Reproductive Manipulation Effects of wAna Genome Integration

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Introduction

Drosophila is a genus of vinegar flies that includes the well-researched model organism, *Drosophila melanogaster*. A prevalent genus of alpha-proteobacteria, *Wolbachia*, is known to infect many *Drosophila* species. *Wolbachia* are intracellular bacteria that are often maternally transferred throughout a population. *Wolbachia* reside in over 50% of all insect species. Observing the effects of this infectious bacteria within a group closely related to *D. melanogaster* can help biologists build a greater understanding of *Wolbachia* within all of its hosts.

In many of its hosts, *Wolbachia* are known to cause reproductive manipulations. A reproductive manipulation is a mechanism enacted on a host by its parasite or symbiont to move the population infection frequency in favor of the infection. The most common type of reproductive manipulation found in *Drosophila* is cytoplasmic incompatibility. Cytoplasmic incompatibility (CI) occurs when there is a significant increase in offspring mortality when the incompatible cross, infected male with uninfected female, reproduces. The infected females are able to protect from the CI caused by infected males, which leads to successful progeny. This causes a frequency increase of infected mothers within the population since their offspring survive to pass the infection to the next generations. *Drosophila ananassae* is a species that

exhibits CI, and stable maternal transfer of the *Wolbachia* strain, *wAna* in natural populations. *Wolbachia* typically reside in the cytoplasm of their hosts. Yet, research has shown that within *D. ananassae*, some flies have the entire *wAna* genome integrated into their nuclear genome (Dunning Hotopp, 2007). Currently, there are no published results about the effects of integrated *Wolbachia* on their hosts. This paper aims to reveal potential phenotypic effects of *Wolbachia*-integrated genomes by researching possible effects concerning cytoplasmic incompatibility. Can females with the *Wolbachia*-integrated genomes protect from CI as the cytoplasm infected females can? Can males with integrated genomes induce CI like cytoplasm males?

Materials and Methods

The lines of *D. ananassae* in this experiment were sent to Professor Michael Turelli's lab at the University of California, Davis from Cornell University to conduct these CI experiments. The first step of the experiment was molecular analysis to verify the *Wolbachia* infection status of the lines. For this experiment, three infection types were analyzed: cytoplasmic infected (C), genome-integrated infected (I), uninfected (U). Cornell sent cytoplasmic infected lines HNL0501 and RC102, genome-integrated lines TBU3 and T18, and the uninfected line 14024-0371.15. To test for *Wolbachia* infection status, polymerase chain reaction was performed on fly samples to test for the gene *wsp*. Then, gel electrophoresis was utilized to determine the presence of *wsp* within the sample. *Wsp* is a gene in *Wolbachia* that does not naturally appear in the genome of eukaryotes. Therefore, if *wsp* was discovered within a sample, the *Wolbachia* genome resides in the host. A *wsp* band was observed whether the *Wolbachia* was cytoplasmic or integrated. To verify the genome-infected lines, these samples were cleared with tetracycline, which to kill the free-living *Wolbachia*. If the presence of *wsp* persisted, the line was considered *Wolbachia* integrated.

Next, two separate CI assays were performed. In these assays, virgin females were collected for three days to ensure they had not mated outside of the assay. Then, the virgin females were placed in a vial with a male based on infection status. These pairs were placed in different vials for 5 days to allow the female to lay enough eggs to reliably score CI. Then for each cross, we calculated the number of hatched and unhatched eggs for each pair to determine the average fraction of hatched eggs. Unhatched eggs indicated death of the offspring. Therefore, hatched eggs represented the successful progeny in the experiment.

For these CI experiments, crosses were observed for the six types of mating that can occur between males (M) and females (F) of different infection statuses: IMxUF, UMxIF, CMxUF, UMxCF, IMxCF, CMxIF. The CMxUF cross is the known incompatible cross from CI, which results in relatively low hatched eggs. The reciprocal cross, UMxCF, does not induce egg mortality due to the male's uninfected status (UM). For the remaining crosses in the experiment, there is no known outcome of the offspring mortality rate, yet we can make some predictions based on known patterns. Every cross with UM should not exhibit a bias toward any infection

status. On the other hand, cytoplasm infected males (CM) are known to cause CI, therefore low hatch means should occur in these crosses. If integrated females (IF) do protect from CI, we would expect to observe a significant difference between the average number of hatched eggs in the CMxIF and CMxUF crosses. While if integrated males (IM) were to cause CI, we would expect to see significant differences in the frequency of hatched eggs between IMxUF and UMxIF crosses. These pairwise comparison between crosses would allow us to understand the interactions between *Wolbachia*'s genome and its hosts.

