Diet and sex affect Mediterranean fruit flies' mortality rate.

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Abstract:

Statistical studies on male-female mortality differences in nonhuman species are important since they provide insights into both the nature of age-specific gender differences and the concept of survival in difficult and differing environments. Our study, based on over 400,000 medflies, showed that on both a normal diet (sucrose plus protein) and on protein deprivation, females have a higher life expectancy than males. Our results indicate that the primary source of variability in the mortality rate stems from the random effects associated with cage assignments during the experimental studies. Additionally, our findings revealed a positive correlation between the mortality rate and the number of Mediterranean fruit flies present in the cage. The former two results suggested the use of balanced experimental designs while investigating male-female medflies mortality differentials under diet restriction.

Keywords: Mediterranean fruit flies, Ceratitis capitata, sex differential.

1 Introduction

The Mediterranean fruit fly (Ceratitis capitata), hereafter medfly, is considered one of the most destructive pests worldwide. Originating from Africa, through international trade, medflies have spread to the Middle East, southern Europe, the Caribbean, Central America, and Australia [Ri12]. As a highly invasive species, it is resilient to many environmental conditions and attacks more than 260 fruits, vegetables, and nuts. It can be especially damaging to citrus, stone fruits, pome fruits, tomatoes, and figs. Thus, in the interest of preserving fruit and vegetable production worldwide, it is important to study the impact of environmental and genetic factors on medfly survival. Longevity, life span, and life expectancy are several ways medfly survival is measured in the literature. Current research indicates that these measures of survival heavily depend on genetics and environmental factors such as sex, diet, and temperature [Mü97]. On average in a controlled environment, medflies' median survival age is approximately 40 days [Ca08].

Diet quality and diet restriction have impacts on medfly survival and life expectancy. Researchers found quality of protein and carbohydrates impacted certain phases of development in medflies. Lower quality and quantity of protein specifically had the most impact on medflies during the larval phase, the stage where 90 % of medflies' growth occurs. Thus, reduced quantity and quality protein can cause stunted growth and higher mortality rates [Ka02]. The impact on protein deprivation alone was also found to reduce life expectancy in both male and female adult medflies [Mü97].

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In addition to diet, genetic factors such as sex also play a role. In [Mü97] the authors reported a reversal in sex differential of medflies subject to dietary restriction. The same authors established that on a full nutrient diet containing both protein and sugar, female medflies had a greater life expectancy than males; however, on a nutrient deprived diet (containing only sugar), males had a greater life expectancy than females [Mü97]. The reversal of the male-female life expectancy differential was linked egg-laying and physiological processes undergo by female medflies under protein deprivation. Studies suggest environmental factors such as temperature and cage density impact the longevity of medflies. Despite medflies' resilience, researchers found that lower temperature ranges (between 15-30 degrees Celsius) indicated higher survival rates in medflies [Di20]. Temperatures above 35 degrees Celsius impacted the development of medflies at immature stages [Ri12].

Cage density may also impact medflies' survival. In "Sex Differentials and Selective Survival in Large Medfly Cohorts: Implications for Human Sex Mortality Differentials" researchers found medflies maintained in solitary confinement lived longer than medflies maintained in cages, suggesting cage density impacts medfly survival. In the experiment for the grouped flies, cages contained both male and female flies, and there were approximately 7,200 flies in each cage. Temperature and diet were fixed, and their diet consisted of sugar and water ad libitum. For the cohorts of medflies maintained in cages, they also found males lived longer than females, but while in solitary confinement, male and female life spans were similar (Carey 1995). These findings suggest that females may be more sensitive to environmental changes than male flies. However, the validity of these findings are unclear since medflies were maintained on a diet of sugar and water, and research has shown that compared to males, females' survival is more impacted by protein deprivation [Mü97]. The diet restriction may have given males a survival advantage over females since females may be more impacted by nutritional deficiencies. Thus, contrary to Carey et al.'s findings, the impact of cage density on male and female medflies is unclear.

The impact of cage density on medfly survival becomes increasingly complex depending on how survival is measured (i.e. longevity vs life expectancy), which can favor one sex over another. Further, measuring survival also depends on the specific point in time the medflies' mortality rate is calculated. In "Mortality Dynamics of Density in the Mediterranean Fruit Fly," Carey et al. found sex dependent mortality crossovers dependent on the density of cages at a specific point in time. On day 0, the life expectancy for males was greater than females, but on day 40 females had a higher life expectancy than males [CL95]. These findings conflicted with another study that found increased density lead to greater reduction in the probability of survival in males over females [GFC02]. However, in Gaskin's study, medflies were maintained on a sugar and protein diet, while in Carey et al.'s study, medflies were on a diet of sugar and water.

