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Facultatively ectoparasitic mites as vectors for entomopathogenic bacteria in Drosophila

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ABSTRACT

Opportunistic bacterial infections are common in insect populations but there is little information on how they are acquired or transmitted. We tested the hypothesis that *Macrocheles* mites can transmit systemic bacterial infections between *Drosophila* hosts. We found that 24% of mites acquired detectable levels of bacteria after feeding on infected flies and 87% of infected mites passed bacteria to naïve recipient flies. The probability that a mite could pass *Serratia* from an infected donor fly to a naïve recipient fly was 27.1%. These data demonstrate that *Macrocheles* mites are capable of serving as vectors of bacterial infection between insects.

1. Introduction

Opportunistic bacterial infections in wild-caught insects are widespread (Lysenkol, 1985) although we have little insight into how those infections are acquired. Injuries that compromise the cuticle, including those caused by ectoparasitic mites, can be exploited by environmental bacteria. Such injuries may be common in natural insect populations (e.g., Cherrill and Brown, 1997; Gilad et al., 2022; Subasi et al., 2023). Ectoparasitic mites have been documented to vector insect viruses (Dainat et al., 2009) though most of this research has focused on the *Varroa*-honey bee system (Traynor et al., 2020). Bacterial cells have also been found to colonize *Varroa* mite wound sites on honey bees and their larvae (Kanbar and Engels, 2003; Ramsey et al., 2019). Wild *Drosophila hydei* have been captured harboring *Macrocheles* sp. mites infected by endosymbiotic *Spiroplasma* bacteria (Osaka et al., 2013) and Jaenike et al. (2007) provided evidence for *M. subbadius* mites as vectors of *S. poulsonii* between *D. nebulosa* hosts. The mite *Coccipolipus hippodamiae* has been shown to transfer vertically-transmitted endosymbiotic bacteria between ladybeetle hosts (Shaikevich et al., 2023). Here, we directly test whether a phoretic mite, *M. muscaedomesticae,* can transmit pathogenic bacterial infection from one *D. melanogaster* to another. *Drosophila* flies regularly encounter macrochelid mites in nature, as they both utilize habitats with decomposing food resources (Wade and Rodriguez, 1961)

2. Methods

2.1. Fly, mite and bacterial culture maintenance

The *M. muscaedomesticae* mite culture was established from roughly 10 female mites attached to *Drosophila* spp. flies collected from compost in Gainesville, FL USA in the spring of 2019. Mites were maintained in ventilated 4 L plastic containers with wheat bran and wood chips moistened with distilled water inoculated with wild-caught Rhabditid nematodes from M. Polak at the University of Cincinnati (OH, USA) as a food source. Fresh ingredients and a sprinkle of nutritional yeast were added weekly. Mites were maintained at ambient lab conditions (23 °C, 33 % RH). Host flies (*D. melanogaster*, strain Canton S) were maintained on molasses-agar food at ambient lab conditions (details in supplemental materials). We used *Serratia* sp. (hereafter "*Serratia*") as a model bacterial pathogen as it is a common disease-causing agent in insects (Poinar et al., 1979). Our *Serratia* culture was isolated from a wild-derived *M. muscaedomesticae* colony, from which individual mites were noticed to be dead with a characteristic red bacterial infection.