Results

In order to prove cytoplasmic incompatibility occurs within a population, the data needs to show a statistically sound reduction in hatch rates between the incompatible cross and the reciprocal cross. R studio was utilized to analyze the data from this experiment. In the first assay, twenty crosses were conducted for each mating type, which resulted to a total of one-hundred twenty crosses. The lines used in this assay were infected line (HNL0501), integrated line (TBU3), and uninfected line (14024-0371.15). Vials with dead mates or contamination were removed from the analysis. CI has been observed in *D. ananassae* populations, therefore the cytoplasmic by uninfected crosses should display the mechanisms that have been observed within these populations. A Mann-Whitney U test was performed to determine statistical significance of the relationship between the crosses by pair-wise comparison. In this test, our null hypothesis suggests that the observed means of the compared samples are statistically similar. The alternative hypothesis claims that the differences in the observed means are statistically significant In other words, the alternative hypothesis confirms our observations when the p-value is less than 0.01, $P < 0.01$.

The mean for CMxUF (Table 1), the known incompatible cross, was 0.342, while, the mean for UMxCF was 0.585, which resulted in a p-value < 0.01. Therefore, there is statistical significance in CMxUF having a lower mean than UMxCF. For the other crosses, we did not know what to expect, but, surprisingly, the IMxCF had a mean of 0.374. This is quite close to the mean of the incompatible cross, although CF should have a big advantage in this population. When tested for statistical significance, the p-value was 0.241. Although it does support the IMxCF having a greater mean, the results do not seem significant. Due to the interesting results and low sample size of the first assay, a second assay was conducted.

In the second assay of the *D. ananassae* experiment, the same uninfected line (14024-0371.15) was used, while we used different lines for the cytoplasm infection sample (RC102) and genome-integrated sample (T18). In this assay, we conducted 25 crosses for each mating, which produced one-hundred fifty crosses total. Once again, vials with contamination or dead females were not considered in the analysis.

For this assay, the results were a bit more conventional. The mean difference between the known CI crosses, CMxUF and UMxCF, were statistically significant, $P < 10^{-5}$. Also, the mean (Table 2) for the incompatible cross was substantially lower than all other crosses as expected. When comparing CMxUF (mean = 0.044) to CMxIF (mean = 0.466) to see if integrated females protect from CI, we find $P< 10^{-5}$. This outcome supports the protection of integrated females.

We also looked at the IMxUF (mean = 0.386) and UMxIF (0.482) to determine if there is a significant difference in these reciprocal crosses to determine if males cause CI in females. A substantial difference in reciprocal Although the mean for IMxUF was lower than the mean for UMxIF, P= 0.1513. Then, we performed a pairwise comparison of IMxUF and MxCF to verify any difference in the hatch rates of integrated male offspring. The mean for IMxUF was 0.386, while the mean for IMxCF was 0.368. The p-value for this comparison was 0.4485. This result did not support a statistically significant difference between the means of these crosses. In future assays, a look at IMxIF might help to understand the possible effects of IM mating. At the moment, the data does not support CI caused by integrated males.

Discussion

We found no evidence that integrated males can cause CI. Yet, CI caused by integrated male would not seem plausible in nature. Selection by the hosts would push for less effects of CI in the population. If the parasite genome resides in the hosts, selection should push for low expression levels of the genes that would cause CI. This may or may not be the answer, but we see that integrated genomes can help us better understand the conflict between host and parasite selection, and the conditions that allow for one to dominate over the other within a population.

The integration of the entire *Wolbachia* genome into then *D. ananassae* genome is an exciting occurrence that we are yet to know the full consequence of. When looking at the effects of integrated infections on the mechanism of CI in this experiment, we see significant evidence that females with integrated Wolbachia are protected from CI caused by cytoplasmically infected males. This could explain why integrated *Wolbachia* genomes are found in most of *D. ananassae*'s geographic range (Choi and Aquadro, 2014). The integrated genome potentially provides the advantage of protection from CI without housing bacteria in most cells. This result would support the claim that the host will evolve to suppress CI (Turelli, 1994). This mechanism would seem to be favored by selection on host genes because this would increase compatibility in infected male and uninfected female matings, which would reduce the spread of the parasite.

Tables

Table 1. Means and Standard Deviations for Assay 1			
Crosses	Mean	SD	Sample size
CMxUF	0.342	0.175	11
UMxCF	0.585	0.263	16
IMxUF	0.325	0.322	14
UMxIF	0.302	0.322	16
IMxCF	0.374	0.315	8
CMxIF	0.281	0.278	14

Table 2. Means and Standard Deviations for Assay 2			
Crosses	Mean	SD	Sample size
CMxUF	0.044	0.0801	19
UMxCF	0.452	0.198	14
IMxUF	0.386	0.297	21
UMxIF	0.482	0.386	20
IMxCF	0.368	0.351	15
CMxIF	0.466	0.246	18

References

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