Non-consumptive effects of an ectoparasitic mite on a *Drosophila* host

by

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Abstract

Parasite ecologists have increasingly recognized that parasites have ecologically significant roles beyond infection. One mechanism by which parasites influence their communities outside infection is by imposing trade-offs on potential hosts. In predator-prey ecology “non-consumptive effects” (NCEs) describe the negative effects predators have on prey outside of infection, e.g. increased stress, reduced feeding opportunity, lower mating success. Currently, it is not known the extent to which parasites impact potential hosts though analogous NCEs. In this thesis, I investigated short and long term NCEs experienced by *Drosophila nigrospiracula* exposed to, but not infected by, its natural ectoparasite *Macrocheles subbadius*. I also investigated how potential hosts vary in the NCEs they experience based on sex and mating status. Flies varied in NCEs (physiological and behavioural) based on sex and mating status, at least in the short term. Moreover, individual female flies had reduced fecundity and survival during chronic mite exposure; however these changes may not scale up to production-level effects based on current simulations. In the short term, fly resistance against mite infection trades off with dispersal ability and reproduction. Thus, there is a need to study how individual hosts and host populations compensate for NCEs and how this varies among different host groups. Additional research on parasite NCEs across different scales (ecological, generational) and fly lifespan may show the lifetime impacts of NCEs are larger than suggested here. This thesis contributes to our attempts to extend “the ecology of fear” to host-parasite interactions.

Preface

Section 1 is a general introduction to the topic, the model system, and objectives. Section 6 is a summary and synthesis. Sections 2, 3, 4, and 5 are edited and formatted versions of previously published manuscripts:

2.1 is an adaptation of “Current parasite resistance trades off with future defences and flight performance”. Published in *Behavioural Ecology and Sociobiology (2019)*. Collin Horn and Lien Luong. I conceptualised the study, collected and analysed all data, and wrote the manuscript.

2.2 is an adaptation of “Trade-offs between reproduction and behavioural resistance against ectoparasite infection”. Published in *Physiology and Behaviour (2021).* Collin Horn and Lien Luong. I conceptualised the study, collected and analysed all data, and wrote the manuscript.

3.1 is an adaptation of “Extending the ecology of fear: parasite-mediated sexual selection drives host response to parasites”. Published in *Physiology and Behaviour (2020)*. Collin Horn, Monika, Mierzejewski, Maesha Elahi, Lien Luong. I conceptualised the study, collected metabolic data, analysed all data, and wrote the manuscript.

3.2 is an adaptation of “Scared of the dark? Phototaxis as Behavioural Immunity in a Host-Parasite System”. Published in *Biology Letters (2022).* Collin Horn, Jacob Wasylenko, Lien Luong. I conceptualised the study, collected phototaxis data, analysed all data, and wrote the manuscript.

4.1 is an adaptation of “Proximity to parasites reduces host fitness independent of infection in a Drosophila-Macrocheles system”. Published in *Parasitology (2018).* Collin Horn and Lien Luong. I conceptualised the study, collected and analysed all data, and wrote the manuscript.

4.2 is an adaptation of “More to fear than fear itself? Relative contributions of parasite consumptive and non-consumptive effects to host population suppression: Insights from modelling a fly-mite interaction”. This manuscript is accepted at *Oecologia* (2022). Collin Horn[e], Darcy Visscher[e], Lien Luong (e = equal contributors). I collaborated with DRV to conceptualise the study, I reviewed the literature and determined parameters, I set the direction for modelling, and co-wrote the manuscript.

5.1. Is an adaptation of “Endosymbiotic Male-Killing Spiroplasma Affects the Physiological and Behavioural Ecology of Macrocheles-Drosophila Interactions”. Published in *Applied and Environmental Microbiology* (2022). Collin Horn, Taekwan Yoon, Monika Mierzejewski, Lien Luong. I designed the study and experiments, conducted metabolic experiments, analysed all data, and wrote the manuscript.

Dedication

To my parents Karen and Dave, Aunt Cor, and Monika.

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Chapter 1: Introduction

1.1 Background

Parasitism may be the most common lifestyle among animals, and almost all free-living organisms face the risk of infection [1; 2; 3]. In turn, hosts have adapted to living in infectious environments by developing many forms of immunity to resist infection [reviewed in 4]. Immunity research has traditionally been studied in terms of cellular, biochemical, and physical barrier (e.g. skin, cuticle) mechanisms that prevent and limit infection [5; 6; 7]. Maintaining and deploying these mechanisms can be costly and impose both energetic costs as well as carrying the risk of immunopathology, i.e. harm to the host from its own immune system [8; 9]. For example, the energetic costs of an immune response to sham infection can be deadly to caloric-restricted *Bombus terrestris* [8].

Host behaviour is increasingly recognized as an important mechanism by which hosts resist and tolerate infection [4; 10; 11]. This "behavioural immunity" includes identifying infected conspecifics, avoiding parasite/pathogen infectious, grooming; and thermotaxis, (i.e. selectively moving along temperature gradients) [12; 13; 14; 15; 16]. Trade-offs between behavioural immunity, especially grooming, and other life history traits have been observed [16; 17]. Bats, *Myotis myotis*, invest a substantial portion of their daily energy expenditures in anti-mite grooming, sometimes to the point of running an energy deficit which resulted in weight loss [18]. Given the ubiquity of parasites, trade-offs between immunity (physiological/cellular and behavioural) and other host traits are likely pervasive.

Other natural enemies have impacts on potential victims through trade-offs [19]. The "ecology of fear" framework was developed to describe the impacts of predators on potential prey outside of direct attack. Even if the individual prey is not consumed, it may still experience prolonged stress, decreased feeding success, depressed immunity, reduced growth, and ultimately lower fitness [20; 21]. Because they occur outside of predation, i.e. consumption, these negative effects from mere predator exposure are often called trait-mediated or non-consumptive effects (NCEs) [19]. The survival of leverets from wild mother hares exposed to predators is reduced by predator exposure, even if the predator is removed post-birth [22]. This reduction may be driven by predator-induced increases in stress hormones that have deleterious effects on reproduction [23.]. Survival of invertebrates can also be affected by predator presence: dragonfly larvae exposed to predators across a barrier are less likely to survive to adulthood [21].

Theoretical and experimental work suggests these individual-level NCEs can scale up and impact prey population structure and growth even if predation itself is minimal or absent [24; 25]. For example, copepod sex ratios can be altered by the presence of predatory opossum shrimp, and this change is not due to differential predation [26]. Furthermore, the growth rate of aphid populations was decreased by the presence of predators (damsel bugs) with surgically removed mouthparts [27]. A simulation study found that predator-mediated decreases in body mass, caused by predator presence, could reduce porcupine population size independent of predation [25]. In fact, over half the effects of predators on prey population

growth may be through NCEs [24]. Integrating the ecology of fear can lead to a better understanding of host-predator interactions and improve the accuracy of population models [20].

Recent work has extended the ecology of fear framework to other natural enemies, e.g. parasitoids and parasites [28; 29]. This expansion of parasite ecology includes considering if parasites have impacts on potential hosts outside of infection, i.e. outside the consumptive effects of feeding on host tissues [30; 31]. The NCEs of parasites/pathogens have also been referred to as an "ecology of disgust" [30; 31]. Incidental attacks by parasitoid wasps against a non-competent aphid species induce defensive behaviours (e.g. escape dropping) that ultimately reduced aphid population growth despite the lack of infection [28]. That parasitoids elicit similar NCEs as predators is perhaps unsurprising; both predators and parasitoids reduce victim fitness to zero [32]. As a result, prey and the hosts of parasitoids should evolve strong adaptations to avoid predation/parasitoidism. Although hosts defend themselves from parasite infection through costly immunity (physiological and behavioural), there is limited evidence for parasites having analogous NCEs on potential hosts. Unlike predators and parasitoids, parasites do not necessarily kill or castrate hosts, and potential hosts may invest relatively little in parasite-resistance and resultantly experience fewer NCEs [32]. Trade-offs between parasite resistance and other beneficial host traits (e.g. dispersal), however, could drive non-consumptive effects [33]. Given that parasitism is a near universal threat to free-living organisms [26; 34], parasites could exert widespread NCEs that add up to substantial ecological effects. In particular, if parasites exert fitness-level effects on potential hosts through NCEs, then the ecology of fear could have implications for the ecology and coevolution of host-parasite systems.

An important aspect of the ecology of fear that has been largely overlooked is the role of intra-specific variation [35; 36]. For example, male crickets have larger increases in CO_2 production (a proxy measure of metabolic rate and energy consumption) upon exposure to the faeces of a native predator (skink) than female crickets [35]. Metabolic rate captures both the behavioural and physiological costs of anti-predator responses in a shared biological currency (energy) [37]. Likewise, male snails lost weight during predator exposure, while females did not [36]. Sex-dependent changes in metabolism upon exposure to predator cues suggests inter-sex variation in how much energy potential prey invest in anti-predator resistance [35; 36].

Sex-biased infection is commonly observed in vertebrates and sometimes in invertebrates [38, 39]. These biases can be driven by differences in exposure, resistance, and/or preferences by parasites for certain hosts (i.e. host preference) [40; 41; 42]. As a result, both vertebrate and invertebrate hosts show sex differences in investment in immunity [reviewed in 43; 44]. Thus, it is reasonable to expect potential hosts to vary in the NCEs they experience due to parasite exposure. Similarly to how predator-mediated increases in metabolic rates suggest investment in anti-predator defences [35], increases in metabolic rate during parasite exposure may also indicate investment in resistance by potential hosts [45]. Thus, comparison of host metabolic rates in the presence and absence of parasites may provide insight into intra-specific variation in NCEs hosts experience.

In chapters 2-4, I investigated the non-consumptive effects of the mite *Macrocheles subbadius* (Berlese 1904) (Mesostigmata: Macrochelidae) on a host fly, *Drosophila nigrospiracula* Patterson and Wheeler 1942 (Diptera: Drosophilidae). This fly is cactiphilic and lives on rotting *Carnegiea gigantea* in the South Western United States and Northern Mexico [46]. This species feeds Flies leave sites as they age/dessicate and colonise near-by fresh rots. *M. subbadius* are facultative ectoparasites that feed on fly hemolymph during infection and also use *D. nigrospiracula* for dispersal between habitats (ephemeral *C. gigantea* rots which desiccate over time) [47]. While free-living the mites feed on small invertebrates, e.g. nematodes (lab observations). Infection can more than halve fly longevity and fecundity [47]. The consumptive effects of this mite have been well studied by Polak [47,], but if the mite has NCEs on the fly is not known. Flies defend themselves against mite infection through energetically limited behavioural defences, e.g. grooming, kicking, jumping, bursts of flight [47; 48]. The ability to resist mite infection is heritable, and primarily behavioural [49]. Melanization also occurs at the site of mite attachment. Changes in metabolism (energy use) of *Drosophila* are often studied using respirometry: measuring the rate of gas production/consumption as a proxy measure of metabolic rate [50]. The metabolic rate, measured as rate of CO_2 production, of congeneric flies (*D. hydei*) is increased by exposure to mites across a mesh screen, i.e. without infection, suggesting energetic trade-offs between parasite resistance and other traits are possible [45]. Appendix A1 provides additional methodological details.

As a facultative ectoparasite, *M. subbadius* can be cultured separately from *D. nigrospiracula*. Flies exhibit a strong response to the presence of mites, and infection is readily observable. Likewise, the generally short life-span makes observing flies for their entire life feasible and therefore, measuring both short and long-term NCEs of mite exposure is possible. These traits make this system highly tractable to physiological and behavioural studies.

1.2 Objectives

My research pursued three main questions: 1) Does ectoparasite resistance have trade-offs with other fly traits? 2) How do hosts vary intra-specifically in the NCEs they experience? and 3) Do the NCEs experienced by hosts during parasite exposure impact the survival and fecundity of hosts?

Chapter 2.1 examines how anti-mite behaviour (induced grooming) increases the metabolic rate of flies. The metabolic rate of a con-generic fly is increased by proximity to *M. muscaedomesticae* across a mesh barrier [45]. This energetic cost suggests the potential for parasite resistance to trade-off with other energetically demanding activities, such as flight and reproduction [45]. *Drosophila* rely on flight to disperse between ephemeral habitats (such as cactus rots) [46; 51]. Thus, I test the hypothesis that anti-mite behaviour (namely grooming] trades-off with flight endurance (measured with a hover assay, [48]). Chapter 2.2 investigates the energetic cost of reproduction in *Drosophila* and its potential consequences on host ability to resist mite infection. Given the centrality of reproduction to fitness, trade-offs between mating and ectoparasite resistance may have ecological and evolutionary implications.

Chapter 3.1 tests for intra-specific host variation in expression of NCEs. Male and female prey can vary in the NCEs induced by predators that they experience [35]. I test if male and female hosts vary in the NCEs (using respirometry) they experience due to the presence of parasites. I also aim to extend this understanding by testing if parasite-mediated sexual selection may drive these sex differences by comparing mated and unmated flies during mite exposure. Understanding intra-specific variation in NCEs is necessary for fully understanding the impacts of parasites on host populations. Anti-mite behaviours likely vary in their energetic costs (e.g. grooming, flying, or walking away), and intra-specific differences in resistance may be dependent on the type of resistance. In particular, I investigated differences in mite-mediated phototaxic behaviour of mated and unmated flies in chapter 3.2. Phototaxis behaviour is likely linked to important thermal regulation and feeding behaviours in desert flies exploiting a food source that rapidly dessicates.

Chapter 4.1 tests if parasite NCEs can have fitness-level impacts on hosts. The presence of predators can lower prey fitness (measured as survival/longevity and reproductive output) separate from predation. Analogous effects of parasites on potential hosts, however, have not been observed. Given how wide-spread parasites are [2], their potential NCEs may be having broad, yet underestimated, impacts. I tested if chronic exposure to mites reduces the lifetime fecundity and lifespan of individual female flies. Chapter 4.2 is a collaborative study to determine if individual-level NCEs can scale up into population-level effects, e.g. effects on population growth [25], through statistical modelling.

Chapter 5 is a preliminary investigation on a fly-mite-endosymbiont interaction. We tested if *Spiroplasma poulsonii*, a male-killing endosymbiotic bacteria of flies, impacts the endurance (a proxy measure of resistance) of *Drosophila melanogaster*, and whether it alters the host preference of *Macrocheles subbadius*. We found flies infected with *S. poulsonii* had weaker endurance, which could have potential implications for future NCEs by affecting the cost-benefit ratios of resistance.

1 References

1. Windsor, D.A., 1998. Most of the species on Earth are parasites. International Journal for Parasitology 28, 1939-1941. doi: 10.1016/s0020-7519(98)00153-2
2. Poulin, R., Morand, S. 2000. The diversity of parasites. Quarterly Review of Biology 75: 277-293.
3. Dobson, A., Lafferty, K.D., Kuris, A.M., Hechinger, R.F., Jetz, W., 2008. Homage to Linnaeus: How many parasites? How many hosts? Proceedings of the National Academy of Sciences of the United States of America 105, 11482-11489. doi: 10.1073/pnas.0803232105
4. Schulenburg, H., Kurtz, J., Moret, Y., Siva-Jothy, M.T., 2009. Introduction. Ecological immunology. The Royal Society, Philosophical Transactions of the Royal Society B, pp. 3-14.
5. Dimopoulos, G., 2003. Insect immunity and its implication in mosquito-malaria interactions. Cellular Microbiology 5, 3-14. doi: 10.1046/j.1462-5822.2003.00252.x
6. Marshall, J.S., Warrington, R., Watson, W., Kim, H.L., 2018. An introduction to immunology and immunopathology. Allergy Asthma and Clinical Immunology 14. doi: 10.1186/s13223-018-0278-1
7. Paludan, S.R., Pradeu, T., Masters, S.L., Mogensen, T.H., 2021. Constitutive immune mechanisms: mediators of host defence and immune regulation. Nature Reviews Immunology 21, 137-150. doi: 10.1038/s41577-020-0391-5
8. Moret, Y., Schmid-Hempel, P., 2000. Survival for immunity: the price of immune system activation for bumblebee workers. Science 290, 1166-1168. doi: 10.1126/science.290.5494.1166
9. Lazarro, B.P., Tate, A.T., 2022. Balancing sensitivity, risk, and immunopathology in immune regulation. Current opinion in insect science 50, 100874.
10. de Roode, J.C., Lefevre, T., 2012. Behavioral Immunity in Insects. Insects 3, 789-820.
11. Rakus, K., Ronsmans, M., Vanderplasschen, A., 2017. Behavioral fever in ectothermic vertebrates. Developmental and Comparative Immunology 66, 84-91. doi: 10.1016/j.dci.2016.06.027
12. Fritzsche, A., Allan, B.F., 2012. The Ecology of Fear: Host Foraging Behavior Varies with the Spatio-temporal Abundance of a Dominant Ectoparasite. Ecohealth 9, 70-74. doi: 10.1007/s10393-012-0744-z
13. Arnold, P.A., White, C.R., Johnson, K.N., 2015. Drosophila melanogaster does not exhibit a behavioural fever response when infected with Drosophila C virus. Journal of General Virology 96, 3667-3671. doi: 10.1099/jgv.0.000296
14. Hunt, V.L., Zhong, W.H., McClure, C.D., Mlynski, D.T., Duxbury, E.M.L., Priest, N.K., 2016. Cold-seeking behaviour mitigates reproductive losses from fungal infection in Drosophila. Journal of Animal Ecology 85, 178-186. doi: 10.1111/1365-2656.12438
15. Masuzzo, A., Maniere, G., Viallat-Lieutaud, A., Avazeri, E., Zugasti, O., Grosjean, Y., Kurz, C.L., Royet, J., 2019. Peptidoglycan-dependent NF-kappa B activation in a small subset of brain octopaminergic neurons controls female oviposition. Elife 8. doi: 10.7554/eLife.50559

16. Daversa, D.R., Manica, A., Cenis, H.B., Lopez, P., Garner, T.W.J., Bosch, J., 2021. Alpine Newts (Ichthyosaura alpestris) Avoid Habitats Previously Used by Parasite-Exposed Conspecifics. Frontiers in Ecology and Evolution 9. doi: 10.3389/fevo.2021.636099
17. McCullough, E.L., Chou, C.C., Backwell, P.R.Y., 2020. Cost of an elaborate trait: a trade-off between attracting females and maintaining a clean ornament. Behavioral Ecology 31, 1218-1223. doi: 10.1093/beheco/araa072
18. Giorgi, M.S., Arlettaz, R., Christe, P., Vogel, P., 2001. The energetic grooming costs imposed by a parasitic mite (Spinturnix myoti) upon its bat host (Myotis myotis). Proceedings of the Royal Society B-Biological Sciences 268, 2071-2075. doi: 10.1098/rspb.2001.1686
19. Peacor, S.D., Werner, E.E., 2008. Nonconsumptive effects of predators and trait-mediated indirect effects, Encyclopedia of Life Sciences (ELS). John Wiley & Sons, Ltd, Chichester, United Kingdom, pp. 1-8.
20. Peckarsky, B.L., Abrams, P.A., Bolnick, D.I., Dill, L.M., Grabowski, J.H., Luttbeg, B., Orrock, J.L., Peacor, S.D., Preisser, E.L., Schmitz, O.J., Trussell, G.C., 2008. Revisiting the classics: Considering nonconsumptive effects in textbook examples of predator-prey interactions. Ecology 89, 2416-2425. doi: 10.1890/07-1131.1
21. McCauley, S.J., Rowe, L., Fortin, M.J., 2011. The deadly effects of "nonlethal" predators. Ecology 92, 2043-2048. doi: 10.1890/11-0455.1
22. MacLeod, K.J., Krebs, C.J., Boonstra, R., Sheriff, M.J., 2017. Fear and lethality in snowshoe hares: the deadly effects of non-consumptive predation risk. Oikos 127, 375-380. doi: 10.1111/oik.04890
23. Sheriff, M.J., Krebs, C.J., Boonstra, R., 2009. The sensitive hare: sublethal effects of predator stress on reproduction in snowshoe hares. Journal of Animal Ecology 78, 1249-1258. doi: 10.1111/j.1365-2656.2009.01552.x
24. Preisser, E.L., Bolnick, D.I., 2008. Themany faces of fear: comparing the pathways and impacts of nonconsumptive predator effects on prey populations. Plos One 3, 1-8. doi: 10.1371/journal.pone.0002465
25. DeWitt, P.D., Visscher, D.R., Schuler, M.S., Thiel, R.P., 2019. Predation risks suppress lifetime fitness in a wild mammal. Oikos 128, 790-797. doi: 10.1111/oik.05935
26. Heuschele, J., Ceballos, S., Borg, C.M.A., Bjaerke, O., Isari, S., Lasley-Rasher, R., Lindehoff, E., Souissi, A., Souissi, S., Titelman, J., 2014. Non-consumptive effects of predator presence on copepod reproduction: insights from a mesocosm experiment. Marine Biology 161, 1653-1666. doi: 10.1007/s00227-014-2449-z
27. Nelson, E.H., Matthews, C.E., Rosenheim, J.A., 2004. Predators reduce prey population growth by inducing changes in prey behavior. Ecology 85, 1853-1858. doi: 10.1890/03-3109
28. Fill, A., Long, E.Y., Finke, D.L., 2012. Non-consumptive effects of a natural enemy on a non-prey herbivore population. Ecological Entomology 37, 43-50. doi: 10.1111/j.1365-2311.2011.01333.x

29. Koprivnikar , J., Penalva, L., 2015. Lesser of Two Evils? Foraging Choices in Response to Threats of Predation and Parasitism. Plos One 10: 1-11.
30. Weinstein, S.B., Buck, J,C., Young, H.S., 2018. A landscape of disgust. Science 359: 1213-1214.
31. Doherty, J.F., Ruehle, B., 2020. An Integrated Landscape of Fear and Disgust: The Evolution of Avoidance Behaviors Amidst a Myriad of Natural Enemies. Frontiers in Ecology and Evolution 8. doi: 10.3389/fevo.2020.564343
32. Lafferty, K.D., Kuris, A.M., 2002. Trophic strategies, animal diversity and body size. Trends in Ecology & Evolution 17, 507-513. doi: 10.1016/s0169-5347(02)02615-0
33. Lazzaro, B.P., Tate, A.T., 2022. Balancing sensitivity, risk, and immunopathology in immune regulation. Current opinion in insect science 50, 100874-100874. doi: 10.1016/j.cois.2022.100874
34. Poulin, R., Morand, S., 2000. The diversity of parasites. Quarterly Review of Biology 75, 277-293. doi: 10.1086/393500
35. Lagos, P.A., Herberstein, M.E., 2017. Are males more scared of predators? Differential change in metabolic rate between males and females under predation risk. Physiology & Behavior 173, 110-115. doi: 10.1016/j.physbeh.2017.02.002
36. Donelan, S.C., Trussell, G.C., 2020. Sex-specific differences in the response of prey to predation risk. Functional Ecology 34, 1235-1243. doi: 10.1111/1365-2435.13569
37. Lighton, J.R.B., 2008. Measuring Metabolic Rates: A Manual For Scientists. Oxford University Press, New York, United States of America.
38. Polak, M., Markow, T.A., 1995. Effect of ectoparasitic mites on sexual selection in a sonoran desert fruit-fly. Evolution 49, 660-669. doi: 10.2307/2410319
39. Sheridan, L.A.D., Poulin, R., Ward, D.F., Zuk, M., 2000. Sex differences in parasitic infections among arthropod hosts: is there a male bias? Oikos 88, 327-334. doi: 10.1034/j.1600-0706.2000.880211.x
40. Wedekind, C., Jakobsen, P.J., 1998. Male-biased susceptibility to helminth infection: an experimental test with a copepod. Oikos 81, 458-462. doi: 10.2307/3546767
41. Christe, P., Glaizot, O., Evanno, G., Bruyndonckx, N., Devevey, G., Yannic, G., Patthey, P., Maeder, A., Vogel, P., Arlettaz, R., 2007. Host sex and ectoparasites choice: preference for, and higher survival on female hosts. Journal of Animal Ecology 76, 703-710. doi: 10.1111/j.1365-2656.2007.01255.x
42. Alsaiyah, M.A.M., Ebssa, L., Zenner, A., O'Callaghan, K.M., Griffin, C.T., 2009. Sex ratios and sex-biased infection behaviour in the entomopathogenic nematode genus Steinernema. International Journal for Parasitology 39, 725-734. doi: 10.1016/j.ijpara.2008.11.003
43. Zuk, M., Stoehr, A.M., 2002. Immune defense and host life history. American Naturalist 160, S9-S22. doi: 10.1086/342131
44. Kelly, C.D., Stoehr, A.M., Nunn, C., Smyth, K.N., Prokop, Z.M., 2018. Sexual dimorphism in immunity across animals: a meta-analysis. Ecology Letters 21, 1885-1894. doi: 10.1111/ele.13164

45. Luong, L.T., Horn, C.J., Brophy, T., 2017. Mitey costly: energetic costs of parasite avoidance and infection. Physiological and Biochemical Zoology 90, 471-477. doi: 10.1086/691704
46. Johnston, J.S., Heed, W.B., 1976. Dispersal of desert-adapted Drosophila: the Saguaro-breeding D. nigrospiracula. American Naturalist 110, 629-651. doi: 10.1086/283095
47. Polak, M., 1996. Ectoparasitic effects on host survival and reproduction: The Drosophila-Macrocheles association. Ecology 77, 1379-1389. doi: 10.2307/2265535
48. Luong, L.T., Heath, B.D., Polak, M., 2007. Host inbreeding increases susceptibility to ectoparasitism. Journal of Evolutionary Biology 20, 79-86. doi: 10.1111/j.1420-9101.2006.01226.x
49. Polak, M., 2003. Heritability of resistance against ectoparasitism in the Drosophila-Macrocheles system. Journal of Evolutionary Biology 16, 74-82. doi: 10.1046/j.1420-9101.2003.00500.x
50. Lighton, J.R.B., Halsey, L.G., 2011. Flow-through respirometry applied to chamber systems: Pros and cons, hints and tips. Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology 158, 265-275. doi: 10.1016/j.cbpa.2010.11.026
51. Markow, T.A., 1988. Reproductive behavior of Drosophila melanogaster and Drosophila nigrospiracula in the field and in the laboratory. Journal of Comparative Psychology 102: 169-173.

Chapter 2: Trade-offs between resistance and other host traits

Chapter 2 Introduction

In this chapter I considered trade-offs between parasite resistance and other host traits. Anti-parasite behaviour is energetically costly, and this creates an opportunity to trade-off with energetically demanding activities. First, we consider a short term trade-off between induced grooming and future mite resistance and flight (2.1). Then we consider a long term trade-off between mating, another process requiring substantial energy/resource investment, and the ability to resist mite infection (2.2). We observed both short- and long- term trade-offs: flies induced to groom had reduced dispersal ability, and mated females were less able to resist infection than unmated females. We discuss these energetically mediated trade-offs in light of the ecology, in particular dispersal, and potential co-evolution of our host and parasite.

2.1: Grooming trades-off with future mite resistance and dispersal

Introduction

Parasites have negative and sometimes dramatic effects on host behaviour, survival, and reproduction [1; 2]. Not surprisingly, hosts have evolved physiological and behavioural defence mechanisms to mitigate these fitness costs [3; 4]. Behavioural responses such as parasite avoidance and anti-parasite grooming are widespread in nature, and in particular among insects [4; 5]. Anti-parasite behaviours are often automatic and can be induced by parasite exposure and/or proximity to parasitic cues [6; 7; 8]. Furthermore, behavioural defences are time consuming and energetically costly [5; 9], which diverts time and resources away from essential activities such as feeding and reproduction and can ultimately lead to reduced growth, longevity, and fecundity [9; 10; 11]. Giorgi et al. [12] measured the energy expended on grooming by bat hosts when infested with mites. These measurements did not distinguish between the energetic costs of grooming and possible metabolic changes resulting from infection [12; 13]. In order to determine if/how defensive grooming trades-off with other host activities, we need to measure the energetics of self-grooming separately from the effects of parasite infection. In this study, we investigate whether energetic trade-offs can have effects on host investment in future parasite avoidance and resistance, as well as other energetically demanding activities such as flight.

Insects groom in response to numerous stimuli including noxious chemicals, pathogens (e.g., bacteria), ectoparasites, dust, and other irritants [14; 15; 16]. Fruit flies can distinguish between harmful [e.g., quinine and bacterial compounds] and non-harmful (e.g., water and sugar) substances and mount a significantly stronger grooming response upon contact with the former, even among decapitated flies [16; 17]. Grooming also serves to distribute antibacterial/antifungal compounds, improving insect resistance to bacterial and fungal disease [5]. Additionally, grooming aids in the maintenance of insect sensory systems, e.g., by keeping olfactory sensors on the antenna clean [18].

Drosophila nigrospiracula (Diptera: Drosophilidae) is a cactiphilic fruit fly and naturally associated with the facultative ectoparasite *Macrocheles subbadius* (Acari: Macrochelidae) [19]. Mites feed on fly

hemolymph and use flies for dispersal between temporally transitive habitats (rotting cacti) [14]. Resistance against mites is primarily behavioural; flies groom and undergo bursts of flight to avoid mite infection [14]. Moreover, direct contact between *Drosophila* hosts and mites is not necessary to induce defensive behaviours [8]. Flies exposed to mites across a mesh screen exhibit elevated levels of CO_2 production, presumably due to an increase in grooming/escape activity [8]. We explicitly test this hypothesis by measuring the energetic cost of grooming. Furthermore, we hypothesise that the ability of hosts to mount an effective defence against parasitism is depleted by previous energetically demanding activities. We predict that flies induced to groom via an irritant will be more susceptible to infection and harbour a higher mite burden upon subsequent exposure to mites compared to flies allowed to rest.