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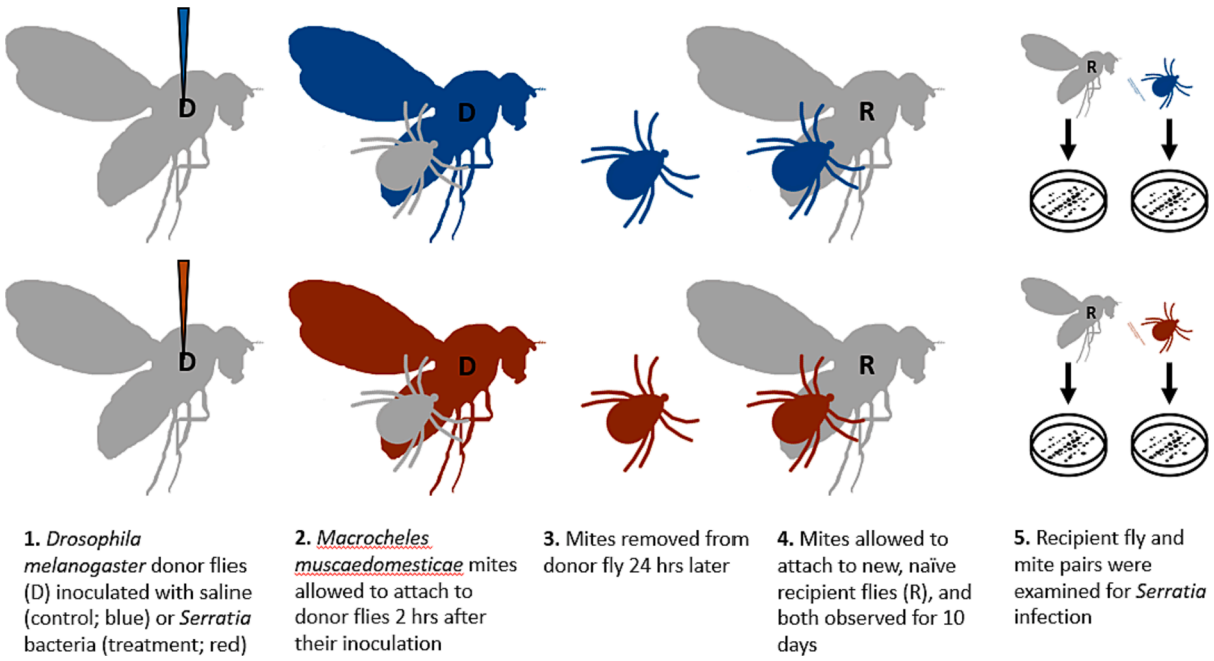

Fig. 1. Schematic representation experimental design. Fly silhouettes by Ramiro Morales-Hojas from PhyloPic. Mite silhouettes by Melissa Schmitt from the Noun Project. Petri dishes by Anthony Bossard from the Noun Project.

Bacterial colonies were recovered from a dead mite by crushing it on a Lysogeny broth (LB) agar plate and incubating for 24 h at ambient lab conditions. Red bacterial colonies were isolated, DNA extracted and amplified, and their 16 s rDNA was sequenced via Genewiz (supplemental materials). Comparing our sequences to the NCBI database via BLAST determined to be 99.1 % – 100 % identical to *Serratia* sp. (Supplemental Table S3). A source stock was frozen at −80 °C in glycerol and a streak plate maintained at 4 °C was used as the source of bacteria for infection experiments.

2.2. Experimental infection

We followed the procedure outlined in Khalil et al. (2015) to infect *D. melanogaster*. Two mL of LB was inoculated with a single *Serratia* colony and grown overnight (~16–18 h) at 37 °C with shaking, then 600 µL was transferred to a 1.5 mL microcentrifuge tube to pellet for 3 min at 5000 RPM in a microcentrifuge. The supernatant was discarded and the pelleted bacteria were resuspended in sterile phosphate-buffered saline (PBS). We measured optical density at 600 nm and diluted the solution to $OD_{600} = 0.6$ to infect flies with the septic pinprick method (see Khalil et al., 2015 for details). Essentially, a minutien pin was dipped into solution (either sterile PBS or *Serratia*-PBS) and then used to puncture the thorax of anesthetized flies. Flies were then placed into a vial containing molasses-agar food to recover from CO_2 anesthesia.

