees, no study has yet examined whether the provision of nesting structures actually increases local bee populations in comparison to control populations, establishing that bees are nest site limited (Westerfelt, Weslien, and Widenfalk 2018; Roulston and Goodell 2011). In order to conduct such a study, I must first test that nesting structures installed in anthropogenic habitat are not a population sink, attracting nesting female bees to a potentially unfit environment for larval survival, which could theoretically harm, rather than protect, local wild bee populations.

In Hungarian apple orchards, it has been found that when comparing nesting structures installed in paired apple orchard and natural habitat sites, higher colonization rates and counts of bee and wasp live offspring were observed in semi-natural habitat (Bihaly et al. 2020). This is possibly due to a higher prevalence of floral resources in semi-natural habitat when compared to orchard floral resources (Bihaly et al. 2020).

In fruit orchards, a wide variety of pollinators such as butterflies, wasps and of course, bees, both domestic and wild, can be found. *Osmia* spp. is a common wild bee genus in fruit orchards that distinguishes itself from other bees, like honey bees, by its ability to fly at cooler temperatures (Isaacs et al. 2017; Natalie K. Boyle and Pitts-Singer 2019) and deposit higher pollen loads on a wide-range of flowers (Földesi et al. 2015). To test whether similar observations can be made in orchards within the greater Ottawa area, nesting structure survival rates of *Osmia* spp. (“mason bees”) offspring in orchard and natural habitats will be compared with one another.



Figure 1: Osmia Entering Nesting Structure

### 1.3 Hypothesis

The installation of nesting structures in apple orchards, are potential population sinks rather than populations sources, conceivably due to lesser floral resource availability over the entire growing season. As a result, nesting structures in natural habitat will have a greater ratio of survival of *Osmia spp.* offspring when compared to apple orchard habitat.

### 1.4 Prediction

If nesting structures placed in natural environments have a significantly greater *Osmia spp.* survival ratio than orchard environments, then it is likely that nesting structures placed in orchard environments are an *Osmia spp.* population sink.

## 2. Methods

### 2.1 Data Collection

This study was conducted from May to August 2017 in 7 apple orchards and 8 natural habitats (6 on National Capital Commission (NCC) grounds and 2 on City of Ottawa grounds) around the Ottawa-area for a total of 15 sites. Overall, 75 nesting blocks were installed in both natural and orchard habitats with 5 blocks per site. Each block had 10 nests or holes.

Throughout these 4 months, various insects, mostly wasps and bees, inhabited the holes, and some nested within them, laying their eggs. The eggs developed first as larvae, then as pupae. In September, the blocks were collected and stored in a laboratory environment where the insects over-wintered (hibernated). Before their expected scheduled emergence, the nests were inspected and *Osmia spp.* cell survival count was estimated using visual markers such as nest material and cell size.

### 2.2 Data Set

The “Osmia Survival.xlsx” data set contains the data that will be used for subsequent statistical analyses. It includes information such as the original number of cells observed in the straw (*No.cells*), the initial perceived number of surviving cells (*No.surv.cells*) as well as a validated revised number of surviving *Osmia spp.* cells (*No.surv.osmia*).

It is important to note that the number of surviving cells does not account for bees that died over winter due to unfavorable storage conditions. Therefore, we are only accounting for deaths of bees that have clearly died as larvae either through parasitization or other un-diagnosed causes. Deaths at the larval stage can only be attributed to causes occurring during the growing season or associated with either orchard or natural conditions. By the developmental stage of adult eclosion, nests were already transferred to a laboratory environment. Any deaths occurring at the adult stage are therefore ignored.

### 2.2 Statistical Methods

To explore frequentist and bayesian statistics, as an exercise, I will be running the same model using these two different approaches. Because the response that is measured is the proportion of surviving *Osmia* offspring, the data will is not normally distributed but binomially distributed. This was verified using both a histogram of the distribution of the frequency of *Osmia spp.* survival ratios and a q-q plot. Thus, all models will be run using a binomial distribution.

### 2.2.1 Frequentist Approach

Knowing that the data was hierarchical with random effects of block nested within site, I wanted to test the significance of these terms. To do this, I ran a simple generalized linear model (GLM). Afterwards, I accounted for the hierarchical structure of the data and ran a generalized linear mixed model (GLMM) using block nested within site as a random effect.

**2.2.1.1 Frequentist Key Assumptions** The main assumption of a GLMM, is that the chosen probability distribution and associated link function is appropriate for the data. As previously stated, due to the use of proportional data, the distribution used was binomial and the link function was the logarithm of the odds (logit). Another assumption is that the random effects are normally distributed. Finally, it is assumed that there is no over-dispersion or under-dispersion. This refers to the constancy of the variance and was verified by comparing the ratio of the residual deviance to the degrees of freedom.

