A Multipart Analysis of Protein Deprivation on Mortality Rate in Male and Female Medflies

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Motivation

The Mediterranean fruit fly (*Ceratitis capitata*), hereafter Medfly, is considered as one of the most destructive pests worldwide. The Medfly attacks more than 260 fruits, vegetables, and nuts. It can be especially damaging to citrus, stone fruits, pome fruits, tomatoes, and figs, just to name a few. In [2] the authors showed using *drosophila melanogaster* as a model organism that temperature, sex, and diet all impact their longevity. Furthermore, it has been shown that on average in a controlled environment, Medflies' median survival is around 80 days [4]. Moreover, in [3], it was reported that on a full nutrient diet containing both protein and sugar, female flies had a greater life expectancy than male flies; however, on a nutrient deprived diet containing only sugar, male flies had a greater life expectancy than female flies. This phenomena was referred to as a "Reversal of Life Expectancy Sex Differential".

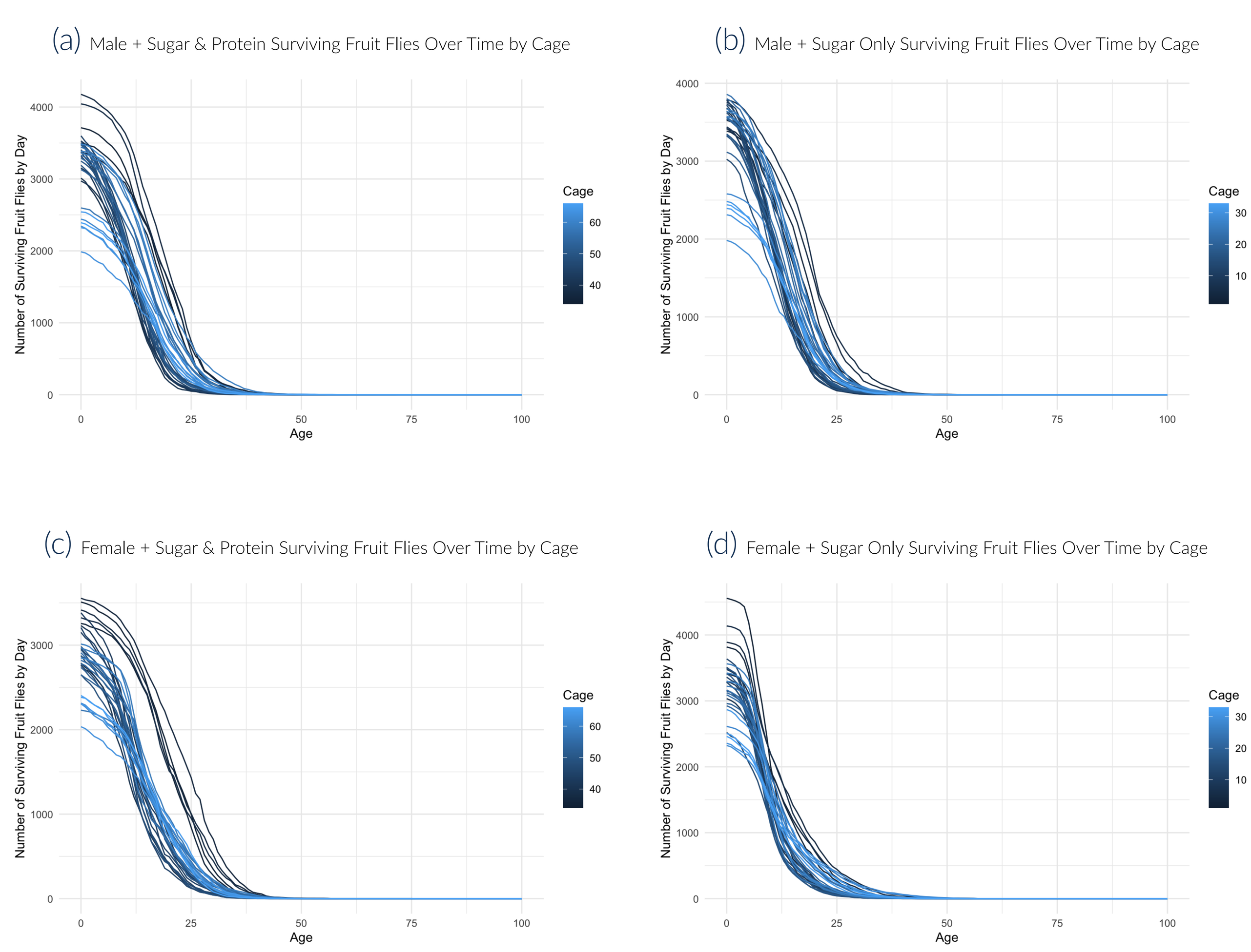
Goal

The main goal of this project was to quantify the effects (main and interactions) of nutrition and sex on mortality. The Medflies are used as prototype in our analysis.