Flight plays an important role in insect dispersal and migration but requires considerable energetic investment [20; 21]. Energy spent grooming may therefore trade-off with flight capability. While studies have shown that parasite infection can reduce flight endurance, for example, there was a 20% reduction in flight distance in monarch butterflies infected with *Ophryocystis elektroscirrha* [22], there is little direct evidence that anti-parasite behaviours *per se* trade-off directly with flight capacity. Flight performance is related to short- and long- term parasite resistance in two important ways. First, flight endurance in *D. nigrospiracula* serves as an indicator of general physical endurance and hence a fly's ability to mount a sustained defence against mites [23]. Second, the ability to disperse may limit future infections by allowing hosts to escape habitats with high parasite density [24]. We predict that flies induced to groom will subsequently have lower flight endurance than flies previously at rest.

Methods

Fly and mite cultures

Drosophila nigrospiracula Patterson and Wheeler lab populations were founded from approximately 150 adult male and 150 adult female flies collected in 2015 from necrotic cacti (*Carnegiea gigantea*) in the Sonoran Desert [AZ, USA]. Culture medium consisted of instant potato flakes, *Drosophila* medium [Formula 4-24 Instant Drosophila Medium, Carolina Biological Supply Company, Burlington, NC], and nutritional yeast. Autoclaved necrotic cactus was added as a nutritional supplement. Newly eclosed adult flies were transferred to agar medium in sex-specific vials. Flies were kept in incubators [Percival Scientific, Peny, IA, USA] at 24℃ and 50% relative humidity with a 12L:12D cycle. Unmated adult female flies were selected at random from the stock populations for the experiments below.

Macrocheles subbadius Berlese cultures were initiated from ~200-300 adult mites from wild caught *D. nigrospiracula*. Mites were maintained on a 2:1 wheat bran to wood chips medium. Free-living bacteriophagic nematodes were cultured in the same media as a food source. Mite cultures were maintained at 26°C and 70% relative humidity, with a 12L:12D cycle (Percival Scientific, Perry, IA, USA). Adult female mites used in the following experiments were collected at random from the stock culture using a Berlese funnel and transferred to moist plaster-of-paris until use.

Respirometry

Respirometry provides a way to determine the short-term energetic costs of grooming. The carbon dioxide (CO_2) output of organisms is linked to energy at fixed ratios by the cellular respiration equation [25]. Therefore, CO_2 production can be used as a relative measure of organisms energy usage [25]. We measured CO_2 production with a flow-through respirometer using a Li-7000 infrared CO_2 analyzer (Li-COR Biosciences, Lincoln, NE). Incurrent atmospheric air was pumped through an ascarite-drierite column at 30 mL/min to remove both CO_2 and water vapour. Respirometry chambers were purged for half a minute to vacate atmospheric gases from the system before recording data. Using a MAVen-FT system (Sable Systems, Las Vegas, NV], purified air was directed to either an experimental chamber or a baseline chamber (Fig. 2.1.1). The actual flow rate was measured in real time and used in the calculation of CO_2 production rate [26]. Excurrent gas was again scrubbed of water vapour with a magnesium perchlorate column before being analysed by the Li-7000. The Li-7000 used a reference cell, consisting of CO_2-purged bone-dry air, to measure gas concentrations from the MAVEn. The analyzer was periodically spanned with dry 20 ppm CO_2 at 30 mL/min (Praxair, Danbury, CT).

We recorded the CO_2 production of each fly for 5 min, at 1 observation/second. Baseline measurements were recorded for the 120 s before and after each assay. The baseline values were used to correct for drift in measurements over the recording period. Following recording, mean respiration rate (average of ~300 observations for each fly) was calculated and used in analysis. Metabolic measurements were recorded at ambient lab conditions (20-24°C and 21-32% relative humidity).

Energetic cost of grooming

In this experiment, we measured the energetic cost of grooming independent of infection using respirometry. Female flies were age-matched and assigned randomly to either a grooming group or a control group. Flies in both groups were anaesthetised with CO_2 simultaneously, and the ventral side of flies in the grooming group were lightly dusted with volcanic ash (Natural Dust Bath; Sunseed, Weston OH). While anaesthetised, flies were held upright with forceps and pulled across a layer of volcanic ash (on weigh paper) once. This method limited the amount of the body dusted and increased consistency between trials. Control flies were handled identically but streaked against irritant-free weigh paper instead. All flies were given 10 min to recover from anaesthesia before being transferred to individual respirometer chambers (4.5-cm length x 0.5-cm diameter glass cylinders) to measure CO_2 production. This experiment was conducted over 8 replicate blocks, each consisting of three controls and three flies exposed to the irritant. Trials were conducted across multiple days leading to variation in fly age between blocks but not within blocks.

A second experiment tested if grooming was the underlying cause of increased respiration, and not the irritant *per se*. Flies were exposed to volcanic ash but were prevented from grooming by restraining their movement. Female flies were exposed to the irritant or left unperturbed, as above, but placed into a sarcophagus that prevented grooming. Following exposure, flies were transferred to a

pipette tip and gently pushed using a paint brush until the legs and wings of the fly were held against the thorax. The pipette tip was placed into a piece of rubber tubing sealed with mesh to prevent fly escape while allowing air flow. The entire sarcophagus was placed into the respirometry chamber such that the fly faced down stream. The rubber tubing created a seal that forced air to pass through the sarcophagus and held the fly in the path of the air flow. Following the experiment, chambers were checked to confirm that the flies were still restrained. Seven replicate blocks were conducted, each containing 3 control flies and 3 dusted flies.

Behavioural response to irritants and mites

We compared the behavioural response of flies to irritants and mites as recent research has emphasised the specificity of fly responses to different threats [7; 17]. Flies were exposed to either an irritant or mites and their behaviour recorded, along with a control group (mite and irritant free) as a baseline. In all conditions, flies were anaesthetised with CO_2 and assigned to either an exposed [volcanic ash or mites] group or control group. Flies were then moved to individual micro-arenas and given 5 min to recover. Flies were observed for 5 s of every minute for 30 min; at each observation, flies were recorded as grooming (e.g., rubbing abdomen, thorax, or head with legs, wing flicking), moving, or resting. Ambulatory movement was recorded because activity throughout the chamber may indicate escape behaviour [27]. Flight was not observed as the chambers were too restrictive. Resting was defined as remaining stationary while not engaging in grooming behaviour. Because flies were visually marked with the irritant or mites, it was not possible to blind the recorder as to which group was being observed.

In the behavioural response to irritants experiment, flies in the irritant group were dusted with volcanic ash as described above. Flies were observed in glass tubes (4.5-cm length, 0.5-cm diameter) also used for respirometry..

In the behavioural response to mites experiment, a smaller observation chamber was used in order to ensure contact between mites and flies. Both the mite group and control were placed in a 200-mL pipette tip cut in half (~2 cm long, ~0.5-cm diameter) and plugged with cotton at each end. For the treatment group, three adult female mites were placed in the pipette tip, while the control group was not exposed to mites.

Grooming and subsequent susceptibility to infection

In order to test if flies that spent time grooming were more vulnerable to infection, the irritant-exposed and respective control group from the above behavioural response experiment were transferred individually to an Eppendorf tube plugged with cotton (0.3 mL of space for fly movement) containing 5 adult female mites. During the 1 h exposure period, the Eppendorf tubes were covered with an opaque box since *M. subbadius* are more likely to infect *D. nigrospiracula* in the dark (pers. obs, chapter 3.2). The number of mites attached at the end of the hour was recorded; flies were frozen at -20°C and later weighed to obtain dry body mass (Mettler Toledo XP105, Columbus, OH).

Grooming and flight endurance

This experiment tested the trade-off between grooming behaviour and future flight performance of *D. nigrospiracula*. Flight endurance was measured as time to exhaustion when hovering on a tether [23; 28]. The tethers were made from a 2.5-cm-long fishing line (Powerflex tippet 0.102 mm; Rio Products, Idaho Falls, IA) glued (Elmer's rubber cement) to the blunt end of an insect pin (size 0 with a right-angle bend at the end 3 mm). CO_2-anaesthetised female flies were attached to the line by dipping the bent end in a UV curing adhesive (KOA I Oosp; Kemxert Corp, York, PA). The tether was then secured to the dorsal thorax of the fly with two, 20 s pulses of UV light (LED-200 UV curing system; Electro-lite, Bethel CT). Flies were allowed 20 min to recover during which time they were positioned to stand on their own legs against a foam block; the tether was then secured horizontally to a second foam block so that the weight of the tether was not borne by the flies. Following the rest period, flies were then randomly assigned to a control group or a grooming group that was induced to groom with an irritant applied to the ventral thorax and abdomen.

Following 30 min of resting or grooming, flies were assayed for flight endurance. The pins at the end of the tethers were inserted into a foam plank attached to a suspended wooden support beam [28]. Flies were induced to hover, if they did not begin spontaneously, by allowing them to perch on a platform which was then pulled away rapidly. If flies ceased hovering and did not resume within 10 s after prodding the legs with a paint brush and a puff of air, then the fly was considered exhausted and the trial was ceased [28]. Time hovered was recorded to the nearest second and was converted into minutes before statistical analysis. In order to be considered as having successfully hovered, a fly had to maintain flight for at least 1 min (see results for flight success rates). This criterion removed flies that endured wing damage and other bodily harm during tethering that could disrupt flight as well as flies that, although alive and intact, could not be induced to fly under tethered conditions [28]. Flight assays were conducted under ambient conditions (19-23°C and 3-19% relative humidity) with a combination of natural and fluorescent light.

Statistical analyses

All statistics were performed in R using the R studio environment [29]. We used backwards model reduction to arrive at a minimal model (glm function). An initial model was generated including all independent variables using a distribution based on visual inspection of the data. Starting with the least significant factor, terms were removed and competing models were compared with an ANOVA (F test). We tested for normalcy of model residuals (shapiro.test), and we checked for outliers (Cook's distance > 0.5).

The respiration of grooming flies was modelled with independent variables: treatment group (irritant or control), fly body mass, and age. Respiration in the restraining control was modelled with treatment and replicate, while the post hoc comparison between free flies and restrained flies considered

only treatment. Intensity of infection following grooming was modelled with group (irritant or control) and body mass. Flight endurance (i.e.,hovering time) was modelled with group (irritant or control), fly mass, and age; while the probability of initiating flight between the irritant and control group was compared using a proportion test (prop.test, R Core Team Stats). Behaviour counts during irritant exposure and mite exposure were analysed with the Mann-Whitney-Wilcoxon test using treatment group as the explanatory variable (wilcox.test, R Core Team Stats). Data is available at the Open Science Foundation (doi: 10.17605/OSF.IO/K2PXV).

Results

Energetic cost of grooming

Flies exposed to an irritant (N = 24) produced 30.2% more CO_2 than control flies (N = 23) (Fig. 2.1.2). The irritant group had a significantly higher mean rate of CO_2 production (0.194 ± 0.026 uL/min) compared to the control group (0.143 ± 0.017 uL/min; F = 4.49, P = 0.040). The mean body mass of flies in the grooming group was 2.14 ± 0.06 mg and for the control group was 2.16 ± 0.06 mg; however, fly mass was not a significant predictor of respiration in this experiment (F = 0.93, P = 0.34). Age was also considered as a covariate: in the irritant group the mean age was 8.5 ± 1.4 days post-eclosion while in the grooming group, the mean age was 7.8 ± 1.2 days. Age was a significant predictor of respiration and retained as a covariate (F = 18.10, P = 0.0010). These results show that grooming is energetically costly for flies independent of parasite exposure or infection; energetic costs may cause trade-offs, as measured in subsequent experiments.

The follow-up experiment (flies exposed to volcanic ash and restrained) suggests grooming contributes to the rise in respiration rate, not solely irritant exposure itself. The group exposed to an irritant N = 21) had a CO_2 production rate of 0.124 ± 0.018 uL/min, while the unexposed group (N = 21) produced CO_2 at a rate of 0.0987 ± 0.005 uL/min (Fig. 2.1.2). This 22.7% difference was not significantly different (F = 3.69, P = 0.063). Replicate was also a significant predictor of CO_2 production (F = 2.70, P = 0.030). Post hoc analysis between the restrained flies (N = 21) and free flies (N = 23) (both without irritant) found that free flies produced CO_2 at a greater rate, 0.0987 ± 0.005 uL/min and 0.143 ± 0.017 uL/min respectively. This was a statistically significant 36.7% difference (F = 17.25, P < 0.001).

Behavioural response to irritants and mites

As expected, exposure to an irritant was sufficient to induce grooming behaviours in flies. Fly behaviour was categorised as grooming, moving, or resting at each observational period. Exposed flies (N = 20) displayed on average 5.05 ± 0.56 bouts of grooming per trial, nearly 10 times more than the control group (0.55 ± 0.29; N = 20); this difference in frequency of grooming behaviour was statistically significant (Fig. 2.1.3a, wilcox.test, P < 0.001). Flies in the irritant group (1.97 ± 0.25 times) were observed moving less often than the control group (2.9 ± 0.73 times); this difference was also statistically significant (Fig. 2.1.3a, wilcox.test, P = 0.026). Time spent grooming and moving about necessarily

affected time spent at rest; hence, we did not statistically test differences in rest time. Nevertheless, the control group spent more time at rest than the irritant group (26.55 ± 0.54 and 22.95 ± 0.72 scans, respectively). Exposure to an irritant increased the amount of time spent grooming while decreasing time spent walking around/exploring the chamber.

In the behavioural response to mites experiment, we found a statistically significant difference in the number of grooming bouts observed between the mite-exposed group (N = 20) and the control group (N = 20). Flies exposed to mites displayed 8.05 ± 0.53 bouts of grooming behaviour while unexposed flies exhibited 0.35 ± 0.15 bouts of grooming, and this difference was statistically significant (Fig. 2.1.3b, wilcox.test, $P < 0.001$). We also observed 62.9% more ambulatory events in the exposed group than the control group, 4.45 ± 0.74 and 2.32 ± 0.43 events respectively. This difference in the number of times flies were observed walking around the arena was also statistically significant (Fig. 2.1.3b, wilcox.test, $P = 0.018$). Necessarily, the control group was observed at rest more often (27.3 ± 0.15) than the exposed group (17.5 ± 0.73). Flies increased the amount of time engaged in grooming and locomotion upon exposure to mites.

Grooming and subsequent susceptibility to infection

Susceptibility to infection was assayed among flies that were either induced to groom (N = 20) or left untreated (N = 20). Flies induced to groom became infected with 0.90 ± 0.19 mites/fly on average while flies in the control group were infected with 0.40 ± 0.15 mites/fly. Flies that were induced to groom were subsequently more susceptible to mite attack, resulting in higher parasite burdens ($F = 5.51$, $P = 0.025$). The grooming group had a mean mass of 1.97± 0.08 mg and the control group had a mean mass of 2.12 ± 0.08 mg. Fly body mass and infection intensity were weakly but positively correlated ($F = 3.8$, $P = 0.059$).

Grooming and flight endurance

We tested whether time spent grooming prior to flight affected the flight endurance of flies. Flies induced to groom with an irritant (N = 33) flew on average 20.4 ± 1.7 min while control flies (N = 35) flew 27.7 ± 3.3 min (30.3% less, $F = 4.3$, $P = 0.042$; Fig. 2.1.4). The mean control fly age was 20.3 ± 1.2 days, while the mean treatment fly was 21.3 ± 1.2 days old. The mean body mass of flies in the control group was 2.54 ± 0.05 mg and 2.58 ± 0.06 mg in the treatment group. However, including fly age ($F = 0.46$, $P = 0.50$) or body mass ($F = 0.51$, $P = 0.48$) did not improve the model. Flies in the control group (38%) were not more likely to initiate flight than flies in the treatment group (32%, prop.test, $P = 0.43$). These results show that induced grooming reduced flight endurance, at least in the short-term.

Discussion

Our results support the hypothesis that current investment in defensive behaviours (i.e., grooming) is energetically costly, with negative consequences for future resistance against parasite

infection and for flight endurance. Exposure to an irritant increased the frequency of grooming and the rate of respiration, and the increase was larger when flies were free to groom, suggesting an energetic cost of grooming independent of infection. Moreover, flies induced to groom for 30 min before parasite exposure were less able to resist infection and as a result accumulated more mites than control flies. Given that flies defend themselves against mites primarily through energetically demanding behavioural defences, the short-term energetic costs of grooming likely rendered hosts more susceptible to future infection. Time spent grooming also adversely affected endurance during hovering, suggesting energy expended on grooming subsequently reduces the energy available for flight.

Variation between potential hosts in how easily they exhaust and/or recover from exhaustion may explain variation in fly susceptibility to infection [30; 31]. The aggregation of *Macrocheles* spp. within host populations is influenced by a number of host traits (e.g., size, sex, body condition, metabolic state, and previous infection) [26; 32; 33]. Heterogeneity in host defensive behaviours may be in part driving aggregation, if traits that predict susceptibility to infection reflect endurance generally. Given that the ability of *D. nigrospiracula* to defend itself against mite infection is heritable [34; 35], genetic variation may exist in the degree of exhaustion following grooming. Therefore, selection experiments on fly resistance may in fact be selecting for flies with a higher threshold for exhaustion and/or faster recovery following energetically demanding activities, allowing for a more sustained defense [34; 35].

We also show that grooming trades-off with other host behaviours such as flight and by extension dispersal. Luong et al. [35] found that inbred *D. nigrospiracula* are less resistant to infection compared to control flies, and inbred flies are more easily exhausted during hovering assays. Furthermore, flies forced to hover until exhaustion were less able to defend themselves against infection [35]. These results in conjunction with our findings suggest that similar genetic and/or physiological mechanisms may limit both parasite defence and flight endurance. It is possible that the presence of the irritant may have obstructed flight, but exposure to an irritant did not significantly alter the probability of initiating flight ($P = 0.43$).

Unsurprisingly, grooming alone had a smaller impact on flight endurance (30.3% reduction) than infection itself (53-57% reduction, [35]) or even prior mite exposure ~50% reduction, [28]). The energetic cost associated with anti-parasitic grooming may contribute to the decreased flight endurance observed in previous studies, but grooming alone cannot entirely explain the negative effects of mites on host flight. A recent study showed that European shags infected with gastrointestinal nematodes expend more energy on flight, but do not increase their total daily energy expenditures suggesting there is a limit to host energy expenditure [36]. Consequently, infected birds spent less time in flight in order to remain within the constraint of maximum daily energy flux [36]. Our observations are consistent with and generalise those results, as energetic costs associated with defensive behaviours had knock-on effects on other activities even in the absence of parasite exposure. The results reported here suggest that grooming and other defensive activities, without infection, may partially account for the energetic costs and substantial reduction in flight capacity brought about by other parasites [22; 28].

Trade-offs between grooming and flight may affect dispersal as *D. nigrospiracula* travel upwards of 900 m between cactus rots [37]. Organisms have finite time and resources available to expend on self-grooming [38; 39], but the degree to which investing in resistance abilities, separate infection, impacts flight/dispersal is underexplored. Trade-offs between parasite resistance and dispersal have potentially important implications for host ecology and evolution [40; 41; 42]. Genetic mixing of *D. nigrospiracula* populations is maintained in part by the ability of flies to disperse between sites, which average 121 m apart [37; 43]. Reduced flight endurance resulting from trade-offs with energetically costly defences against parasites may contribute to reduced gene flow between fly sub-populations. Thus, populations under strong selection pressure from parasites may experience fragmentation with the potential for parasite-mediated speciation [44; 45].

Flies in our experiments responded differently to the presence of mites and irritants. In both cases, exposed flies increased grooming behaviour relative to control flies; however, exposure to an irritant resulted in decreased ambulatory/exploratory movement relative to control flies. In contrast, exposure to mites led to increased movement at least for the short duration of the trial. This disparity suggests *D. nigrospiracula* are specific in their response to the threat experienced, in agreement with previous studies of *Drosophila* [7; 17]. Neurobehavioral research suggests that fly grooming in response to mites and irritants are neurologically and biomechanically distinct behaviours and as such may require different energy investments [7]. Different movements may be optimal for removal of irritants and ectoparasites. More generally, we found that fly responses to mites and irritants differ in time allocation as well the type of grooming. However, both grooming in response to an irritant and to mites are energetically costly and may cause trade-offs with other energetically demanding activities (see results; [8]). Future research could compare the size of trade-offs caused by different types of fly grooming.

Interestingly, fly responses to ectoparasites and predators are similar [27]. In a study on the responses of *Drosophila* to predatory spiders, fruit flies increased their movement [27]. The authors suggest that the flies were searching for an escape from the environment with predators [27]. Beyond simple parasite avoidance, increased movement may indicate an attempt to disperse from a high-risk environment. Dispersal can reduce the impacts of predators on prey populations [46] and is hypothesised to do the same in host populations under the pressure of parasitism [26; 47]. Increased movement in response to the presence of mites may indicate that flies use a mixed escape and defensive strategy in response to ectoparasites [27; 48]. In contrast, increased movement could be ineffective or counter-productive when challenged with an irritant. Noteworthily, de la Flor et al. [27] found that over time flies came to tolerate the predation risk posed by spiders and reduced their defensive movements. The flies in our study may become habituated to the risks of parasitism if it is persistent, and perhaps moderate their defensive behaviours. Additional research should examine whether the defensive efforts of flies decrease with repeated or extended exposures to mites. We hypothesise that eventually the energetic costs of grooming will outweigh the costs of infection, and flies will either disperse or tolerate infection.

In our experiments, flies were exposed to mites in isolation; however, in nature, *D. nigrospiracula* populations are densely populated at necrotic cacti [19; 37]. Flies, consequently, are exposed to other flies and mites simultaneously. The threat of ectoparasites may be similar to the threat of predators to prey [47; 49]. However, the presence of conspecific prey can either increase or decrease the expression of defensive strategies by individual prey depending on the species and environmental context [50; 51]. In either case, the behaviour of individual flies may simplify the fly response to mites. Future research should therefore compare the behaviour of individual hosts exposed to parasites with the behaviour of groups of hosts exposed to parasites.

In conclusion, we detected an energetic cost of grooming in *D. nigrospiracula* and showed that current investment in grooming made flies more susceptible to future infection and reduced flight endurance. Hosts experience a dilemma in parasitic defense: invest in current resistance with potential consequences for future resistance to infection and reduced ability to escape parasite risk. This dilemma raises important questions of when hosts should choose to resist parasites, tolerate infection, or risk dispersing to a new habitat based on fluctuating cost-benefit ratios.

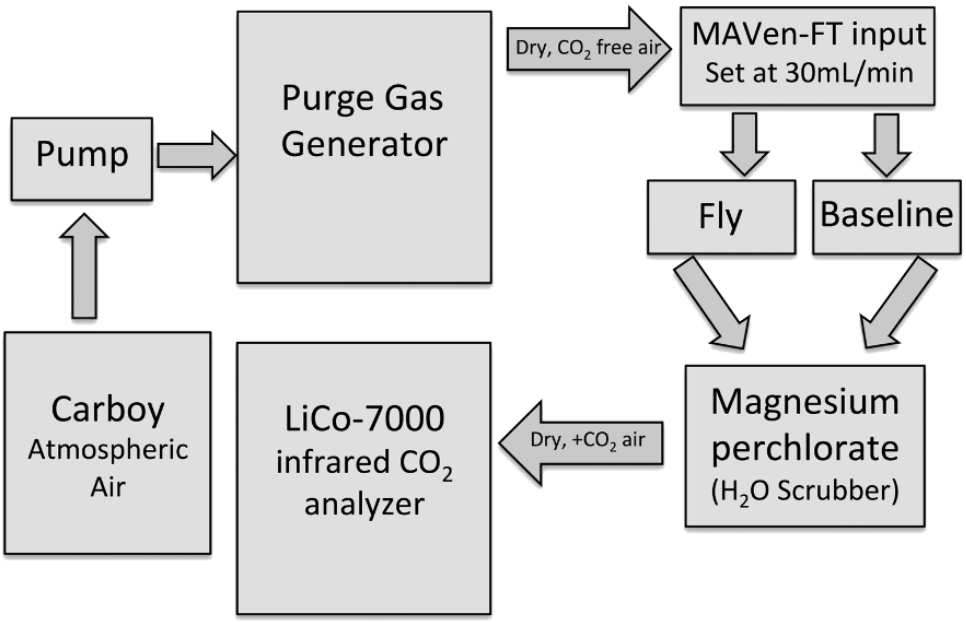


Figure 2.1.1: Simplified diagram of respirometer system used to measure carbon dioxide production (a proxy measure of fly metabolic rate).

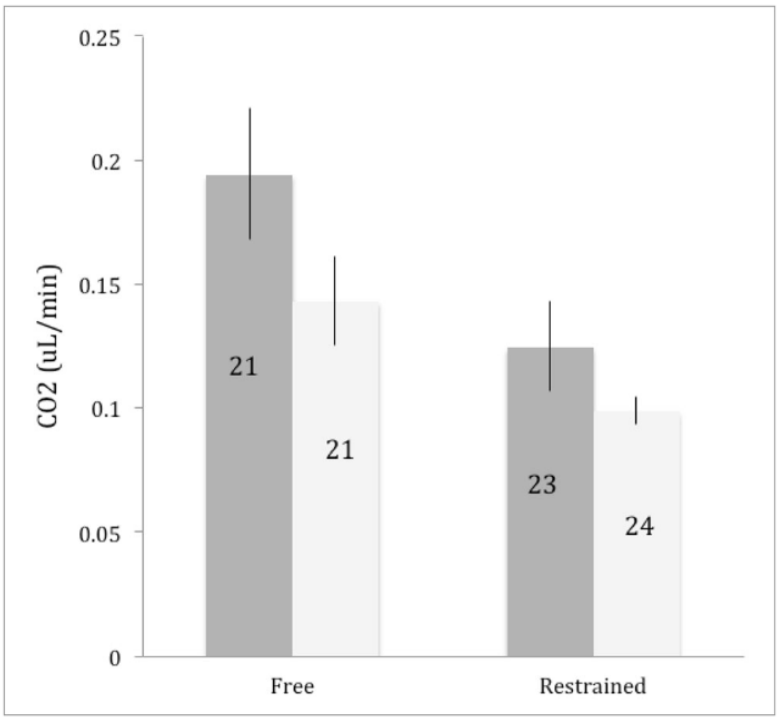


Figure 2.1.2: Mean respiration rates of flies exposed to volcanic ash (dark bars) or left at rest (light bars) while restrained or free to groom. Measurements were recorded in a flow-through respirometer. Each fly was monitored for 5 min and one measurement was recorded every second. Error bars represent ± 1 standard error, and data labels (in bar) represent sample size.

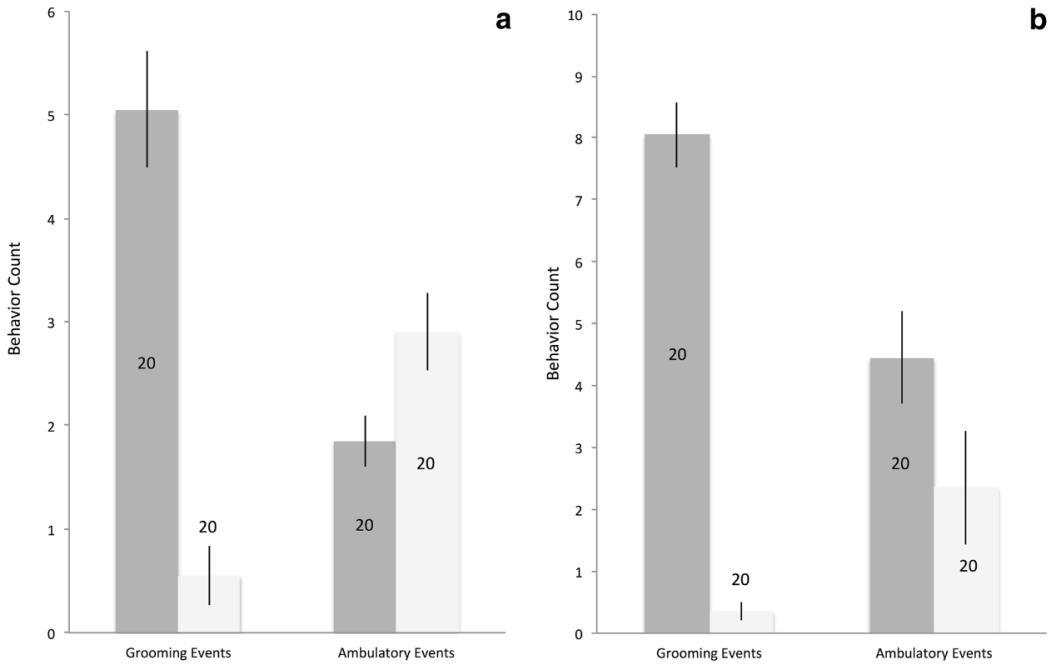


Figure 2.1.3: (a) Mean number of times behaviour was observed during a 30-min reporting period with scans every minute during irritant exposure. Flies were exposed to an irritant or were unexposed controls in glass observation chambers. Flies were scored as grooming, moving, or at rest at each scan. Dark bars represent the irritant-exposed group and light bars represent the control group. Error bars represent ± 1 standard error. (b) Mean number of times behaviour was observed during a 30-min reporting period with scans every minute during exposure to mites. Flies were exposed to three mites or were unexposed in cropped pipette tip observation chambers. Flies were scored as grooming, moving, or at rest at each scan. Dark bars represent the mite-exposed group and light bars represent the control group. Error bars represent ± 1 standard error, and data labels (in/above bar)represent sample size.

