Report on Figures and Data Analysis

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Comparing colony productivity and pathogen infection between California-bred and New England-bred honey bees (Apis mellifera)

Metadata:

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Data Set: These data were collected in 2016 in New York state by Andre Burnham and the Hamilton College bee research team from two yards in NY owned by one beekeeper who volunteered for the study.

Data Source: 2016 Hamilton Queen Origin Research Project

Funding Source: Hamilton College undergraduate research grants

Data Collection: Weights of colonies were obtained by summing the weights of individual hive bodies and supers for each colony at each yard (add more about protocols for other assays).

Columns: (from left to right) FieldID: Id including number location and treatment, Time: Generic Time Steps (T1-T5), Origin (California and Local), Yard (1 or 2),Nosema (spores per bee) only three time steps,Varroa (mites per 300 bees),Brood (frames of brood in colony),NosemaPA (binary data for nosema),VarroaPA (binary data for mites),MassDay(days after requeening for mass),NosemaDay days after requeening for nosema),VarroaDay days after requeening for varroa),BroodDay days after requeening for brood),MassDate (dates sampled),NosemaDate(dates sampled),VarroaDate(dates sampled),BroodDate (dates sampled)

Rows: Data points for all columns in order from each collection event

Missing values: NA

SUMMARY

Background

Honey bees (Apis mellifera) are global pollinators that have been on the decline due to various environmental and pathogen-related factors (vanEngelsdorp et al. 2009). However, recent studies have suggested that local environmental adaptations in A. mellifera phenotypes could be tied to colony productivity, survivability, and other fitness-related factors and thus hinder colony performance when migratory beekeeping practices are exercised (Costa et al. 2012, Buechler et al. 2014). The behavioral and morphological characteristics of these colony ecotypes are largely a result of genotypic variation in drones and the queen bee, a colony's only reproducing female. In this study, we tested the null hypothesis that queen bee ecotypes from very contrasting climates produce offspring that are equally productive and resistant to pathogens when observed in the same geographical region. In particular, we compared queens grafted in Vermont with queens reared by one of the largest queen breeders in California. We conducted our experiment in Central New York, which we determined to be a fair representation of the Vermont bees' local environment.

Results

We demonstrated that local colonies grew significantly more in total colony biomass than those of the Californian bees during specific time periods (T2, 7 July 2016; and T4, 9 August 2016). We also found that over five time periods (from 30 May 2016 to 3 August 2016), locally-raised bees reared significantly more brood than the Californian bees. However, no notable correlation with queen bee origin and mass of pollen collected was observed. In assessing resistance to parasites and pathogens, we found no significant difference over time in levels of the ectoparasite Varroa destructor. Using semi-quantitative PCR to evaluate the levels of deformed wing virus (DWV), black queen cell virus, (BQCV), and Israeli acute paralysis virus (IAPV) (three common RNA viruses in A. mellifera), we found that the levels of viral infection for BQCV and IAPV were unaffected after requeening the two groups with our experimental queens. However, our results demonstrated higher infection levels of DWV in CA colonies than in local colonies from T1 to T2 (15 June 16 and 13 July 2016). Levels of Nosema sp. (a microsporidian) in both the local and CA colony groups differed through three time periods. From T1 to T2 (16 June 2016 and 19 July 2016), Nosema spore loads significantly decreased in the local bees, while spore loads in the CA colonies increased. The third time period (9 August 2016) also showed significant decreases in local colony infection, while CA colonies continued to have high spore loads.