According to a 2023 study from the University of Michigan [Ge23], researchers discovered that Drosophila melanogaster (a model organism closely related to the medfly) reacts to the presence of dead members of their species (conspecifics) in their environment. This reaction includes aversion behaviors, physiological changes, and a decrease in lifespan. The study

shows that for the flies to exhibit these responses, both their visual and olfactory senses need to be functional. Specifically, merely seeing dead conspecifics was enough to trigger aversive responses and changes in the metabolome (a comprehensive set of metabolites) in the fly's head. The study further identifies that these effects are mediated by specific neural pathways involving the serotonin receptor 5-HT2A, as both genetic and pharmacological reduction of this signaling pathway mitigated the impacts on aversion and lifespan [Ge23].

The analysis of survival of male and female medflies are complex due to the different measurements of survival (longevity vs life expectancy) and the handling of the medflies (diet, temperature, and cage density). Because of the importance of understanding the nature of differences in male and female mortality differentials in nonhuman species and the different sources of variability of the data due the complexity of the experiment, we investigated herein sex mortality differentials in medflies. Our broad objective in this article is to present some important considerations that should be taken into account when analyzing mortality rates in the medfly, which in turn can help designing future experiments. We have organized our findings around the following questions: Do female medflies outlive their male counterparts as predicted in the literature [Au11; Ha48; HA90; HM69] ? What are the different sources of variability in the data ? Is there a reversal of life expectancy sex differential observed using the mortality rate as previously reported in [Mü97]? Lastly, can one quantify the effects (main and interactions) of nutrition and gender on medflies mortality rate ?

2 Data Materials and Statistical Methods

2.1 Data materials

The data is collected from Müller's paper, "Early mortality surge in protein deprived females causes reversal of sex, " published in 1997. A total of 416,289 medflies were maintained in a medfly mass-rearing facility in Metapa, Chiapas, Mexico. Medflies were kept on two different diets: protein-plus-sugar and protein-deprived diet. The protein-plus-sugar diet consisted of three parts sucrose and one part yeast hydrolysate, while the protein-deprived diet consisted of sucrose. Approximately half of the males and females were divided between the two diets. They were kept in 15x60x90cm aluminum cages, where 33 cages contained male and female flies on the protein-plus-sugar diet, and 33 cages contained male and female flies on the protein-deprived diet. Each cage contained a mix of male and female flies, with approximately 6,000 flies in each cage. The flies were kept on a cycle of 12 hours of light exposure and 12 hours of darkness at about 24 degrees Celsius and 65% humidity. Every day, over the span of 100 days, the dead flies were removed from the cages and their sex was recorded.

2.2 Statistical Methods

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

First, we computed the average rate of change R, as

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

Second, we estimated the mortality rate, β_1, by fitting the following simple linear model to the observed counts. The model is,

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

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 diet and sex on medflies' mortality rate. The analysis we used include, K-means analysis, Linear model, and Mixed effect model.

We fit a mixed effect model with interaction to the mortality rates. Since the goal is to make inferences about the specific sex and diet at hand, we treat sex and diet as fixed effects. We assume that there is a population cage effect (think of an average profile across all potential cages), but each cage is allowed to have its own random deviation. We thus define the observed l^{th} rate of death of sex i on diet j in cage k by Y_{ijkl} and formulate our proposed model as follows,

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

where,

- μ is the population mean rate of death of a randomly selected cage, randomly selected from the whole population of all cages
- α_i is the fixed effect of sex i, $i = 1, 2$
- β_j is a fixed effect of diet j, $j = 1, 2$
- $(\alpha\beta)_{ij}$ is the corresponding interaction (fixed effect), $(i, j) \in$
- $\delta_{k(j)}$ is the random effect of cage k. "general rate of death of diet nested in cage"$k(j)$, $k = 1, \ldots, 66$.

- $\delta_{k(j)} \, i.i.d \sim N(0, \sigma_\delta^2)$

- $\epsilon_{ijkl} \, i.i.d \sim N(0, \sigma_\epsilon^2)$

- In addition, all random terms are assumed to be independent.