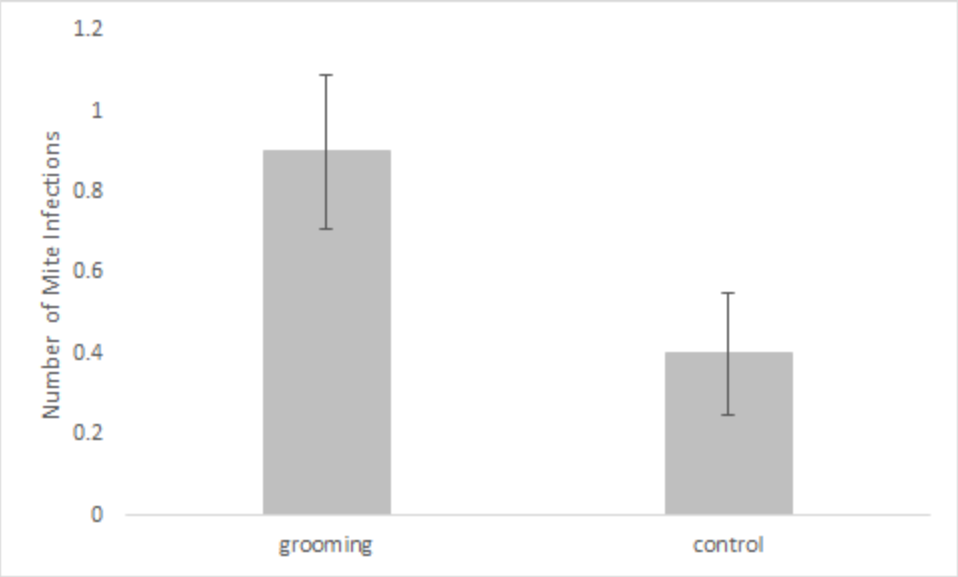


Figure 2.1.4: Mite infections by previous activity of potential hosts. Flies were either induced to groom with volcanic ash or left at rest for 30 minutes. Error bars represent ± 1 standard error.

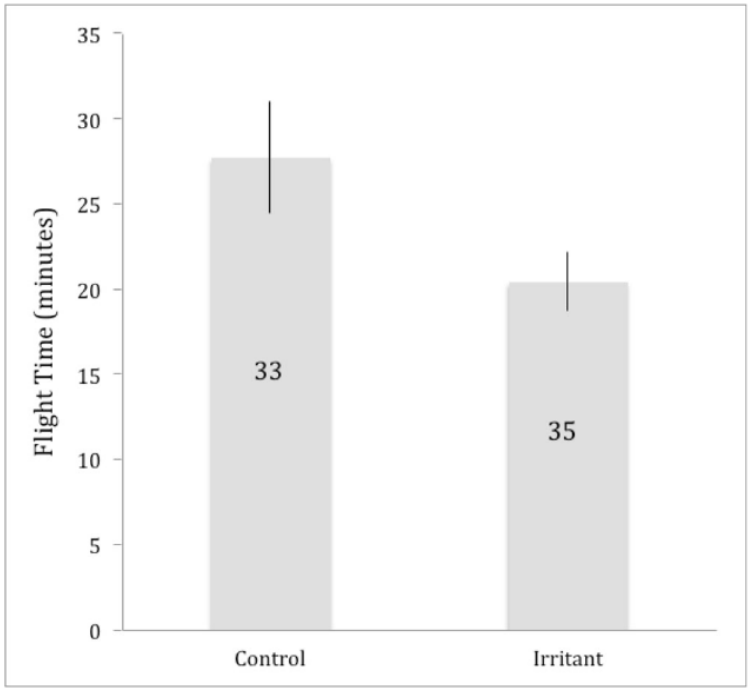


Figure 2.1.5: Flight endurance (measured as hovering time) of flies either induced to groom or left at rest prior to the hovering assay. Flies were attached to a tether using UV curing adhesive allowed to rest for 20 min, then induced to groom with volcanic ash for 30 min. Following the grooming period flies were induced to hover while suspended from a board, and flight time was recorded to the nearest second. Error bars represent ± 1 standard error, and data labels (in bar) represent sample size.

2.1 References

1. Poulin R, Morand S (2000) The diversity of parasites. Q Rev Biol 75: 277–293
2. Robar N, Burness G, Murray DL (2010) Tropics, trophics and taxonomy: the determinants of parasite-associated host mortality. Oikos 119: 1273–1280
3. Sheldon BC, Verhulst S (1996) Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. Trends Ecol Evol 11:317–321
4. Schulenburg H, Kurtz J, Moret Y, Siva-Jothy MT (2009) Introduction ecological immunology. Philos T Roy Soc B 364:3–14
5. Zhukovskaya M, Yanagawa A, Forschler BT (2013) Grooming behavior as a mechanism of insect disease defense. Insects 4:609–630
6. Rohr JR, Swan A, Raffel TR, Hudson PJ (2009) Parasites, info disruption, and the ecology of fear. Oecologia 159:447–454
7. Li JF, Zhang W, Guo ZH, Wu S, Jan LY, Jan YN (2016) A defensive kicking behavior in response to mechanical stimuli mediated by *Drosophila* wing margin bristles. J Neurosci 36:11275–11282
8. Luong LT, Horn CJ, Brophy T (2017b) Mitey costly: energetic costs of parasite avoidance and infection. Physiol Biochem Zool 90:471– 477
9. Garrido M, Adler VH, Pnini M, Abramsky Z, Krasnov BR, Gutman R, Kronfeld-Schor N, Hawlena H (2016) Time budget, oxygen con- sumption and body mass responses to parasites in juvenile and adult wild rodents. Parasite Vector 9:120
10. Auld S, Penczykowski RM, Ochs JH, Grippi DC, Hall SR, Duffy MA (2013) Variation in costs of parasite resistance among natural host populations. J Evol Biol 26:2479–2486
11. Horn CJ, Luong LT (2018) Proximity to parasites reduces host fitness independent of infection in a *Drosophila-Macrocheles* system. Parasitology 145:1564–1569 1–6
12. Giorgi MS, Arlettaz R, Christe P, Vogel P (2001) The energetic grooming costs imposed by a parasitic mite (*Spinturnix myoti*) upon its bat host (*Myotis myotis*). P Roy Soc B-Biol Sci 268:2071–2075
13. Robar N, Murray DL, Burness G (2011) Effects of parasites on host energy expenditure: the resting metabolic rate stalemate. Can J Zool 89:1146–1155
14. Polak M (1996) Ectoparasitic effects on host survival and reproduction: the *Drosophila-Macrocheles* association. Ecology 77:1379–1389
15. Barradale F, Sinha K, Lebestky T (2017) Quantification of *Drosophila* grooming behavior. Jove-J of Vis Exp 50, 55231.
16. Yanagawa A, Neyen C, Lemaitre B, Marion-Poll F (2017) The gram- negative sensing receptor PGRP-LC contributes to grooming induction in *Drosophila*. PLoS One 12:e0185370
17. Yanagawa A, Guigue AMA, Marion-Poll F (2014) Hygienic grooming is induced by contact chemicals in *Drosophila melanogaster*. Front Behav Neurosci 8:254

18. Boroczky K, Wada-Katsumataa A, Batchelor D, Zhukovskaya M, Schal C (2013) Insects groom their antennae to enhance olfactory acuity. P Natl Acad Sci USA 110:3615–3620
19. Markow TA (1988) Reproductive behavior of *Drosophila melanogaster* and *Drosophila nigrospiracula* in the field and in the laboratory. J Comp Psychol 102:169–173
20. Beenakke AM (1969) Carbohydrate and fat as a fuel for insect flight. A comparative study. J Insect Physiol 15:353–361
21. Niven JE, Scharlemann JPW (2005) Do insect metabolic rates at rest and during flight scale with body mass? Biol Lett 1:346–349
22. Bradley CA, Altizer S (2005) Parasites hinder monarch butterfly flight: implications for disease spread in migratory hosts. Ecol Lett 8:290– 300
23. Luong LT, Heath BD, Polak M (2007) Host inbreeding increases susceptibility to ectoparasitism. J Evol Biol 20:79–86
24. Nolan MP, Delaplane KS (2017) Parasite dispersal risk tolerance is mediated by its reproductive value. Anim Behav 132:247–252
25. Lighton JRB (2008) Measuring metabolic rates: a manual for scientists. Oxford University Press, New York, USA
26. Horn CJ, Mierzejewski MK, Luong LT (2018) Host respiration rate and injury-derived cues drive host preference by an ectoparasite of fruit flies. Physiol Biochem Zool 91:896–903
27. de la Flor M, Chen LJ, Manson-Bishop C, Chu TC, Zamora K, Robbins D, Gunaratne G, Roman G (2017) *Drosophila* increase exploration after visually detecting predators. PLoS One 12:e0180749
28. Luong LT, Penoni LR, Horn CJ, Polak M (2015) Physical and physiological costs of ectoparasitic mites on host flight endurance. Ecol Entomol 40:518–524
29. R Studio Team (2015) R Studio: integrated development for R. RStudio, Inc, Boston, MA
30. Polak M, Markow TA (1995) Effect of ectoparasitic mites on sexual selection in a sonoran desert fruit-fly. Evolution 49:660–669
31. Poulin R (2007) Are there general laws in parasite ecology? Parasitology 134:763–776
32. Campbell EO, Luong LT (2016) Mite choice generates sex- and size- biased infection in *Drosophila hydei*. Parasitology 143:787–793
33. Luong LT, Brophy T, Stolz E, Chan SJ (2017) State-dependent parasitism by a facultative parasite of fruit flies. Parasitology 144:1468– 1475
34. Polak M (2003) Heritability of resistance against ectoparasitism in the *Drosophila-Macrocheles* system. J Evol Biol 16:74–82
35. Luong LT, Polak M (2007) Costs of resistance in the *Drosophila-Macrocheles* system: a negative genetic correlation between ectoparasite resistance and reproduction. Evolution 61:1391–1402
36. Hicks O, Burthe SJ, Daunt F, Newell M, Butler A, Ito M, Sato K, Green JA (2018) The energetic cost of parasitism in a wild population. P Roy Soc B-Biol Sci 285:8

37. Johnston JS, Heed WB (1976) Dispersal of desert-adapted *Drosophila*: the *Saguaro*-breeding *D. nigrospiracula*. Am Nat 110:629–651
38. Rigby MC, Hechinger RF, Stevens L (2002) Why should parasite resistance be costly? Trends Parasitol 18:116–120
39. Hawlena H, Bashary D, Abramsky Z, Krasnov BR (2007) Benefits, costs and constraints of anti-parasitic grooming in adult and juvenile rodents. Ethology 113:394–402
40. Boots M, Haraguchi Y (1999) The evolution of costly resistance in host-parasite systems. Am Nat 153:359–370
41. Lefevre T, de Roode JC, Kacsoh BZ, Schlenke TA (2012) Defence strategies against a parasitoid wasp in *Drosophila*: fight or flight? Biol Lett 8:230–233
42. Klemme I, Karvonen A (2017) Vertebrate defense against parasites: interactions between avoidance, resistance, and tolerance. Ecol Evol 7:561–571
43. Pfeiler E, Ngo NM, Markow TA (2005) Linking behavioral ecology with population genetics: insights from *Drosophila nigrospiracula*. Hereditas 142:1–6
44. Raeymaekers JAM, Hablutzel PI, Gregoir AF, Bamps J, Roose AK, Vanhove MPM, Van Steenberge M, Pariselle A, Huyse T, Snoeks J, Volckaert FAM (2013) Contrasting parasite communities among allopatric colour morphs of the Lake Tanganyika cichlid *Tropheus*. BMC Evol Biol 13:41
45. Thornhill R, Fincher CL (2013) The parasite-driven-wedge model of parapatric speciation. J Zool 291:23–33
46. Geraldi NR, Macreadie PI (2013) Restricting prey dispersal can overestimate the importance of predation in trophic cascades. PLoS One 8: 1–9
47. Raffel TR, Martin LB, Rohr JR (2008) Parasites as predators: unifying natural enemy ecology. Trends Ecol Evol 23:610–618
48. Eilam D (2005) Die hard: a blend of freezing and fleeing as a dynamic defense - implications for the control of defensive behavior. Neurosci and Biobehav Rev 29:1181–1191
49. Peckarsky B, Cowan C, Penton M, Anderson C (1993) Sublethal consequences of stream-dwelling predatory stoneflies on mayfly growth and fecundity. Ecology 74:1836–1846
50. Tollrian R, Duggen S, Weiss LC, Laforsch C, Kopp M (2015) Density- dependent adjustment of inducible defenses. Sci Rep-UK 5:12736
51. James WR, McClintock JB (2017) Anti-predator responses of amphipods are more effective in the presence of conspecific chemical cues. Hydrobiologia 797:277–288

2.2: Mating trades-off of with mite resistance in female flies

Introduction

Trade-off theory considers how, given organisms' limited resources (e.g. energy, time, nutrients), investment in one beneficial trait may require reductions in other positive traits [1; 2]. Quintessential examples of life history trade-offs are between longevity and reproduction, as well as between offspring quantity and quality [3; 4; 5]. The cost to benefit ratios in these trade-offs may vary within individuals, between populations, or both [5, 6]. Parasites are a risk to nearly all free-living species and have the potential to impose ecological and evolutionary trade-offs on hosts [7]. Anti-parasite defences (both physiological and behavioural) are plastic responses that may carry costs for hosts: requiring energy investment, reducing time for feeding, and limiting access to valuable resources [8; 9; 10]. Trade-off theory, therefore, can provide a useful framework for understanding variation in host anti-parasite behaviour and by extension heterogeneity in infection outcomes [1].

Reproduction is costly for both invertebrates and vertebrates: it can impair microbial immunity; reduce time available for feeding, grooming, and other forms of maintenance, as well as requiring energetic investment [11; 12; 13; 14; 15; 16]. Trade-offs between reproduction and disease resistance have been observed in many insects (Reviewed in [17]). For example, mating generally reduces the survival of *Drosophila melanogaster* challenged with gram negative bacteria [18; 19]; likewise, mating reduces the ability of aphids to resist parasitoid wasp attack [20]. On the other hand, some studies have observed increased microbial resistance following mating [17]. Currently, there is a paucity of evidence that mating impacts susceptibility of hosts to ectoparasite attack (such as mites). However, given the high energetic costs of both reproduction and ectoparasites, potential trade-offs could have substantial implications for host fitness and populations. In this study, we test if mating has a deleterious effect on the ability of a fly host to resist mite infection.

We use the *Drosophila nigrospiracula - Macrocheles subbadius* system to investigate the trade-off between mating and ectoparasite defence. *D. nigrospiracula* is a cactiphilic fly that feeds on plant exudate (*Carnegiea gigantea*) and reproduces on rotting plant tissue [21; 22]. *M. subbadius* is a facultative ectoparasite of *D. nigrospiracula* that feeds on fly hemolymph and uses flies for dispersal from ephemeral environments [23; 24]. Infection can more than halve the longevity and fecundity of infected females [24]. Consequently, *D. nigrospiracula* have evolved strong defensive responses to mites. These behavioural defences are energetically demanding, as measured by increases in fly metabolic rate during mite exposure [25]. Furthermore, mating is costly for *Drosophila* [26; 27]. Female flies increase investment in egg development following copulation [28; 29]. Additionally, mated *D. simulans* and *D. nigrospiracula* females have higher metabolic rates than unmated conspecifics, suggesting an energetic cost of reproduction [25; 30]. Because reproduction and resistance are both energetically demanding in this system, it provides an opportunity to test for physiological trade-offs between reproduction and ectoparasite resistance [25; 31].

Previous research showed that flies experience a genetic trade-off between resistance and reproduction; flies from lines that underwent laboratory selection for increased mite resistance produce fewer eggs [32; 33]. However, whether there are within-fly trade-offs between mating (copulation and egg development) and the ability to resist mites is unknown. We hypothesise that mated flies will be more susceptible to infection due to trade-offs between behavioural parasite resistance and reproduction. In turn, we predict that mated female flies exposed to mites will have higher levels of infection than unmated females exposed to mites. Given that mites prefer to infect mated female flies over unmated females [25], we also investigated a potential underlying mechanism for the trade-off: that mating reduces overall endurance among female flies as measured by negative geotaxis assays [34].

Methods

Lab populations of *Drosophila nigrospiracula* Patterson and Wheeler originated from >300 flies of each sex collected from the Sonoran desert (Arizona, USA). Flies were cultured on a 3:1 mix of instant mashed potato flakes to *Drosophila* medium (Formula 4–24 Instant *Drosophila* Medium; Carolina Biological Supply Company, Burlington, NC). *D. nigrospiracula* larvae require host plant tissue which was added to the media (autoclaved *Carnegiea gigantea*), and nutritional yeast was sprinkled as a supplement [35]. Since *D. nigrospiracula* females are not sexually mature until 4–5 days post emergence [22], newly emerged adults were transferred to agar medium and aged to maturity in sex separate vials. Fly culture incubators were set at 24℃ and 50% relative humidity with a 12L:12D cycle (Percival Scientific, Perry, IA, USA).

Macrocheles subbadius Berlese cultures were initiated from ~250 female mites collected from wild caught *D. nigrospiracula*. Mite cultures were maintained on a 2:1 mix of wheat bran to wood chips and co-cultured with free-living nematodes as a food source. Mite culture incubators were set at 26 ◦C and 70% relative humidity, with a 12L:12D cycle (Percival Scientific, Perry, IA, USA). Mites for experiments were collected from stock culture using a Berlese funnel and stored on moistened plaster-of-paris until use.

Female flies in experiments 2.1-2.4 were assigned to 1 of 3 groups for experiments: 1) Newly-mated females held with males for 72 h; 2) mated-with-rest females held with males for 48 h then transferred to female only vials for 24 h; and 3) control groups consisting of unmated females. Flies were held at a 2:1 (male to female) ratio in agar vials, except for the unmated control group which was kept at equal density but in female only vials. When flies were moved to new vials, all groups were treated the same way (e.g. same density ±1 fly / vial). Groups were age-matched.

Experiment 1: newly-mated versus unmated female susceptibility to infection

This experiment tested if newly-mated or unmated female flies experienced higher infection abundances (average number of mites per fly) following exposure to multiple mites. Mating was carried out as described above, then individual flies were exposed to 5 adult female mites in micro-arenas

(cropped pipette tip). Both ends of the micro-arena were plugged with cotton, and the micro-arena was covered with an opaque box to exclude light. Flies could not escape but were able to groom and pick at mites. Mites were given an hour to attach to the fly, following which the number of mites was counted under a dissecting microscope. Individual flies were the unit of replication. The dependent variable was the number of mites each fly was infected with (0–5), and the independent variables were the mating status of the fly (newly-mated or unmated) and fly age (glm, family=poisson). Backward model selection was performed by successively removing the least significant independent variable ($P < 0.1$), and comparing the new model with the previous using the anova function (R Stats).

Experiment 2: mated-with-rest versus unmated female susceptibility to infection

This experiment aimed to test if male harassment could explain the increased susceptibility of females to parasitism. Mating was conducted as above, but after 2 days, female flies were removed and placed in female only vials for 24 h. This rest period provided female flies an opportunity to recover from any short-term consequences of male harassment, namely expensive bursts of movement and increased vigilance [36]. Mated-with-rest and unmated females were exposed individually to 5 mites as in Exp. 1. Individual flies were the unit of replication. The dependent variable was the number of mites each fly was infected with (0–5), and the independent variables were the mating status of the fly (mated-with-rest or unmated) and fly age. Backward model selection was otherwise performed as in Experiment 1.

Experiment 3: newly-mated versus mated-with-rest female susceptibility to infection

This experiment directly compared the effect of mating status (newly-mated flies) with male harassment (mated-with-rest), as well as unmated controls. The resistance assay was performed as above. In this experiment, flies were frozen after the assay so their mass could be recorded postmortem. Individual flies were the unit of replication. The dependent variable was the infection status of the fly (infected or uninfected), and the initial independent variables were fly group (newly-mated, mated-with-rest, or unmated), fly age and fly frozen mass. Backward model selection was otherwise performed as in (experiment 1).

Experiment 4: mating status and fly endurance in negative geotaxis assays

This experiment tested if mated females have lower endurance than unmated flies, which may mechanistically explain why mated flies accumulated more infections. Energetically demanding activity can be used as a proxy for resistance capacity [31; 37]. This approach has two advantages 1) it can be directly observed and quantified and 2) it eliminates the confounding variables of mite preferences [25]. Negative geotaxis assays measure energetically demanding induced climbing to evaluate the physical endurance of flies [34; 38]. Individual females (either newly-mated or unmated) were placed into agar vials with a mark 6 cm above the base. Following a minimum of 1 hour for acclimatisation, the base of the vial was tapped against a cork board to knock the fly to the bottom of the vial and a timer was started.

When flies ascended to the 6 cm line the knockdown was repeated. We defined exhaustion as when flies no longer ascended within 10 s of the drop. We recorded the number of times the fly climbed to the line (cycles) and the length of time until exhaustion (seconds, s). The unit of replication was the individual fly. The dependent variable was either the number of cycles flies climbed (count #) or the duration of climbing (seconds), and the independent variable was fly mating status (mated or unmated). Backwards model selection (glm function) was performed as in Experiment 1.

Experiment 5: trade-off between ectoparasite resistance and climbing behaviour

This experiment tested if exhausted flies (as in the negative geotaxis assays) were more susceptible to infection. By testing for a trade-off between climbing endurance and parasite resistance, we sought to confirm that climbing ability is a robust proxy of parasite resistance. Mated female flies were induced to climb in vials and repeatedly knocked down until they would no longer climb (i.e., exhausted, as in experiment 4); control flies were placed into identical individual vials but were not induced to climb. These flies frequently live high on rotting arms of cacti,and this strong negative geotaxis instinct is likely part of their habitat finding (or returning if dislodged) behaviour. After exhaustion, individual flies were moved into micro-arenas containing an adult female mite. Micro-arenas were covered for 2 h then each fly was checked for infection. The unit of replication was individual flies. Infection outcome for each fly (infected, uninfected) was modelled with the independent variable exhaustion group (exhausted via geotaxis or left at rest) (glm, family=binomial), and the model was compared to the null model with the anova function (R stats, test="Chisq").

Data availability

Original data is available in Dryad (doi: 10.5061/dryad.6m905qg0c).

Results

Results 1: newly-mated versus unmated female susceptibility to infection

This experiment tested whether newly-mated females or control unmated females had more infections upon exposure to 5 mites. The number of mite infections ranged from 0 to 4, and a Poisson distribution (glm, family = "poisson") was used to model parasitism. Mating status and fly age were used to model the number of infections each fly experienced. The newly-mated flies (N = 24) had 1.4 ± 0.3 (mean ± SE) mites on average, and the unmated group had a mean 0.71 ± 0.18 mites (N = 21). Mating status was a statistically significant predictor of the number of infections (X^2 = 4.7, P = 0.03). The mean age in the mated group was 13.8 ± 1.1 days (N = 24) and the mean age of the unmated group was 14.0 ± 1.2 days (N = 21); age was not a significant predictor of parasite abundance in this experiment (X^2 = 0.03, P = 0.86).

Results 2: mated-with-rest versus unmated female susceptibility to infection

This experiment tested if male harassment might explain the decreased resistance among mated females by comparing the susceptibility of mated-with-rest flies (N = 24) with unmated controls (N = 18). The number of infections ranged from 0 to 4, and a poisson distribution was used to model the number of mite infections each fly experienced. Mating status and age were considered as predictors of the number of mites. On average, the mated group had 1.17 ± 0.23 mites (N = 24) while the unmated group accumulated 0.50 ± 0.20 mites (N = 18). Mating status was a statistically significant predictor of mite infections (X^2 = 5.5, P = 0.019) (Fig. 2.2.1B). Mated flies, even when given a rest from male harassment, were more susceptible to infection than unmated females. The mean age of the mated group was 15.3 ± 1.2 days old (N = 24) and the mean age of the unmated group was 15.0 ± 1.4 days old (N = 18); age was not a significant predator of parasite abundance in this experiment (X^2 = 0.22, P = 0.63).

Results 3: newly-mated versus mated-with-rest female susceptibility to infection

This experiment directly compared the infection outcomes of newly-mated females (N = 43), mated-with-rest females (N = 45), as well as unmated controls (N = 43) in a single experiment. Overall infection intensity was lower in this experiment than the pairwise experiments, and, therefore, the outcomes were better described as a binary result for each fly (infected or uninfected). The prevalence (# infected flies / total # flies) of infection among unmated controls was 0.28 ± 0.07 (proportion ± SE, N = 43), whereas the proportion of newly-mated group and the mated-with-rest group that became infected were 0.43 ± 0.08 (N = 43) and 0.47 ± 0.07 (N = 45) respectively. Visual inspection of infection outcomes (Fig. 2.2.1C) shows minimal difference in infection outcomes between the newly-mated and mated-with-rest groups (i.e., a recovery period had little effect). Nor was there a significant difference between models that used mating status (mated versus unmated) or group (newly-mated, mated-with-rest, or unmated) as predictors (X^2 ~ 0.00, P = 0.97).

Since no substantial difference was observed between the newly mated and mated-with-rest groups they were pooled into a single “mated” group. After pooling, the prevalence among all mated females was 0.45 ± 0.05 (N = 88). Therefore, we modelled the dependent variable (fly status: infected/uninfected), using mating status, frozen fly mass (mg), and fly age. Mating status (mated or unmated) was a marginal predictor of infection outcome (X^2 = 3.0, P = 0.08). Mated flies, pooling newly-mated and mated-with rest, had higher infection prevalence than unmated females, i.e. mated flies had weaker resistance regardless of post-mating recovery time (Fig. 2.2.1C). Fly mass was also a marginally significant predictor of infection (X^2 = 3.0, P = 0.08); flies that became infected were slightly lighter than uninfected flies, 2.09 ± 0.05 mg and 2.21 ± 0.06 mg respectively. Since flies were frozen immediately after the assays, this difference in mass is unlikely to be a result of mite feeding.

Unlike in the two-group experiments (results 1 & results 2 of this chapter), fly age was a significant predictor of infection outcome in this experiment (X^2 = 4.5, P = 0.03). We considered if age in this model was incidentally detecting stochastic day-to-day variance in mite infectivity by replacing fly age

with the categorical variable “assay date”, then repeating model reduction as above. Assay date was a marginal predictor in these new models (X^2 = 11.0, P = 0.08). When model reduction was performed with “assay date” in lieu of “fly age”, fly mass was a substantially weaker predictor (X^2 = 1.2, P = 0.18), but mating status was a significant predictor (X^2 = 3.8, P = 0.05). In both analyses, mated females were more vulnerable to infection than unmated females.

Results 4: mating status and fly endurance in geotaxis assays

In this experiment, we tested if mated females were more readily exhausted in negative geotaxis endurance assays than unmated controls. By extension we considered if increased susceptibility, not just mite proclivity to infect [25], contributed to increased infection among mated females relative to unmated females (see 3.3, Fig. 2.2.1). We measured the number of knockdowns before exhaustion (cycles) as well as the length of time until exhaustion (seconds), each modelled separately as dependent variables with the predictor “mating status”. Mated females (N = 27) climbed for fewer cycles, 24.6 ± 4.0 cycles, and for less time, 134.5 ± 16.7 s, on average than unmated controls (N = 27), which climbed for 35.4±.4.0 cycles and 187.3 ± 20.4 s. Cycles until exhaustion was modelled with the glm function (family = “poisson”) and time until exhaustion was modelled with the lm function. Mating status was a significant predictor of cycles until exhaustion (Δdeviance = 51.6, P<0.001) and of time until exhaustion (F = 3.9, P = 0.05). Mated flies exhausted faster in endurance assays than unmated conspecifics (Fig 2.2.2).

Results 5: trade-off between ectoparasite resistance and climbing behaviour

This experiment tested if flies exhausted via climbing (N = 30) are more susceptible to parasitism than flies left at rest (N = 30). Post knock-down, 8/30 (27%) of exhausted flies were infected upon exposure, whereas only 2/30 (7%) of flies left at rest became infected (Fig. 2.2.3). Infection outcome for each fly (infected, uninfected) was modelled with the independent variable exhaustion group (exhausted via geotaxis or left at rest) and backwards model reduction was performed. Group was a significant predictor of infection outcome (X^2 = 4.6, P = 0.03). This result suggests climbing endurance is a reasonable proxy measure of resistance capability.

Discussion

We set out to test the hypothesis that mating increases fly susceptibility to ectoparasite infection due to trade-offs between reproduction and parasite defence. Mated female *D. nigrospiracula* consistently acquired more infections than unmated females (Fig. 2.2.1). Additionally, harassment by male flies was not a substantial contributor to the observed increase in susceptibility, because flies that had time to recover after mating had similar infection rates as newly-mated flies (Fig. 2.2.1C). We also sought to rule out the alternate hypothesis that mite preferences for mated females might be solely driving this disparity [25]. In order to do so, we tested the endurance of mated and unmated females in negative geotaxis assays to confirm that reduced fly endurance following mating (a proxy measure of infection resistance,

[37]) was contributing to increased susceptibility. Mated females had reduced endurance relative unmated controls (Fig. 2.2.2). Furthermore, flies exhausted through negative geotaxis had increased susceptibility to infection (Fig. 2.2.3). Together, these results suggest that mating reduces energy available for maintaining defensive behaviour, which in turn increases susceptibility to infection among mated females.

Previous studies of insects have generally found a negative relationship between mating and resistance to bacterial and parasitoid attack, and our results expand these trade-offs to ectoparasite systems [17; 20; 27]. However, in a minority of systems mating increases resistance to bacterial infection [17; 27]. Mating can stimulate female insect antimicrobial peptide production and males may also transfer antimicrobial compounds during copulation as part of a “nuptial gift” [39; 40]. Consequently, mating can, in some circumstances/species, actually improve microbial immunity [17]. On the other hand, antimicrobial compounds do not assist with ectoparasite defences, which are primarily behavioural in *Drosophila* [24]. Thus, trade-offs between resistance and reproduction are unlikely to be masked in ectoparasite-host systems. Additional studies could use ectoparasite-host systems to investigate immunity-mating trade-offs with fewer confounding factors.