2.3. Transfer of bacteria via mites

The experiment had four steps (Fig. 1; see Supplemental Material for additional details): 1) Donor flies were experimentally inoculated with either PBS or *Serratia* and allowed 2 h to recover. 2) A single mite was placed in an exposure chamber with a single fly for 1 hr. Exposure chambers were made with a 200 µL micropipette tip cut in half and stopped with cotton. mites that attached remained with the donor fly for 24 h. Mites that did not attach were removed from the experiment. 3) Mites were removed from donor flies and moved into an exposure chamber with a single naïve recipient fly for one hour, after which attachment was recorded and then the mite-fly pair were transferred to a fly food vial and monitored for another 10 days. 4) After 10 days, mites and recipient flies were examined for *Serratia* infection. This experiment occurred over two separate blocks during Spring and Fall of 2020. We determined infection status of mites and recipient flies using three methods: 1), flies and attaching mites (n = 18) were crushed with sterile forceps and placed on LB agar and monitored for bacterial growth; 2) mites from step 3 (Fig. 1; n = 22) were surface sterilized before plating to determine whether mites were systemically infected with *Serratia* or superficially contaminated with it; 3) fly hemolymph (n = 22; 10 control and 12 infected) was extracted and plated to determine infection status and confirm that the infection was systemic. Six red colonies grown from infected recipient flies were sent for 16 s sequencing and 5/6 were confirmed to match our isolated *Serratia*. One colony could not be sequenced because of poor sample quality (Supplemental materials). Thus, we felt confident that flies and mites that grew red bacterial colonies were infected with the experimental *Serratia*. Those that failed to grow red colonies were considered uninfected.

2.4. Statistical analysis

Given our small sample sizes, we chose a model selection technique to calculate likelihoods of infection at each stage, while incorporating potential predictors. This was preferred over frequency-based probabilities as it made potential explanations comparable. To determine the probability of transmitting *Serratia* at each stage (donor fly to mite and mite to recipient fly) as well as the overall probability of infection passing between flies via the mites (donor fly to recipient fly), we built binomial generalized linear models with infection status as a response variable, treatment as an independent variable, and replicate block as a fixed effect because there are only 2 levels of block. To determine model fit, we assessed all three models against their relevant block-only model. Model fit was assessed using an Akaike Information Criterion (AICc) approach, corrected for small sample sizes in the R package "AICcmodavg" (Mazerolle, 2020). The model with the lowest AICc score that differed from the next best model by greater than 2 was interpreted as the best model.

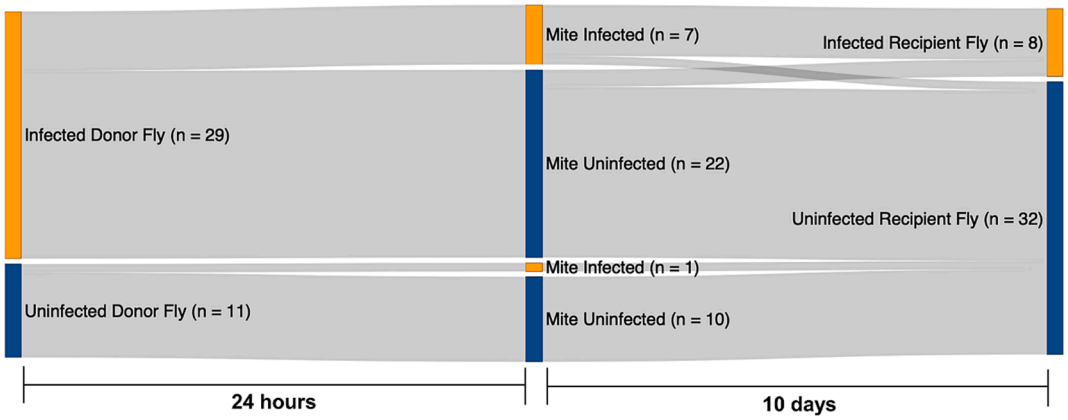

Fig. 2. Schematic representation of transmission events. Sankey diagram showing the proportions of infected (orange) and uninfected (blue) individuals and how they are related through time. That is, connections between the three time points represent the relationships between dono flies, mites, and recipient flies. Sample sizes represent the number of *M. muscaedomesticae* mites that did or did not show detectable *Serratia* infection after attachment to either an infected or uninfected donor fly. On the right are the number of naïve recipient flies that showed *Serratia* infection after the attachment of either an infected or uninfected mite. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3. Results and discussion

