A POSSIBLE NOVEL STRATEGY FOR REPRODUCTIVE MANIPULATION BY AN ENDOSYMBIONT

A Senior Scholars Thesis

by

AMANDA RAE HALTOM

Submitted to the Office of Undergraduate Research
Texas A&M University
in partial fulfillment of the requirements for the designation as

UNDERGRADUATE RESEARCH SCHOLAR

April 2010

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Associate Dean for Undergraduate Research: Robert C. Webb

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ABSTRACT

A Possible Novel Strategy for Reproductive Manipulation by an Endosymbiont.
(April 2010)

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Reproductive manipulation is commonly seen in insects infected by maternally inherited endosymbionts. These endosymbionts have adopted several strategies to manipulate their hosts in order to guarantee their transmission into the next host generation. Cytoplasmic incompatibility (CI) is the most common method of manipulation and occurs when an infected male mates with an uninfected female. Such matings result in embryonic mortality of offspring, which is observed as a decrease in the hatch rate of eggs, and provides infected females in a population a reproductive advantage over uninfected females. Another form of manipulation is the selective killing of the male offspring of infected females. This type of manipulation, referred to as male killing, occurs in several strains of the bacterial endosymbiont *Spiroplasma,* which infects several *Drosophila* species. However, not all *Spiroplasma* that infect *Drosophila* cause male death, but other forms of manipulation have not yet been examined. Here, we determined whether the non-male killing *Spiroplasma* strain *Hyd 1*(native to *Drosophila hydei*) induces CI in *D. melanogaster*. We ran several crosses of *D. melanogaster*

infected with *Spiroplasma* strain *Hyd1*, and found no evidence of CI since hatch rates did not differ from control crosses. Instead, we observed a significant decrease in egg production by females from crosses in which only the male was infected in comparison to the other crosses. Although this strain of *Spiroplasma* does not exhibit the conventional manipulation strategy of CI, these results could indicate an alternative strategy for achieving the goal of increasing the endosymbiont's frequency in a host population by decreasing the fecundity of uninfected female hosts.

DEDICATION

To my mother, for always being emotionally supportive; to my father, for always being financially supportive; and to my sisters and brother

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NOMENCLATURE

CI	Cytoplasmic Incompatibility
Un	Uninfected

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CHAPTER I

INTRODUCTION

Approximately 65% of all known insect species harbor heritable bacterial endosymbionts (5). These bacteria need a method for persisting in their hosts, such as a high transmission rate, increasing their hosts' defenses against parasites and other dangers, or reproductive manipulation of their hosts (13). The four types of reproductive manipulation are parthenogenesis (16), feminization (13), male killing (14), and cytoplasmic incompatibility (8). Flies in the genus *Drosophila* are known to harbor two endosymbiotic bacteria that are maternally transmitted (heritable): *Spiroplasma* and *Wolbachia* (7, 4). *Spiroplasma* is known to infect 19 species of *Drosophila* (17). Several strains of *Spiroplasma* induce the male killing phenotype, in which male offspring of infected females die during development. As a result, many researchers have focused on the maternal effects of the endosymbiont. However, CI is maintained in the population through males. Examples of male killing strains of *Spiroplasma* are MSRO (*melanogaster* sex ratio organism) native to *D. melanogaster* and NSRO (*nebulosa* sex ratio organism) native to *D. nebulosa.* Non-male killing strains include *hyd 1* and *hyd 2*, native to *D. hydei.* Other possible mechanisms of reproductive manipulations of Spiroplasma have not yet been tested.

Cytoplasmic incompatibility (CI) is a method of reproductive manipulation in

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This thesis follows the style of *Journal of Bacteriology.*

which a cross between an infected male and a female of a different infection status is incompatible (3). This infection is caused by maternally inherited endosymbiotic bacteria in many insect taxa. CI exhibits two types of manipulation: unidirectional (Fig. 1) and bidirectional (11). Unidirectional incompatibility is defined as an incompatible cross in which the male insect has a particular infection strain that the female does not have. Bidirectional incompatibility is defined as an incompatible cross in which both the female and male have an infection but different strains. Such an infection will be maintained in a population because infected females will have a greater reproductive advantage over uninfected females as they can have viable progeny despite the infection status of their mate. The extent of this reduced fecundity exhibited by incompatible crosses varies and lies on a scale from partial to extreme CI (1, 6, 7, 9, 13, 15), The exact molecular mechanism of CI currently remains to be elucidated.