The physiological or biochemical mechanism(s) by which mating impacts endurance, and consequently ectoparasite resistance, is currently unknown. Sex peptide, first identified in *D. melanogaster* and widely conserved amongst insects, is transferred from male to female flies in seminal fluid and alters the physiology and behavior of the recipient female [41; 42]. Sex-peptide encourages egg development and prevents reabsorption of developing oocytes, potentially limiting energy available for non-reproductive activities [29; 43]. The behavioural impacts of sex peptide include the induction of ovipositing behaviours and the suppression of copulatory behaviours [44]. Future experiments could tease apart the impacts of egg development/investment and mating *per se* by exposing females to seminal fluid without viable sperm, e.g. from sterile males [45].

We did not observe a substantial difference in susceptibility to infection between newly-mated females and females that were given a rest period after mating to recover from male harassment. Beetle females experience deleterious fitness impacts due to male harassment, independent of copulation, but these effects only manifest in wet environments [46]. Thus, deleterious impacts of male harassment may be environment dependent. Alternatively, the presence/severity of fitness impacts of females due to male harassment may depend on the mating behaviour of the species [47]. For example, the relative interspecific impact of male poeciliid harassment on females depends on the prevalence of sneak copulations in the species [47]. Comparatively, female fish of species that exhibit more courtship behaviour experience fewer deleterious impacts of male exposure [47]. Female *D. nigrospiracula* are choosy, and selectively mate with courting males [22]. Thus, our results are consistent with previous observations that the negative impacts of male harassment are lower in species with courtship and choosy females. Further research is needed to test if male harassment negatively impacts female ectoparasite resistance in host species with different mating systems.

Mated female flies had reduced climbing capacity in the negative geotaxis endurance assays, showing reduced endurance contributed to increased susceptibility (Fig. 2.2.2). However, in nature both mite proclivity to infect and reduced resistance may contribute to increased infection intensities/prevalence among mated females [25]. This current study did not test the relative contributions of mite proclivity and reduced resistance to infection outcomes. Future experiments could attempt to quantify the relative contributions by restraining potential hosts to eliminate behavioural resistance [48].

In addition to mating (Fig. 2.2.1), exhaustion via climbing also made flies more susceptible to infection (Fig. 2.2.3). This is consistent with observations that *D. nigrospiracula* defend themselves through energetically demanding activity, and resistance can be exhausted via forced hovering or grooming [31; 37]. Here we established induced climbing is a proxy measure of resistance capacity. This proxy could be used to investigate other trade-offs that would be confounded by mite preference. For example some ectoparasites perform better on starved hosts relative to well fed hosts, but starvation also reduces energy available for defence [49].

Host defences may be limited by available energy and, therefore, ultimately connected to metabolic rate [50]. Another study found that mated *D. nigrospiracula* females have higher resting metabolic rates than unmated conspecific females [25]. Although the metabolic impacts of parasite infection is well studied, the implications of metabolic rate for future parasite resistance have received relatively little attention [51; 52.]. We posit two hypotheses regarding the relationship between metabolic rate and future parasite resistance: 1) higher metabolic rates suggest more free energy available for defence, or 2) higher metabolic rates suggest energy consumption that cannot be used for other activities (such as parasite resistance). Taken together with our results here, increased metabolic rates among mated *D. nigrospiracula* relative to unmated conspecifics provides support for the hypothesis that increased metabolic rate represents energy that cannot be used for defence against ectoparasites [25]. Alternatively, mites may use CO_2 to locate/discriminate between hosts and increased MR makes it easier for mites to find hosts. Comparative research on additional host-parasite systems could test this hypothesis explicitly and if it is a general trend among parasitic symbioses.

Since reproduction is a primary determinant of fitness, potential hosts may reduce investment in parasite resistance to maximise reproductive effort [53; 54; 55]. However, parasitism is a near universal risk to free-living organisms [7]. Thus, this trade-off can have substantial impacts on the ecology and evolution of the host-parasite relationships. Insects can vary in their reproductive timing [56; 57; 58]. If mating leads to disproportionate infection and density-dependent mortality/morbidity [59; 60], then the pressures of parasitism could ultimately impact the evolution of host reproductive timing. Long-term studies of the impact of parasitism on reproduction and vice-versa, across host lifespans and generations is, therefore, warranted. Since mite resistance is a heritable trait with genetic trade-offs between reproductive ability and resistance ability, the *Drosophila-Macrocheles* association is a potential avenue to investigate these questions [32].

Conclusions

Mated females had more mite infections than unmated females, regardless of whether they had a chance to recover from male harassment. Reduced endurance among mated females likely contributed to increased susceptibility. Potential hosts balance infection risk with reproductive output, and how those competing forces influence host-parasite interactions may have widespread ecological and ultimately coevolutionary consequences.

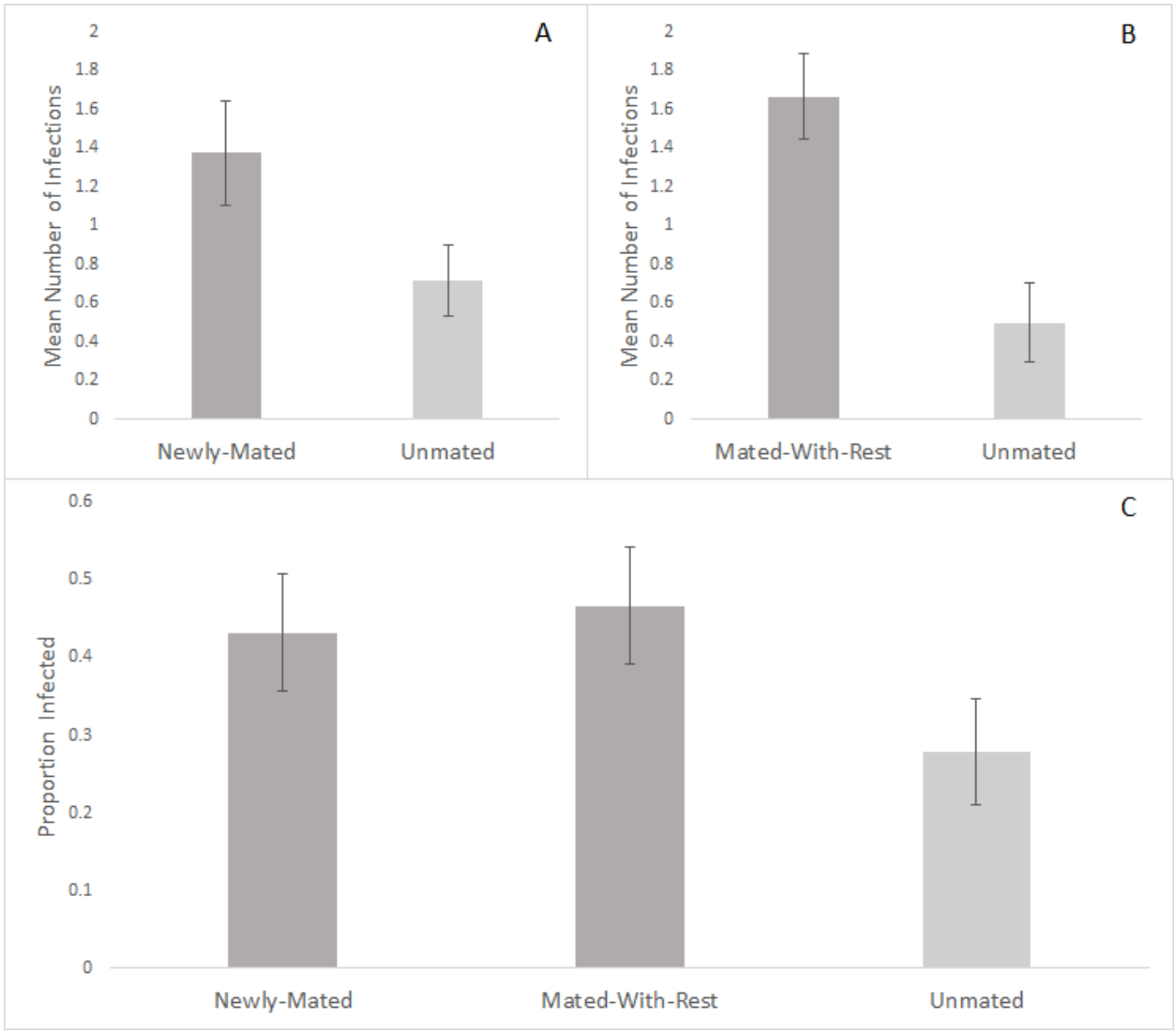


Figure 2.2.1: Mean number of mite infections among new-mated (72-hours with males), mated-with-rest (48 h with males then 24 separate), or unmated female flies exposed individually to 5 mites. Error bars represent standard error of the mean (A, B), or the standard error of the proportion (C).

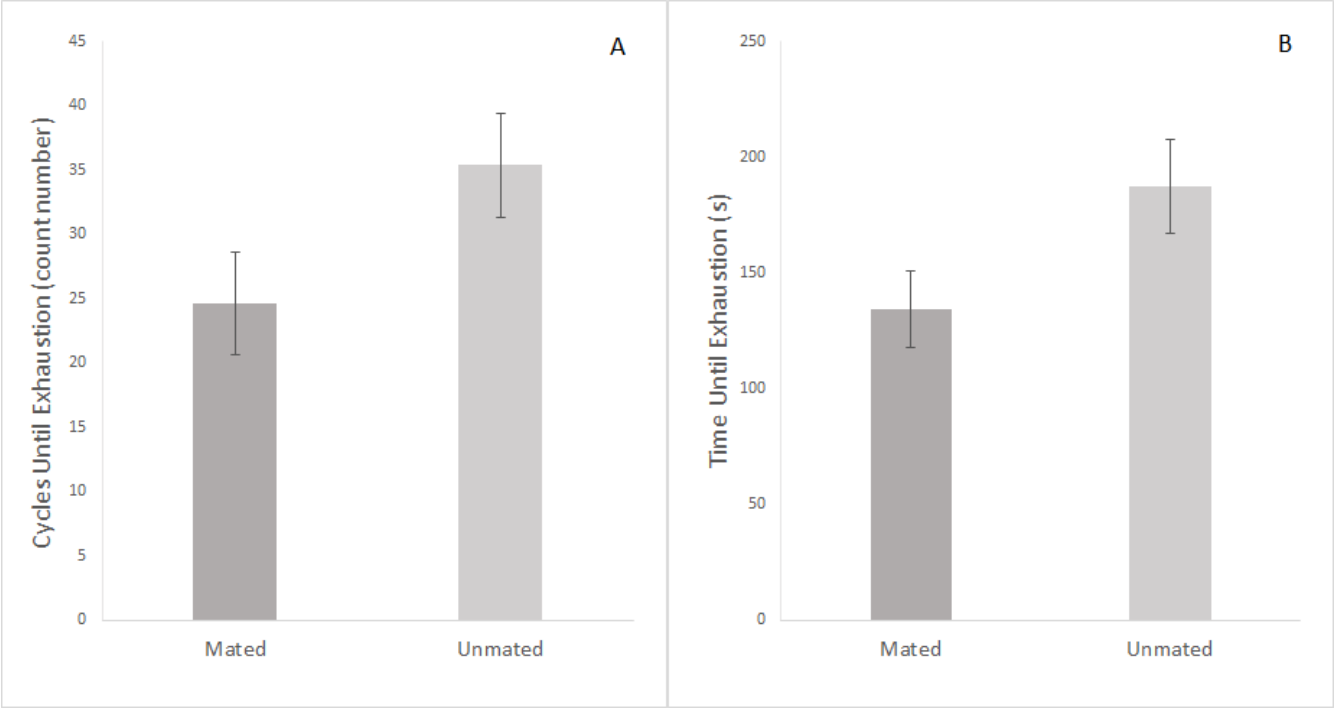


Figure 2.2.2: (A) The number of knockdown cycles until mated or unmated female flies were exhausted in individual negative geotaxis endurance assays. (B) The length of time (seconds) until mated or un-mated female flies were exhausted in the negative geotaxis endurance assays. Error bars represent standard error of the mean.

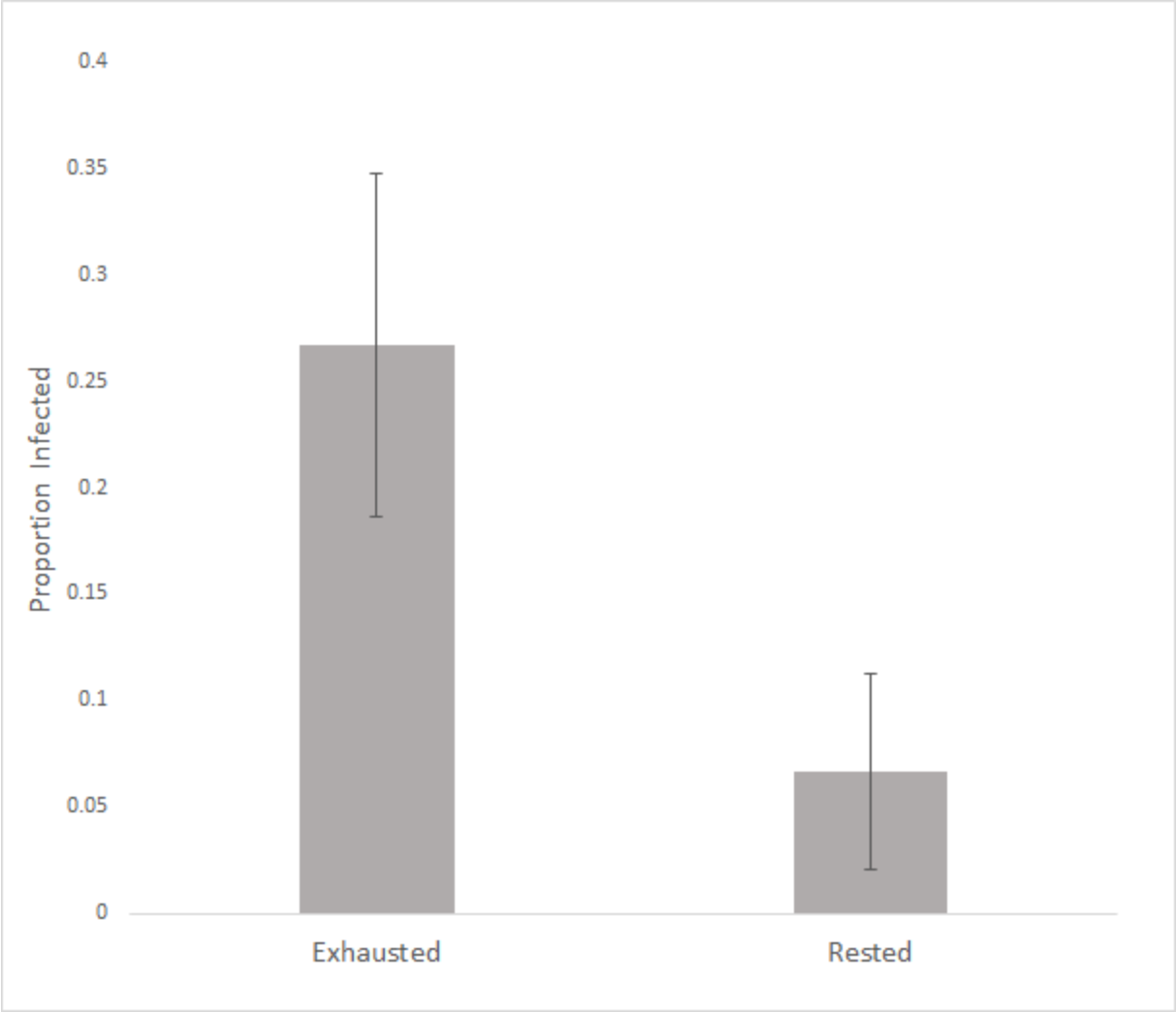


Figure 2.2.3: Infection outcomes of individual female flies exposed to a mite following either exhaustion by geotaxis or left at rest. Error bars represent the standard error of the proportion.

2.2 References

1. M.R.Hutchings, J.Judge,I. J.Gordon, S.Athanasiadou, I.Kyriazakis, Use of trade-off theory to advance understanding of herbivore-parasite interactions, Mamm. Rev. 36 (2006) 1–16, https://doi.org/10.1111/j.1365-2907.2006.00080.x.
2. S.C. Stearns, Trade-offs in life-history evolution, Funct. Ecol. 3 (1989) 259–268, https://doi.org/10.2307/2389364.
3. R.M. Cox, E.U. Parker, D.M. Cheney, A.L. Liebl, L.B. Martin, R. Calsbeek, Experimental evidence for physiological costs underlying the trade-off between reproduction and survival, Funct. Ecol. 24 (2010) 1262–1269, https://doi.org/ 10.1111/j.1365-2435.2010.01756.x.
4. A. Koliada, K. Gavrilyuk, N. Burdylyuk, O. Strilbytska, K.B. Storey, V. Kuharskii, O. Lushchak, A. Vaiserman, Mating status affects *Drosophila* lifespan, metabolism and antioxidant system, Comparat. Biochem. Physiol. a-Molecular Integrat. Physiol. 246 (2020), https://doi.org/10.1016/j.cbpa.2020.110716.
5. T.Mappes,E.Koskela, Genetic basis of the trade-off between off spring number and quality in the bank vole, Evolution (N Y) 58 (2004) 645–650, https://doi.org/ 10.1111/j.0014-3820.2004.tb01686.x.
6. G.S. Betini, A.G. McAdam, C.K. Griswold, D.R. Norris, A fitness trade-off between seasons causes multigenerational cycles in phenotype and population size, Elife 6 (2017), https://doi.org/10.7554/eLife.18770.
7. R. Poulin, S. Morand, The diversity of parasites, Q. Revi. Biol. 75 (2000) 277–293, https://doi.org/10.1086/393500.
8. H. Hawlena, D. Bashary, Z. Abramsky, B.R. Krasnov, Benefits, costs and constraints of anti-parasitic grooming in adult and juvenile rodents, Ethol. 113 (2007) 394–402, https://doi.org/10.1111/j.1439-0310.2007.01332.x.
9. L.E.Nadler,E.Bengston,E.J.Eliason,C.Hassibi,S.H.Helland-Riise,I.B.Johansen, G.T. Kwan, M. Tresguerres, A.V. Turner, K.L. Weinersmith, O. Overli, R. F. Hechinger, A brain-infecting parasite impacts host metabolism both during exposure and after infection is established, Funct. Ecol. 35 (2021) 105–116, https://doi.org/10.1111/1365-2435.13695.
10. S.S. French, D.F DeNardo, M.C. Moore, Trade-offs between the reproductive and immune systems: Facultative responses to resources or obligate responses to reproduction? American Naturalist 170 (2007) 79–89, https://doi.org/10.1086/ 518569.
11. P. Bergeron, V. Careau, M.M. Humphries, D. Reale, J.R. Speakman, D. Garant, The energetic and oxidative costs of reproduction in a free-ranging rodent, Funct. Ecol. 25 (2011) 1063–1071, https://doi.org/10.1111/j.1365-2435.2011.01868.x.
12. K.M. Fedorka, J.E. Linder, W. Winterhalter, D. Promislow, Post-mating disparity between potential and realized immune response in *Drosophila melanogaster*, Proceed. Roy. Societ. B-Biological Sci. 274 (2007) 1211–1217, https://doi.org/ 10.1098/rspb.2006.0394.

13. S. Paukku, J.S. Kotiaho, Cost of reproduction in *Callosobruchus maculatus*: effects of mating on male longevity and the effect of male mating status on female longevity, J. Insect Physiol. 51 (2005) 1220–1226, https://doi.org/10.1016/j. jinsphys.2005.06.012.
14. B.C. Sheldon, S. Verhulst, Ecological immunology: costly parasite defenses and trade-offs in evolutionary ecology, Trends in Ecology & EvolutionTrends Ecol. Evol. (Amst.) 11 (1996) 317–321, https://doi.org/10.1016/0169-5347(96)10039- 2.
15. S. Wigby, T. Chapman, Sex peptide causes mating costs in female *Drosophila melanogaster*, Curr. Biol. 15 (2005) 316–321, https://doi.org/10.1016/j. cub.2005.01.051.
16. D.R. Ardia, K.A. Schat, D.W. Winkler, Reproductive effort reduces long-term immune function in breeding tree swallows (*Tachycineta bicolor*), Proceedings of the Royal Society B-Biological Sciences 270 (2003) 1679–1683, https://doi.org/ 10.1098/rspb.2003.2424.
17. R.A. Schwenke, B.P. Lazzaro, M.F. Wolfner, Reproduction-Immunity trade-offs in insects, Annu. Rev. Entomol. 61 (61) (2016) 239–256, https://doi.org/10.1146/ annurev-ento-010715-023924.
18. K.M. Fedorka, M. Zuk, Sexual conflict and female immune suppression in the cricket, *Allonemobious socius*, J. Evol. Biol. 18 (2005) 1515–1522, https://doi.org/ 10.1111/j.1420-9101.2005.00942.x.
19. S.M. Short, M.F. Wolfner, B.P. Lazzaro, Female Drosophila melanogaster suffer reduced defense against infection due to seminal fluid components, J. Insect Physiol. 58 (2012) 1192–1201, https://doi.org/10.1016/j.jinsphys.2012.06.002.
20. D.M.Gwynn, A.Callaghan, J.Gorham, K.F.A.Walters, M.D.E.Fellowes, Resistance is costly: trade-offs between immunity, fecundity and survival in the pea aphid, Proceed. Roy. Society B-Biological Sci. 272 (2005) 1803–1808, https://doi.org/ 10.1098/rspb.2005.3089.
21. M.R. Frank, J.C. Fogleman, Involvement of cytochrome-p450 in host-plant utilization by sonoran desert *Drosophila*, Proc. Natl. Acad. Sci. U.S.A. 89 (1992) 11998–12002, https://doi.org/10.1073/pnas.89.24.11998.
22. T.A. Markow, Reproductive behavior of *Drosophila melanogaster* and *Drosophila nigrospiracula* in the field and in the laboratory, J. Comp. Psychol. 102 (1988) 169–173.
23. L.T. Luong, D. Subasinghe, A facultative ectoparasite attains higher reproductive success as a parasite than its free-living conspecifics, Experimental and Appl. Acarol. 71 (2017) 63–70, https://doi.org/10.1007/s10493-016-0098-2.
24. M. Polak, Ectoparasitic effects on host survival and reproduction: the *Drosophila macrocheles* association, Ecol. 77 (1996) 1379–1389, https://doi.org/10.2307/ 2265535.
25. C.J. Horn, M.K. Mierzejewski, M.E. Elahi, L.T. Luong, Extending the ecology of fear: parasite-mediated sexual selection drives host response to parasites, Physiol. Behav. 224 (2020), https://doi.org/10.1016/j.physbeh.2020.113041.

26. T. Chapman, J. Hutchings, L. Partridge, No reduction in the cost of mating for *Drosophila melanogaster* females mating with spermless males, Proceed. Royal Societ. B 253 (1993) 211–217, https://doi.org/10.1098/rspb.1993.0105.
27. K. Oku, T.A.R. Price, N. Wedell, Does mating negatively affect female immune defences in insects? Anim. Biol. 69 (2019) 117–136, https://doi.org/10.1163/ 15707563-20191082.
28. M. Soller, M. Bownes, E. Kubli, Mating and sex peptide stimulate the accumulation of yolk in oocytes of *Drosophila melanogaster*, Europ. J. Biochem. 243 (1997) 732–738, https://doi.org/10.1111/j.1432-1033.1997.00732.x.
29. M. Soller, M. Bownes, E. Kubli, Control of oocyte maturation in sexually mature *Drosophila* females, Dev. Biol. 208 (1999) 337–351, https://doi.org/10.1006/ dbio.1999.9210.
30. J.T. Giesel, C.A. Lanciani, J.F. Anderson, Metabolic-rate and sexual-activity in *Drosophila simulans*, J. Insect Physiol. 35 (1989) 893–895, https://doi.org/ 10.1016/0022-1910(89)90106-6.
31. C.J. Horn, L.T. Luong, Current parasite resistance trades off with future defenses and flight performance, Behav. Ecol. Sociobiol. (Print) 73 (2019), https://doi.org/10.1007/s00265-019-2697-5.
32. L.T. Luong, M. Polak, Costs of resistance in the *Drosophila-Macrocheles* system: a negative genetic correlation between ectoparasite resistance and reproduction, Evolut. (N. Y.) 61 (2007) 1391–1402, https://doi.org/10.1111/j.1558- 5646.2007.00116.x.
33. L.T. Luong, M. Polak, Environment-dependent trade-offs between ectoparasite resistance and larval competitive ability in the *Drosophila-Macrocheles* system, Heredity (Edinb) 99 (2007) 632–640, https://doi.org/10.1038/sj. hdy.6801040.
34. M.J. Tinkerhess, L. Healy, M. Morgan, A. Sujkowski, E. Matthys, L. Zheng, R. J. Wessells, The *Drosophila* pgc-1 alpha homolog spargel modulates the physiological effects of endurance exercise, PLoS ONE 7 (2012), https://doi.org/ 10.1371/journal.pone.0031633.
35. M.K.Mierzejewski, C.J.Horn, L.T.Luong, Ecology of fear: environment-dependent parasite avoidance among ovipositing drosophila, Parasitol. 146 (2019) 1564–1570, https://doi.org/10.1017/s0031182019000854.
36. Y. Takahashi, M. Watanabe, Female reproductive success is affected by selective male harassment in the damselfly by *Ischnura senegalensis*, Anim. Behav. 79 (2010) 211–216, https://doi.org/10.1016/j.anbehav.2009.10.032.
37. L.T. Luong, B.D. Heath, M. Polak, Host inbreeding increases susceptibility to ectoparasitism, J. Evol. Biol. 20 (2007) 79–86, https://doi.org/10.1111/j.1420- 9101.2006.01226.x.
38. Barone M.C., Bohmann D., 2013. Assessing neurodegenerative phenotypes in *Drosophila* dopaminergic neurons by climbing assays and whole brain immunostaining. Jove-Journal Visualiz. Exper.. doi: 10.3791/50339.

39. S.H. Liu, H.F. Li, Y. Yang, D. Wei, H.B. Jiang, W. Dou, G.R. Yuan, J.J. Wang, Antimicrobial peptide gene bdpho responds to peptidoglycan infection and mating stimulation in oriental fruit fly, *Bactrocera dorsalis* (hendel), AMB. Express 8 (2018), https://doi.org/10.1186/s13568-017-0533-8.
40. O. Lung, L. Kuo, M.F. Wolfner, *Drosophila* males transfer antibacterial proteins from their accessory gland and ejaculatory duct to their mates, J. Insect Physiol. 47 (2001) 617–622, https://doi.org/10.1016/s0022-1910(00)00151-7.
41. P.S. Chen, E. Stummzollinger, T. Aigaki, J. Balmer, M. Bienz, P. Bohlen, A male accessory-gland peptide that regulates reproductive-behavior of female *Drosophila melanogaster*, Cell 54 (1988) 291–298, https://doi.org/10.1016/0092-8674(88) 90192-4.
42. N. Yapici, Y.J. Kim, C. Ribeiro, B.J. Dickson, A receptor that mediates the post- mating switch in *Drosophila* reproductive behaviour, Nat. 451 (2008), https://doi. org/10.1038/nature06483, 33-U31.
43. T. Chapman, J. Bangham, G. Vinti, B. Seifried, O. Lung, M.F. Wolfner, H.K. Smith, L. Partridge, The sex peptide of *Drosophila melanogaster*: female post-mating responses analyzed by using rna interference, Proc. Natl. Acad. Sci. U.S.A. 100 (2003) 9923–9928, https://doi.org/10.1073/pnas.1631635100.
44. I.U. Haussmann, Y. Hemani, T. Wijesekera, B. Dauwalder, M. Soller, Multiple pathways mediate the sex-peptide-regulated switch in female *Drosophila* reproductive behaviours, Proceed. Roy. Societ. B-Biological Sci. 280 (2013), https://doi.org/10.1098/rspb.2013.1938.
45. L. Xue, M. Noll, *Drosophila* female sexual behavior induced by sterile males showing copulation complementation, Proceed. Nat. Acad. Sci. U.S.A. 97 (2000) 3272–3275, https://doi.org/10.1073/pnas.060018897.
46. Iglesias-Carrasco M Jigisha, A. Vincent, M.L. Head, Disentangling the costs of mating and harassment across different environments, Anim. Behav. 165 (2020) 79–88, https://doi.org/10.1016/j.anbehav.2020.05.005.
47. M. Dadda, Female social response to male sexual harassment in poeciliid fish: a comparison of six species, Front. Psychol. 6 (2015), https://doi.org/10.3389/ fpsyg.2015.01453.
48. E.O. Campbell, L.T. Luong, Mite choice generates sex- and size-biased infection in *Drosophila hydei*, Parasitol. 143 (2016) 787–793, https://doi.org/10.1017/s0031182016000305.
49. B.R. Krasnov, I.S. Khokhlova, M.S. Arakelyan, A.A. Degen, Is a starving host tastier? reproduction in fleas parasitizing food-limited rodents, Funct. Ecol. 19 (2005) 625–631.
50. J.H. Brown, J.F. Gillooly, A.P. Allen, V.M. Savage, G.B. West, Toward a metabolic theory of ecology, Ecol. 85 (2004) 1771–1789, https://doi.org/10.1890/03-9000.
51. O. Hicks, S.J. Burthe, F. Daunt, M. Newell, A. Butler, M. Ito, K. Sato, J.A. Green, The energetic cost of parasitism in a wild population, Proceed. Roy. Societ. B- Biological Sci. 285 (2018) 8, https://doi.org/10.1098/rspb.2018.0489.