In this setup, for both sexes there is (over the whole population) an average preference profile with respect to the two different diet types given by $E(Y_{ijkl}) = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij}$. Every cage can have its individual random deviation from this profile. This deviation consists of a general shift $\delta_{k(j)}$ (general rate of death liking of a cage). For the variance of Y_{ijkl} we have $Var(Y_{ijkl}) = \sigma_\delta^2 + \sigma_\epsilon^2$. This implies that observations from different cages ($k \neq k'$) are uncorrelated while observations from the same cages ($k = k'$) are correlated. Thus, the correlation structure is obtained as,

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

The correlation within the same cage is well known as the interclass correlation (ICC) where a large value ($\sigma_\delta^2 >> \sigma_\epsilon^2$) indicated that observations within the same cage are more similar than observations from different cages. Parameter estimation for the variance components σ_δ^2 and σ_ϵ^2 is typically obtained suing the so called restricted maximum likelihood (REML) [JN07]. The parameter μ is estimated with the maximum likelihood assuming that the variances are known.

To further investigate the similarity between the mortality rate among the cages, we make use of the well-known method for partitioning method namely the K-means algorithm. The K-means method is one of the most widely used algorithms for geometric clustering. It was originally proposed by Forgy in 1965 [Fo65] and McQueen in 1967 [Ma67] and is often known as Lloyd's algorithm [Ll]. It is a local search algorithm aiming at partitioning n data points into k clusters.

We conducted a comprehensive cluster analysis using the K-means method to discern whether the speed at which medflies died was influenced by their cage assignments, potentially indicating environmental or handling disparities across the cages. The method aiming to group cages with similar mortality rates using the Elbow Method.

3 Results

3.1 Mortality rate for both male and female Medlies under protein deprivation.

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%, see Table 1. 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. Our results did establish a reversal of the male–female life expectancy differential under dietary manipulation.

Tab. 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 ± 0.14	3.02 ± 0.29

Note: Values are expressed as mean ± standard deviation.

3.2 Clustering Analysis suggests tree main cage clusters for the four differing cohorts.

Before we discuss clustering results in this section, it's worth noting that there is a large discrepancy in the number of flies within cages ranging from 8096 (Cage 1) to 4022 (Cage 63) (see Table 4). Moreover, lower numbered cages within the same diet treatment contain more medflies than higher numbered cages. For example, for males on the sugar only diet, Cage 1 contains 3539 flies and Cage 33 contains only 2393 flies. For males on the sugar plus protein diet, Cage 34 has 3712 flies and Cage 66 has only 2392 flies. The same pattern was observed in the other cohorts. We conducted a two sample T-test to compare the mean number of flies in Cages (1:33) to that of Cages (34:66). The average number of flies in the lower numbered cages was estimated as $\bar{x}_1 = 6556.121$ whereas the average number of flies in higher numbered cages was $\bar{x}_1 = 6058.697$ for a difference of 497.424. The test statistics were obtained as $t_s = 2.2549$ for a p-value of 0.0138. Hence based on this result, we concluded that the average number of flies in higher numbered cages was larger than that of the lower numbered cages at a level of significance $\alpha = 0.05$. We constructed a 95% confidence interval for the difference in means and concluded that we are 95% confident that the mean number of flies in lower numbered cages is higher than the mean number of flies in higher numbered cages by number between 56.6648 and 938.1837.
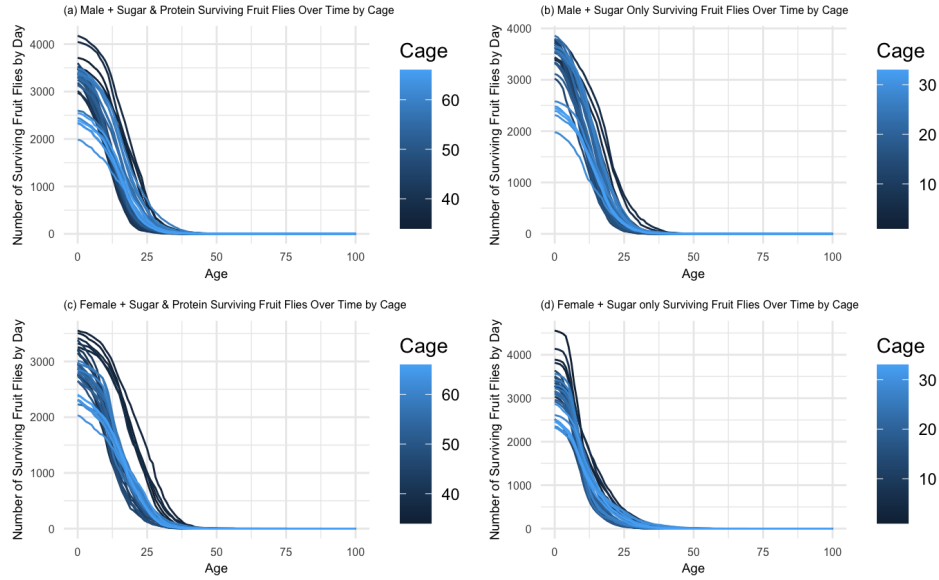
Figure 1: Surviving Fruit Flies Over Time by Cage

