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Regulation of the post-mating immune response in female *Drosophila melanogaster*

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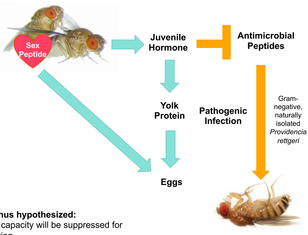
BACKGROUND

When challenged with a systemic infection, mated female *Drosophila melanogaster* have higher pathogen burden, greater risk of death, and lower capacity to induce antimicrobial gene expression. During mating, the male transfers seminal fluid proteins that change female physiology and behavior. One such seminal fluid protein, Sex Peptide, induces the female to produce Juvenile Hormone (JH)[1]. JH promotes vitellogenesis: the uptake of yolk proteins 1, 2, and 3 into oocytes[2]. We and others have previously shown that JH is immunosuppressive and decreases resistance to bacterial infection[3]. We thus hypothesize that JH signaling might control resource allocation between reproduction and immunity. Previous studies showed that females were less resistant to bacterial infection at 2.5 and 26.5 hours after mating but did not test whether a mated female would eventually recover virgin levels of immunity[4,5]. We tested the permanence of the post-mating effect on immune defense by infecting females 2, 4, 7, and 10 days after mating.



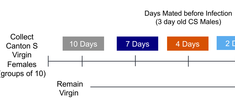
We thus hypothesized:

1) Female immune capacity will be suppressed for 10 days after mating

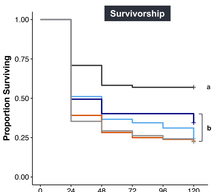
2) Females who mated twice will become more susceptible to infection than females mated once

CAN FEMALES RECOVER THEIR IMMUNE RESPONSE?

Virgin Canton S flies were collected and aged for 3 days. All female flies were split into groups of 10 and randomly assigned to one of 5 treatment groups. On the day of mating, 15 3-day old Canton S males were added to the vial. After 8 hours, males were removed. All groups were infected on the same day with the same *Providencia rettgeri* culture.



Survivorship



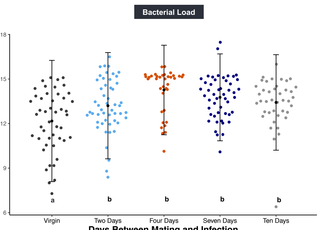
Sample Size

Days Between Mating and Infection	Number of Flies
Virgin	79
Two Days	90
Four Days	92
Seven Days	87
Ten Days	99

Cox proportional hazards model. (Time of Death, Alive/Dead) ~ Days between Mating and Infection + Round. Pairwise log-rank tests, Bonferroni corrections, p<0.05 cutoff. Letters represent degree of difference of significance.

All mated flies were less likely to survive than virgin flies, but there was no effect of the time between mating and infection.

Bacterial Load



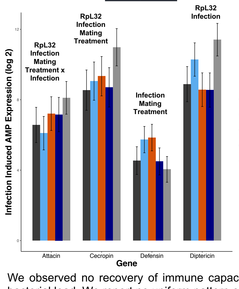
Sample Size

Days Between Mating and Infection	Number of Flies
Virgin	47
Two Days	51
Four Days	37
Seven Days	49
Ten Days	41

Tukey HSD Corrections on GLM: ln(CFU) ~ Days between Mating and Infection, p<0.05 cutoff. Letters represent degree of difference of significance.

All mated flies had higher bacterial loads than virgin flies, but there was no effect of the time between mating and infection.

AMP Expression



For each gene, the following model was applied.

Y= μ + RpL32 + Mating.Status + Infection.Status + Mating.Status x Infection.Status.

The least squared means of the interaction term was extracted from the model for each treatment group. A measure of induction was determined by subtracting the infected samples from the uninfected controls. Infection induced AMP gene expression is reported as fold change. Significant model terms are reported above each gene, p<0.05 cut off.