52. N. Robar, D.L. Murray, G. Burness, Effects of parasites on host energy expenditure: the resting metabolic rate stalemate, Canad. J. Zoology-Revue Canadien. De Zoologie 89 (2011) 1146–1155, https://doi.org/10.1139/z11-084.
53. M.Boots,Y.Haraguchi,The evolution of costly resistance in host-parasite systems, Am. Natural. 153 (1999) 359–370, https://doi.org/10.1086/303181.
54. B. Koskella, Resistance gained, resistance lost: an explanation for host-parasite coexistence, PLoS Biol. 16 (2018), e3000013, https://doi.org/10.1371/journal. pbio.3000013.
55. D. Nordling, M. Andersson, S. Zohari, L. Gustafsson, Reproductive effort reduces specific immune response and parasite resistance, Proceed. Roy. Societ. B- Biological Sci. 265 (1998) 1291–1298, https://doi.org/10.1098/rspb.1998.0432.
56. D. Berger, M. Olofsson, M. Friberg, B. Karlsson, C. Wiklund, K. Gotthard, Intraspecific variation in body size and the rate of reproduction in female insects - adaptive allometry or biophysical constraint? J. Anim. Ecol. 81 (2012) 1244–1258, https://doi.org/10.1111/j.1365-2656.2012.02010.x.
57. J.Ellers,M.Jervis,Bodysizeandthetimingofeggproductioninparasitoidwasps, Oikos 102 (2003) 164–172, https://doi.org/10.1034/j.1600-0706.2003.12285.x.
58. Y. Tsuda, Reproductive strategy of insects as adaptation to temporally varying environments, Res. Popul. Ecol. (Kyoto) 24 (1982) 388–404, https://doi.org/ 10.1007/bf02515584.
59. T. Brophy, L.T. Luong, Ectoparasite-induced increase in *Drosophila* host metabolic rate, Physiol. Entomol. 46 (2021) 1–7, https://doi.org/10.1111/phen.12334.
60. M. Polak, W.T. Starmer, Parasite-induced risk of mortality elevates reproductive effort in male *Drosophila*, Proceed. Roy. Society B-Biological Sci. 265 (1998) 2197–2201.

Chapter 3: Host variation in non-consumptive effects

Chapter 3 Introduction

In this section I tested the hypothesis that hosts will vary in the NCEs they experience based on their traits (specifically, sex and mating status). We considered changes in metabolic rate due to increased defensive behaviours and stress (3.1), and phototaxis behaviour during mite exposure (3.2). Flies varied in their metabolic responses to mite presence. Flies with higher costs of infection (from parasite-mediated sexual selection) had the strongest responses, specifically male and unmated females had stronger responses to mites than female and mated females respectively (3.1). On the other hand, unmated females did not have a noticeably larger change in phototaxis during mite exposure compared to mated females. Thus intra-specific variation in responses to parasites, and by extension resultant NCEs, may be dependent on the behaviours and potentially environment observed.

3.1: Sex and mating status differences in metabolic responses to mites

Introduction

The ecology of fear describes the negative effects of predators on their potential prey outside of direct attacks, i.e. non-consumptive effects (NCEs) [1; 2; 3; 4]. Even if the individual prey is not consumed, it may still experience prolonged stress, decreased feeding success, depressed immunity, reduced growth, and ultimately lower fitness [5; 6; 7; 8]. Males and females often differ in their predation risk [9; 10]. Consequently, they may exhibit dissimilar responses to predation risk and resultant non-consumptive costs [11; 12; 13]. For example, male crickets experienced larger increases in metabolic rate (MR) when exposed to predator-derived fecal cues than female crickets [12]. These sex-biased outcomes show that the NCEs of predators are not borne equally by members of the prey population [12].

A growing number of studies have attempted to apply the ecology of fear framework to describe host-parasite interactions outside of infection [14; 15; 16; 17]. Tadpoles show comparable avoidance of parasite infectious stages and predator cues [18], albeit in a hierarchical manner [19]. Likewise, large tick populations can cause grazing small mammals and ungulates to leave foraging sites earlier than sites lacking ticks [20; 21; 22]. These studies suggest that ectoparasites can cause would-be hosts to forgo foraging at otherwise preferred sites and potentially cause trade-offs between nutrition and parasite avoidance [20; 22].

Variation in the risks and costs of infection may influence the evolution of different parasite avoidance strategies, and it is reasonable to also expect intraspecific variation in NCEs [14; 23]. Individuals differ in their responses to infection risk by sex and reproductive status, and these differences could lead to variation in NCEs [24; 25; 26]. However, unlike studies examining predator-prey systems [12; 13], little research has directly tested how parasite-mediated NCEs vary within host species.

Here we measured intraspecific variation in NCEs in terms of host metabolic changes (measured as rates of CO_2 production). Avoiding parasites and predators can be energetically costly [12; 27], likely due to increases in activity, immunity, and/or stress [28; 29]. Because of these energetic costs, MR

measurements integrate the costs of behavioural and physiological responses into a shared currency [30].

We investigated the NCEs an ectoparasitic mite, *Macrocheles subbadius*, has on *Drosophila nigrospiracula* [31]. Mites have consumptive effects on flies when they feed on host hemolymph [32]. Flies defend themselves primarily through bouts of intense grooming, kicking, jumping, or bursts of flight, and melanization is also observed at mite attachment sites [16; 32; 33]. Mite infection can more than halve fly longevity as well as reduce the fecundity of infected female flies by up to two-thirds [32; 34]. Additionally, exposure to mites also elicits non- consumptive effects [35]. Flies have elevated MR when exposed to mites across a mesh screen and when induced to groom, [27; 29]. Chronic parasite exposure also causes fitness losses among would-be hosts females reared adjacent to mites had ~13% fewer adult offspring than unexposed flies and a ~23% reduction in lifespan [36]. Because hosts in this system are known to experience NCEs from exposure to mites, without infection, it provides an opportunity to investigate variation in non-consumptive interactions. We anticipate variation between flies in the strength of NCEs they experience based on the relative costs and risks of infection they experience.

Male and female *D. nigrospiracula* both experience intensity-dependent reductions in longevity and reproductive success due to infection with *M. subbadius* [32; 34; 37]. However, parasite-mediated sexual selection is significantly stronger for male flies than female flies [37]. Mite infection reduces the odds of copulating more for male than female flies, and fewer infections are required to completely exclude male flies from mating than female flies [37]. These differences may be partially due to mites physically obstructing mating attempts by infected males [38]. We, therefore, hypothesise that males are under selection to mount stronger anti-parasite responses than females due to asymmetrical costs of infection. Consequently, the cost of infection hypothesis predicts that male flies will experience larger increases in MR (a measure of energetic expenditure) upon exposure to mites than female flies.

If asymmetrical costs resulting from parasite-mediated sexual selection drive variation in responses to mites, then we also anticipate that unmated females will have stronger responses to mites than mated females. Mite infection reduces the odds of copulation for female flies, albeit less than males; however, sexual selection is tautologically reduced in already mated females [32; 37]. As such, unmated females are likely adapted to respond more strongly to mites than mated females [37]. Therefore, the cost of infection hypothesis also predicts that unmated female flies will experience a larger increase in MR compared to mated females upon exposure to mites.

Sex-biased infections are commonly observed in vertebrate [39; 40], invertebrate [41; 42], and even dioecious plant systems [43; 44]. Differential risk of infection between sexes can occur because many ectoparasites actively seek out and selectively attack hosts based on host characteristics [39; 41; 45]. Currently, whether *M. subbadius* prefers to infect male or female *D. nigrospiracula* is not known, but it is known *M. subbadius* distinguishes between potential hosts based on MR [46]. The congeneric mite *M. muscaedomesticae* preferentially infects female *D. hydei* over male flies in pair-wise choice tests [41]. Similarly, we predict *M. subbadius* will prefer to infect female *D. nigrospiracula* over males. In preliminary

preference experiments, we found that *M. subbadius* selectively infects female *D. nigrospiracula* over males (Fig. 3.1.1). Since the preferred sex experiences a higher risk of infection, we hypothesize that the preferred sex will be under stronger selection to respond to parasites [47; 48]. The asymmetrical risk of infection hypothesis predicts that female flies — the preferred host of the parasite — will exhibit a larger increase in MR upon parasite exposure than males. Further trials showed that mites have a weaker preference for mated females over unmated females (Fig 3.1.1). Thus, the asymmetrical risk hypothesis also predicts that mated females will have a stronger response to mite exposure than unmated females.

We experimentally tested two hypotheses by comparing the MR of male and female flies as well as mated versus unmated female flies at rest and during mite exposure. **H1: The asymmetrical costs hypothesis** predicts that male flies will experience larger increases in MR when exposed to mites than female flies, and unmated females will have larger increases in MR than mated females. **H2: The asymmetrical risks hypothesis** predicts that female flies will experience a larger mite-induced increase in MR than male flies, and that mated females will exhibit a larger increase in MR than unmated females. In order to elucidate the relative contribution of physiological and behavioural changes to inter-group differences in MR, we also measured fly activity simultaneously with respiration.

Methods

Mite and cultures

Drosophila nigrospiracula Patterson and Wheeler cultures were founded from approximately 150 flies of each sex collected from cactus (*Camegiea gigantea*) rots located in the Sonoran Desert (Phoenix, Arizona). Flies were cultured on a 3:1 mix of instant potatoes to *Drosophila* formula (Formula 4-24 Instant *Drosophila* Medium, Carolina Biological Supply Company, Burlington, NC, USA). Because *D. nigrospiracula* larvae fail to pupate in the absence of host plant material, autoclaved cactus was added to culture bottles [16]. Nutritional yeast was added to the media as a supplement. Following emergence, adult flies were moved to sex-separate agar vials before reproductive maturity [49]. Flies were stored in incubators at 24ºC and 50% relative humidity until they were used in the experiments below (Percival Scientific, Perry, IA, USA).

Macrocheles subbadius (Berlese) cultures were founded from approximately 200 adult female mites collected from wild caught *D. nigrospiracula*. Mites were reared on a 2:1 wheat bran to wood chips mixture moistened with distilled water and co-cultured with bacteriophagic nematodes as a food source. Mite cultures were stored in incubators at 26 ºC and 70% relative humidity (Percival Scientific, Perry, IA, USA). Adult female mites, the infectious stage, were collected from the stock culture using Berlese funnels and stored in specimen cups lined with moistened paper towel or Plaster-of-Paris until experiments. The number of mites used in each experiment was chosen based on the size of the lab population.

Host preferences of mites

Host sex

This experiment tested whether *M. subbadius* preferentially infected female or male *D. nigrospiracula* in choice-tests. Age-matched unmated male and female flies were anaesthetised with CO_2 and glued (Elmer's rubber cement) to cotton in order to eliminate behavioural resistance [41]. Following a recovery period, a fly was transferred to each arm of a y-shaped maze (Fisherbrand Tubing, Y Polypropylene Connectors). To control for idiosyncratic biases, the male and female flies alternated between the left and right arms. A single adult female mite was then placed in the free arm and the maze was sealed with cotton. We placed mazes under an opaque box to exclude light as mites are more likely to infect in the dark (pers. obs). After 1 h of mite exposure, the y-maze was inspected. A trial was considered successful if the mite infected either fly, then the sex of the infected fly was recorded. In a subset of successful trials (19 of 31) the masses of both flies were recorded before the assay. Between assays, the y-mazes were washed with detergent, sterilised with 70% ethanol, then rinsed with distilled water to remove chemical cues left behind by mites or flies. Appendix 1 considers if mites have a preference for larger females over smaller females.

A second experiment tested if the preference for female flies was driven by sexual dimorphism in size, since female *Drosophila* are on average larger than conspecific males ([41], 3.1.1). In this follow-up experiment male and female flies were size (mass) matched to within 5% and assigned as matched-pairs to the y-mazes. The choice tests were otherwise conducted as above. Binomial tests (binom.test) were used to test if flies disproportionately infected male or female flies (R Stats package).

Host reproductive status

Pair-wise choice tests, as in the section above, were used to determine if mites preferred to infect mated or unmated females. Flies were mated by placing them into agar vials at a 2:1 ratio of males to females. This biased ratio ensured nearly all female flies were mated following the 72-hour mating period. Pairwise choice tests were conducted 72 h post-mating. Mated and unmated females were size matched (mass difference within 5%) before each assay. Each matched-pair was then transferred to a y-maze. A single adult female mite was introduced to the maze and allowed 2 h to infect while the y-maze was covered with an opaque box. A binomial test (binom.test) was used to test if flies disproportionately infected mated or unmated females.

Fly responses to mite exposure

Sex differences

To test which fly sex has a stronger response to parasites we measured the rate of CO_2 production, a proxy for metabolic rate, of male and female flies either unexposed or exposed to mites using flow-through respirometry (illustrated in [27]). Unexposed flies were in otherwise empty respirometry chambers, whereas flies in the exposed condition were in chambers with 3 mites. During the exposed

trials, an extra chamber containing only 3 mites was also measured. A Li-7000 infrared analyzer was used to measure the concentration of CO_2 produced by individual flies (Li-COR Biosciences, Lincoln, NE). A MAVen FT system (Sable Systems, Las Vegas, NV) was used to direct inflow air, at 30 mL/min, through either a respirometry chamber or the baseline. The real time flow rate was recorded by the MAVen-ET and used in the calculation of CO_2 production rate (calculation described in [46]). In order to improve sensitivity, incoming air was purged of CO_2 and water vapour using a purge gas generator (FT-IR Purge Gas Generator 75-45, Parker Canada Division, Milton Ontario). Excurrent air was scrubbed of water vapour by passing the excurrent air through a magnesium per- chlorate column before analysis with the Li-7000. A reference cell of bone-dry CO_2-free air also produced via the purge generator was used to enable the Li-7000 to measure the excurrent gas. The Li-7000 was spanned periodically with dry 20 ppm CO_2 at 30 mL/min (Praxair, Danbury, CT) and zeroed before each assay. Female and male flies were placed in alternating experimental chambers and the CO_2 production of each fly was recorded sequentially for 300 s (1 observation / second). The 300 observations were averaged to calculate a mean respiration rate for each fly using the Expedata software. Following respirometry measurements, the flies were frozen so that mass could be measured afterwards.

We recorded the activity of flies using an infrared monitor simultaneously with MR in order to test if changes in activity (measured in arbitrary voltage) accounted for differences between male and female MR (Sable Systems, Las Vegas, NV). Activity measurements for flies within the same replicate block were conducted concurrently. The activity monitor primarily detects translocation and large movements, which increase during parasite exposure [27].

Following each assay, we visually inspected the flies for mites. Infection occurred at negligible rates and at sub-pathological intensities (2 flies across all experiments acquired 1 mite each) [29]. Thus, the MR changes we detected are primarily due to fear, not infection. This low rate of infection is likely due to ample space permitting fly defences, the presence of light that inhibits mite infection (overhead lights were off, but ambient light could not be fully excluded with the activity monitor in place), and/or relatively short exposure times precluding infection.

We analysed MR using the lmer and glmer (family = Gamma) functions (lme.4 package), based on visual inspection of the data. Backwards model selection was performed by sequentially removing fixed effects from the model. Model comparison was carried out with the anova function (test = X^2) for lmer models and the Wald t-statistic for glmer models. If there was a significant difference between models with and without the explanatory variable the variable was retained. CO_2 production was modeled with the fixed predictors: fly sex, mass, activity, and chamber. Replicate block was included as a random effect within the experiment date. Linear rescaling of CO_2 production was necessary in some glmer models. We examined the residuals of models for normality (shapiro.test, R Core Team Stats). We considered the potential of non-singularity in mixed effect models by examining models that only used replicate block or date, and models based on single random effects always lead to the inclusion of the same fixed effects as models based on both.

Mating status

We tested if mated or unmated females had larger changes in MR during mite exposure by measuring CO_2 production of flies either exposed to mites or left undisturbed using flow-through respirometry. Flies were mated as above. Respiratory and activity measurements were conducted as in the sex-difference experiments, except for this mite exposure condition using 5 mites. Backwards model selection was performed as above.

Results

Host preferences

Mites have a size-mediated preference for female flies over male flies

This experiment tested if *M. subbadius* prefers to infect male or female flies in pairwise choice-tests. Female flies were infected in 22 of 31 successful trials (71%), significantly more often than the male flies (29%) (binom.test, P = 0.029). Males on average weighed (Fig. 3.1.1) 2.35 ± 0.09 mg and females weighed 2.86 ± 0.15 mg, a 19.6% difference.

A follow-up experiment was conducted to test if the observed preference for female flies was driven by sexual dimorphism in mass by size-matching male and female flies. Size matching essentially eliminated the observed difference in mass between the sexes: females in this experiment averaged a mass of 2.22 ± 0.09 mg (N = 29) and males on average weighed 2.20 ± 0.09 mg (N = 29). The largest difference in size between a paired male and female fly was 3.1% and the mean percent difference was less than 1%. The female fly was infected in 13 of 29 successful trials (45%) and the male was infected in 16 trials (55%) (Fig. 3.1.1). When flies were size-matched, there was no significant preference for either sex (binom.test, P = 0.55). Taken together our results show mites selectively infect female flies because females are larger than males. An additional experiment also found that mites prefer larger female flies over smaller female flies (Appendix 1).

Mites prefer to infect mated female flies over unmated *females*

We tested if flies preferentially infected mated or unmated female flies in size-matched preference experiments. In 24 of 37 (65%) trials, the mite infected the mated fly whereas only 35% of mites preferentially infected the unmated fly (binom.test, P = 0.099) (Fig. 3.1.1). The confidence interval (0.47 - 0.80) of this weak preference slightly overlapped 0.5 (95% CI, Fig. 3.1.1). Following size-matching, the average mass of the mated flies was 2.56 ± 0.04 mg and the average mass of the unmated female was 2.53 ± 0.04 mg. On average there was a 1.3% difference in mass between the mated and unmated fly in a y-maze and the largest difference was 4.2%.

Fly responses to mite risk

Male flies have stronger responses to mite risk than female flies

Unexposed females (N = 36) had substantially higher CO_2 production rates than males (N = 36), 0.063 ± 0.003 µL/min versus 0.053 ± 0.003 µL/min respectively (16.3% higher), and sex was a significant predictor of MR among unexposed flies (χ^2 = 6.34, P = 0.012) (Fig. 3.1.2a). Because of sexual dimorphism, we performed backwards model selection on initial models of MR with mass and sex separately to avoid colinearity. There was a 9.5% difference in mass between male and female flies in this experiment. Males had an average mass of 2.20 ± 0.08 mg and females had an average mass of 2.43 ± 0.09 mg, but mass was not a significant predictor of MR (χ^2 = 0.43, P = 0.51). Respirometry chamber was a significant predictor of MR (χ^2 = 12.3, P = 0.0004).

The MR of the flies exposed to mites was best described using a gamma distribution (glmer, family = Gamma). In the exposed treatment, the MR of male (0.073 ± 0.008 µL/min) and female (0.073 ± 0.007 µL/min) flies were nearly identical, and sex was not a significant predictor of MR (Wald t = 0.24, P = 0.81, Fig 3.1.2a). However, since control females started off with a higher MR, the relative increase in MR upon exposure was higher among males than females. When exposed to mites, the rate of CO_2 production increased by 15.1% among females and 31.3% in males. Flies exposed to mites had higher MR than unexposed flies overall, but, as predicted in the cost of infection hypothesis, male flies showed a stronger response compared to female flies upon exposure to mites.

Female flies in the exposed experiment on average had 24% higher masses than male flies, 2.48 ± 0.06 mg and 1.95 ± 0.03 mg, respectively. Due to this sexual dimorphism, we modeled MR with either mass or sex. In models with mass as a predictor, but not sex, mass significantly predicted MR in the exposed condition (Wald t = 2.27, P = 0.023). Chamber was a significant predictor in the exposed condition model of MR (Wald t = 5.84, P <0.001); we ruled out leaks by inspecting real-time flow-rates, and this likely represents additional acclimation/stress between chambers due to sequential measurements. On average the chamber with three mites (N= 4) produced 0.00052 ± 0.00006 µL/ min of C02, and as such the respiration of the mites was a negligible contributor to the difference in metabolic rates between the exposed and unexposed conditions.

Activity was recorded simultaneously with CO_2 production. In the control condition (no mites), the level of activity among females (0.037 ± 0.007 V, N = 36) did not differ substantially from males (0.040 ± 0.00B V, N = 36, Fig. 3.1.2b), and activity was not a significant predictor of MR (χ^2 = 0.11, P = 0.74). In the exposed treatment, activity was a significant predictor of MR (Wald t = 4.47, P<0.0001). This result was sensitive to the inclusion of a single male fly, the removal of which led to activity not being a significant predictor of exposed MR (Wald t = 1.301, P = 0.19). There was no substantial difference in activity between male (0.13 ± 0.04 V, N = 28) and female (0.11 ± 0.01 V, N = 28) flies, even if the male with the highest activity is removed (male: 0.10 ± 0.01 v, N = 27) (Fig 3.1.2b). Unsurprisingly, the presence of mites increased fly activity 3-4 times compared with unexposed flies. However, male and female flies had comparable activity at rest or when exposed to mites.

Unmated females have stronger responses to mite risk than mated females

The MR of unexposed flies was best described with a normal distribution, thus the lmer function was used for modelling. Mating status was a moderate predictor of MR when flies were at rest (χ^2 = 3.23, P = 0.07) (Fig. 3.1.2c). In the unexposed experiment, the mated group (0.057 ± 0.004 µL/min, N = 27) produced 24.8% more CO_2 than the unmated group (0.042 ± 0.004 µL/min, N = 28). Mass did not differ substantially between the unmated and mated groups (2.54 ± 0.06 mg and 2.52 ± 0.07 mg, respectively), or significantly predict unexposed MR (χ^2 = 0.72, P = 0.40).

The MR of the flies exposed to mites was best described using a gamma distribution (glmer, family = Gamma). Upon exposure to mites, mated flies (0.081 ± 0.005 µL/m, N = 31) produced CO_2 at nearly the same rate (Fig. 3.1.2c) as unmated flies (0.079 ± 0.004 µL/min, N = 33), and mating status was not a significant predictor of MR (Wald t = -1.08, P = 0.28). Among flies that were mated, exposure to mites resulted in a 34.8% rise in CO_2 production compared to the unexposed experiment. By comparison, unmated flies responded to mite exposure by increasing CO_2 production by 61.2% over the unexposed group.

In the unexposed condition, unmated flies were 9% heavier than mated flies, 3.19 ± 0.06 mg (N = 35) and 2.91 ± 0.05 mg (N = 35), respectively; however, mass was not a significant predictor of exposed MR (Wald t = 1.06, P = 0.29). On average the chamber with five mites (N = 5) produced 0.000084 ± 0.000013 µL/min of CO_2, and as such the respiration of the mites was a negligible contributor to the difference in metabolic rates between the exposed or unexposed experiments.

The activity of mated and unmated females was recorded simultaneously with MR when exposed or unexposed to mites. When flies were not exposed to mites, activity did not substantially differ between unmated and mated groups: 0.025 ± 0.008 V and 0.017 ± 0.003 V respectively (Fig. 3.1.2d). Nor did activity significantly predict MR when flies were not exposed (χ^2 = 0.03, P = 0.87). By contrast, mated and unmated female flies exposed to mites were 8-9 times more active than flies not exposed to mites, and activity was a significant predictor of MR (Wald t = 4.45, P < 0.0001, Fig. 3.1.2d). However, the activity levels of the unmated (0.16 ± 0.02 V, N = 35) and mated (0.16 ± 0.02 V, N = 35) groups were indistinguishable during mite exposure (Fig. 3.1.2d).

Discussion and conclusion

We set out to test two hypotheses which made mutually-exclusive predictions: 1) asymmetrical costs primarily drive selection for stronger responses to parasite exposure; or 2) asymmetrical risks primarily drive selection for stronger responses to parasite exposure. The latter hypothesis predicted a stronger response (i.e. increase in MR upon exposure to mites) among females compared to males, and that mated females would have stronger responses than unmated females. This prediction was not borne out, even though mites have a preference for female flies over male flies, and a moderate preference for mated females over unmated females independent of size. In other words, although the risk of infection is

greater for female flies than males, and more for mated females than unmated females, it did not determine the relative magnitude of the host response to parasitic threat. By contrast, the asymmetrical cost hypothesis predicted a stronger response among male flies than female flies, and a stronger response among unmated females relative to mated females. Our results support the asymmetrical costs hypothesis: male flies had a larger increase in metabolic rate than female flies when exposed to mites, 31.3% versus 15.1% respectively. Furthermore, unmated females had larger increases in MR, 61.2%, when exposed to mites than mated females, 34.8%. Hence unequal costs of infection, arising from parasite-mediated sexual selection, are likely driving the relative strength of responses of potential fly hosts to mites.

Fly activity was a significant predictor of MR in the mite-exposed treatments, and activity was 3-9 times higher on average among flies exposed to mites than flies at rest. Activity was not a significant predictor of MR among unexposed flies. Intraspecific differences in MR may be present under conditions of high activity or recovery but absent during routine conditions [50; 51; 52]. Our results are consistent with the general finding that energetically demanding activities correlate more strongly with overall MR than low energy activities [53]. The increase in activity observed here may be adaptive as activity can aid in parasite avoidance, particularly those that attack via the integument [7; 27; 54]. However, there was no substantial difference in activity levels between male and female or mated and unmated flies under any condition we tested. While activity may explain why flies exposed to mites generally have higher MR than unexposed flies, it does not fully account for the sex and mating status differences observed here.

Differences in NCEs experienced by potential hosts may instead be due to physiological (e.g. stress) or immunological mechanisms (e.g. upregulated melanization) [13; 55; 56; 57]. In *Drosophila*, the stress hormone octopamine appears to increase MR since octopamine knock-out flies have significantly reduced CO_2 production [58]. Similarly, mammalian stress hormones are positively correlated with MR. Short-term stress, e.g. during initial [59] exposure, can be adaptive and assists crickets and tadpoles in evading predation [60; 61]. Comparably, stress may also help hosts avoid infection by mobile parasites, particularly ectoparasites [14; 62].

Predation risk can also alter immune responses in insects [63; 64]. Dragonfly larvae exposed to cannibalistic conspecifics had increased melanization upon sham infection with microfilaments — potentially because melanization promotes both wound healing and immune function [64]. Predator presence can increase melanization responses in potential prey even when those predators are caged [63]. In our study, flies may respond to the presence of mites with preemptive increases in immunity which could impose an energetic cost. Potential changes could occur in components of the phenoloxidase (PO) system which governs melanin production by PO via activation of PO zymogens (see mini-review [65]). Future research should test how the mere presence of mites impacts the stress and immune systems of flies [35].

Although hosts may benefit from stress responses and/or preemptive priming of immune systems (e.g. melanization) to parasite and predator exposure in the short term, these responses likely decrease

with time since long-term stress can negatively impact fitness [8; 61; 66]. For example, exposure to predator cues increases tadpole oxygen consumption initially, but with repeated exposures oxygen consumption is suppressed [66]. A meta-analysis of vertebrate species enduring parasite infection found that stress hormones are highest early in infection, and, although still generally higher than uninfected con- specifics, this difference decreased over time [67]. Additionally, short term evasion strategies, e.g. bolting, may differ from longer-term strategies, e.g. hiding, and require different energy investment [66]. Thus, it is important for future studies on parasite-mediated NCEs to consider a range of non-consumptive effects across different time scales.

Our results suggest that, at least in the short term, mated and unmated female flies experience unequal NCEs from parasite exposure. However, previous research did not find a significant difference in longevity between mated female flies and unmated female flies chronically exposed to mites [36]. This relationship may be obscured by the complex and not well-understood link between mating and survival in *Drosophila* [68]. Alternatively, NCEs, and potentially intraspecific variation in NCEs, can be increased or decreased depending on the environment [16; 69; 70; 71]. Differential fitness impacts of NCEs may only manifest under poor conditions where organisms cannot easily compensate, e.g. with increased feeding. As a result, short-term NCEs on MR may not always predict relative long-term NCEs on longevity and may be environment dependent.

Organisms vary intra- and inter- specifically in how they compensate for long-term risk. Compensatory physiological changes can help damselfly larvae cope with reduced feeding during predator exposure [72]. Male and female prey can differ in how much they compensate for risk. For example, female lizards, *Podarcis hispanicus*, habituate to the presence of predators more than conspecific males [73]. Variation in compensation has implications for the ecology of prey species as interspecific differences in habituation to predation risk may drive competitive advantages among amphipod prey [74]. Future research should consider intra- and inter- specific variation in habituation to the presence of parasites and consequent implications for unequal long-term NCEs.

Community ecology and parasitology have increasingly recognized that parasites have important impacts on host populations and their communities outside of infection [75; 76; 77]. Infection does not impact all hosts equally, and our results suggest parasites also do not have equal NCEs on all potential hosts. Male flies and unmated females had stronger bioenergetic responses to parasites than female and mated flies respectively. These unequal reactions were not fully explained by inter-group differences in activity. Our results are compatible with the hypothesis that costs of infection primarily determine the relative strength of response to parasite exposure. Further research should investigate the specific mechanisms that drive this intraspecific variation in NCEs. Organisms face a complex landscape of fear, and how they navigate that landscape will depend on optimal investment in defence based on their relative costs of infection.