3.1. Infection of naïve recipient flies via mites

We collected data from 40 complete trials which included a donor fly, recipient fly, and a mite that attached to both the donor and recipient. Of the 29 mites fed on infected flies, seven acquired detectable levels of *Serratia* and six of those subsequently passed the infection to a naïve recipient fly. Therefore, mites were 24 % likely to acquire *Serratia* infection from infected donor flies and infected mites transferred *Serratia* to uninfected flies with a likelihood of 87 % (Fig. 2). Of the 22 mites that did not pick up detectable levels of *Serratia* from their infected donor fly, one subsequently passed an infection to a naïve recipient fly (Fig. 2). One mite that attached to a control donor fly tested positive for *Serratia* although its recipient fly did not become infected. Although we are not certain, it is possible that this mite was naturally infected with *Serratia* before our experiment, as the *Serratia* strain we used was originally isolated from dead mites in culture. Further, there were two mites that fed on an infected donor fly and transmitted the infection to their recipient fly, though *Serratia* infection was not detected in the mite. This may have been a consequence of bacteria not growing to a detectable threshold in the mite, generating a false negative (Abad-Franch et al., 2014). Cumulatively, the likelihood that a recipient fly ultimately became infected from an infected donor via passage through a mite was 27.1 %, while no recipient fly infections came from uninfected donor flies (Supplemental Table 1.3). Mites with detectable infection had a 74 % chance of passing on infection while mites without a detectable infection had a 6.4 % probability (see supplemental material for complete model selection results).

Historically, there has been no consensus on whether and which *Macrocheles* spp. mites fed on their hosts (Wade and Rodriguez, 1961) or simply attached to them for transportation (Farish and Axtell, 1971). More recent data suggests that species of *Macrocheles* do feed on their hosts but the evidence is indirect (Jaenike et al., 2007; Durkin et al., 2019), and Subasi et al. (2023) demonstrated that *Macrocheles* spp. mites leave melanized wounds on their attachment site. Although additional assays are required to definitively test whether *Macrocheles* spp. mites obtain nutrition from feeding on their host, evidence is now unequivocal that they can cause damage to their hosts and thereby transmit pathogens via contaminated body parts such as cuticle or chelicerae.

Infected recipient flies survived much longer (7.87 ± 3.4 SD days) than experimentally infected donor flies, which survived roughly 24 h after infection (data not shown). This may be explained by a difference in infectious dose transmitted by mites compared to by experimental manipulation. It could also be explained by the location of the infection, since Chambers et al. (2014) demonstrated that *D. melanogaster* experimentally infected with bacterial pathogens in the abdomen survived longer and with lower bacterial load compared to flies infected via the thorax. Our experimentally infected flies were punctured in the thorax whereas *Macrocheles* mites typically attach to the abdomen of their host. Lastly, infestation by ectoparasitic mites can trigger immune responses in *D. melanogaster* (Benoit et al., 2020), which may influence resistance against bacterial pathogens.

Our data suggest that facultative parasites may play a more important role in pathogen transmission dynamics than we currently recognize.

CRediT authorship contribution statement

Emily K. Stone: Investigation, Methodology, Writing – original draft. **Emily S. Durkin:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Visualization, Writing – original draft, Writing – review & editing. **Andrew Cook:** Formal analysis, Visualization. **Elise A. Richardson:** Investigation, Methodology. **Brian P. Lazzaro:** Supervision, Writing – original draft, Writing – review & editing. **Carl N. Keiser:** Conceptualization, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jip.2024.108084.

References

Abad-Franch, F., Valenca-Barbosa, C., Sarquis, O., Lima, M.M., 2014. All that glisters is not gold: sampling-process uncertainty in disease-vector surveys with false-negative and false-positive detections. PLoS Neglected Tropical Diseases 8 (9), e3187.

Benoit, J.B., Bose, J., Bailey, S.T., Polak, M., 2020. Interactions with ectoparasitic mites induce host metabolic and immune responses in flies at the expense of reproduction-associated factors. Parasitology 147 (11), 1196–1205.

Chambers, M.C., Jacobson, E., Khalil, S., Lazzaro, B.P., 2014. Thorax injury lowers resistance to infection in *Drosophila melanogaster*. Infection and Immunity 82, 4380–4389. https://doi.org/10.1128/IAI.02415-14.

Cherrill, A., Brown, V., 1997. Sublethal injuries in a field population of the bush cricket decticus verrucivorus (L.) (tettigoniidae) in southern England. Journal of Orthoptera Research 6, 175. https://doi.org/10.2307/3503550.