To evaluate model fit, a likelihood ratio test using the *drop1* function was conducted. Out of curiosity, the GLMM and GLM versions of the model output (one with the random effect term of block nested within site and one without) were compared via a likelihood ratio test and also by simply comparing their Aikake Information Criteria (AIC) from their model outputs. The normality of the random effects was assessed visually using a q-q plot (*Figure\_*). While this is not an explicit assumption of GLMM, the normality of the residuals were evaluated visually using a residuals versus fitted values plot (*Figure\_*).

### 2.2.2 Bayesian Approach

Two Monte Carlo Markov Chains (MCMC) were run following the same model structure described previously (random effects of block nested within site and the binomial probability distribution). The first chain consisted of a test run and was sequenced using the default settings of the MCMCglmm package (sample size of 1,000, number of iterations of 13,000, burnin of 3,000, thin of 10). The second chain was run with a larger sample size and number of iterations as well as adjusted priors (sample size of 4,000, number of iterations of 2,020,000, burnin of 20,000, thin of 500, V=1, nu=1).

**2.2.2.1 Bayesian Key Assumptions** Posterior trace and density plots were used to visually diagnose the variance and autocorrelation of each iterative value in the chains. The *autocorr.diag* functions were used to assess the autocorrelation of the chain in further detail. The *raftery.diag* function was used to diagnostically determine the optimal sample size for the final chain (sample size of 4,000).

## 3. Results

### 3.1 General Results

At the end of August 2017, 71 out of the initial 75 installed nests were occupied, with 37 occupied blocks in natural habitats and 34 occupied blocks in orchard habitats by various species of bees and wasps.

In **Figure 2**, a simple comparison of the means of *Osmia* spp. survival across habitat types shows that there is not much apparent difference in survival rates among different natural versus orchard populations.

### 3.2 Statistical Results

Summary of Statistical results and comparison between bayesian and frequentist results.

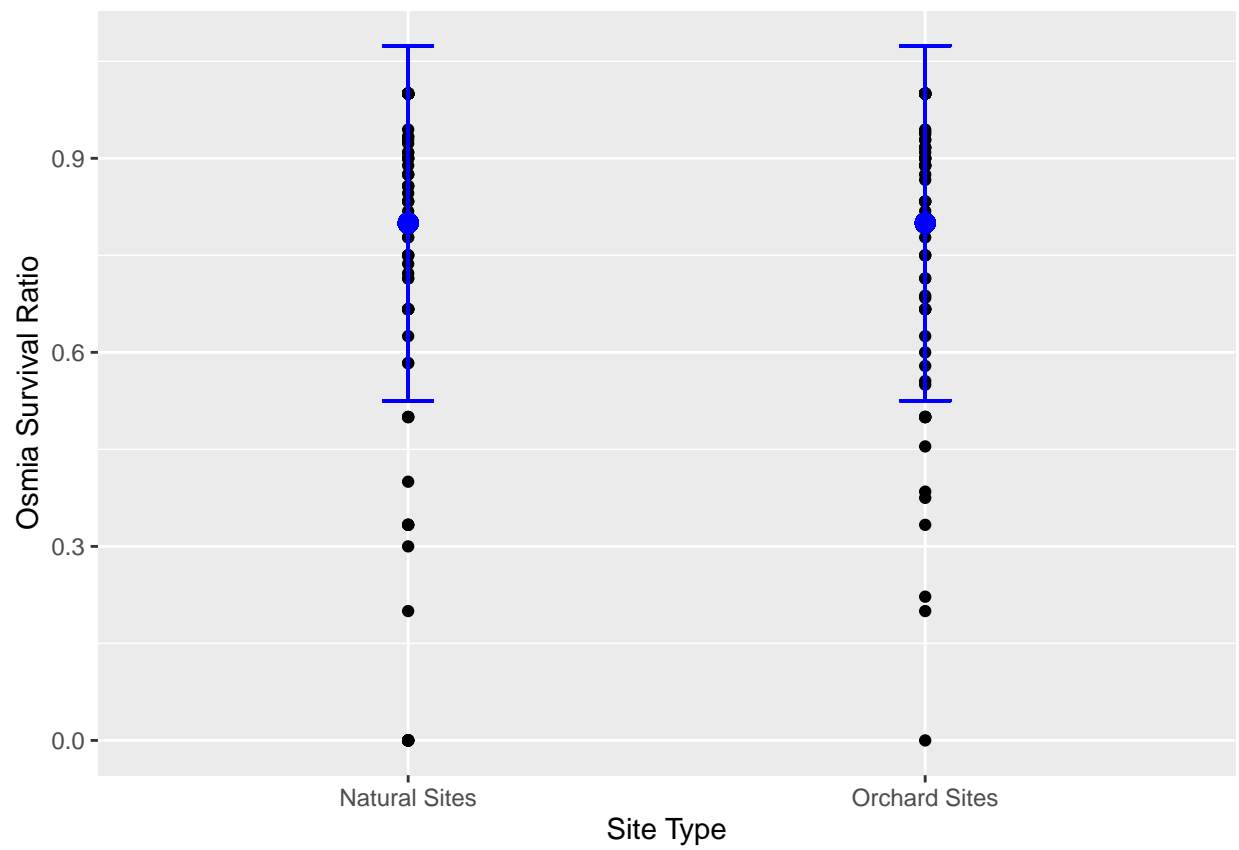


Figure 2: Plot of Osmia Survival per Site Type

### 3.2.1 Frequentist Results

FIX TABLES!