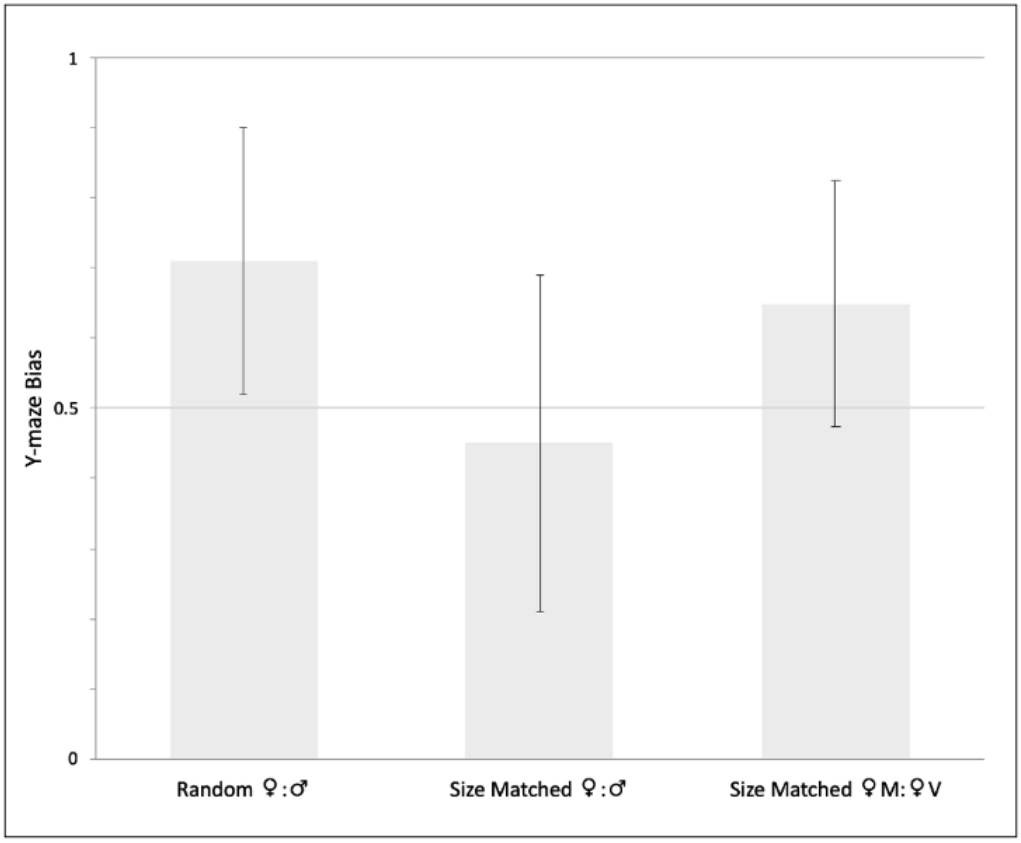


Figure 3.1.1: Host preferences from y-maze experiments. Biases (A:B) represent the proportion of y-mazes in which the group A fly was infected. Flies were either selected at random or size-matched within 5% of mass. All flies were unmated, except when mites were given a choice between mated females (♀M) and unmated females (♀V). Error bars represent 95% CI (R, binom.test).

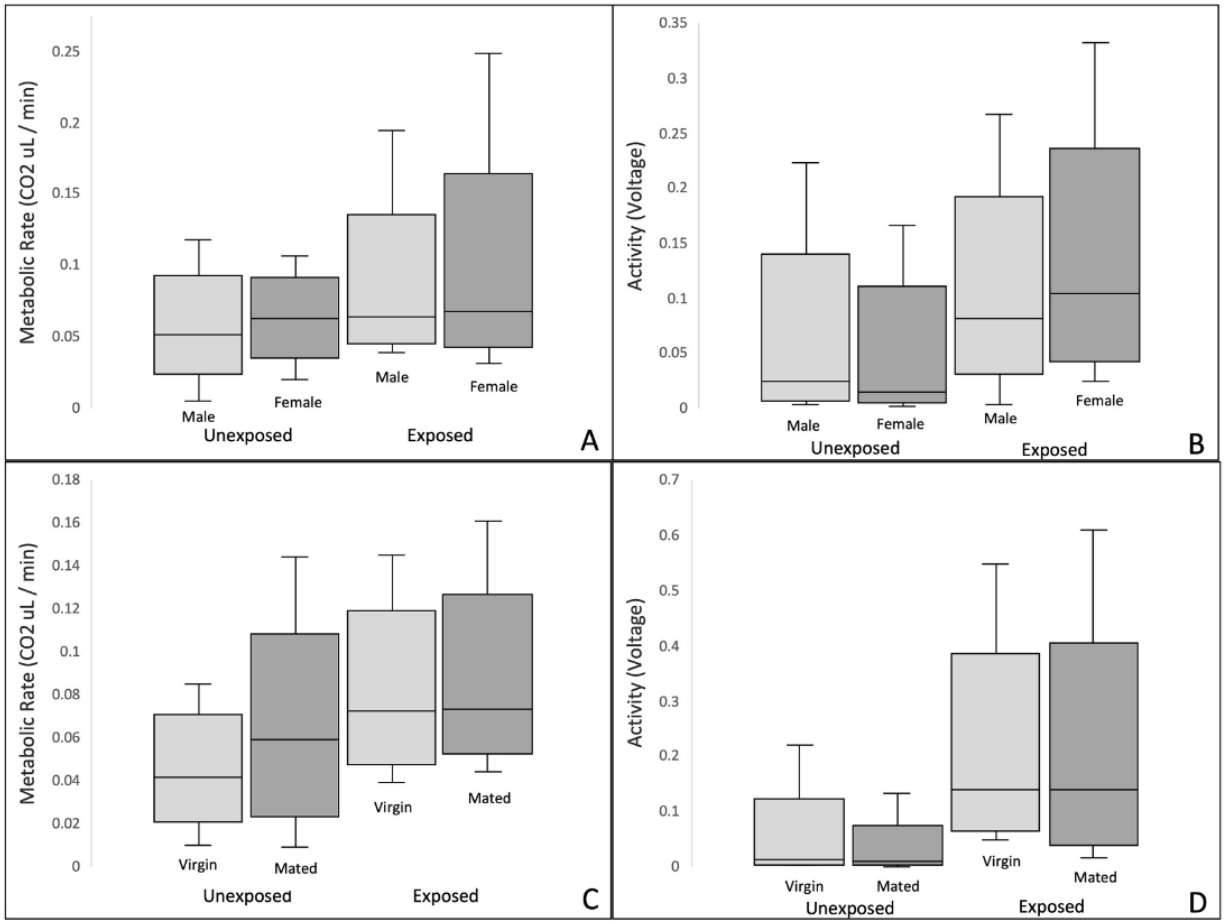


Figure 3.1.2: Fly responses to mite exposure. A) Metabolic rates of male and female flies at rest or exposed to mites. B) Activity of male and female flies at rest or exposed to mites. One male fly was removed due to high activity. C) Metabolic rates of unmated and mated females at rest or exposed to mites. D) Activity of unmated and mated females at rest or exposed to mites. Boxplots represent minimum, 25th percentile, median, 75th percentile and maximum.

3.1 References

1. M. Clinchy, M.J. Sheriff, L.Y. Zanette, Predator-induced stress and the ecology of fear, Funct. Ecol. 27 (2013) 56–65, https://doi.org/10.1111/1365-2435.12007.
2. A.J. Gallagher, S. Creel, R.P. Wilson, S.J. Cooke, Energy landscapes and the land- scape of fear, Trends Ecol. Evol. 32 (2017) 88–96, https://doi.org/10.1016/j.tree. 2016.10.010.
3. S.D. Peacor, E.E. Werner, Nonconsumptive effects of predators and trait-mediated indirect effects, Encyclopedia of Life Sciences (ELS), John Wiley & Sons, Ltd., Chichester, United Kingdom, 2008, pp. 1–8.
4. B. Peckarsky, C. Cowan, M. Penton, C. Anderson, Sublethal consequences of stream- dwelling predatory stoneflies on mayfly growth and fecundity, Ecology 74 (1993) 1836–1846, https://doi.org/10.2307/1939941.
5. C. Navarro, F. de Lope, A. Marzal, A.P. Moller, Predation, host immune response, and parasitism, Behav. Ecol. 15 (2004) 629–635, https://doi.org/10.1093/beheco/arh054.
6. S.J. McCauley, L. Rowe, M.J. Fortin, The deadly effects of "nonlethal" predators, Ecology 92 (2011) 2043–2048, https://doi.org/10.1890/11-0455.1.
7. J. Koprivnikar, T.M.Y. Urichuk, Time-lagged effect of predators on tadpole beha- viour and parasite infection, Biol. Lett. 13 (2017), https://doi.org/10.1098/rsbl. 2017.0440.
8. DeWitt P.D., Visscher D.R., Schuler M.S., Thiel R.P. Predation risks suppress lifetime fitness in a wild mammal. Oikos 128 (2019) 1–8.
9. L. Acharya, Sex-biased predation on moths by insectivorous bats, Anim. Behav. 49 (1995) 1461–1468, https://doi.org/10.1016/0003-3472(95)90067-5.
10. K. Norrdahl, E. Korpimaki, Does mobility or sex of voles affect risk of predation by mammalian predators? Ecology 79 (1998) 226–232.
11. S.L. Ball, R.L. Baker, Predator-induced life history changes: antipredator behavior costs or facultative life history shifts? Ecology 77 (1996) 1116–1124, https://doi. org/10.2307/2265580.
12. P.A. Lagos, M.E. Herberstein, Are males more scared of predators? Differential change in metabolic rate between males and females under predation risk, Physiol. Behav. 173 (2017) 110–115, https://doi.org/10.1016/j.physbeh.2017.02.002.
13. S. Slos, L. De Meester, R. Stoks, Food level and sex shape predator-induced phy- siological stress: immune defence and antioxidant defence, Oecologia 161 (2009) 461–467, https://doi.org/10.1007/s00442-009-1401-2.
14. J.C.Buck,S.B.Weinstein,H.S.Young, Ecological and evolutionary consequences of parasite avoidance, Trends Ecol. Evol. 33 (2018) 619–632, https://doi.org/10.1016/j.tree.2018.05.001.
15. Daversa D.R., Hechinger R.F., Madin E., Fenton A., Dell A.I., Ritchie E., Rohr J., Rudolf V.H.W., Preprint 2019. Beyond the ecology of fear: non-lethal effects of predators are strong whereas those of parasites are diverse. Preprint at bioRxiv. doi.org/10.1101/766477.

16. M.K. Mierzejewski, C.J. Horn, L.T. Luong, Ecology of fear: environment-dependent parasite avoidance among ovipositing *Drosophila*, Parasitology 146 (2019) 1564–1570, https://doi.org/10.1017/s0031182019000854.
17. T.R. Raffel, L.B. Martin, J.R. Rohr, Parasites as predators: unifying natural enemy ecology, Trends Ecol. Evol. 23 (2008) 610–618, https://doi.org/10.1016/j.tree.2008.06.015.
18. J.R. Rohr, A. Swan, T.R. Raffel, P.J. Hudson, Parasites, info-disruption, and the ecology of fear, Oecologia 159 (2009) 447–454, https://doi.org/10.1007/s00442- 008-1208-6.
19. J. Koprivnikar, L. Penalva, Lesser of two evils? Foraging choices in response to threats of predation and parasitism, PLoS One 10 (2015), https://doi.org/10.1371/ journal.pone.0116569.
20. B.F. Allan, T.S. Varns, J.M. Chase, Fear of parasites: lone star ticks increase giving- up densities in white-tailed deer, Isr. J. Ecol. Evol. 56 (2010) 313–324, https://doi. org/10.1560/ijee.56.3-4.313.
21. S.B.S. Baleba, B. Torto, D. Masiga, M.N. Getahun, C.W. Weldon, Stable Flies, *Stomoxys calcitrans* L. (Diptera: muscidae), improve offspring fitness by avoiding oviposition substrates with competitors or parasites, Front. Ecol. Evol. 8 (2020) 1–5, https://doi.org/10.3389/fevo.2020.00005.
22. A. Fritzsche, B.F. Allan, The ecology of fear: host foraging behavior varies with the spatio-temporal abundance of a dominant ectoparasite, Ecohealth 9 (2012) 70–74, https://doi.org/10.1007/s10393-012-0744-z.
23. S.B. Weinstein, J.C. Buck, H.S. Young, A landscape of disgust, Science 359 (2018) 1213–1214, https://doi.org/10.1126/science.aas8694.
24. C.J. Cloutier, M. Kavaliers, K.P. Ossenkopp, Rodent sex differences in disgust behaviors (anticipatory nausea) conditioned to a context associated with the effects of the toxin LiCl: inhibition of conditioning following immune stimulation with lipo-polysaccharide, Pharmacol. Biochem. Behav. 152 (2017) 4–12, https://doi.org/10.1016/j.bb.2016.08.006.
25. D Gálvez, M Chapuisat, Immune priming and pathogen resistance in ant queens, Ecology and Evolution 4 (10) (2014) 1761–1767, https://doi.org/10.1002/ece3.1070.
26. C.N. Keiser, V.H.W. Rudolf, M.C. Luksi, J.B. Saltz, Sex differences in disease avoidance behavior vary across modes of pathogen exposure, Ethology 126 (2019) 304–312, https://doi.org/10.1111/eth.12969.
27. C.J. Horn, L.T. Luong, Current parasite resistance trades off with future defenses and flight performance, Behav. Ecol. Sociobiol. 73 (2019), https://doi.org/10.1007/s00265-019-2697-5.
28. D. Hawlena, O.J. Schmitz, Herbivore physiological response to predation risk and implications for ecosystem nutrient dynamics, Proc. Natl. Acad. Sci. U.S.A. 107 (2010) 15503–15507.
29. L.T. Luong, C.J. Horn, T. Brophy, Mitey costly: energetic costs of parasite avoidance and infection, Physiol. Biochem. Zool. 90 (2017) 471–477, https://doi.org/10.1086/691704.
30. Lighton, J.R.B., 2008. Measuring Metabolic Rates: A Manual for Scientists. Oxford University Press, Oxford New York.

31. A. Perez-Leanos, M.R. Loustalot-Laclette, N. Nazario-Yepiz, T.A. Markow, Ectoparasitic mites and their *Drosophila* hosts, Fly 11 (2017) 10–18, https://doi.org/10.1080/19336934.2016.1222998.
32. M. Polak, Ectoparasitic effects on host survival and reproduction: the *Drosophila- Macrocheles* association, Ecology 77 (1996) 1379–1389, https://doi.org/10.2307/2265535.
33. D.V. Beresford, J. Sutcliffe, The effect of *Macrocheles muscaedomesticae* and *M. subbadius* (Acarina: macrochelidae) phoresy on the dispersal of *Stomoxys calcitrans* (Diptera: muscidae), Syst. Appl. Aracol. 23 (2009) 1–30, https://doi.org/10.11158/saasp.23.1.1 Special Publications.
34. M. Polak, W.T. Starmer, Parasite-induced risk of mortality elevates reproductive effort in male *Drosophila*, Proc. R. Soc. B Biol. Sci. 265 (1998) 2197–2201.
35. J.B.Benoit, J.Bose, S.Bailey, M.Polak, Interactions with ectoparasitic mites induce host metabolic and immune responses in flies at the expense of reproduction-associated factors, Parasitology (2020) 1–10, https://doi.org/10.1017/S0031182020000918 In press.
36. C.J. Horn, L.T. Luong, Proximity to parasites reduces host fitness independent of infection in a *Drosophila-Macrocheles* system, Parasitology 145 (2018) 1564–1569, https://doi.org/10.1017/s0031182018000379.
37. M. Polak, T.A. Markow, Effect of ectoparasitic mites on sexual selection in a sonoran desert fruit-fly, Evolution 49 (1995) 660–669, https://doi.org/10.2307/2410319.
38. Michal Polak, Lien T Luong, William T Starmer, Parasites physically block host copulation: a potent mechanism of parasite-mediated sexual selection, Behavioral Ecology 18 (15) (2007) 952–957, https://doi.org/10.1093/beheco/arm066.
39. P. Christe, O. Glaizot, G. Evanno, N. Bruyndonckx, G. Devevey, G. Yannic, P. Patthey, A. Maeder, P. Vogel, R. Arlettaz, Host sex and ectoparasites choice: preference for, and higher survival on female hosts, J. Anim. Ecol. 76 (2007) 703–710, https://doi.org/10.1111/j.1365-2656.2007.01255.x.
40. S. Morand, J.G. De Bellocq, M. Stanko, D. Miklisova, Is sex-biased ectoparasitism related to sexual size dimorphism in small mammals of Central Europe? Parasitology 129 (2004) 505–510, https://doi.org/10.1016/ensam.inra.fr.
41. E.O. Campbell, L.T. Luong, Mite choice generates sex- and size-biased infection in *Drosophila hydei*, Parasitology 143 (2016) 787–793, https://doi.org/10.1017/s0031182016000305.
42. L.A.D. Sheridan, R. Poulin, D.F. Ward, M. Zuk, Sex differences in parasitic infections among arthropod hosts: is there a male bias? Oikos 88 (2000) 327–334, https://doi.org/10.1034/j.1600-0706.2000.880211.x.
43. K.K. Moritz, C. Bjorkman, A.L. Parachnowitsch, J.A. Stenberg, Female *Salix viminalis* are more severely infected by *Melampsora* spp. but neither sex experiences associational effects, Ecol. Evol. 6 (2016) 1154–1162, https://doi.org/10.1002/ece3.1923.
44. K.J. Yule, K.C. Burns, Parasite - offspring competition for female resources can explain male-biased parasitism in plants, Biol. Lett. 15 (2019), https://doi.org/10.1098/rsbl.2018.0761

45. T. Szentivanyi, O. Vincze, P. Estok, Density-dependent sex ratio and sex-specific preference for host traits in parasitic bat flies, Parasit. Vectors 10 (2017), https://doi.org/10.1186/s13071-017-2340-0.
46. C.J. Horn, M.K. Mierzejewski, L.T. Luong, Host respiration rate and injury-derived cues drive host preference by an ectoparasite of fruit flies, Physiol. Biochem. Zool. 91 (2018) 896–903, https://doi.org/10.1086/697466.
47. E.W. Daly, P.T.J. Johnson, Beyond immunity: quantifying the effects of host anti- parasite behavior on parasite transmission, Oecologia 165 (2011) 1043–1050, https://doi.org/10.1007/s00442-010-1778-y.
48. J.Koprivnikar, M.R.Forbes, R.L.Baker, On the efficacy of anti-parasite behaviour: a case study of tadpole susceptibility to cercariae of *Echinostoma trivolvis*, Can. J. Zool. Rev. Can. Zool. 84 (2006) 1623–1629, https://doi.org/10.1139/z06-158.
49. T.A. Markow, Reproductive behavior of *Drosophila melanogaster* and *Drosophila nigrospiracula* in the field and in the laboratory, J. Comp. Psychol. 102 (1988) 169–173.
50. I.C. Caballero, A.J. Sakla, J.T. Detwiler, M. Le Gall, S.T. Behmer, C.D. Criscione, Physiological status drives metabolic rate in Mediterranean geckos infected with Pentastomes, Plos One 10 (2015) e0144477, https://doi.org/10.1371/journal.pone.0144477.
51. M. Walkey, R.H. Meakins, An attempt to balance the energy budget of a host- parasite system, J. Fish Biol. 2 (1970) 361–372.
52. B.W.M. Wone, P. Madsen, E.R. Donovan, M.K. Labocha, M.W. Sears, C.J. Downs, D.A. Sorensen, J.P. Hayes, A strong response to selection of mass-independent maximal metabolic rate without a correlated response in basal metabolic rate, Heredity 114 (2015) 419–427, https://doi.org/10.1038/hdy.2014.122.
53. K.J. Mathot, N.J. Dingemanse, S. Nakagawa, The covariance between metabolic rate and behaviour varies across behaviours and thermal types: meta-analytic insights, Biol. Rev. 94 (2019) 1056–1074.
54. R.L.Baker, B.P.Smith, Conflict between antipredator and antiparasite behaviour in larval damselflies, Oecologia 109 (1997) 622–628, https://doi.org/10.1007/s004420050125.
55. K.A. Sloman, G. Motherwell, K.I. O'Connor, A.C. Taylor, The effect of social stress on the standard metabolic rate (SMR) of brown trout, Salmo trutta, Fish Physiol. Biochem. 23 (2000) 49–53, https://doi.org/10.1023/a:1007855100185.
56. D.F. Garcia-Diaz, J. Campion, F.I. Milagro, A. Lomba, F. Marzo, J.A. Martinez, Chronic mild stress induces variations in locomotive behavior and metabolic rates in high fat fed rats, J. Physiol. Biochem. 63 (2007) 337–346, https://doi.org/10.1007/bf03165765.
57. S. Slos, R. Stoks, Predation risk induces stress proteins and reduces antioxidant defense, Funct. Ecol. 22 (2008) 637–642, https://doi.org/10.1111/j.1365-2435.2008.01424.x.

58. Y. Li, J. Hoffmann, F. Stephano, I. Bruchhaus, C. Fink, T. Roeder, Octopamine controls starvation resistance, life span and metabolic traits in Drosophila, Sci. Rep. 6 (2016), https://doi.org/10.1038/srep35359.
59. C.G. Haase, A.K. Long, J.F. Gillooly, Energetics of stress: linking plasma cortisol levels to metabolic rate in mammals, Biol. Lett. 12 (2016), https://doi.org/10.1098/rsbl.2015.0867.
60. S.A. Adamo, I. Kovalko, B. Mosher, The behavioural effects of predator-induced stress responses in the cricket (*Gryllus texensis*): the upside of the stress response, J. Exp. Biol. 216 (2013) 4608–4614, https://doi.org/10.1242/jeb.094482.
61. P.S. Kulkarni, N.P. Gramapurohit, Effect of corticosterone on larval growth, anti- predator behaviour and metamorphosis of *Hylarana indica*, Gen. Comp. Endocrinol. 251 (2017) 21–29, https://doi.org/10.1016/j.ygcen.2016.09.001.
62. E.A. Krusemark, W. Li, Do all threats work the same way? Divergent effects of fear and disgust on sensory perception and attention, J. Neurosci. 31 (2011) 3429–3434, https://doi.org/10.1523/jneurosci.4394-10.2011.
63. T.M. Duong, S.J. McCauley, Predation risk increases immune responses in a larval dragonfly (*Leucorrhinia intacta*), Ecology 97 (2016) 1605–1610, https://doi.org/10.1890/15-1964.1.
64. R.L. Murray, S. Tah, J. Koprivnikar, L. Rowe, S.J. McCauley, Exposure to potentially cannibalistic conspecifics induces an increased immune response, Ecol. Entomol. 45 (2020) 355–363, https://doi.org/10.1111/een.12806.
65. I. González-Santoyo, A. Córdoba-Aguilar, Phenoloxidase: a key component of the insect immune system, Entomol. Exp. Appl. 142 (2011) 1–16, https://doi.org/10.1111/j.1570-7458.2011.01187.x.
66. U.K. Steiner, J. Van Buskirk, Predator-induced changes in metabolism cannot ex- plain the growth/predation risk tradeoff, PLoS ONE 4 (2009), https://doi.org/10.1371/journal.pone.0006160.
67. K. O'Dwyer, F. Dargent, M.R. Forbes, J. Koprivnikar, Parasite infection leads to widespread glucocorticoid hormone increases in vertebrate hosts: a meta-analysis, J. Anim. Ecol. 89 (2019), https://doi.org/10.1111/1365-2656.13123.
68. T.A. Markow, “Cost” of virginity in wild *Drosophila melanogaster* females, Ecol. Evol. 1 (2011) 596–600, https://doi.org/10.1002/ece3.54.
69. S.C. Donelan, J.H. Grabowski, G.C. Trussell, Refuge quality impacts the strength of consumptive effects on prey, Ecology 98 (2016) 403–411, https://doi.org/10.1002/ecy.1647.
70. M.F.Kersh-Becker,J.S.Thaler, Plant resistance reduces the strength of consumptive and non-consumptive effects of predators on aphids, J. Anim. Ecol. 84 (2015) 1222–1232, https://doi.org/10.1111/1365-2656.12371.
71. O.J. Schmitz, A.E. Rosenblatt, The temperature dependence of predation stress and prey nutritional stoichiometry, Front. Ecol. Evol. 14 (2017) 1–8, https://doi.org/10.3389/fevo.2017.00073.

72. M. Van Dievel, L. Janssens, R. Stoks, Short- and long-term behavioural, physiological and stoichiometric responses to predation risk indicate chronic stress and compensatory mechanisms, Oecologia 181 (2016) 347–357, https://doi.org/10.1007/s00442-015-3440-1.
73. I. Rodriguez-Prieto, J. Martin, E. Fernandez-Juricic, Individual variation in behavioural plasticity: direct and indirect effects of boldness, exploration and sociability on habituation to predators in lizards, Proc. R. Soc. B Biol. Sci. 278 (2011) 266–273, https://doi.org/10.1098/rspb.2010.1194.
74. L. Jermacz, J. Kobak, Keep calm and don't stop growing: non-consumptive effects of a sympatric predator on two invasive Ponto-Caspian gammarids *Dikerogammarus villosus* and *Pontogammarus robustoides*, PLoS One 12 (2017), https://doi.org/10.1371/journal.pone.0182481.
75. K.D. Lafferty, A.P. Dobson, A.M. Kuris, Parasites dominate food web links, Proc. Natl. Acad. Sci. U.S.A. 103 (2006) 11211–11216, https://doi.org/10.1073/pnas.0604755103.
76. K.M. McKee, J. Koprivnikar, P.T.J. Johnson, M.T. Arts, Parasite infectious stages provide essential fatty acids and lipid-rich resources to freshwater consumers, Oecologia 192 (2020) 477–488, https://doi.org/10.1007/s00442-019-04572-0.
77. V. Nehring, H. Teubner, S. König, Dose-independent virulence in phoretic mites that parasitize buring beetles, Int. J. Parasitol. 49 (2019) 759–767, https://doi.org/10.1016/j.ijpara.2019.05.011.

3.2: Exposure to mites decreases photophobia independent of mating-status

Background

Nearly all free-living organisms face the risk of parasite/pathogen infection, and have evolved a variety of immune responses to minimise the deleterious effects of infection [1]. Although immunity research has traditionally focused on cellular, physiological/biochemical and barrier mechanisms that prevent infection, behavioural immunity, defined as host behaviours that minimise the risks or costs of infection, has increasingly been studied as a mechanism of host defence [2; 3; 4; 5]. For example, *Drosophila melanogaster* experiencing *Metarhizium robertsii* infection move to cooler habitats, reducing fungal virulence [6]. Thermotaxis (selectively moving along temperature gradients), grooming and microbe sensing have been the focus of most studies on behavioural immunity, with other mechanisms being potentially overlooked [7; 8; 9; 10].

In particular, phototaxis (selectively moving along light–dark gradients) as a potential mechanism of behavioural immunity remains underexplored, despite light conditions influencing infection outcomes [11; 12]. Light intensity impacts prevalence of infection among snails challenged with schistosomes [12]. However, phototaxis can also affect many species' survival, body condition, UV stress and egg hatch rate, among other traits [13]. For example, positive phototaxis may help mayfly larvae survive winter stress [14]. Therefore, altered host phototaxis as a form of behavioural immunity could carry ecologically significant, yet underestimated, trade-offs.

In this study, we tested if cactiphilic *Drosophila nigrospiracula* alter their phototaxic behaviour during exposure to a natural ectoparasite, the haemolymph-feeding mite *Macrocheles subbadius*. The flies live on rotting cacti, and negative phototaxis may aid thermoregulation and feeding [15; 16]. Mite infection substantially reduces host survival and fecundity [17], and flies deploy energetically demanding behaviours, eg. grooming and flight, to resist infection [18; 19].

In preliminary experiments, flies were less likely to become infected when challenged with mites in the light than when challenged in the dark, suggesting the risk of infection is lower in the light. We experimentally tested the hypothesis that phototaxis is a mechanism of behavioural immunity. Specifically, we predicted that when flies are subject to a risk of parasitism (i.e. mites are present) they will spend more time in the light side of phototaxis chambers than when mites are absent. Mated *D. nigrospiracula* females invest less energy in anti-parasite defences than unmated females [20]. Thus, we also anticipated that mated females would experience reduced parasite-mediated phototaxis relative to unmated females.

Methods

(a) Cultures

Drosophila nigrospiracula Patterson and Wheeler (Diptera: Drosophilidae) and *Macrocheles subbadius* (Berlese) (Mesostigmata: Macrochelidae) cultures are fully described in [21]. Flies were moved from stock cultures to separate-sex agar vials before maturity [15]. Female flies are the preferred host of

the mites and are more photophobic than males [16; 20]. Therefore, we tested if mites impacted the phototaxis of female flies (mated and unmated) in the experiments below. Female flies for mated groups were moved to vials at a 2:1 ratio of males to females 72 h before experiments (~14 males to 7 females), and unmated females were simultaneously transferred to female-only vials at the same density (number of flies per vial). Adult female mites (the infectious stage) were collected using Berlese funnels.

(b) Risk of infection in light and dark environments

First, we tested if *D. nigrospiracula* were more likely to be infected by mites when exposed in the light or in the dark. Individual female flies were placed with a single adult female mite in micro-arenas (cropped pipette tips) sealed with cotton wool (1 fly and 1 mite per arena). Micro-arenas were small enough to prevent escape, but flies could groom and kick mites. Micro-arenas were haphazardly assigned to either the light or dark condition. The light condition was under ambient lab light (fluorescent), whereas the dark condition was under an opaque box that fully excluded light. After 1 h flies were checked for infection.

(c) Phototaxis of flies exposed and unexposed to mites

This experiment tested if *D. nigrospiracula* changed its phototaxis behaviour under risk of infection, i.e. in the presence of mites. We used phototaxis chambers to measure the phototaxic behaviour of mated and unmated female flies in the presence and absence of mites [16]. Chambers consisted of linear transparent tubing with half of the length blacked out with two layers of opaque tape. A fly was placed in the midpoint of a chamber and the ends sealed with cotton wool. In the unexposed condition, the fly was in the chamber alone. In the exposed condition, a single adult female mite was also in the chamber. Once every minute (for 10-15 min) chambers were inspected, and the location of the fly and/or mite was recorded at the moment of observation. Following each assay, the fly (and mite when applicable) was given an individual phototaxis score (the proportion of observations at which the fly (mite was in the dark: number of dark observations / total number of observations). A phototaxis score of 0 indicates the fly /mite was entirely in the light at all observations, and a score of 1 indicates the fly / mite was in the dark at all observations. An additional trial observed individual mites in identical assays (for 10 or 20 min), but there was no time-based difference in behaviour. We also measured the phototaxic behaviour of males in the absence of mites.