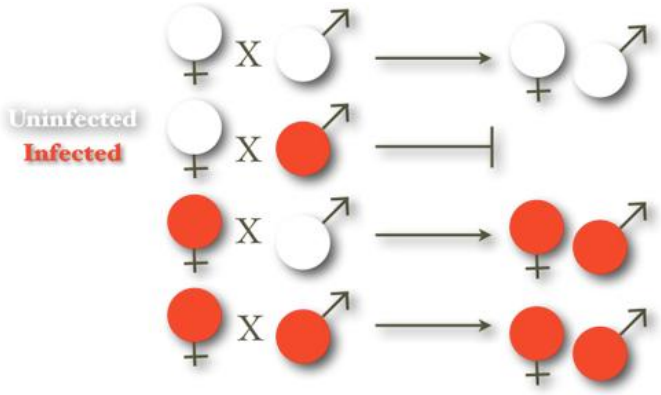

Fig. 1: Unidirectional cytoplasmic incompatibility

http://dobsonserv.ca.uky.edu/DobsonSite/CI1_files/shapeimage_2.png

In the 1950s, CI was first observed in the mosquito *Culex pipiens* when crosses of

different populations demonstrated reduced fecundity (3). The mosquito's endosymbiont *Wolbachia* is the cause of this CI as discovered years later (19). Since then, *Wolbachia* has been studied extensively and demonstrates all four types of reproductive manipulation—parthenogenesis, CI, male killing, and feminizing. One hypothesized mechanism for CI is that *Wolbachia* modifies its host sperm during spermatogenesis (2). The mod/resc model proposed by Werren (18) explains this phenomenon. He hypothesized that *Wobachia* induces a change in its host sperm while also modifying the eggs of the female host, preparing them to "rescue" the embryo should one of the eggs be fertilized with a modified sperm.

Spiroplasma has not yet been tested for any other strategy of reproductive manipulation other than male killing. This endosymbiont was tested for evidence of CI by crossing *D. melanogaster* with and without *hyd 1*.

CHAPTER II

METHODS

To test the hypothesis that *Spiroplasma* is able to induce cytoplasmic incompatibility in *Drosophila*, one strain of *Spiroplasma* was used, *hyd* 1, a non male killing strain native to *D. hydei*. *D. melanogaster* strain Canton-S was artificially infected with this strain through the use of microinjection. The crosses that were performed are as follows:

Unidirectional (male x female):

hyd 1 x *hyd 1*	&	*hyd 1* x uninfected
uninfected x *hyd 1*	&	uninfected x uninfected (control)

Infected individuals of *D. melanogaster* (1 female and 1 male) were aged for 7-10 days and were placed in a vial to mate, with 5 replicates per cross. The flies in each replicate were placed in a new vial every 24 hours for 5 days, and the eggs were counted each day. At 48 hours, unhatched eggs were counted to determine the hatch rate. The number of pupae in each vial was recorded as well as the sex ratio of the adults when they emerged. Each cross was compared to 5 replicates of control crosses (uninfected x uninfected) to determine the results. Significant differences in hatch rate and total laid eggs were determined using the ANOVA statistical test.

CHAPTER III

RESULTS

In the summer of 2009, I performed the first run of crosses with the 7 day old males, excluding the uninfected x *hyd 1* cross. The female in the cross *hyd 1 x hyd 1* laid significantly more eggs than the *hyd 1* x uninfected cross, and the control results were inconclusive. I determined the hatch rate of these crosses, and it was not significantly different (Fig 2).

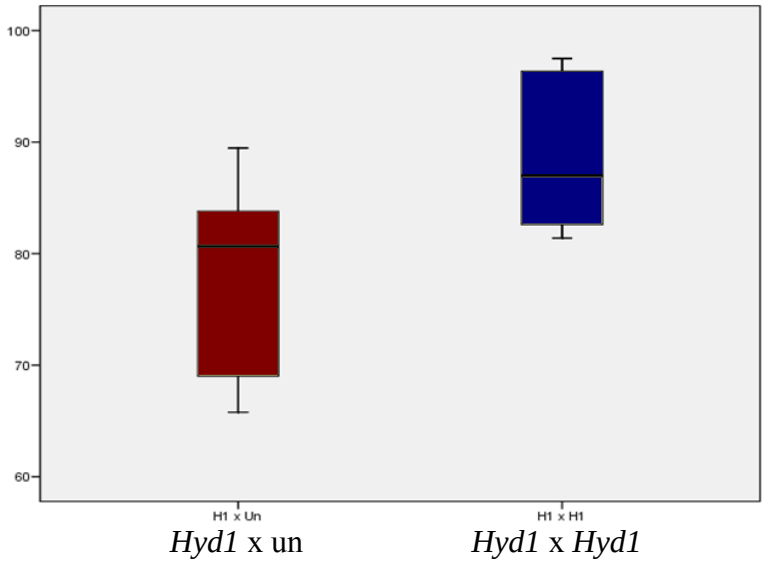

Fig. 2. Hatch rates of different crosses. *Hyd 1* (H1) x uninfected cross (left, n= 5) and *Hyd 1* x *Hyd 1* cross (right., n=5). Hatch rate=number of hatched eggs/total eggs laid.