AIC	765.46
BIC	779.02
Pseudo-R <sup>2</sup> (fixed effects)	0.00
Pseudo-R <sup>2</sup> (total)	0.19

Fixed Effects				
	Est.	S.E.	z val.	p
(Intercept)	1.78	0.22	8.03	0.00
s.typeo	0.05	0.32	0.15	0.88

Random Effects		
Group	Parameter	Std. Dev.
Block_ID:s.ID	(Intercept)	0.84
s.ID	(Intercept)	0.25

Grouping Variables		
Group	# groups	ICC
Block_ID:s.ID	57	0.17
s.ID	15	0.02

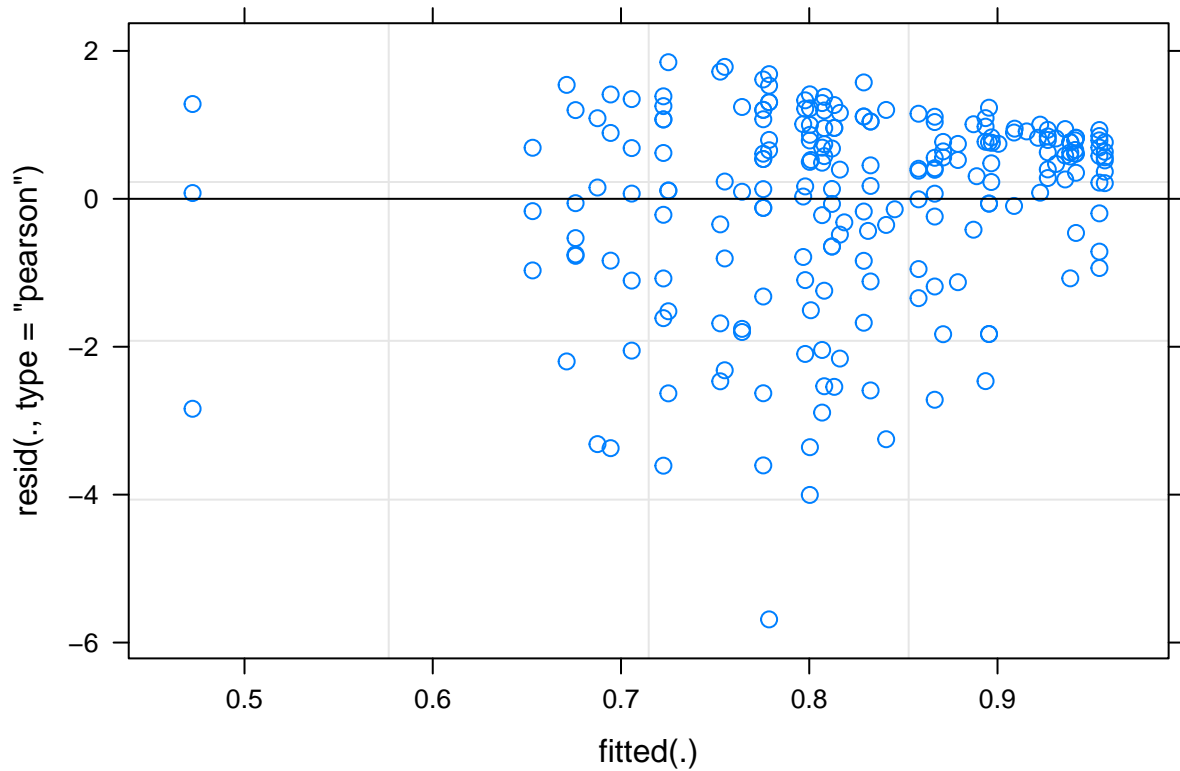
Observations	219
Dependent variable	No.surv.osmia/No.cells
Type	Mixed effects generalized linear model
Family	binomial
Link	logit

AIC	765.46
BIC	779.02
Pseudo-R <sup>2</sup> (fixed effects)	0.00
Pseudo-R <sup>2</sup> (total)	0.19

Fixed Effects				
	Est.	S.E.	z val.	p
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The GLMM output shows that when controlling for differences in site and block characteristics, there is no evidence that there is a difference in *Osmia* spp. larvae survival among orchard and natural habitat types.