Conclusion

The significant differences we observed in colony weight and brood production between California-bred and northern-bred bees suggest that locally-bred A. mellifera have stronger nectar foraging and brood-rearing tendencies than their Californian counterparts. The lower Nosema sp. spore and DWV loads in the local colonies that we demonstrated strongly suggest that local bees are more resistant to certain pathogen strains and perhaps have heightened immune gene expression when compared with bees from another climate. To our knowledge, this is the first study to demonstrate significant differences in Nosema sp. infections between different A. mellifera ecotypes. Overall, our results present evidence that offspring of locally-raised queens from the Northeast have better adapted for survival and productivity in northern regions as compared to queens and worker bees from California, a climatically contrasting environment. These environmental adaptions relating to A. mellifera fitness could be tied to future colony survivability.

```r
# Preliminaries:

# Clear memory of characters:
ls()
```

```
## character(0)
```

```r
rm(list=ls())

# Set Working Directory:
setwd("~/AndreCollaborations/QueenExperimentBurnham")

# Read in Data:
QueenDF <- read.table("2016QueensHam.csv", header=TRUE, sep = ",", stringsAsFactors = FALSE)




# These dataframes are seperate becuase they do not follow that sampling
# pattern that the other four varaibles in the previous data frame do (i.e.5
# time steps):
PollenDF <- read.table("PollenQueens.csv", header=TRUE, sep = ",")
VirusDF <- read.table("RNAVirus.csv", header=TRUE, sep = ",")

# load plyr for data manipulation and ggplot plotting package and other
```

```r
# related packages:
library(plyr)
library(ggplot2)
library(grid)
library(dplyr)
library(scales)
```

```
## Warning: package 'scales' was built under R version 3.3.2
```

Colony Mass:

```r
# MASS - use repeated measures anova to look at differences between local
# and california through time:
aov.out <- aov(Mass~Origin * Time + Error(FieldID), data=QueenDF)

# look at summary of model:  SIGNIFICANT
summary(aov.out)
```

```
##
## Error: FieldID
##             Df Sum Sq Mean Sq F value  Pr(>F)
## Origin       1   3189    3189   8.123 0.00719 **
## Time         1   2538    2538   6.463 0.01546 *
## Origin:Time  1      8       8   0.019 0.89012
## Residuals   36  14135     393
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Error: Within
##             Df Sum Sq Mean Sq F value  Pr(>F)
## Time         1   9482    9482  124.89 < 2e-16 ***
## Origin:Time  1    832     832   10.96 0.00137 **
## Residuals   85   6453      76
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Frames of Brood:

```r
# BROOD - use repeated measures anova to look at differences between local
# and california through time:
aov.out <- aov(Brood ~ Origin * Time + Error(FieldID), data=QueenDF)

# look at summary of model: SIGNIFICANT
summary(aov.out)
```

```
##
## Error: FieldID
##             Df Sum Sq Mean Sq F value  Pr(>F)
## Origin       1  18.78  18.784   6.619 0.01436 *
## Time         1  25.45  25.451   8.968 0.00494 **
## Origin:Time  1   1.60   1.602   0.564 0.45739
## Residuals   36 102.16   2.838
```

```
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Error: Within
##              Df Sum Sq Mean Sq F value   Pr(>F)
## Time          1  11.40  11.403   7.115 0.008624 **
## Origin:Time   1  19.93  19.931  12.436 0.000584 ***
## Residuals   129 206.75   1.603
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