Data Materials and Methods

The dataset we analyzed here was first introduced in [3]. The dataset consists of a total of 416,289 Medflies, where about half are male. The Medflies were maintained in 66 aluminum cages of the same size (15 cm $\times$60 cm $\times$90 cm), each cage containing about 6000 male and female Medflies. Out of the 66 cages, 33 were assigned to Medflies on a full diet (protein + sugar) and 33 to a sugar only diet. Note that diets were assigned alternatively. Each day (over the span of 100 days), the dead flies were removed from the cage and the sex was determined thereof.

Figure 1. Survival curves for Male and Female Medflies



For each cage, we summarized the observed counts and quantified the patterns obtained over the study period of 100 days by computing two statistics.

- We computed the average rate of change R, as

$$R = \frac{\sum(Surviving\ flies_{day} - Surviving\ flies_{day-1})}{Total\ number\ of\ days}$$

- We estimated the mortality rate, β_1, by fitting the following simple linear model to the observed counts. The model was,

$$y_i = \beta_0 + \beta_1 x_i + \epsilon_i$$

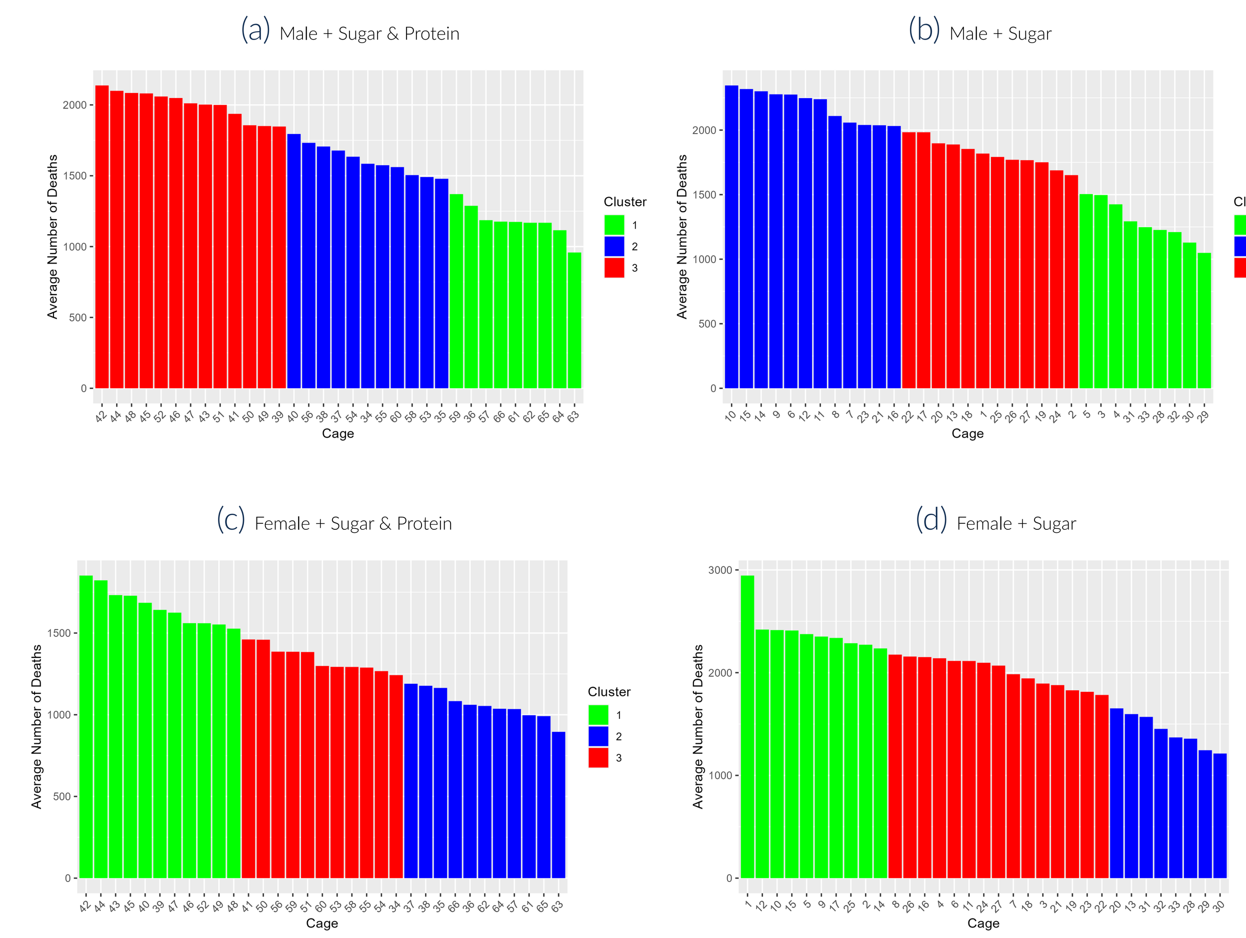
where y_i represents the counts at day x_i, and ϵ_i represents the error component.