**3.2.1.1 Key Assumptions** The likelihood ratio test demonstrates that model fit is marginally improved when the site type term is included with a marginal difference in AIC of 1.97. Because of this, it is most likely that site type has little predictive influence on *Osmia* spp. survival. When comparing the mixed effect model (GLMM) with the model without the nested random effects of site and block ID (GLM), the inclusion of these nested random effects improves model fit (GLMM AIC of 765.5 while GLM AIC of 820.78). This is further confirmed by the likelihood ratio test which compares the GLM, the single-random effect GLMM and the nested random effect GLMM. The test shows that both GLMM models are preferable to the non-mixed model. However, since the non-nested model has issues with convergence and over-dispersion, the model which includes the nested term is still preferable.

Even with this nested final model, while the ratio of residual deviance to degrees of freedom was above a value of 1 (1.92), over-dispersion was not prominent enough to justify changing to a more complex probability distribution such as a quasi-binomial distribution. In my final model, there was a hint of over-dispersion since the residual deviance (413.40) was greater than the number of degrees of freedom (216).

Returning to the initial assumptions of a GLMM that the residuals of the random effects should be normal and homoscedastic, in **Figure 3**, the residuals are constant throughout all levels of the random effect. Moreover the q-q plots in **Figure 4** show that the nested random effects of site and block ID are appropriately normal.

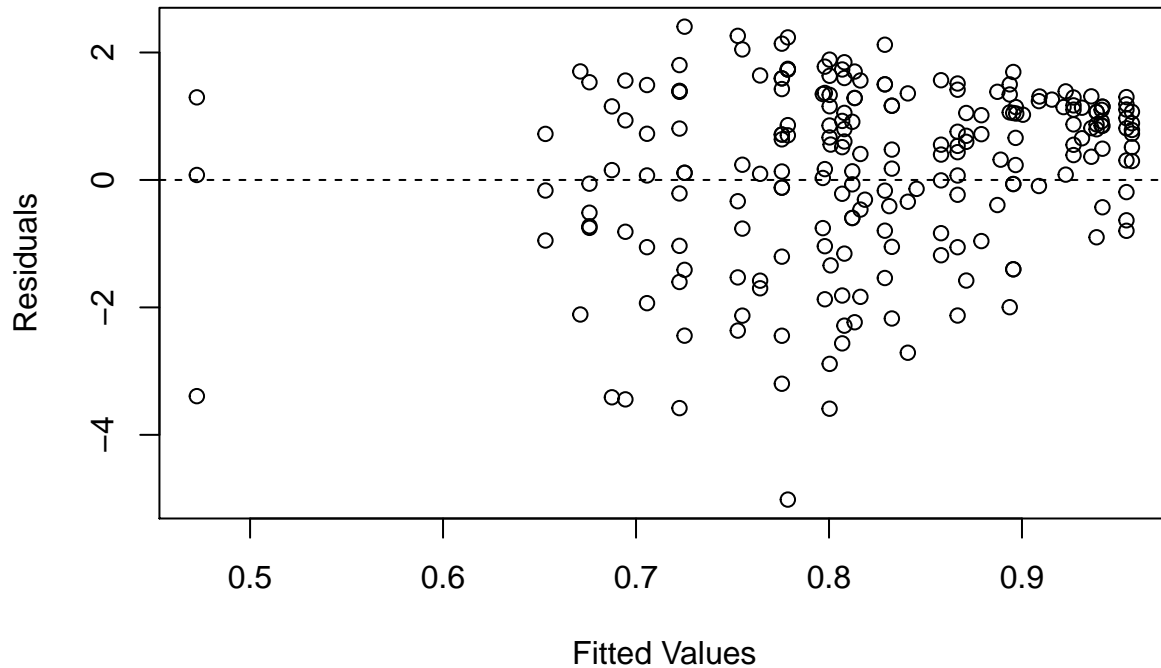


Figure 3: Residuals versus Fitted Values of Final Osmia Survival Model

### 3.2.2 Bayesian Results

```
## (Intercept)      s.typeo
## 1.975085211 -0.006145925

##          lower    upper
## (Intercept) 1.4378072 2.684517
## s.typeo     -0.7547036 1.051921
## attr("Probability")
## [1] 0.95

##          s.ID Block_ID:s.ID      units
## 0.004327554 0.007743126 2.000558672
```