The second run of crosses, performed in spring 2010, exhibited the same results as

the first run in that the cross between infected male and uninfected female laid significantly fewer eggs (Fig. 3). Hatch rate was not calculated due to the lack of difference in hatch rate in the first run.

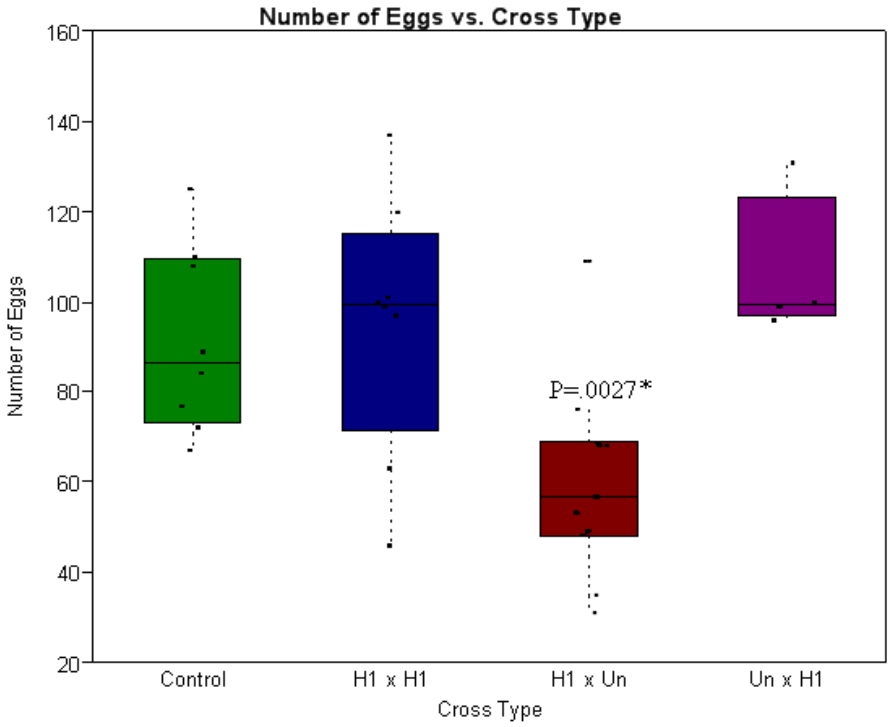

Fig 3. Number of total eggs laid for the 3 crosses and the control. P value for the significantly different cross is given. (Control, n=8; H1 x H1, n=10; H1 x Un, n=11; Un x H1, n=5)

CHAPTER IV

SUMMARY AND CONCLUSIONS

An estimated 65% of all insects are infected with one or more reproductive parasites (5). CI is the most common strategy employed by these bacterial endosymbionts which are maternally inherited to ensure their transmission within a host population. Because CI reduces the fitness of uninfected females and hence confers a reproductive advantage to infected females, the frequency of the endosymbiont increases in a host population (12). Many strains of *Spiroplasma*, though not all, use "male-killing" to alter host reproduction. To date, this is the only form of reproductive manipulation known in this endosymbiont. The non male-killing strain *Hyd 1* is maintained in high frequencies (~68%) in some populations of its native host, albeit not causing the "male-killing" phenotype (10). We explored the possibility of this common strain of *Spiroplasma* causing CI, but we found no evidence for it since the hatch rate between the crosses did not differ. We did, however, find a significant difference in fecundity for crosses between an infected male and an uninfected female in comparison to all other combinations of crosses.

Although infection status had no effect on hatch rate, as it would in the case of CI, matings between infected males and uninfected females seem to be unfavorable. Under this mechanism, uninfected females produce fewer eggs than infected females, which would benefit the endosymbiont by increasing its frequency in the host population in a manner similar to CI. Our results suggest a possible novel strategy of reproductive

manipulation, which involves an interaction that causes a decrease in egg production or suppressed egg laying by the female. However, our results are demonstrated in an artificial host. A possible mechanism of this interaction is that the male's ejaculate contains proteins that stimulate the female's immune system, therefore directing energy toward antimicrobial peptides instead of egg laying, resulting in fewer eggs. If this mechanism is the case, then the uninfected female's egg laying would slowly increase if she mated with an uninfected male after mating with an infected male. Another possible mechanism is that the peptides in the male's ejaculate are modified by *Spiroplasma* and suppress egg laying in uninfected females. This modification is rescued by the *Spiroplasma* in the egg of the female. Both of these mechanisms would benefit the endosymbiont in that they would decrease the proportion of uninfected progeny in the population. To gain a better understanding of this reproductive manipulation method, our future goals are to perform the same crosses in *hyd 1*'s natural host, *D. hydei*. We also hope to cross an infected male and an uninfected female, then crossing the female with an uninfected male three days later to determine if she immediately regains her full egg laying capacity.