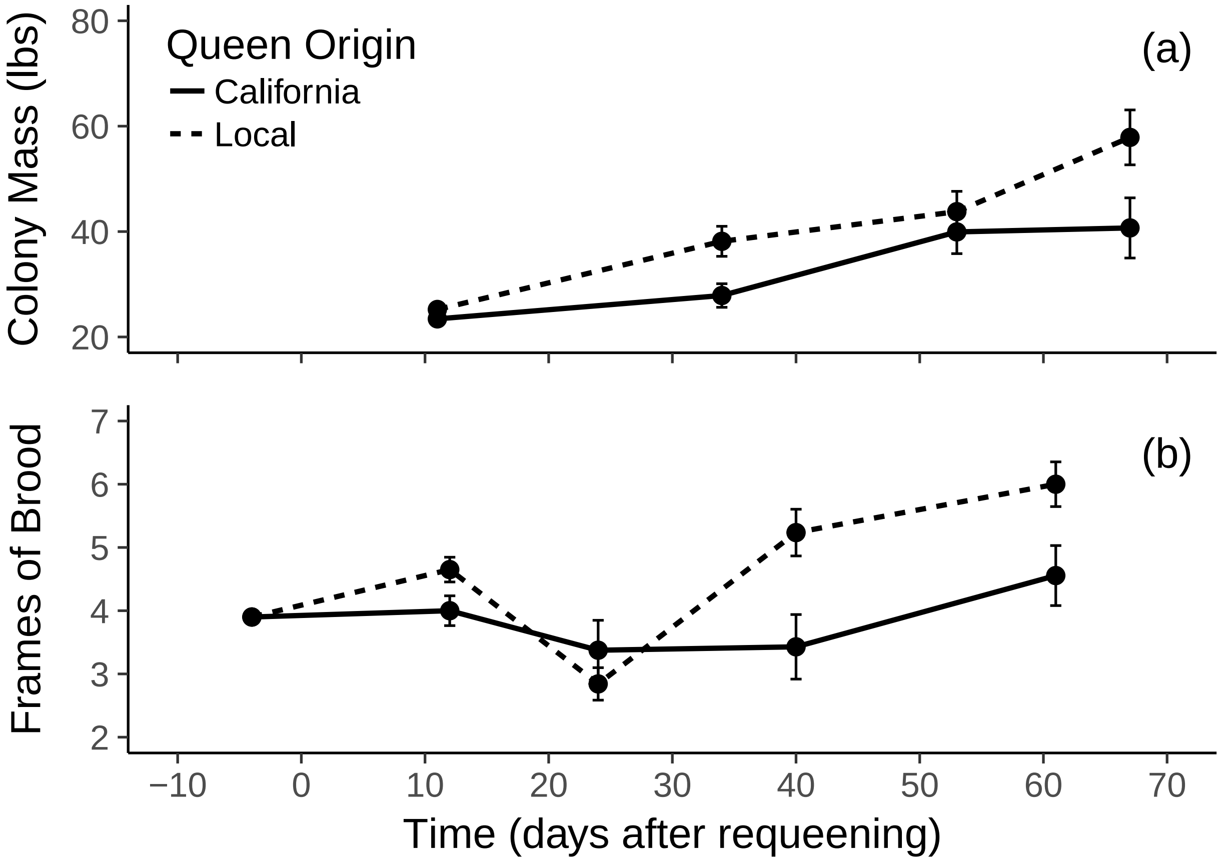
Frames of Brood & Colony Mass Through Time by Queen Origin

```
##       Origin MassDay  n     mean        sd       se
## 1 California      11 20 23.43000  5.096655 1.139647
## 2 California      34 16 27.86250  8.960571 2.240143
## 3 California      53 14 39.93333 15.427573 4.123192
## 4 California      67  9 40.68889 17.132750 5.710917
## 6      Local      11 20 25.21000  4.309341 0.963598
## 7      Local      34 19 38.14737 12.422711 2.849965
## 8      Local      53 17 43.77647 15.910355 3.858828
## 9      Local      67 17 57.87059 21.473465 5.208080
```

```
##        Origin BroodDay  n     mean        sd         se
## 1  California       -4 20 3.900000 0.3077935 0.06882472
## 2  California       12 20 4.000000 1.0540926 0.23570226
## 3  California       24 16 3.375000 1.8929694 0.47324236
## 4  California       40 14 3.428571 1.9100659 0.51048658
## 5  California       61  9 4.555556 1.4240006 0.47466687
## 6       Local       -4 20 3.900000 0.3077935 0.06882472
## 7       Local       12 20 4.650000 0.8750940 0.19567696
## 8       Local       24 19 2.842105 1.1186876 0.25664454
## 9       Local       40 17 5.235294 1.5218990 0.36911472
## 10      Local       61 17 6.000000 1.4577380 0.35355339
```

```
##
## Attaching package: 'gridExtra'
```

```
## The following object is masked from 'package:dplyr':
##
##     combine
```



Varroa:

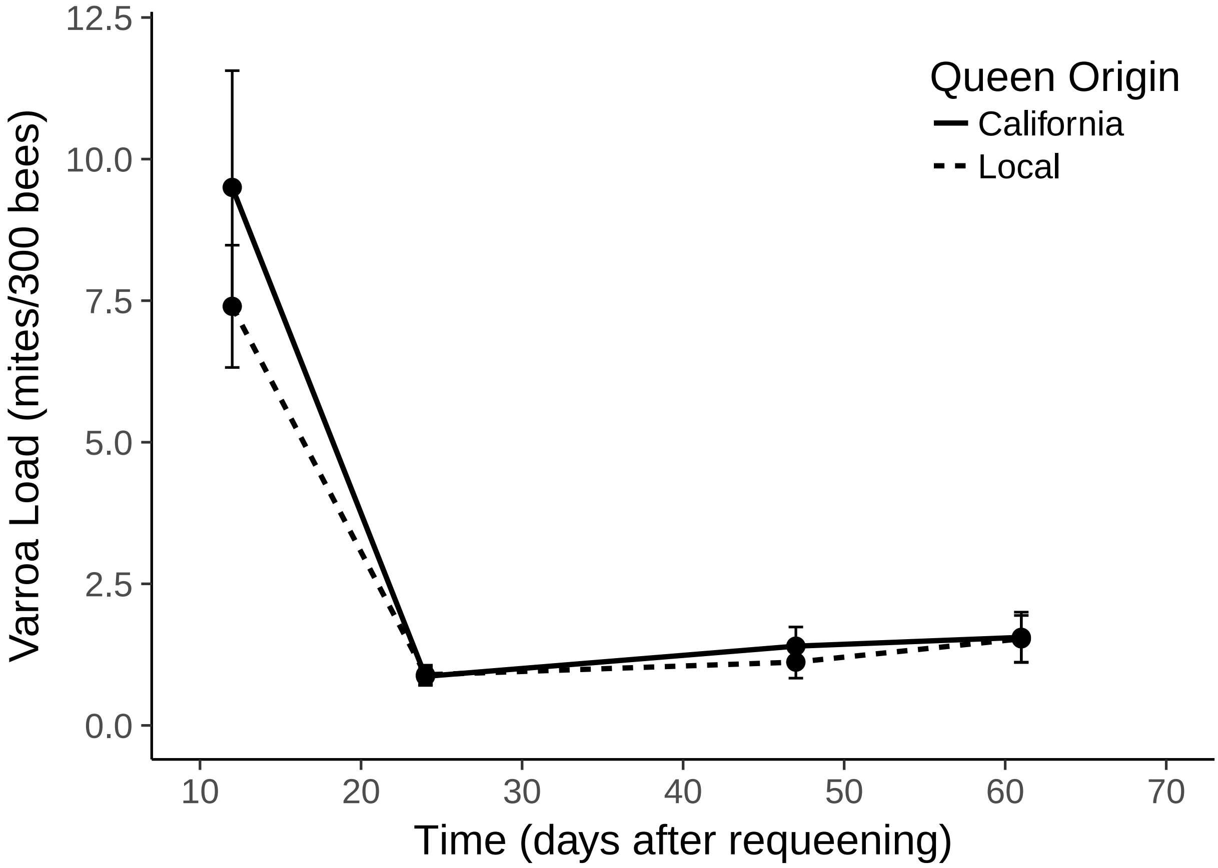
```
# VARROA - use repeated measures anova to look at differences between local
# and california through time:
aov.out <- aov(Varroa ~ Origin * Time + Error(FieldID), data=QueenDF)

# look at summary of model:  NOT SIGNIFICANT
summary(aov.out)
```

```
##
## Error: FieldID
##             Df Sum Sq Mean Sq F value Pr(>F)
## Origin       1   43.7   43.75   3.189 0.0831 .
## Time         1    1.1    1.13   0.082 0.7763
## Origin:Time  1   14.6   14.57   1.062 0.3100
## Residuals   34  466.5   13.72
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Error: Within
##             Df Sum Sq Mean Sq F value   Pr(>F)
## Time         1  855.6   855.6  37.460 2.77e-08 ***
## Origin:Time  1  100.2   100.2   4.387   0.0392 *
## Residuals   85 1941.6    22.8
```

```
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Varroa Mites through Time by Queen Origin