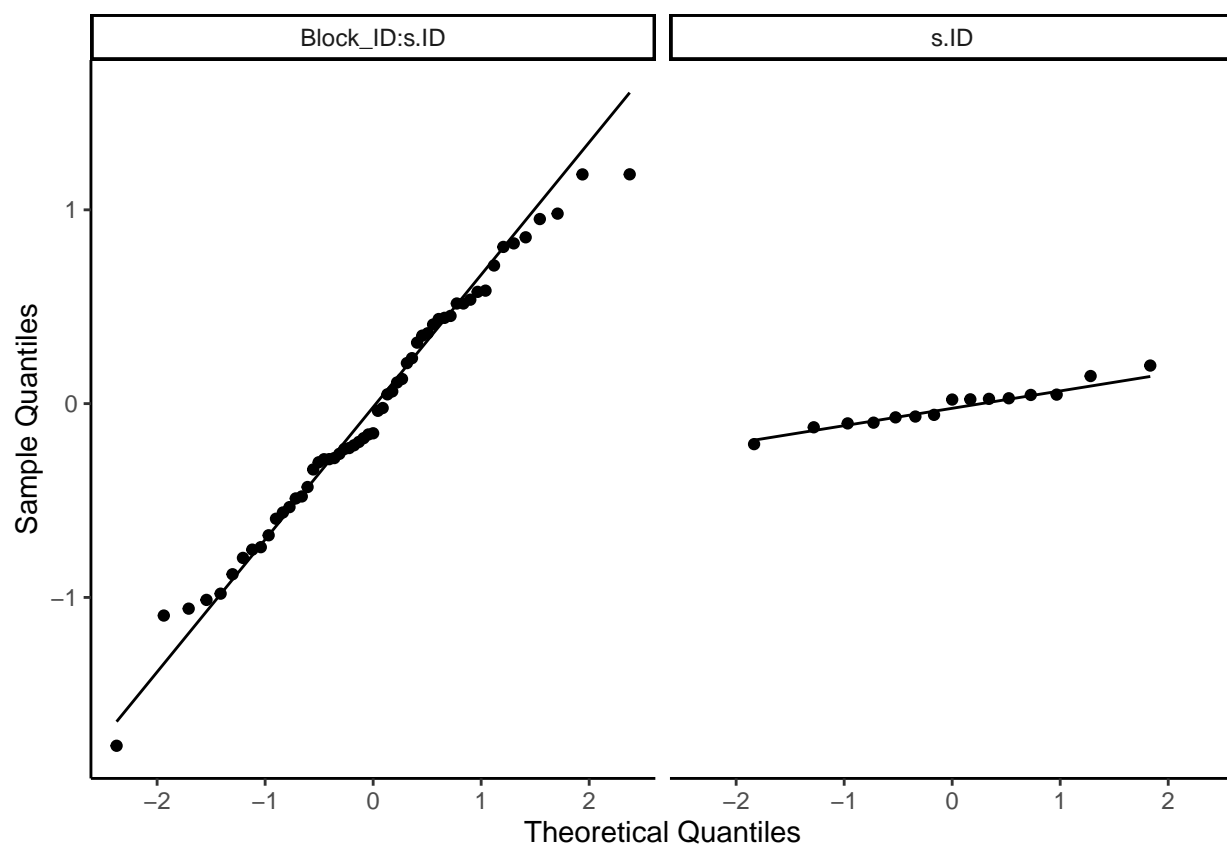


Figure 4: Q-Q Plots of Nested Random Effects of Block and Site ID (BLUPs)

```
##               lower      upper
## s.ID          5.400409e-09 0.8590605
## Block_ID:s.ID 4.668243e-09 1.4283633
## units         1.084530e+00 3.0244161
## attr(,"Probability")
## [1] 0.95
```

Graphics with data and model outputs Tables reporting statistics Text describing results

**3.2.2.1 Bayesian Key Assumptions** From a visual assessment of the posterior trace and density plots of the first chain (**Figure 5**), there was high evidence of autocorrelation and variability, especially for the random effects. There was also some degree of autocorrelation since most lag iterations had autocorrelation values exceeding 0.1. Moreover, the *raftery.diag* function recommended a sample size of at least 3,746. To remedy this, for the second and final chain, sample size was increased from 1,000 to 4,000, number of iterations was increased from 13,000 to 2,020,000 and priors were adjusted ( $V=1$ ,  $\nu=1$ ).