(d) Statistical analyses

The proportion of female flies infected in dark micro-arenas was compared with the proportion infected in the light micro-arenas using a two-proportion Z-test (prop-test, R Stats). The mite infecting the fly was defined as a success. In total, 39 light micro-arenas and 39 dark micro-arenas were tested. Because the number of successes was low (less than 5) in the light micro-arenas, Yates continuity correction was applied.

The phototaxis behaviour of flies in response to mites was analysed using generalised linear models (glm, family = quasibinomial, link = logit). The response variable was phototaxis score. The estimated dispersion was substantially greater (~6.1) than the 1 assumed by the binomial model, suggesting the quasibinomial was justified. To avoid pseudoreplication, the unit of replication was individual flies (1 phototaxis score per fly).

The primary analysis tested the phototactic of mated and unmated females in the presence or absence of mites. First, we tested the interaction between mating and exposure using the model Score ~ Mating x Exposure. Since the interaction was not significant (P = 0.31, see Results), we examined the main effects using the model Score ~ Mating + Exposure. We inspected residuals for normalcy and homoscedasticity. We also examined the phototaxis of male flies unexposed to mites. In a separate model, we tested the effect of fly sex (pooling mated and unmated females) on the phototaxis score of flies not exposed to mites: Score ~ Sex. We also recorded the position of mites when they were present. One-sample non-parametric tests (one-sample Wilcoxon signed rank) were used to test if mite groups (alone, with mated females, or with unmated females) had median phototaxis scores different front 0.5, i.e. if mites showed positive or negative phototaxis (Wilcox.test function, Ho: median phototaxis score = 0.5). Each group was tested separately versus the null hypothesis.

Results

(a) Risk of infection in light and dark environments

First, we tested if a higher proportion of flies became infected upon mite exposure under light or dark conditions. In light micro-arenas, 3/39 flies became infected (8%). In dark micro-arenas, 12/39 flies became infected (31%). A significantly higher proportion of flies were infected in the dark micro-arenas than the light micro-arenas (prop.test, X^2 = 5.3, P = 0.02). Infection risk was higher in the dark.

(b) Phototaxis of flies exposed and unexposed to mites

This experiment tested the hypothesis that mite exposure would alter the phototaxis behaviour of female flies and tested whether mating impacts parasite-mediated phototaxis. The interaction between mating status (mated/ unmated) and mite exposure was not significant (coeff = 0.71, t = 1.02, P = 0.31). In the main effects model, exposure was a significant predictor of phototaxis (coeff= 1.38, t = 4.14, P < 0.0001); whereas, mating status was a marginal predictor of phototaxis (coeff = 0.51, t = 1.70, P =0.09). The regression coefficients suggest mite exposure (1.38) substantially increased time spent in the light, while mating (0.51) caused a smaller reduction in photophobia (Fig 3.2.1).

We also tested if fly sex had a significant impact on phototaxis in the absence of mites (Fig 3.2.2). Among unexposed flies, sex (pooling unmated and mated females) was a significant predictor of phototaxis (coeff= -1.69, t = -4.16, P < 0.0001). The regression coefficient (-1.69) suggests males were substantially less photophobic than females.

In the absence of mites, unmated females (N = 30) had a mean phototaxis score of 0.91 ± 0.03 (mean ± 1 s.e.), and mated females (N = 30) had a mean phototaxis score of 0.79 ± 0.07. Male flies (unexposed to mites) (N = 30) had a mean phototaxis score of 0.52 ± 0.07. In the presence of mites, unmated females (N = 28) and mated females (N = 27) had mean phototaxis scores of 0.64 ± 0.06 and 056 ± 0.06, respectively.

(c) Phototaxis behaviour of mites

The phototaxis score of mites in phototaxis chambers was recorded either alone, with mated female flies, or with unmated females. Each group of mites was tested with one-sample Wilcoxon-signed rank test (wilcox.test, Ho: median phototaxis score = 0.5). Mites held with unmated female flies (N = 28) and mated female flies (N = 27) had mean phototaxis scores of 0.54 ± 0.04 and 0.48 ± 0.03, respectively. Neither the mites with unmated flies (P = 0.36) or the mites with mated flies (P = 0.79) showed significant negative or positive phototaxis. Mites without flies had a mean photo- taxis score of 0.59 ± 0.04; this phototaxis score was significantly different from 0.5 (P = 0.04).

(d) Data availability

Raw data are available at Dryad (doi:10.5061/dryad.sxksn0341).

Discussion

We aimed to test the hypothesis that flies alter their phototaxis as a mechanism of behavioural immunity. Specifically, we predicted female flies would spend more time in the light section of phototaxis chambers when exposed to mites than when not exposed. In preliminary experiments we tested the risk of infection under different light conditions. Flies were more likely to be infected by mites if exposed in the dark than in the light. In phototaxis experiments mite exposure significantly reduced the photophobia of female flies. Moving to light conditions, i.e. where infection was less likely, during mite exposure suggests phototaxis is a mechanism of *D*. *nigrospiracula* behavioural immunity.

Reduced infection rates among females exposed to mites in the light compared with in the dark could indicate (1) flies have stronger defences in the light and/or (2) mites are less likely to or less capable of successfully infecting a host in light environments. Despite lacking eyes, some mites are responsive to light [22]. However, Macrochelidae are not known to have explicit light-sensing organs [23], despite some early suggestions that *Macrocheles* may have light-sensitive behaviour [24]. Congeneric *Macrocheles muscaedomesticae* are not more or less infectious in the light [25]. When held with flies, mites did not show significant positive or negative phototaxis, and without flies mites only showed weak positive phototaxis. Thus, decreased infection risk in the light is likely fly-mediated.

Female flies became more positively phototaxic during mite exposure (Fig 3.2.1). Shifting to light environments could improve fly ectoparasite resistance since flies use a combination of visual, olfactory and contact cues to detect mites [18; 26]. Moving into the light may aid visual identification and

subsequent avoidance of mites. Alternatively, light conditions may alter fly behaviour in ways that make infection less likely. Because flies defend themselves against mites through bursts of activity (jumping, kicking, grooming), shifting to brighter environments may functionally improve resistance if light encourages fly activity [17; 26]. The correlation between light levels and activity is generally positive [27; 28; 29]. Wheeler et al. [27] reported higher activity in *D. melanogaster* in the light than the dark; however, other studies suggest flies are most active in lower intensity morning and evening light [28; 29]. Additionally, dark conditions can induce rest/sleep states that may increase vulnerability to infection [30]. Thus, positive phototaxis during parasite exposure may increase the efficacy of the broader defensive behavioural syndrome [31].

In desert flies, including *D. nigrospiracula*, phototaxis is likely linked to heat stress [16]. Furthermore, the primary food source of *D. nigrospiracula* is fluid cactus exudate, which desiccates quickly [15]. Thus, shifting to light environments as part of behavioural immunity could impose thermal and nutritional trade-offs on flies. Since female flies have hierarchical preferences for habitats, future studies could consider whether mite-mediated phototaxis occurs when the environment simultaneously varies in food availability, suitability for eggs, conspecific presence, etc. [32; 33].

Drosophila phototaxis, both negative and positive, is heritable and responds strongly to artificial selection [34; 35; 36]. Mite infection can more than halve fly survival and fecundity [17]. Therefore, shifting to lighter environments during mite exposure may give flies a fitness advantage. It is possible that parasites can select for light-seeking/avoiding personalities in potential hosts [37; 38]. Field studies should consider this possibility by testing if fly populations adapted to mites show different phototaxis behaviour compared with mite-free populations.

In the absence of mites, fly sex was a significant predictor of photophobia (Fig 3.2.2). Males were significantly less photophobic than females, which broadly agrees with previous observations [16]. The behaviour observed here and in a previous study is consistent with the natural history of the system [15]. Female flies are more likely to be found inside pockets of necrotic cactus, where they feed and lay eggs. On the other hand, males defend territory exterior to the rot to attract mates [15]. Sex differences in phototaxis may facilitate females situating within rot and males at external territories.

Marginally reduced photophobia among mated females relative to unmated females, independent of mite exposure, may represent increased exploratory behaviour while searching for ovipositing sites [32]. Furthermore, copulation *per se* can alter the behaviour of female flies [39; 40]. For example, copulation increases *Drosophila* dispersiveness [39.]. Male-associated compounds may explain these changes. Sex-peptide is transferred from male to female flies during copulation and alters recipient physiology and behaviour [41; 42]. Using sterile males to test if sex-peptide impacts female phototaxis separately from reproduction may be insightful [43; 44].

Since mated female *D. nigrospiracula* invest less energy in active parasite resistance than unmated females, we anticipated that mated females would experience reduced parasite-mediated phototaxis [20]. However, we did not observe a significant interaction between mating status and mite

exposure. Costs of mating (e.g. egg development) may reduce the energy available for active resistance (e.g. grooming, kicking, bursts of flight) [21]. By contrast, phototaxic behaviour may not be as energetically demanding, which may explain the absence of a mating-exposure interaction.

Disease ecology has increasingly emphasised the effects of parasites on their hosts outside infection, especially through trade-offs [45; 46]. Trade-offs associated with parasite exposure can manifest as non-consumptive effects, impacting hosts even when infection does not occur (i.e. ecology of fear, [47]). Our results are consistent with the hypothesis that female flies change their phototaxic behaviour to minimise infection risk. Identifying phototaxis as a mechanism of behavioural immunity opens new directions in the study of these host-parasite interactions.

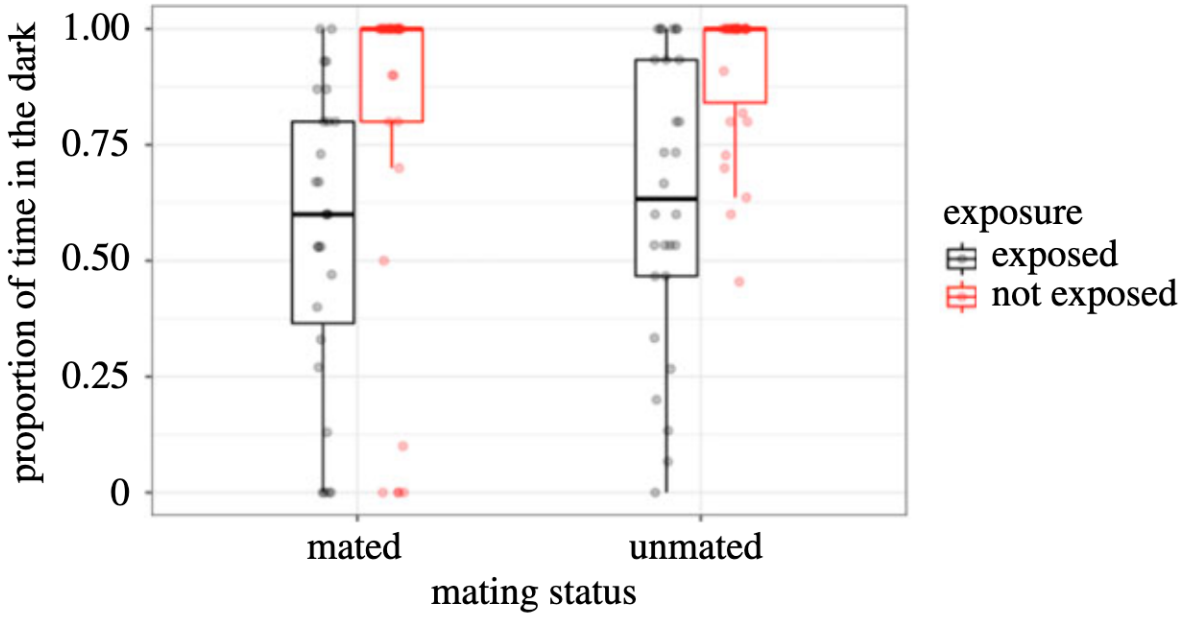


Figure 3.2.1: Phototaxis of mated and unmated female flies exposed and unexposed to mites. Flies were in individual phototaxis chambers either alone or with a single infectious mite. A score of 0 indicates the fly /mite in the light at all observations, and a score of 1 indicates the fly / mite was in the dark at all observations.

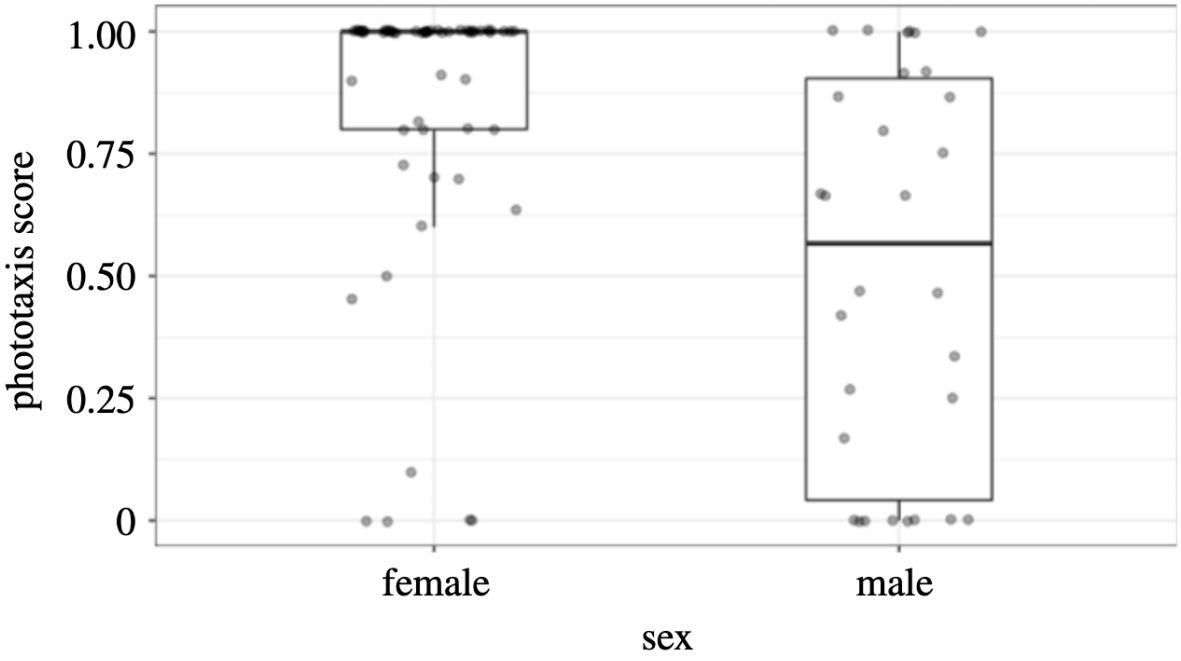


Figure 3.2.2: Phototaxis of flies by sex (pooling mated and unmated females) when not exposed to mites. A score of 0 indicates the fly /mite in the light at all observations, and a score of 1 indicates the fly / mite was in the dark at all observations.

3.2 References

1. Poulin R, Morand S. 2000 The diversity of parasites. Q. Rev. Biol. 75, 277–293. (doi:10.1086/393500)
2. Schulenburg H, Kurtz J, Moret Y, Siva-Jothy MT. 2009 Introduction. Ecological immunology. Phil. Trans. R. Soc. B 364, 3–14. (doi:10.1098/rstb.2008. 0249)
3. de Roode JC, Lefevre T. 2012 Behavioral immunity in insects. Insects 3, 789–820. (doi:10.3390/ insects3030789)
4. Kurz CL, Charroux B, Chaduli D, Viallat-Lieutaud A, Royet J. 2017 Peptidoglycan sensing by octopaminergic neurons modulates *Drosophila* oviposition. eLife 6, e21937. (doi:10.7554/eLife. 21937)
5. Rakus K, Ronsmans M, Vanderplasschen A. 2017 Behavioral fever in ectothermic vertebrates. Dev. Comp. Immunol. 66, 84–91. (doi:10.1016/j.dci.2016. 06.027)
6. Hunt VL, Zhong WH, McClure CD, Mlynski DT, Duxbury EML, Priest NK. 2016 Cold-seeking behaviour mitigates reproductive losses from fungal infection in *Drosophila*. J. Anim. Ecol. 85, 178–186. (doi:10.1111/1365-2656.12438)
7. Arnold PA, White CR, Johnson KN. 2015 *Drosophila melanogaster* does not exhibit a behavioural fever response when infected with *Drosophila* C virus. J. Gen. Virol. 96, 3667–3671. (doi:10.1099/jgv.0.000296)
8. Masuzzo A, Maniere G, Viallat-Lieutaud A, Avazeri E,Zugasti O, Grosjean Y, Kurz CL, Royet J. 2019 Peptidoglycan-dependent NF-κB activation in a small subset of brain octopaminergic neurons controls female oviposition. eLife 8, e50559. (doi:10.7554/eLife.50559)
9. Daversa DR, Manica A, Cenis HB, Lopez P, Garner TWJ, Bosch J. 2021 Alpine newts (Ichthyosaura alpestris) avoid habitats previously used by parasite- exposed conspecifics. Front. Ecol. Evol. 9, 636099. (doi:10.3389/fevo.2021.636099)
10. Gao K, van Wijk M, Dang QTD, Heckel DG, Zalucki MP, Groot AT. 2021 How healthy is your mate? Sex- specific consequences of parasite infections in the moth Helicoverpa armigera. Anim. Behav. 178, 105–113. (doi:10.1016/j.anbehav.2021.06.005)
11. Swartz TE et al. 2007 Blue-light-activated histidine kinases: two-component sensors in bacteria. Science 317, 1090–1093. (doi:10.1126/science.1144306)
12. Steinauer ML, Bonner KM. 2012 Host susceptibility is altered by light intensity after exposure to parasites. J. Parasitol. 98, 1052–1054. (doi:10.1645/ GE-3109.1)
13. Kim KN, Huang QY, Lei CL. 2019 Advances in insect phototaxis and application to pest management:a review. Pest Manag. Sci. 75, 3135–3143. (doi:10.1002/ps.5536)
14. Nagell B. 1977 Phototactic and thermotactic responses facilitating survival of *Cloeon dipterum* (Ephemeroptera) larvae under winter anoxia. Oikos 29, 342–347. (doi:10.2307/3543625)

15. Markow TA. 1988 Reproductive behavior of *Drosophila melanogaster* and *Drosophila nigrospiracula* in the field and in the laboratory. J. Comp. Psychol. 102, 169–173. (doi:10.1037/0735-7036.102.2.169)
16. Markow TA, Fogleman JC. 1981 Behavioral differentiation between two species of cactiphilic *Drosophila*. 1. Adult geotaxis and phototaxis. Experientia 37, 145–146. (doi:10.1007/BF01963198)
17. Polak M. 1996 Ectoparasitic effects on host survival and reproduction: the *Drosophila–Macrocheles* association. Ecology 77, 1379–1389. (doi:10.2307/2265535)
18. Li JF, Zhang W, Guo ZH, Wu S, Jan LY, Jan YN. 2016 A defensive kicking behavior in response to mechanical stimuli mediated by *Drosophila* wing margin bristles. J. Neurosci. 36, 11 275–11 282. (doi:10.1523/JNEUROSCI.1416-16.2016)
19. Horn CJ, Luong LT. 2019 Current parasite resistance trades off with future defenses and flight performance. Behav. Ecol. Sociobiol. 73, 77. (doi:10.1007/s00265-019-2697-5)
20. Horn CJ, Mierzejewski MK, Elahi ME, Luong LT. 2020 Extending the ecology of fear: parasite-mediated sexual selection drives host response to parasites. Physiol. Behav. 224, 113041. (doi:10.1016/j. physbeh.2020.113041)
21. Horn CJ, Luong LT. 2021 Trade-offs between reproduction and behavioural resistance against ectoparasite infection. Physiol. Behav. 239, 113524. (doi:10.1016/j.physbeh.2021.113524)
22. Walter DE, Proctor H. 2013 Systemic and morphological survey. In Mites: ecology, evolution & behaviour: life at a microscale, 2nd edn, pp. 39–68. Dordrecht, The Netherlands: Springer.
23. Niogret J, Lumaret J, Bertrand M. 2006 Semiochemicals mediating host-finding behaviour in the phoretic association between *Macrocheles saceri* (Acari: Mesostigmata) and *Scarabaeus species* (Coleoptera: Scarabaeidae). Chemoecology 16, 129–134. (doi:10.1007/s00049-006-0338-8)
24. Oliver JH, Krantz GW. 1963 *Macrocheles rodriguezi*, a new species of mite from Kansas (Acarina: Macrochelidae) with notes on its life cycle and behavior. Acarologia 5, 519–525.
25. Jalil M, Rodriguez JG. 1970 Studies of behavior of *Macrocheles muscaedomesticae* (Acarina: Macrochelidae) with emphasis on its attraction to house fly. Ann. Entomol. Soc. Am. 63, 738. (doi:10. 1093/aesa/63.3.738)
26. Luong LT, Horn CJ, Brophy T. 2017 Mitey costly: energetic costs of parasite avoidance and infection. Physiol. Biochem. Zool. 90, 471–477. (doi:10.1086/ 691704)
27. Wheeler DA, Hamblencoyle MJ, Dushay MS, Hall JC. 1993 Behavior in light-dark cycles of *Drosophila* mutants that are arrhythmic, blind, or both. J. Biol. Rhythms 8, 67–94. (doi:10.1177/074873049300800106)
28. Rieger D, Fraunholz C, Popp J, Bichler D, Dittmann R, Helfrich-Forster C. 2007 The fruit fly *Drosophila melangaster* favors dim light and times its activity peaks to early dawn and late dusk. J. Biol. Rhythms 22, 387–399. (doi:10.1177/0748730407306198)

29. Lazopulo S, Lopez JA, Levy P, Syed S. 2015 A stochastic burst follows the periodic morning peak in individual *Drosophila* locomotion. PLoS ONE 10, e0140481. (doi:10.1371/journal.pone.0140481)
30. Shaw PJ, Cirelli C, Greenspan RJ, Tononi G. 2000 Correlates of sleep and waking in *Drosophila melanogaster*. Science 287, 1834–1837. (doi:10. 1126/science.287.5459.1834)
31. Boltana S et al. 2013 Behavioural fever is a synergic signal amplifying the innate immune response. Proc. R. Soc. B 280, 20131381. (doi:10.1098/rspb.2013.1381)
32. Mierzejewski MK, Horn CJ, Luong LT. 2019 Ecology of fear: environment-dependent parasite avoidance among ovipositing *Drosophila*. Parasitology 146, 1564–1570. (doi:10.1017/S0031182019000854)
33. Gorostiza EA, Colomb J, Brembs B. 2016 A decision underlies phototaxis in an insect. Open Biol. 6, 160229. (doi:10.1098/rsob.160229)
34. Hadler NM. 1964 Heritability and phototaxis in *Drosophila melanogaster.* Genetics 50, 1269–1277. (doi:10.1093/genetics/50.6.1269)
35. Dobzhansky T, Spassky B. 1969 Artificial and natural selection for two behavioral traits in *Drosophila pseudoobscura*. Proc. Natl Acad. Sci. USA 62, 75–80. (doi:10.1073/pnas.62.1.75)
36. Markow TA. 1975 Genetic analysis of phototactic behavior in *Drosophila melanogaster*. 1. Selection in presence of inversions. Genetics 79, 527–534. (doi:10.1093/genetics/79.3.527)
37. Kain JS, Stokes C, de Bivort BL. 2012 Phototactic personality in fruit flies and its suppression by serotonin and white. Proc. Natl Acad. Sci. USA 109, 19 834–19 839. (doi:10.1073/pnas.1211988109)
38. Barber I, Dingemanse NJ. 2010 Parasitism and the evolutionary ecology of animal personality. Phil. Trans. R. Soc. B 365, 4077–4088. (doi:10.1098/rstb. 2010.0182)
39. Simon JC, Dickson WB, Dickinson MH. 2011 Prior mating experience modulates the dispersal of *Drosophila* in males more than in females. Behav. Genet. 41, 754–767. (doi:10.1007/s10519-011-9470-5)
40. Isaac RE. 2019 The effect of mating and the male sex peptide on group behaviour of post-mated female *Drosophila melanogaster*. Neurochem. Res. 44, 1508–1516. (doi:10.1007/s11064-019-02722-7)
41. Kubli E, Bopp D. 2012 Sexual behavior: how sex peptide flips the postmating switch of female flies. Curr. Biol. 22, R520–R522. (doi:10.1016/j.cub.2012. 04.058)
42. McGeary MK, Findlay GD. 2020 Molecular evolution of the sex peptide network in *Drosophila*. J. Evol. Biol. 33, 629–641. (doi:10.1111/jeb.13597)
43. Xue L, Noll M. 2000 *Drosophila* female sexual behavior induced by sterile males showing copulation complementation. Proc. Natl Acad. Sci. USA 97, 3272–3275. (doi:10.1073/pnas.97.7.3272)

44. Mack PD, Kapelnikov A, Heifetz Y, Bender M. 2006 Mating-responsive genes in reproductive tissues of female *Drosophila melanogaster*. Proc. Natl Acad. Sci. USA 103, 10 358–10 363. (doi:10.1073/pnas. 0604046103)
45. Buck JC, Weinstein SB, Young HS. 2018 Ecological and evolutionary consequences of parasite avoidance. Trends Ecol. Evol. 33, 619–632. (doi:10. 1016/j.tree.2018.05.001)
46. Kirk D, Greischar M, Mideo N, Krkosek M. 2021 Environmental variability affects optimal trade-offs in ecological immunology. Ecosphere 12, e03654. (doi:10.1002/ecs2.3654)
47. Daversa DR, Hechinger RF, Madin E, Fenton A, Dell AI, Ritchie EG, Rohr J, Rudolf VHW, Lafferty KD. 2021 Broadening the ecology of fear: non-lethal effects arise from diverse responses to predation and parasitism. Proc. R. Soc. B 288, 20202966. (doi:10.1098/rspb.2020.2966)

Chapter 4: Parasites have NCEs on the fitness of potential hosts

Chapter 4 Introduction

In this section, I tested if mite NCEs impact the survival and reproduction of female flies. In fitness experiments, we measured the survival and fecundity of female flies exposed to mites for their entire life, but without the risk of infection. Survival (days alive) and number of adult offspring were lower among females chronically exposed to mites (4.1). In a follow up study, we used modelling to predict if host population growth would be suppressed by parasite NCEs based on the parameters we measured in (4.1). Although the consumptive and NCEs of parasites combined reduced the growth of simulated populations, the effects of NCEs alone may not impact host population growth (4.2).

4.1: Chronic mite exposure without infection lowers female fly fitness

Introduction

Parasite-mediated host mortality is generally assumed to be a consequence of direct harm caused by damage to host tissue, leaching of micronutrients and/or perturbations to host energy budgets [1; 2; 3]. However, direct effects during infection or upon contact are not the only effects parasites have on their host populations. Indirect effects that arise during proximity to parasite infective stages can have potentially significant consequences for host ecology and evolution. These include the costs of behavioural defences, parasite avoidance, maintaining immunity and compensatory physiological changes [4; 5]. The indirect effects of parasitism on potential hosts are analogous to the non-consumptive effects (NCEs) observed in predator-prey systems, in which exposure to predators alters traits among prey species even if the prey is not eaten [6].

Non-consumptive effects are trait-mediated as they depend on the presence of predators and the effect their presence has on the traits (physiological and behavioural) of their prey [6]. Changes in the prey include behavioural avoidance of predators and/or risky habitats, elevated stress responses, not exploiting resources fully, altered competitive ability, and physiological changes [7]. Peacor and Werner [6] distinguished NCEs from the indirect effects of predators on a third species, which they call trait-mediated indirect effects: e.g. a species benefits from a predator preferentially consuming its competitor. Non-consumptive effects are known to reduce the fitness of prey species [8; 9]. An early experiment by Peckarsky et al. [8] showed that mayfly larvae exposed to predators with glued mouthparts had 21-25% slower growth rates and produced fewer eggs later in life than control larvae. This area of predator-prey research is often called the 'ecology of fear' due to the physiological stress and neuro-behavioural responses predators cause in prey [7; 10]. Our objective is to test if parasites suffer a trait-mediated reduction in fitness from the presence of parasites.

Current attempts to integrate parasitology and community ecology may underestimate the effects parasites have on host populations by ignoring effects of parasites on host populations when infection does not take place. The direct effects of infection with macroparasites is generally dependent on the intensity of infection, i.e. density-dependent [11]; while indirect changes in host physiology and behaviour

in response to parasites are likely trait-mediated [12]. Recent work has shown that initial contact with parasites has physiological and fitness effects on the host even when infection does not proceed [13; 14]. Contact with the infectious stages of trematodes significantly impacted tadpole fitness, even when infection failed to establish [15]. Comparable results are seen in fungal diseases [13], suggesting adverse effects from parasite contact occur with a wide range of parasites. Furthermore, the mere presence of parasites may adversely affect hosts; e.g. tadpoles increase their activity in response to the parasite-derived chemical cues and avoid the areas containing those cues [16]. We aim to determine if parasite NCEs are sufficient to reduce host fitness without direct contact. On initial consideration, the cost of infection may appear low relative to predation. This relatively low cost may minimise the amount of investment in parasite defences relative to predation defences, and by extension reduce potential NCEs [12]. However, the frequency of parasite exposures for many free-living organisms is greater than the number of potential predation events [12]. As such, hosts may invest more heavily in parasite defences than initially anticipated.