```
##       Origin VarroaDay  n      mean        sd        se
## 1 California        12 20 9.5000000 9.2179492 2.0611961
## 2 California        24 16 0.8666667 0.6399405 0.1599851
## 3 California        47 14 1.4000000 1.2649111 0.3380617
## 4 California        61  9 1.5555556 1.3333333 0.4444444
## 6      Local        12 20 7.4000000 4.8275525 1.0794736
## 7      Local        24 19 0.8947368 0.7374684 0.1691869
## 8      Local        47 17 1.1176471 1.1663165 0.2828733
## 9      Local        61 17 1.5294118 1.6999135 0.4122896
```



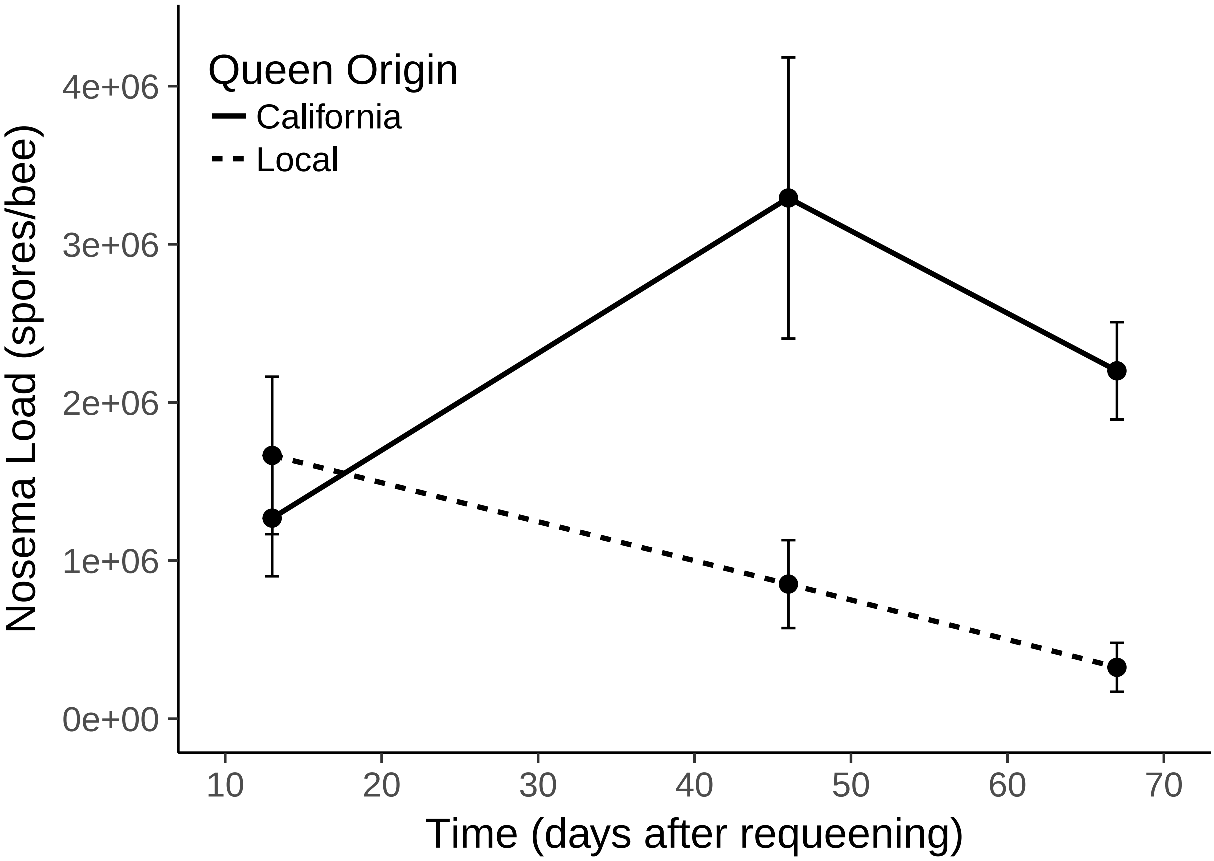
Nosema:

```
# NOSEMA - use repeated measures anova to look at differences between local
# and california through time:
aov.out <- aov(Nosema ~ Origin * Time + Error(FieldID), data=QueenDF)

# look at summary of model: SIGNIFICANT
summary(aov.out)
```

```
##
## Error: FieldID
##             Df    Sum Sq   Mean Sq F value  Pr(>F)
## Origin       1 3.156e+13 3.156e+13   7.972 0.00779 **
## Time         1 2.589e+12 2.589e+12   0.654 0.42413
## Origin:Time  1 9.223e+11 9.223e+11   0.233 0.63234
## Residuals   35 1.386e+14 3.959e+12
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Error: Within
##             Df    Sum Sq   Mean Sq F value   Pr(>F)
## Time         1 5.620e+10 5.620e+10   0.017 0.896098
## Origin:Time  1 4.934e+13 4.934e+13  15.111 0.000275 ***
## Residuals   55 1.796e+14 3.265e+12
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