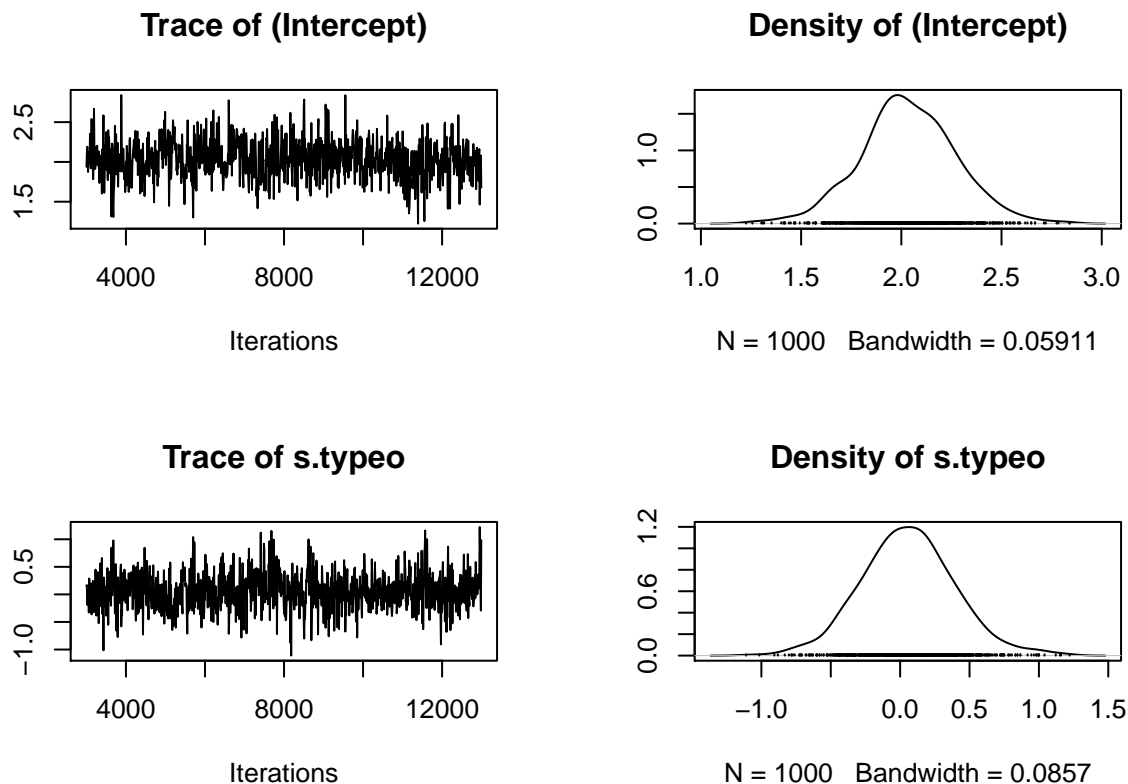


Figure 5: Initial Posterior MCMC Trace and Distribution of the Fixed Effects

A visual assessment of the modified posterior trace and density plots (**Figure 6**) show that there is no identifiable pattern in the chain as what was evident in the first chain and much less proof of autocorrelation. This is further confirmed by the diagnostic autocorrelation test in which all autocorrelation values are well below the 0.1 threshold.