It's worth noting that both measures provided similar results. The results presented here use the mortality rate (β_1). We used various statistical analysis to investigate the relationship between diets and sex on Medflies' mortality rate. The analysis we used include, K-means analysis, Linear model, and Mixed effect model.

Results

Through K-means clustering analysis, we aimed to discern if cage influenced the mortality rate, potentially indicating environmental or handling disparities across the cages. The clustering analysis using the K-means approach suggests three main clusters for each of the four cohorts. We observed an approximate association between cage number and its corresponding cluster. For example, for female flies on the full diet, higher number cages tend to be categorized into cluster 3, with the highest mortality rate prior to day 30. This suggests that cage number could be associated with some other external variables that were not taken into consideration in previous studies.

Figure 2. Clustering of cages per Sex and Diet



Results

We found that mortality rate increased for both male and female flies when they're deprived of protein. The difference was more drastic in female flies, whose mortality rate increased 13.6% on the sugar only diet, while male flies' mortality rate only increased by 0.9%. This finding was statistically significant with ($p - value < 0.001$). Moreover, under the sugar-plus-protein diet, the female mortality rate still exceeded the male mortality rate.

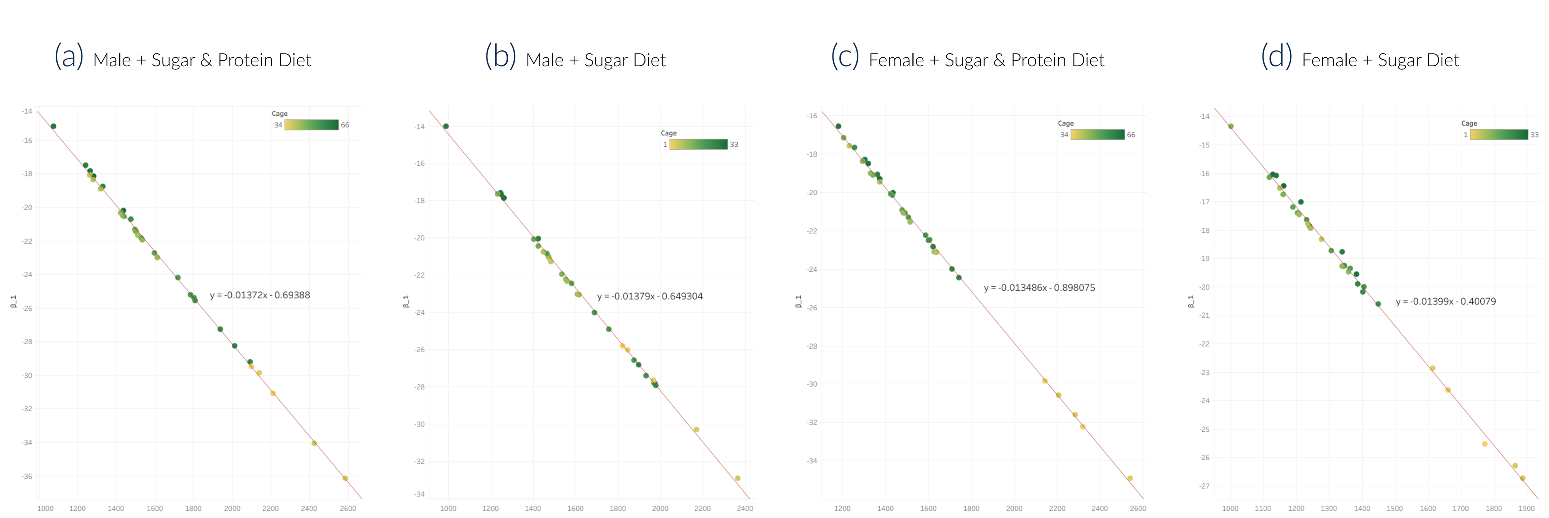
Table 1. Mortality Rates for Male and Female Medflies