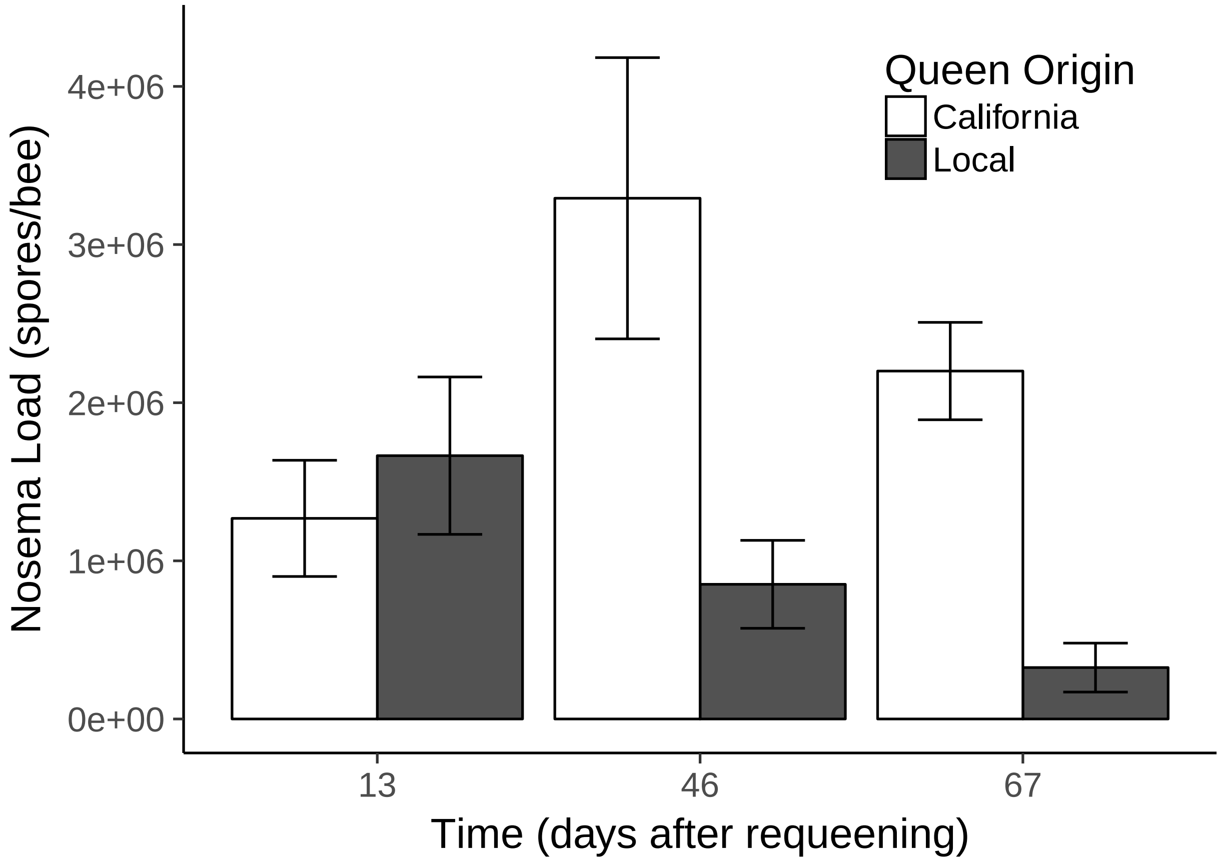
Nosema through Time by Queen Origin

```
##       Origin NosemaDay  n      mean        sd       se
## 1 California        13 20 1268421.1 1643079.8 367403.8
## 2 California        46 14 3292857.1 3327340.5 889269.2
## 3 California        67  9 2200000.0  923901.4 307967.1
## 5      Local        13 20 1665000.0 2225530.7 497643.8
## 6      Local        46 17  851470.6 1146546.1 278078.3
## 7      Local        67 17  325000.0  637806.3 154690.7
```



Nosema through Time by Queen Origin (bar plot)

```
##       Origin NosemaDay  n      mean        sd       se
## 1 California        13 20 1268421.1 1643079.8 367403.8
## 2 California        46 14 3292857.1 3327340.5 889269.2
## 3 California        67  9 2200000.0  923901.4 307967.1
## 5      Local        13 20 1665000.0 2225530.7 497643.8
## 6      Local        46 17  851470.6 1146546.1 278078.3
## 7      Local        67 17  325000.0  637806.3 154690.7
```