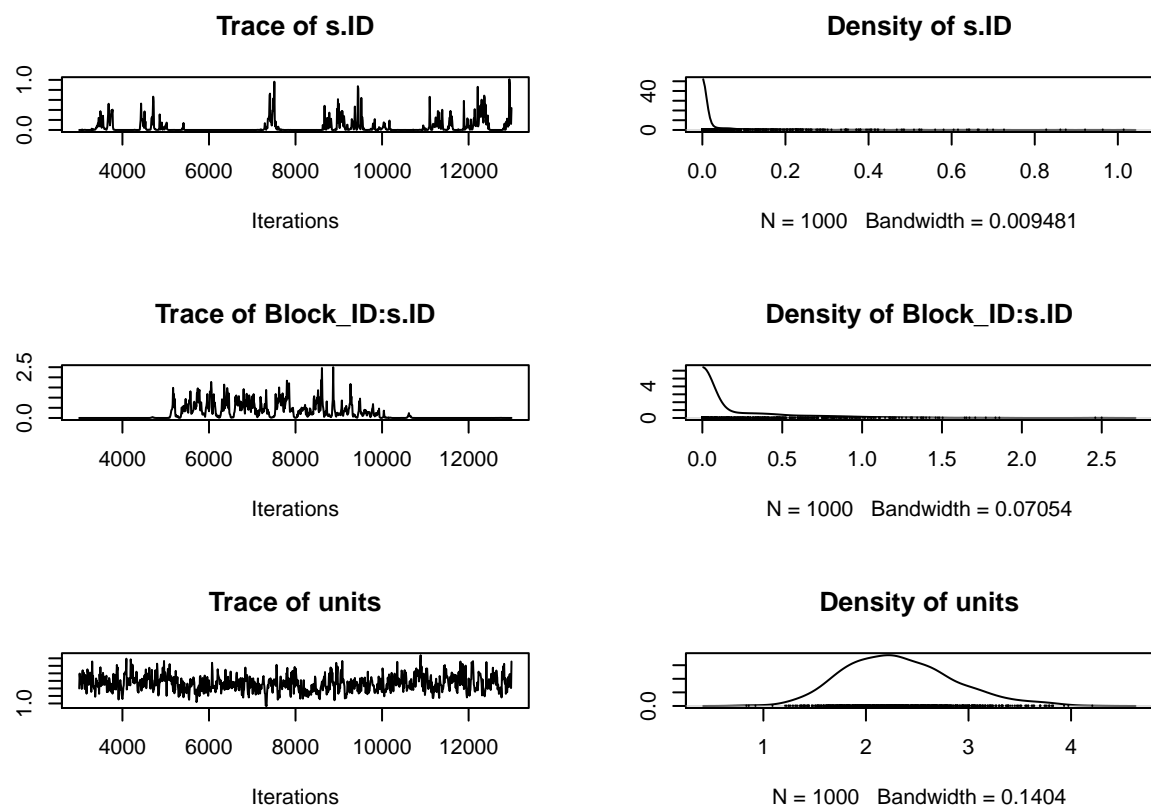


Figure 6: Initial Posterior MCMC Trace and Distribution of the Random Effects

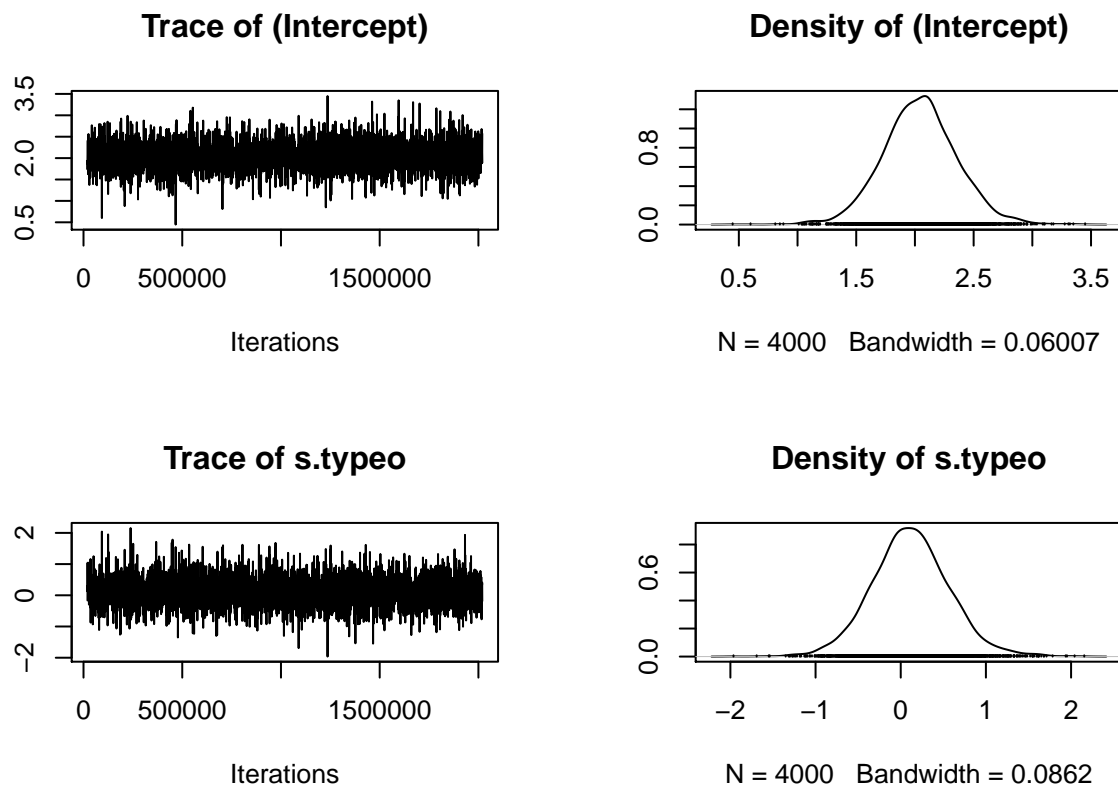


Figure 7: Final Posterior MCMC Trace and Distribution of the Fixed Effects

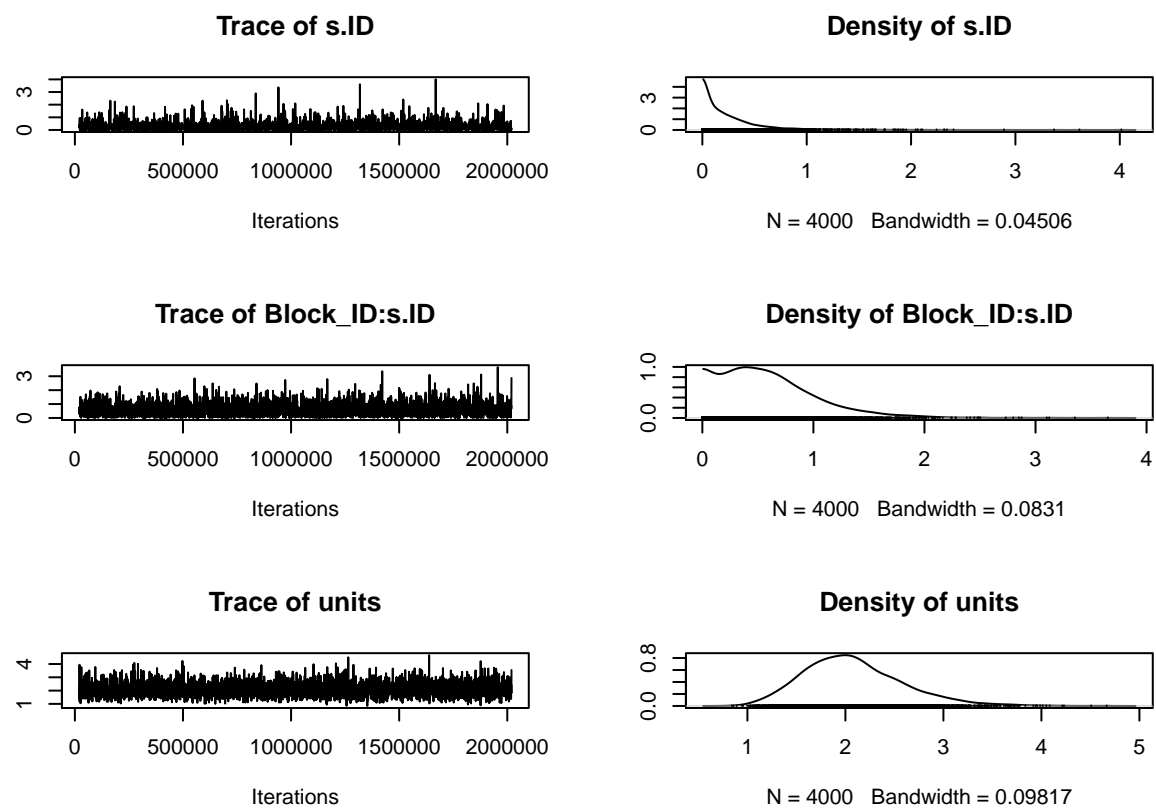


Figure 8: Final Posterior MCMC Trace and Distribution of the Random Effects

## 4. Discussion

### 4.1 Summary

Based on the data collected, there is no evidence for a difference between the survival ratio of *Osmia* spp. in orchard or natural environments.

### 4.2 Implications

While further investigation is needed, this could possibly indicate that the installation of nesting structures pose little risk to the development and survival of *Osmia* spp. offspring and most likely, will not act a population sink.

### 4.3 Limitations of Analysis

Because the hierarchical nature of the data was acknowledged, I was able to avoid issues of autocorrelation within the data structure and issues of non-independent residuals which will lead to an increased rate of false positives (McNeish and Stapleton 2014). However, it must be acknowledged that the number of sites visited in the orchard and natural habitat types is very small (7 and 8, respectively), which overall, leads to bias in the results. It is typically recommended to have a minimum cluster sample size of 30 which is well below the 15 sites or clusters from this data set. Some authors even argue that mixed models become potentially untrustworthy with small sample sizes and should not be run if cluster sizes are below 10 (McNeish and Stapleton 2014). A power analysis using the *simr* package shows that the power to reject the null hypothesis of no effect of site type on *Osmia* survival given this particular set-up is 6.10% which is very low especially when considering that most studies aim for a power of 80% (Green and MacLeod 2016). This low power is most likely due to small sample size.