Dainat, B., Ken, T., Berthoud, H., Neumann, P., 2009. The ectoparasitic mite tropilaelaps mercedesae (Acari, laelapidae) as a vector of honeybee viruses. Insectes Sociaux 56, 40–43. https://doi.org/10.1007/s00040-008-1030-5.

Durkin, E.S., Proctor, H., Luong, L.T., 2019. Life history of macrocheles muscaedomesticae (parasitiformes: macrochelidae): new insights on life history and evidence of facultative parasitism on drosophila. Experimental and Applied Acarology. https://doi.org/10.1007/s10493-019-00431-y.

Farish, D.J., Axtell, R.C., 1971. Phoresy redefined and examined in macrocheles muscaedomesticae (acarina:Macrochelidae). Acarologia 13, 16–29.

Gilad, T., Dorfman, A., Subach, A., Scharf, I., 2022. Leg or antenna injury in *cataglyphis* ants impairs survival but does not hinder searching for food. Current Zoology 68, 441–450. https://doi.org/10.1093/cz/zoab027.

Jaenike, J., Polak, M., Fiskin, A., Helou, M., Minhas, M., 2007. Interspecific transmission of endosymbiotic *spiroplasma* by mites. Biology Letters 3, 23–25. https://doi.org/10.1098/RSBL.2006.0577.

Kanbar, G., Engels, W., 2003. Ultrastructure and bacterial infection of wounds in honey bee (Apis mellifera) pupae punctured by varroa mites. Parasitology Research 90, 349–354. https://doi.org/10.1007/s00436-003-0827-4.

Khalil, S., Jacobson, E., Chambers, M.C., Lazzaro, B.P., 2015. Systemic bacterial infection and immune defense phenotypes in *Drosophila melanogaster*. Journal of Visualized Experiments 52613. https://doi.org/10.3791/52613.

Lysenkol, O., 1985. Non-sporeforming bacteria pathogenic to insects: incidence and mechanisms. Annual Review of Microbiology 39, 673–695.

Mazerolle, M.J., 2020. Model Selection and Multimodel Inference Using the AICcmodavg Package. https://doi.org/10.1016/j.ecolmodel.2003.11.004.

Osaka, R., Watada, M., Kageyama, D., Nomura, M., 2013. Detection of spiroplasma from the mite macrocheles sp. (Acari; macrochelidae) ectoparasitic to the fly Drosophila hydei (diptera; drosophilidae): a possible route of horizontal transmission? Symbiosis 60, 79–84.

Poinar, G.O., Wassink, H.J., Leegwater-van der Linden, M.E., van der Geest, L.P., 1979. *Serratia marcescens* as a pathogen of tsetse flies. Acta Tropica 36, 223–227.

Ramsey, S., Ochoa, R., Bauchan, G., Gulbronson, C., Mowery, J.D., Cohen, A., Lim, D., Joklik, J., Cicero, J.M., Ellis, J.D., Hawthorne, D., Van Engelsdorp, D., 2019. *Varroa destructor* feeds primarily on honey bee fat body tissue and not hemolymph. Proceedings of the National Academy of Sciences of the United States of America 116, 1792–1801. https://doi.org/10.1073/pnas.1818371116.

Shaikevich, E.V., Gorbacheva, A.A., Romanov, D.A., 2023. Ectoparasitic mites: vectors of bacterial symbionts among insects. Biology Bulletin 50 (3), 338–347.

Subasi, B. S., Grabe, V., Kaltenpoth, M., Rolff, J. and Armitage, S. A. O. (2023). How frequently are insects wounded in the wild ? A case study using *Drosophila melanogaster*. *bioRxiv*.

Traynor, K.S., Mondet, F., de Miranda, J.R., Techer, M., Kowallik, V., Oddie, M.A.Y., Chantawannakul, P., McAfee, A., 2020. *Varroa destructor*: a complex parasite, crippling honey bees worldwide. Trends in Parasitology 36, 592–606. https://doi.org/10.1016/j.pt.2020.04.004.

Wade, C.F., Rodriguez, J.G., 1961. Life history of macrocheles muscaedomesticae (acarina: macrochelidae), a predator of the house fly. Annals of the Entomological Society of America 54, 776–781. https://doi.org/10.1093/aesa/54.6.776.