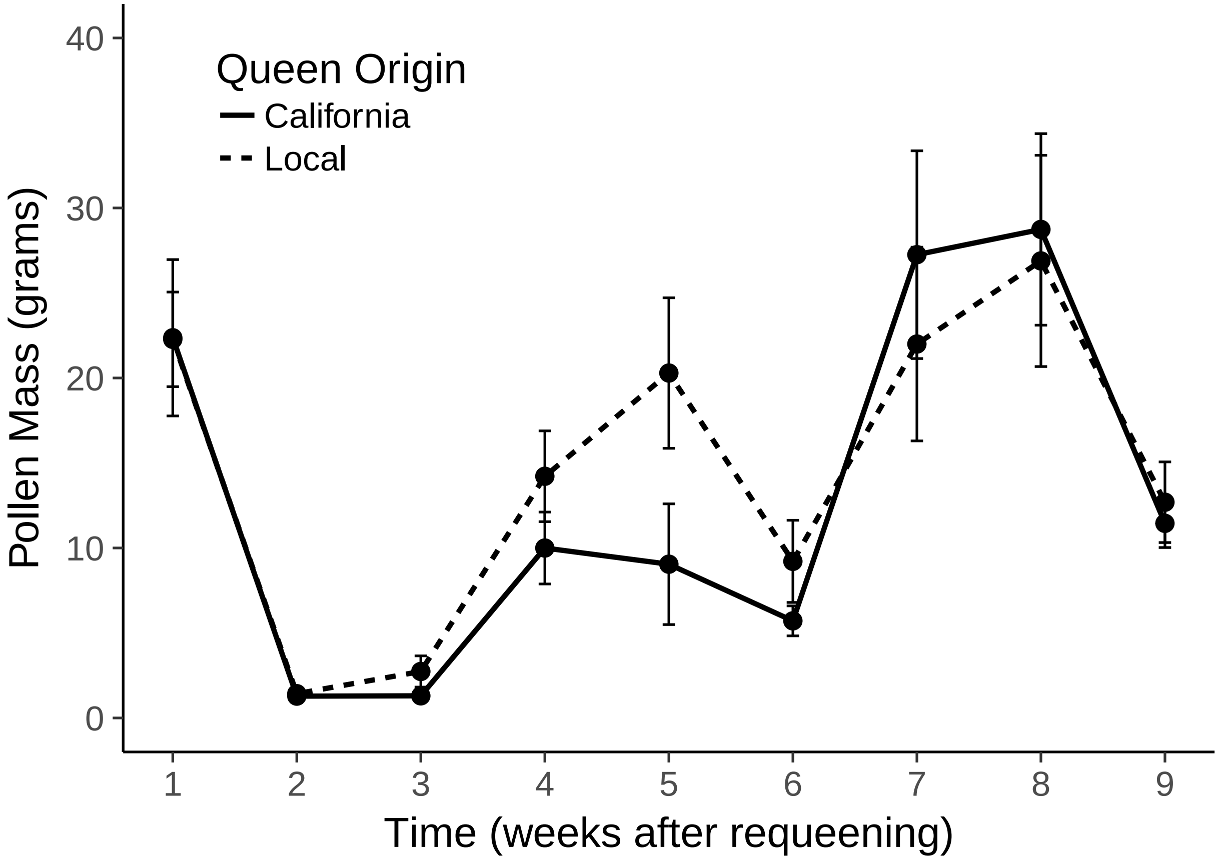
Pollen:

```
# Pollen - use repeated measures anova to look at differences between local
# and california through time:
aov.out <- aov(Pollen ~ Origin * Week + Error(ID), data=PollenDF)

# look at summary of model: NOT SIGNIFICANT
summary(aov.out)
```

```
##
## Error: ID
##              Df Sum Sq Mean Sq F value Pr(>F)
## Origin        1    205   205.2   0.365  0.550
## Week          1    664   663.5   1.180  0.285
## Origin:Week   1    343   343.4   0.611  0.440
## Residuals    35  19677   562.2
##
## Error: Within
##               Df Sum Sq Mean Sq F value Pr(>F)
## Week           1   1206  1206.2   5.084 0.0252 *
## Origin:Week    1    212   211.5   0.892 0.3461
## Residuals    211  50061   237.3
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Weekely Pollen Weight by Queen Origin