For each of the four cohorts, we generated line charts plotting the number of surviving medflies against time, distinguished by cage number (see Figure 1). These visualizations revealed discernible patterns in mortality rates, suggesting that cage number might significantly impact medfly survival. Notably, a preliminary observation indicated that cages with higher numbers within the same diet exhibited a slower rate of fruit fly mortality (darker lines have visually steeper slopes than the lighter lines). To further investigate the relationship between mortality rate and number of flies we plot the mortality rate (β_1) against β_0 where β_0 captures the number of flies at day 1 within a given cage. For all of the four cohorts, we observed a negative linear relationship between β_1 and β_0 where the values are similar (~ 0.9) for the all four groups (see Figure 3b).

Our analysis of the clusters revealed notable differences in the average rates of change in surviving flies. Figure 1 summarizes our findings. It's highlighting the average rate of change in surviving flies for each cage and color-coding the clusters. This visualization of clustering classification revealed a potential relationship between cage number assignment and mortality rate. 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.
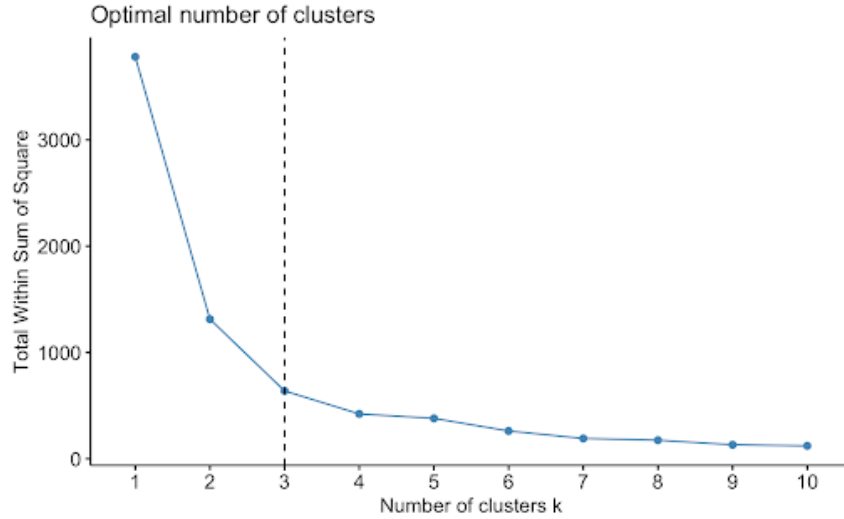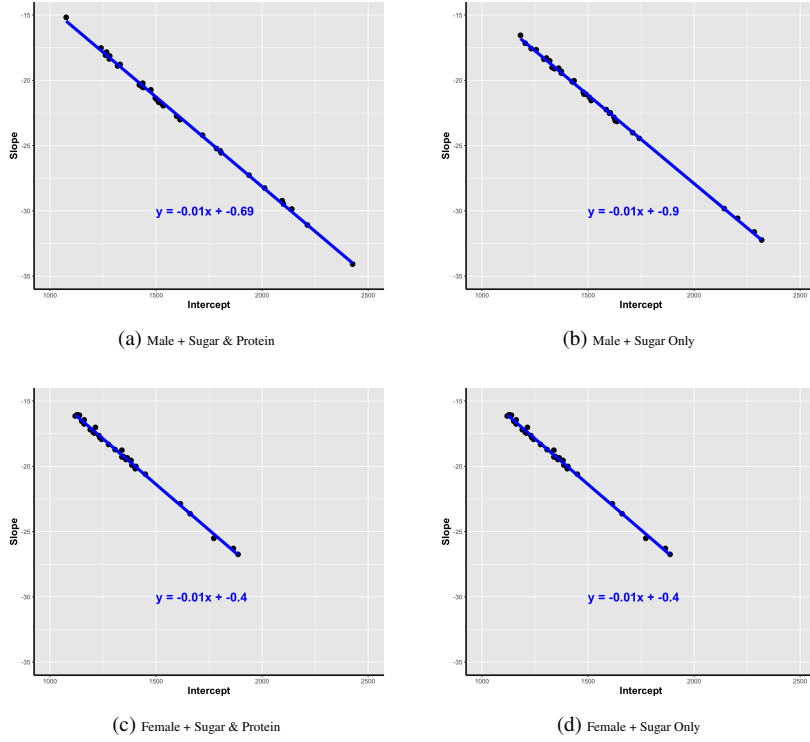
Figure 2: Optimal number of clusters