Diet	No. of cohorts	Mortality Rate, Males	Mortality Rate, Females
Sugar plus protein	33	-23.23 ± 0.88	-22.13 ± 0.81
Sugar only	33	-23.02 ± 0.74	-19.11 ± 0.52
Difference sugar-plus-protein minus sugar only		0.21 ± .14	3.02 ± .29

Results

The mortality rates of flies significantly increased linearly with the number of flies in the cage for all four groups. The estimate of the increasing rate is roughly about 0.014 for each of the four cohorts.

Figure 3. Mortality Rate by Intercept



Results

We fitted the mixed effect model with interaction to the data. Since the goal is to make inference about the specific diet and sex at hand, we treated sex and diet as fixed effects. We assumed that there was a population cage effect (think of an average profile across all potential cages), but each cage was allowed to have its own random deviation. We defined the observed l^{th} mortality rate of sex i on diet j in cage k by Y_{ijkl} and formulated the model as follows,

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \delta_{k(j)} + \epsilon_{ijkl}$$

where

$\delta_{k(j)}\, i.i.d \sim N(0, \sigma_\delta^2)$ $\epsilon_{ijkl}\, i.i.d \sim N(0, \sigma_\epsilon^2)$. The correlation structure is obtained as,

$$Cor(Y_{ijkl}, Y_{i'j'k'l'}) = \begin{cases} 0 & k \neq k' \\ \sigma_\delta^2/(\sigma_\delta^2 + \sigma_\epsilon^2) & k = k', l \neq l' \\ 1 & i = i', j = j', k = k', l = l' \end{cases}$$

- **Random Effect**
  We saw that the largest contribution to the variance is variability between different cages, which contributed to about $15.06/(15.06 + 3.508)$ 81% of the total variance, where 19% was due to the error. This indicated that observations within the same cage are more more similar than observations from different cages.

Table 2. 95% Confidence interval of the estimates.

Parameter	LB (2.5%)	UB (97.5%)
sd$_($$Intercept)|Cage$	3.171	4.661
sigma	1.569	2.210
(Intercept)	-23.594	-20.663
SexM	-1.999	-0.194
DietSugar	0.950	5.095
SexM:DietSugar	-4.091	-1.536

- **Fixed Effect**
  Under fixed effect, the variables Sex, Diet, and their interaction term are all highly significant ($p - value < 0.001$) suggesting that all variables help explain the Medflies' mortality rate. We found the estimate $\hat{\mu} = -22.1283$ which is the estimated mean mortality rate in a randomly selected cage, randomly selected from the whole population of all cages.

Table 3. Coefficient estimates, their standard errors, and corresponding p-values

Parameter	Estimate	Std. error	t Values	$Pr > \|t\|$
(Intercept)	-22.1283	0.7501	-29.500	< 0.00001
SexM	-1.0969	0.4611	-2.379	0.02036
DietSugar	3.0227	1.0608	2.849	0.00561
SexM:DietSugar	-2.8134	0.6521	-4.315	< 0.00001

Conclusion and Future work

- The mortality rate increased with high density. This suggests that environmental and social conditions significantly influence mortality, underscoring the complex interplay between sensory experiences and biological aging processes. This result collaborates the findings in [1].
- We did not observe a "Reversal of Mortality rate Sex Differential" as it was found in [3] where the authors found a reversal of the life expectancy.
- Since the number of flies in each cage differs, and mortality rate is dependant on the number of flies, we should use a measure that does not depend on the number of flies. One approach would be to normalize the counts before computing the rate of death.

References

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