```
##        Origin Week  n      mean        sd        se
## 1  California    1 20 22.365105 20.566750 4.5988650
## 2  California    2 20  1.284077  1.685422 0.3768718
## 3  California    3 20  1.302333  1.671533 0.3737662
## 4  California    4 20  9.997000  9.460188 2.1153625
## 5  California    5 10  9.044625 11.231272 3.5516402
## 6  California    6 20  5.712222  3.945849 0.8823186
## 7  California    7 20 27.251714 27.324256 6.1098894
## 8  California    8 20 28.737889 25.191477 5.6329854
## 9  California    9 20 11.448444  6.365922 1.4234635
## 10      Local    1 20 22.271053 12.444065 2.7825776
## 11      Local    2 20  1.427316  1.357923 0.3036409
## 12      Local    3 20  2.735313  4.109949 0.9190126
## 13      Local    4 20 14.217444 11.946764 2.6713777
## 14      Local    5 10 20.289900 13.998098 4.4265872
## 15      Local    6 20  9.212882 10.811116 2.4174390
## 16      Local    7 20 21.995933 25.477828 5.6970155
## 17      Local    8 20 26.884750 27.790647 6.2141776
## 18      Local    9 20 12.691235 10.607100 2.3718197
```



Viruses

```
# split virus DF by virus for stats:
VirusDF$IntensityMult <- VirusDF$Band_Intensity*10^6
VirusDFsplit <- split(VirusDF, VirusDF$Virus)

#-------------------------------------------------------------------------
# DWV - use repeated measures anova to look at differences between local
# and california through time:
aov.out <- aov(IntensityMult ~ Origin * Time + Error(ID), data=VirusDFsplit$DWV)

# look at summary of model: NOT SIGNIFICANT
summary(aov.out)
```

```
##
## Error: ID
##           Df    Sum Sq   Mean Sq F value Pr(>F)
## Origin     1 1.352e+12 1.352e+12   0.328  0.571
## Time       1 1.026e+12 1.026e+12   0.249  0.621
## Residuals 36 1.485e+14 4.125e+12
##
## Error: Within
##             Df    Sum Sq   Mean Sq F value Pr(>F)
## Time         1 7.022e+10 7.022e+10   0.016 0.9014
## Origin:Time  1 2.285e+13 2.285e+13   5.084 0.0316 *
## Residuals   30 1.348e+14 4.495e+12
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
#-------------------------------------------------------------------------
# BQCV - use repeated measures anova to look at differences between local
# and california through time:
aov.out <- aov(IntensityMult ~ Origin * Time + Error(ID), data=VirusDFsplit$BQCV)

# look at summary of model: NOT SIGNIFICANT
summary(aov.out)
```

```
##
## Error: ID
##           Df    Sum Sq   Mean Sq F value   Pr(>F)
## Origin     1 8.614e+11 8.614e+11   2.131    0.153
## Time       1 1.319e+13 1.319e+13  32.631 1.68e-06 ***
## Residuals 36 1.455e+13 4.042e+11
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Error: Within
##             Df    Sum Sq   Mean Sq F value   Pr(>F)
## Time         1 8.893e+13 8.893e+13 148.971 2.25e-13 ***
## Origin:Time  1 1.234e+12 1.234e+12   2.067    0.161
## Residuals   31 1.850e+13 5.969e+11
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
#----------------------------------------------------------------------------
# IAPV - use repeated measures anova to look at differences between local
# and california through time:
aov.out <- aov(IntensityMult ~ Origin * Time + Error(ID), data=VirusDFsplit$IAPV)

# look at summary of model: SIGNIFICANT
summary(aov.out)
```

```
##
## Error: ID
##           Df    Sum Sq   Mean Sq F value  Pr(>F)
## Origin     1 2.333e+13 2.333e+13  12.153 0.00131 **
## Time       1 1.365e+11 1.365e+11   0.071 0.79129
## Residuals 36 6.911e+13 1.920e+12
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Error: Within
##             Df    Sum Sq   Mean Sq F value Pr(>F)
## Time         1 4.472e+12 4.472e+12   2.828  0.103
## Origin:Time  1 8.007e+11 8.007e+11   0.506  0.482
## Residuals   31 4.902e+13 1.581e+12
```

Viral Load by Virus type at two time points for by queen origin