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. Specifically, cages with lower numbers exhibited accelerated mortality rates, and cages with higher numbers exhibited slower mortality rates. For example, for females on the sugar plus protein diet, Cages 34:38 have mortality rates between -29 to -34, while Cages 45:52 have mortality rates between -17 to -21 (where Cages 34:44 are considered "low" cages, and cages 45-66 are considered "high" cages). A similar pattern is observed for males on the sugar plus protein diet (kept in Cages 34:66) and male and females on the sugar only diet (kept in Cages 1:33).

3.3 Largest contribution to the variance is variability between different cages.

We assumed that the 66 cages are sampled from a larger population of cages hence suggesting the cage effect on the mortality per cage. Thus, we're interested in making a statement about some properties of the whole population and not of the observed cages. We fit the mixed effects model with the *lmer* function in package *lme4*. We estimated the variances components as $\hat{\sigma}_{\delta}^2 = 15.060$ and $\hat{\sigma}_{\epsilon}^2 = 3.508$. These are all point estimates. Thus, the total variability of data is therefore estimated as $15.060 + 3.508 = 18.568$.

Figure 3: Mortality Rate as a function of the y-Intercept.

(a) Male + Sugar & Protein

(b) Male + Sugar Only

(c) Female + Sugar & Protein

(d) Female + Sugar Only

We observe that the largest contribution to the variance is variability between different cages which contributes to about $15.060/18.568 \approx 81\%$ of the total variance. An approximate 95% confidence interval for the standard deviation σ_δ is given $[3.171, 4.661]$. We observe that the estimates of $\hat{\sigma}_\delta$ is therefore quite precise. The corresponding confidence interval for the variance σ_ϵ is $[1.5692.210]$. We therefore conclude that there is statistical evidence that the random interaction term is really needed, as the corresponding confidence interval does not contain zero.

Tab. 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

We are 95% confident the average rate of death of female is larger than that of male medflies by number between 0.1939 and 1.999 ($95\%CI = [0.1939, 1.999]$).

Tab. 3: Coefficient estimates, 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

Under the fixed effects, 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.

4 Conclusion

Studies on the biological basis for sex mortality differentials have historically been based on the assumption that the female longevity advantage is universal. The most important finding was that male and female mortality rate increases with the cage density for all four cohorts. Our study did not establish a reversal of mortality sex differential.

Additionally, in all four cohorts, we observed a linear dependency of the mortality rate and the number of flies. This suggests that environmental and social conditions significantly influence mortality, underscoring the complex interplay between sensory experiences and biological aging processes

Overall, our findings highlight the importance of considering multiple factors—including diet, sex, and environmental conditions—when studying the survival dynamics of medflies. This comprehensive approach not only clarifies the specific conditions under which sex-specific survival advantages emerge but also suggests broader implications for pest management strategies and biological research.

5 Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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Tab. 4: Number of flies by cage.

Cage	Number of flies	Cage	Number of flies
1	8096	34	7222
2	7232	35	6758
3	7799	36	6919
4	7414	37	7593
5	7924	38	5798
6	6923	39	5798
7	6455	40	6203
8	6739	41	5912
9	7238	42	6912
10	7296	43	6465
11	7098	44	6808
12	7319	45	6464
13	5545	46	6146
14	7006	47	6282
15	7120	48	6127
16	6486	49	6138
17	6760	50	6117
18	6481	51	6225
19	6074	52	6462
20	6473	53	5779
21	6925	54	6113
22	6657	55	6110
23	7023	56	6353
24	6795	57	4827
25	7232	58	6326
26	6994	59	6399
27	6933	60	6334
28	5192	61	4756
29	4306	62	4651
30	4668	63	4022
31	5350	64	4628
32	4898	65	4924
33	4901	66	4796