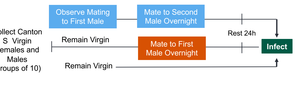
Infection significantly increased expression of all AMP genes measured. However, mating treatment was not uniformly significant.

Conclusions

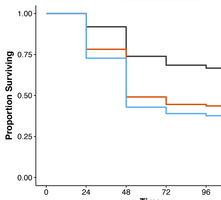
We observed no recovery of immune capacity in mated females over time with either survivorship or bacterial load. We report no uniform pattern of antimicrobial peptide expression across treatment groups. We conclude that mating suppresses the female immune system for at least 10 days after mating.

DO MULTIPLE MATINGS AFFECT IMMUNE PERFORMANCE?

Virgin Canton S flies were collected and aged for 3 days. All female flies were placed into individual vials one day prior to the start of the experiment and randomly assigned to a treatment group. For the two-mating group, the first male had a dominant GFP marker and the second male was a Canton S male. All groups were infected on the same day with the same *Providencia rettgeri* culture. After the experiment, the progeny of each two mating female was scored for GFP status.



Survivorship



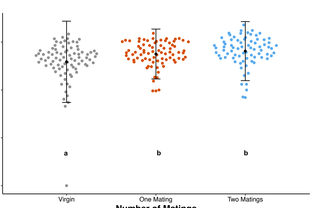
Sample Size

Number of Matings	Number of Flies
Virgin	79
One	76
Two	55

Cox proportional hazards model. (Time of Death, Alive/Dead)~ Number of Matings + Round. Pairwise log-rank tests, Bonferroni corrections, p<0.05 cutoff. Letters represent degree of difference of significance.

Females mated once or twice were less likely to survive infection than virgin females. There was no difference in survivorship when females were mated twice.

Bacterial Load



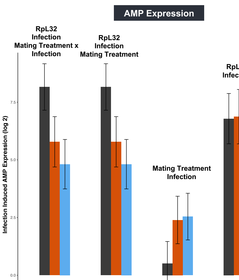
Sample Size

Number of Matings	Number of Flies
Virgin	46
One	48
Two	48

Tukey HSD Corrections on GLM: ln(CFU) ~ Number of Matings, p<0.05 cutoff. Letters represent degree of difference of significance.

Females mated once or twice had higher bacterial loads than virgin females. There was no difference in bacterial load when females were mated twice.

AMP Expression



For each gene, the following model was applied.

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The least squared means of the interaction term was extracted from the model for each treatment group. A measure of induction was determined by subtracting the infected samples from the uninfected controls. Infection induced AMP gene expression is reported as fold change. Significant model terms are reported above each gene, p<0.05 cut off.

Infection significantly increased expression of all AMP genes measured. However, mating treatment was not uniformly significant. Virgins produced more *attacin* and *cecropin* mRNA than either mating group, but produced less *defensin* than mated females and mating treatment was not significant for or *diptericin*.

Conclusion

We found that one or two-mating females were less likely to survive and had higher bacterial loads than virgins. Virgin females produced more *attacin* and *cecropin* mRNA than either the one or two-mating group, but less *defensin*. We conclude that the effects of a single mating are sufficient to suppress the immune response and a second mating does not compound the effect.

FUTURE DIRECTIONS

Does investing resources into eggs through yolk proteins constrain the immune response?

Knockout yolk proteins with CRISPR, measure infection response

Is post-mating immune suppression permanent?

Extend time between mating and infection past known long term effect of Sex Peptide

Do JH titers in mated and virgin females correlate with our understanding of the dynamics of the post-mating immune response?

High performance liquid chromatography on mated vs. virgin, infected vs. uninfected

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2. Soller, M et al. (1999) Dev Bio 208, 337-51.
3. Schwenke, R et al. (2017) Curr Bio 27, 596-601.
4. Short, S et al. (2010) Proc R Soc B Online, 1-9.
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