REFERENCES

1. **Bourtzis, K., A. Nirgianaki, P. Onyango, and C. Savakis.** 1994. A prokaryotic dnaA sequence in *Drosophila melanogaster*: *Wolbachia* infection and cytoplasmic incompatibility among laboratory strains. Insect Mol Biol. **3:**131–142.
2. **Breeuwer, J.A., and H. Werren.** 1993. Cytoplasmic incompatibility and bacterial density in *Nasonia vitripennis*. Genetics **135:**565-574.
3. **Ghelelovitch, S.** 1952. Sur le déterminisme génétique de la sterilité dans le croisement entre differentes souches de *Culex autogenicus* Roubaud. CR Acad Sci Paris **24:**2386–2388.
4. **Haselkorn, T.S., T.A. Markow, and N.A. Moran.** 2009. Multiple introductions of the *Spiroplasma* bacterial endosymbiont into *Drosophila.* Molecular Ecology **18:**1294–1305.
5. **Hilgenboecker, K., P. Hammerstein, P. Schlattmann, A. Telschow, and J.H. Werren**. 2008. How many species are infected with *Wolbachia*? – a statistical analysis of current data. FEMS Microbiol Lett. **281(2):**195–220.
6. **Hoffman, A. A.** 1988. Partial cytoplasmic incompatibility between two Australian populations of *Drosophila melanogaster*. Entomol. Exp. Appl. **48:**61-67.

7. **Hoffmann, A.A., D.J. Clancy, and E. Merton.** 1994. Cytoplasmic incompatibility in Australian populations of *Drosophila melanogaster*. Genetics **136:**993-999.
8. **Hoffman, A.A., and M. Turelli.** 1997. Cytoplasmic incompatibility in insects. pp 42-80 in S.L. O'Neill, A.A. Hoffman, J.H. Werren editors, Influential Passengers. Oxford University Press, USA.
9. **Holden, P.R., P. Jones, and J.F. Brookfield.** 1993. Evidence for a *Wolbachia* symbiont in *Drosophila melanogaster*. Genet. Res. **62:**23-29.
10. **Kageyama, D., H. Anbutsu, M. Watada, T. Hosokawa, M. Shimada, and T. Fukatsu.** 2006. Prevalence of a non-male-killing *Spiroplasma* in natural populations of *Drosophila hydei. Appl Environ* Microbiol **72:**6667–6673.
11. **Laven, H.** 1951. Crossing experiments with *Culex* strains. Evolution **5:**370-375.
12. **Mateos, M., S.J. Castrezana, B.J. Nankivell, A.M. Estes, and T.A. Markow.** 2006. Heritable endosymbionts of *Drosophila.* Genetics **174:**363–376.
13. **O'Neill, S.L., and T.L. Karr.** 1990. Bidirectional incompatibility between conspecific populations of *Drosophila simulans*. Nature **348:**178-180.
14. **Rigaud, T.** 1997. Inherited microorganisms and sex determination of arthropod hosts. pp81-101. In O'Neill SL, Hoffmann AA, Werren JH, editors; Influential Passengers: inherited microorganisms and arthropod reproduction. Oxford University Press, Oxford, UK.

15. **Solignac, M., D. Vautrin, and F. Rousset.** 1994. Widespread occurrence of the proteobacteria *Wolbachia* and partial cytoplasmic incompatibility in *Drosophila melanogaster*. C. R. Acad. Sci. Paris **317:**461-470.

16. **Stouthamer, R., R.F. Luck, and W.D. Hamilton.** 1990. Antibiotics cause parthenogenic *Trichogramma* to revert sex. Proc. Nat. Acad. Sci. **87:**2424-2427.

17. **Watts, T., T.S. Haselkorn, N.A. Moran, and T. A. Markow.** 2009. Variable Incidence of *Spiroplasma* Infections in Natural Populations of *Drosophila* Species. *PLoS ONE* 4(5): e5703. doi:10.1371/journal.pone.0005703

18. **Werren, J.H.** 1997. Biology of Wolbachia. Ann. Rev. Entomol. **42:**587-609.

19. **Yen, J.H., and A.R. Barr.** 1971. New hypothesis for the cause of cytoplasmic incompatibility in *Culex pipiens* L. Nature **232:**657–658.

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