When it comes to a critique of the data, it would have been informative to have more data relating to the cause of death since having this value as a fixed effect would have accounted for some of the over-dispersion detected in the final GLMM. Controlling for cause of death would have also helped with recognizing overarching factors in larval mortality that are not necessarily related to habitat type but rather to normal threats to larval development common across all landscapes. This would have helped with pinpointing the true differences among habitat types which can be overshadowed by these potentially common observable factors. From a different perspective, it would have also helped to determine whether death due to parasitization is more common in orchard habitat types or natural habitat types since this could give some by-proxy insight into parasite population densities.

### 4.4 Future Directions

In the future, it would be useful to rerun this experiment with a larger sample size to address the issue with power. By extension, it would also be interesting to collect some information on *Osmia* spp. survival in urban and suburban habitats in addition to both natural and agricultural habitats since data on *Osmia* spp. populations in these human-dominant landscapes is scarce. Cross-referencing the likelihood of *Osmia* spp. survival in these different habitats can help with re-evaluating the usefulness of installing these nesting structures from a conservation perspective. For instance, if mortality rates are high in suburban landscapes when compared to natural landscapes, nesting structures may be better suited to orchard landscapes where larval mortality has not been shown to differ from natural areas.

Moreover, it would be interesting to expand observations to include other bee and wasp genera and to document their survival. While logistically complicated, since it would involve extensive and detailed monitoring of nesting structures throughout the growing season, comparing the successful attainment of key developmental stages such as hatching, pupation and eclosion across landscape types could be an interesting extension to this question on successful *Osmia* spp. reproduction in anthropogenic environments.

# Data Availability Statement

All data is available in the **Final Report GitHub Repository**.

## References

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