In this study, we experimentally test if exposure to parasites is sufficient to reduce host fitness (i.e. exposure without contact or infection). Our study extends previous research on parasite exposure, by testing if hosts suffer deleterious fitness effects from the presence of parasites even when direct contact does not occur. *Macrocheles subbadius* (Acari: Macrochelidae) is a naturally occurring facultative parasite of *Drosophila nigrospiracula* (Diptera: Drosophilidae) that feeds on fly haemolymph and uses flies for dispersal [17]. Previous studies have found that infection with mites reduces *D. nigrospiracula* longevity and fecundity [17]. Fly hosts typically respond to approaching mites with behavioural defences that are energetically demanding, including bursts of movement and intense grooming behaviour [17; 18]. Previously, we showed that when flies are exposed to mites (sequestered behind a mesh wall), their energy consumption increases, suggestive of increased stress and/or activity linked to the defensive behaviours [18]. The long-term energetic costs of exposure to parasites may divert essential resources away from somatic maintenance and reproduction [19; 20]. We therefore hypothesise that chronic exposure to the infective stages of a parasite will reduce host fitness. Reproduction can impose additional demands on host resources that cause parasites to impact hosts in ways that would otherwise be undetectable [21]. For example, sham infections induce more energetically demanding immune responses in pregnant mice relative to non-pregnant females [21]. Specifically, we predict that exposure to restrained mites will decrease the longevity and fecundity of female flies, and that the effect on longevity will be exacerbated for mated flies. We experimentally manipulated exposure to mites by housing flies with caged mites to assess the impact of parasite proximity on host fitness. To our knowledge, this is the first study to experimentally demonstrate a loss in host fitness due to the indirect effects of exposure *sans* parasite contact.

Materials and methods

Fly and mite cultures

Drosophila nigrospiracaula Patterson and Wheeler were cultured from flies (~120 adults of each sex) collected in the Sonoran desert (Phoenix, Arizona) from necrotic cacti (*Carnegiea gigantea*). Larval stages were kept on a medium consisting of instant potato flakes, *Drosophila* medium (Formula 4-24 Instant Drosophila Medium, Carolina Biological Supply Company, Burlington, NC, USA), nutritional yeast, and autoclaved necrotic cactus. Newly eclosed adult flies were transferred to vials with agar medium within 48 h of emergence. Fly cultures were kept in incubators at 24℃ and 50% relative humidity with a 12 h day-night cycle (Percival Scientific, Perry, IA, USA).

Macrocheles subbadius Berlese (200-300 adult females) were collected from naturally infected wild flies and used to initiate laboratory cultures. Cultured mites were reared on 2:1 wheat bran to wood chips media. Free-living bacteriophagic nematodes (Rhabditida) were co-cultured with the mites as a food source. Mite cultures were maintained at 26°C and 70% relative humidity and a 12 h light cycle. Mites used in the experiments below were collected from the stock culture using a Berlese funnel [22].

Longevity exposure experiment

This experiment was conducted to determine if chronic exposure to mites, without contact, affects fly longevity. Two primary factors were considered, parasite exposure and fly reproductive status. Female flies were therefore assigned randomly to one of four groups: reproducing and exposed to mites, reproducing without mites, unmated and exposed to mites, or unmated without mites. Adult female flies were moved to agar vials upon emergence and housed with or without restrained mites and with or without mates depending on the experimental condition. Exposed groups were housed with mites without a realised risk of infection. This was achieved by creating a small divot (~0.5cm deep and 1.5 cm across) in the underside of the foam plug in the fly vials. Five adult female mites were placed into the divot, which was then covered (using Elmer's super glue) by a small piece of polyester mesh, sequestering the mites. The mesh allowed chemical and visual cues to pass through, but prevented physical contact between flies and mites. With the mites restrained behind the mesh screen, the plug was lowered into the vial, leaving a 2.5 cm space between the agar and the base of the plug (13.3 cm^3), maximising the proximity and likelihood the flies could detect the mites. Control flies had plugs prepared the same way, but without mites, and had identical living spaces.

Fly reproductive status was established by rearing flies with either a male or female companion. Mated females were housed with a male fly and unmated females were reared with another female companion to control for population density effects. Companion flies had the tip of a wing clipped with micro-scissors to distinguish them from the experimental fly. The plug of each vial and the mites within were replaced biweekly, alternating 3 and 4 days between changes (average 3.5 days). *Macrocheles subbadius* can survive 4-5 days without food if kept in a humid environment (pers obs). Agar vials were changed simultaneously with a plug replacement once a week. In order to make sure the reproducing flies

had a constant source of sperm, the companion male was replaced at the same time as the vial change. Since the novelty of new companions may affect fly longevity, we replaced both the male and female companions during the vial change. New companions were all unmated females pulled from stock cultures at random.

Flies in the longevity experiment were kept in an incubator at 26 ºC and 70% RH. Survival was checked every 24 h until the day of death (i.e. days alive). Following death, flies were frozen at -20ºC allowing thorax length to be measured post-mortem as a potential cofactor of longevity. Thoraxes were measured from the most anterior part of the thorax to the scutellum tip [23]. Measurements were made using a Leica MI 20HD camera mounted on a Leica M80 dissecting microscope and processed with the LAS EZ software (Leica Microsystems, Wetzlar, Germany). Two blocks of the experiment were conducted in an identical manner. Both replicate blocks consisted of experimental flies (20 control and 20 exposed flies).

Fecundity-exposure experiment

This experiment measured the lifetime fecundity of flies exposed to mites as compared with unexposed flies. We defined fecundity as the number of offspring to survive to the adult life stage (i.e. offspring that survive to eclosion). Flies were housed in vials with foam plugs containing mites restrained behind a mesh screen or plugs without mites (described above). The experiment commenced 3 days post-eclosion to avoid female flies dying before reproductive maturity [24]. Since *D. nigrospiracula* offspring fail to develop on agar medium, flies in this experiment were maintained on an 18:5 mix of instant potato and fly media supplemented with autoclaved cactus. Incidentally, this medium is not optimal for the survival of adult *D. nigrospiracula*, and as such flies in this experiment survived for less time than flies in the longevity-exposure experiment (see results). To reduce the risk of flies drowning in the relatively wet media, occupancy space was increased to 18.6 cm. Experimental females were maintained in an incubator at 26 ºC and 70% relative humidity. Female flies housed with a single male companion. Experimental flies were transferred to fresh vials every 3-4 days, at which time the male was replaced with a mature unmated male. Survival was checked every 24 h until death. Post-mortem, thorax length was measured (as above). Previously occupied vials were maintained in the incubator until FI emergence. Vials were monitored for 2 weeks following the removal of the adult flies, newly eclosed adults were harvested and counted within 48 h of emergence.

Statistical analyses

All statistical analyses were performed in R using the R studio environment (R studio team, 2013, Version 0.98.932). Linear models for analysis were made using the GLM function (R Core Team, Stats). We analysed the longevity-exposure experiment using two different methods: generalised linear models with stepwise backwards deletion and a Kaplan-Meier technique. Before model reduction, data were normalised using a Box-Cox transformation, $\lambda = 0.38$ (R, MASS Package). The full model included

parasite exposure, mating status, thorax length and block as independent factors. Starting with the least significant variable, factors were removed sequentially. The new model was compared with the previous model with an analysis of variance (ANOVA), and a variable was only retained if there was a significant difference between models (X^2 test, α = 0.05). We compared the survivorship curves for each treatment group using the Survdiff function (R, Survival Package). None of the flies were censored in the longevity-exposure experiment. Since the longevity-exposure experiment was carried out in two replicate batches, block was included as a cofactor.

The fecundity-exposure data were also analysed with backwards model comparison. The glm function was used with a Poisson distribution (R Core Team, Stats), and models were compared with an ANOVA (α = 0.05). Fecundity was the response variable in this experiment; longevity, thorax length and parasite exposure were treated as independent variables. One fly in the control group survived beyond the 28-day experimental period and was censored from the longevity analysis, but not fecundity analysis.

Results

Longevity-exposure experiment

We measured the life span of mated and unmated flies that were either exposed to mites, or not exposed and found only exposure to be a significant predictor of longevity. Exposed flies (N = 40) survived 15.3 ± 1.76 (mean ± S.E.) days post-emergence, a 38% difference compared with unexposed flies (N = 40) that lived 22.4±1.83 days (ΔResidual sum of squares [RSS] = 5.37, P = 0.003). Overall, unmated females (N = 40) lived on average 18.7 ± 1.82 days and mated flies (N = 40) lived 19.2 ± 1.97 days; reproductive status was not a significant predictor of longevity (ΔRSS = 0.85, P = 0.85). The interaction between parasite exposure and mating status was not significant (ΔRSS = 0.064, P = 0.75). Thorax lengths of six flies were not measured due to poor specimen preservation. The mean thorax length in the control group was 1.11 ± 0.007 mm (N = 39) and 1.16 ± 0.016 S.E. mm (N = 35) in the exposed group; thorax length was not a significant predictor of longevity (ΔRSS = 0.835, P = 0.25). Block was also not a significant factor (ΔRSS = 0.982, P = 0.21). Parasite exposure was the only significant predictor of longevity among female flies.

The survival curves of the exposed and unexposed groups were compared using the Survdiff function (Fig. 4.1.1). Flies exposed to mites experienced greater early die off, and although this trend slowed with time the two groups did not achieve parity. The survivorship curves for the exposed group and unexposed group were significantly different (Survdiff, X^2 = 5.1, P = 0.018).

Fecundity-exposure experiment

There was a 13% difference in fecundity between exposed flies and control flies; the unexposed group had a mean fecundity of 36.4 ± 8.0 offspring (N = 20), while the exposed group produced 32.1 ± 6.7 offspring (N = 20). Exposed flies lived on average 10.0 ± 0.87 days (N = 20), a 23.2% difference from the control flies, which survived on average 12.6 ± 1.33 days (N = 19). Thorax length was not a significant

predictor of fecundity (Δdeviance = 0.34, P = 0.56). While exposure status had a significant effect on fecundity (Δdeviance = 18.0, P < 0.001), survival time (Δdeviance > 100, P < 0.001) was a more important factor in predicting fecundity. In other words, exposure to mites strongly impacted longevity, which in turn affected lifetime fecundity (Fig. 4.1.2). Appendix 2 further analyses the effect of the interaction between mite exposure and survival on lifetime fecundity (A2.1).

Data Availability

Data from this study are available at Dryad (doi.org/10.5061/dryad.gmsbcc2pn).

Discussion and conclusion

We tested the hypothesis that chronic proximity to parasites incurs NCEs that adversely affect host fitness. As predicted, female flies exposed to mites suffered reduced survival and lifetime fecundity relative to unexposed females. Not surprisingly, flies that lived longer produced more total offspring. As such, the reduction in fecundity among flies exposed to mites was likely driven by strong effects on longevity. Indeed, longevity was a stronger predictor of fecundity than exposure status. Thus, the effect of exposure on life-time fecundity was primarily longevity mediated, though the fact remains that exposed flies produced fewer offspring. Previous research showed that female *D. nigrospiracula* infected with mites produced 102% fewer eggs than uninfected flies [17]. In that study, the decreased egg output was driven by a 59% reduction in life span among infected flies compared with uninfected flies [17]. Not surprisingly, we observed a smaller effect size as infection likely has a stronger biological effect than exposure alone. Still, the relatively smaller effect size could potentially incur an accumulated cost at the population level.

Previous studies have shown that initial contact with parasites can negatively affect hosts, even if that contact does not lead to a sustained infection [14; 15]. Tadpoles that experienced epidermal damage typical of trematode attack, but did not develop lasting infections, exhibited reduced longevity compared with control tadpoles [15]. Sears et al. [14] showed the fitness cost of coming into contact with parasites depends on the host's relative investment in resisting or tolerating parasites. We extend these findings and show that direct contact between the host and parasite is not necessary for exposure to have a negative effect on host fitness. It is possible that host resistance may influence the extremity of the NCEs, and could be investigated in future research.

The presence of mites and ostensibly cues they produce were sufficient to decrease fly fitness. Currently, we do not know which cue(s) *D. nigrospiracula* use to detect *M. subbadius*, though it is clear that these cues can pass through translucent mesh (see results; [18]). Experimental manipulations of fly vision and olfaction are needed to understand how flies detect mites [25; 26]. Larsson et al. [25] identified Or83b as a gene necessary for olfaction in *Drosophila*, and advances in rapid gene manipulation makes manipulation of fly olfaction viable for future studies [27].

We also predicted that mating status would exacerbate non-consumptive reductions in longevity. We did not find a relationship between mating status and loss of fitness from proximity to parasites. Although in some cases reproduction can make previously undetected parasite effects worse [21; 28], parasite exposure alone was sufficient to reduce host longevity in our study. It is possible that the impact of the non-infective effect was large enough that it masked any interaction between exposure and mating status. Alternatively, environmental factors can mask effects of reproduction/copulation on longevity. The effects of mating on the survival of wild-type flies are not always straightforward [29], and disentangling the effects of copulation, reproduction itself, and parasite exposure is an avenue for future research.

Among the indirect effects of parasites on host fitness, the costs of immune activation are the most well studied; for instance, an immune response against heat-inactivated bacteria reduces the survival of calorically restricted bees [4]. Insects can also experience autoimmune tissue damage [30], so both energetic costs and self-harm from immune activation could mediate reductions in life span from immunity. The ecological consequences of immunity and defence have become the purview of ecological immunology [31; 32]. In their review, Schulenburg et al. [32] categorised costs of host defences into three primary groups: genetic (fixed costs), usage (costs at activation) and immunopathology (self-harm from immune processes). Behavioural defences by *Drosophila* spp. against approaching mites have high energetic costs at activation [18], and thus energetic trade-offs likely contribute to the decrease in fitness observed in the present study. Interestingly, some insects also express an uptick in respiration upon predator exposure [33], suggesting that similar mechanisms may drive the NCEs of parasites and predators.

Other resource-intensive methods of resistance involve the production of costly defensive features. The production of chitin by arthropods is plastic and increases in many arthropods in response to threats from both predators and parasites [34]. Similarly, in insects the hardening of the cuticle via melanization has been shown to either kill or fend off several parasites and pathogens [35]. The energetic and material costs of producing defensive structures and compounds divert resources away from somatic and reproductive activities. Future research should examine if long-term exposure to parasites upregulates the expression of fly genes associated with defensive elements.

Other possible mechanisms underlying the observed loss of fitness include the detrimental effects of vigilance and chronic stress. Maintaining vigilance against impending infection may reduce a fly's ability to forage and/or exploit resources. In predator-prey systems, the need to remain vigilant can reduce prey species fitness relative to competitors, and reduce the efficiency of resource exploitation [6]. In their meta-analysis of non-consumptive effects in arthropods, Buchanan et al. [9] found that predator presence has a significant effect on the feeding behaviour of arthropod prey. Rohr et al. [16] showed that tadpoles change their behaviour and location in response to parasite-derived cues, but did not measure changes in fitness resulting from these behavioural changes. The changes in tadpole behaviour were similar with parasite exposure and predator exposure [16], suggesting that the former may induce changes in hosts similar in

extent to predator exposure [12]. Changes in feeding behaviour and foraging ecology may explain the decrease in fly fitness observed in our study.

The risk of infection may also have implications for fly dispersal, which is known to be influenced by threats of predation [36]. If the mere presence of mites imposes a fitness decrease on flies, it may influence the conditions under which flies will leave a resource patch [6]. Dispersal in turn may also limit the impact of non-infective effects endured by hosts with implications for fly population structures and dispersal patterns [36]. Future studies should integrate parasites into the ecology of fear hypothesis, and examine the indirect effects of parasitism on host population structures outside of infection.

Chronic stress from parasite exposure may impact host fitness. Many prey insects undergo hormonal changes following predator exposure that can affect growth, metamorphosis and immune function [37; 38]. Slos and Stoks [33] found that increases in the stress proteins of larval damselflies following predator exposure is linked with decreases in antioxidative catalase activity. In *Drosophila*, several stress-associated hormones impact long-term survival [37; 38], and if expressed following parasite exposure may explain the decrease in longevity observed here. Threat-induced stress in insects can both increase life-shortening traits and reduce life-sustaining processes. However, there is a paucity of research investigating the link between *Drosophila* stress hormones and the risk of parasitism. Based on general trends in the ecology of fear, we expect that related hormones may be produced in response to both predation and infection risk. Our results suggest studying oxidative-stress associated gene expression during parasite exposure may be a fruitful avenue of future research.

In conclusion, we investigated the fitness costs of chronic exposure to parasites independent of contact or infection with the parasite itself. To our knowledge, no other studies have experimentally shown a decrease in host fitness in the absence of direct contact between hosts and parasites. Our work fits into the growing body of literature expanding the roles of parasites in a community ecology context. Current frameworks may underestimate the effects of parasites on communities by neglecting the NCEs of parasites. Our findings also demonstrate the fruitfulness of testing hypotheses derived from predator-prey models in parasite-host systems and potential unities within natural enemy ecology.

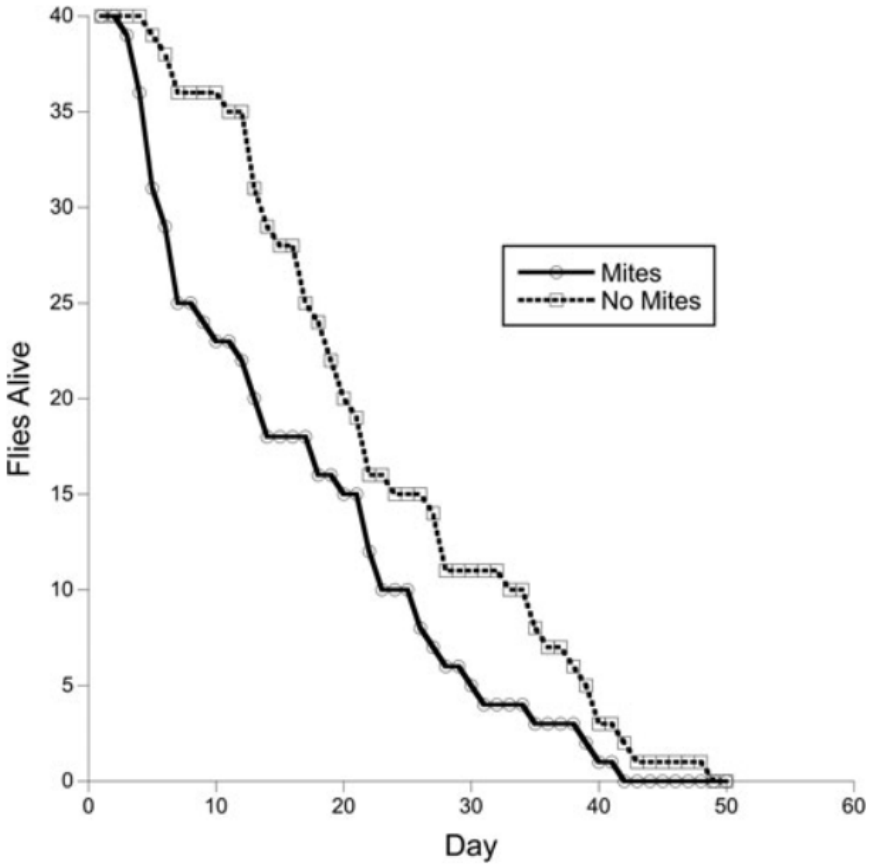


Figure 4.1.1: Survivorship curves for flies that were either exposed to restrained mites (n = 40, solid line) or not exposed at all (n = 40, dashed line). The survivorship curves are significantly different (Survdiff, χ^2 = 5.1, P = 0.018).

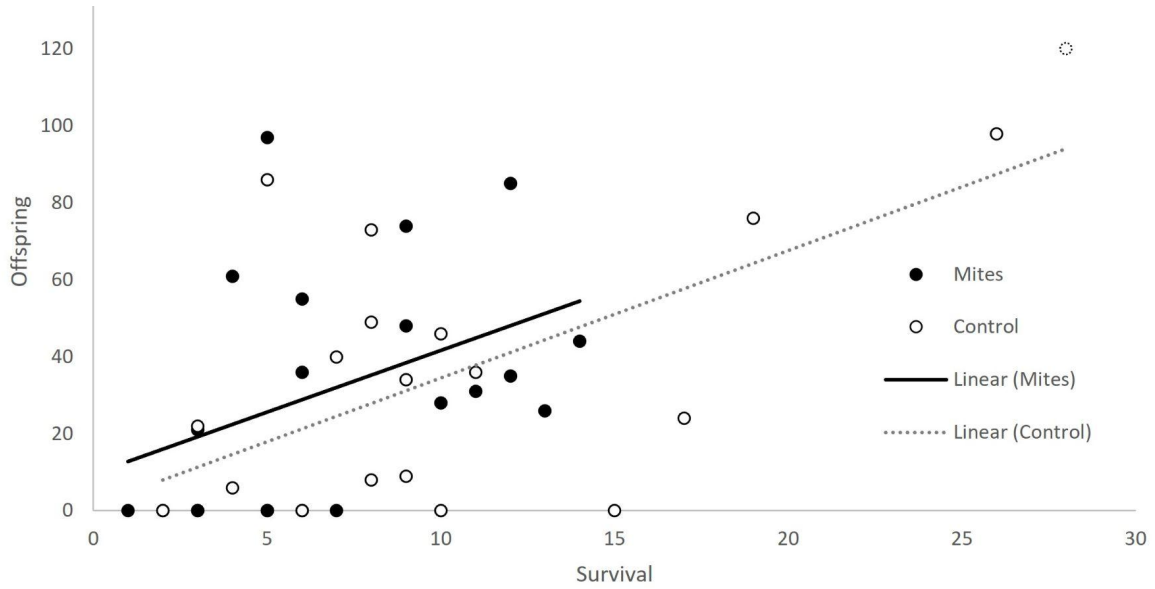


Figure 4.1.2: Reproductive output (measured in adult offspring) of female flies vs. age at death for mite-exposed and unexposed flies. Flies (n = 40) were raised on a fly medium-instant potato mix supplemented with necrotic cactus. One fly in the unexposed condition lived beyond the 28-day trial period, but was treated as if it lived 28 days for this figure (dashed point).

4.1 References

1. Poulin R and Morand S (2000) The diversity of parasites. Quarterly Review of Biology 75, 277–293.
2. Robar N, Burness G and Murray DL (2010) Tropics, trophics and taxonomy: the determinants of parasite-associated host mortality. Oikos 119, 1273–1280.
3. Adelman JS and Hawley DM (2017) Tolerance of infection: a role for animal behavior, potential immune mechanisms, and consequences for parasite transmission. Hormones and Behavior 88, 79–86.
4. Moret Y and Schmid-Hempel P (2000) Survival for immunity: the price of immune system activation for bumblebee workers. Science 290, 1166–1168.
5. Hart BL (2011) Behavioural defences in animals against pathogens and parasites: parallels with the pillars of medicine in humans. Philosophical Transactions of the Royal Society B-Biological Sciences 366, 3406–3417.
6. Peacor SD and Werner EE (2008) Nonconsumptive effects of predators and trait-mediated indirect effects. In Encyclopedia of Life Sciences (ELS).
7. Preisser EL and Bolnick DI (2008) The many faces of fear: comparing the pathways and impacts of nonconsumptive predator effects on prey populations. PLoS ONE 3, 1–8.
8. Peckarsky B, Cowan C, Penton M and Anderson C (1993) Sublethal consequences of stream-dwelling predatory stoneflies on mayfly growth and fecundity. Ecology 74, 1836–1846.
9. Buchanan AL, Hermann SL, Lund M and Szendrei Z (2017) A meta-analysis of non-consumptive predator effects in arthropods: the influence of organismal and environmental characteristics. Oikos 126, 1233–1240.
10. Clinchy M, Schulkin J, Zanette LY, Sheriff MJ, McGowan PO and Boonstra R (2011) The neurological ecology of fear: insights neuroscientists and ecologists have to offer one another. Frontiers in Behavioral Neuroscience 5, 1–6.
11. Wilber MQ, Weinstein SB and Briggs CJ (2016) Detecting and quantifying parasite-induced host mortality from intensity data: method comparisons and limitations. International Journal for Parasitology 46, 59–66.
12. Raffel TR, Martin LB and Rohr JR (2008) Parasites as predators: unifying natural enemy ecology. Trends in Ecology & Evolution 23, 610–618.
13. Rohr JR, Raffel TR, Halstead NT, McMahon TA, Johnson SA, Boughton RK and Martin LB (2013) Early-life exposure to a herbicide has enduring effects on pathogen-induced mortality. Proceedings of the Royal Society B-Biological Sciences 280, 1–7.
14. Sears BF, Snyder PW and Rohr JR (2015) Host life history and host-parasite syntopy predict behavioural resistance and tolerance of parasites. Journal of Animal Ecology 84, 625–636.
15. Rohr JR, Raffel TR and Hall CA (2010) Developmental variation in resistance and tolerance in a multi-host-parasite system. Functional Ecology 24, 1110–1121.

16. Rohr JR, Swan A, Raffel TR and Hudson PJ (2009) Parasites, info-disruption, and the ecology of fear. Oecologia 159, 447–454.
17. Polak M (1996) Ectoparasitic effects on host survival and reproduction: the Drosophila-Macrocheles association. Ecology 77, 1379–1389.
18. Luong LT, Horn CJ and Brophy T (2017) Mitey costly: energetic costs of parasite avoidance and infection. Physiological and Biochemical Zoology 90, 471–477.
19. Auld S, Penczykowski RM, Ochs JH, Grippi DC, Hall SR and Duffy MA (2013) Variation in costs of parasite resistance among natural host populations. Journal of Evolutionary Biology 26, 2479–2486.
20. Lu ZC, Wang YM, Zhu SG, Yu H, Guo JY and Wan FH (2014) Trade-offs between survival, longevity, and reproduction, and variation of survival tolerance in Mediterranean *Bemisia tabaci* after temperature stress. Journal of Insect Science 14, 1–14.
21. Odiere MR, Koski KG, Weiler HA and Scott ME (2010) Concurrent nematode infection and pregnancy induce physiological responses that impair linear growth in the murine foetus. Parasitology 137, 991–1002.
22. Smith RL (1980) Ecology and Field Biology. New York, USA. Harper & Row, Publishers.
23. Bergland AO, Genissel A, Nuzhdin SV and Tatar M (2008) Quantitative trait loci affecting phenotypic plasticity and the allometric relationship of ovariole number and thorax length in *Drosophila melanogaster*. Genetics 180,567–582.
24. Markow TA (1996) Evolution of *Drosophila* mating systems. Evolutionary Biology 29, 73–106.
25. Larsson MC, Domingos AI, Jones WD, Chiappe ME, Amrein H and Vosshall LB (2004) Or83b encodes a broadly expressed odorant receptor essential for *Drosophila* olfaction. Neuron 43, 703–714.
26. Gaudry Q, Nagel KI and Wilson RI (2012) Smelling on the fly: sensory cues and strategies for olfactory navigation in *Drosophila*. Current Opinion in Neurobiology 22, 216–222.
27. Koutroumpa FA, Monsempes C, Francois MC, de Cian A, Royer C, Concordet JP and Jacquin-Joly E (2016) Heritable genome editing with CRISPR/Cas9 induces anosmia in a crop pest moth. Scientific Reports 6, 1–9.
28. Careau V, Thomas DW and Humphries MM (2010) Energetic cost of bot fly parasitism in free-ranging eastern chipmunks. Oecologia 162, 303–312.
29. Markow TA (2011) "Cost" of virginity in wild *Drosophila melanogaster* females. Ecology and Evolution 1, 596-600.
30. Sadd BM, Siva-Jothy MT (2006) Self-harm caused by an insect's innate immunity. Proceedings of the Royal Society B-Biological Sciences 273, 2571-2574.
31. Sadd BM and Schmid-Hempel P (2009) Principles of ecological immunology. Evolutionary Applications 2, 113–121.

32. Schulenburg H, Kurtz J, Moret Y and Siva-Jothy MT (2009) Introduction. Ecological immunology. The Royal Society, Philosophical Transactions of the Royal Society B 364, 3–14.
33. Slos S and Stoks R (2008) Predation risk induces stress proteins and reduces antioxidant defense. Functional Ecology 22, 637–642.
34. Beckerman AP, de Roij J, Dennis SR and Little TJ (2013) A shared mechanism of defense against predators and parasites: chitin regulation and its implications for life-history theory. Ecology and Evolution 3, 5119–5126.
35. Nakhleh J, Moussawi LE and Osta MA (2017) The melanization response in insect immunity. In P. Ligoxygakis (ed). Advances in Insect Physiology, Vol. 52. Cambridge, MA: Academic Press, pp. 83–109.
36. Geraldi NR and Macreadie PI (2013) Restricting prey dispersal can overestimate the importance of predation in trophic cascades. PLoS ONE 8, 1–9.
37. Adamo SA, Easy RH, Kovalko I, MacDonald J, McKeen A, Swanburg T, Turnbull KF and Reeve C (2017) Predator exposure-induced immunosuppression: trade-off, immune redistribution or immune reconfiguration? Journal of Experimental Biology 220, 868–875.
38. Kulkarni PS and Gramapurohit NP (2017) Effect of corticosterone on larval growth, antipredator behaviour and metamorphosis of *Hylarana indica*. General and Comparative Endocrinology 251, 21–29.
39. Ekengren S, Tryselius Y, Dushay MS, Liu G, Steiner HÅK and Hultmark D (2001) A humoral stress response in *Drosophila*. Current Biology 11, 714–718.
40. Kubrak OI, Lushchak OV, Zandawala M and Nassel DR (2016) Systemic corazonin signalling modulates stress responses and metabolism in *Drosophila*. Open Biology 6, 272–283.

4.2: Modelling fly populations experiencing non-consumptive effects

Introduction

Non-consumptive effects (NCEs) are the impacts predators have on potential prey outside of consumption, contrasted with predation itself (i.e. consumption) [1; 2]. This “ecology of fear” can manifest as changes in the physiology, behaviour, and morphology of potential prey under predation risk [3, 4]. NCEs can ultimately reduce the survival and reproductive success of potential prey [3; 5]. Furthermore, NCEs on individuals can scale up to population-level effects [6, 7]. In fact, one meta-analysis suggests NCEs may be responsible for over half of the impact of predators on prey populations [8]. Laboratory studies, mesocosms, and statistical modelling have been used to study the NCEs of predators on prey populations [3; 5; 9]. However, field studies of NCEs are limited to traits that are readily measured, especially among long-lived species where longevity and lifetime fecundity may not be observable in the study period [10]. Given these challenges, few studies have empirically tested for suppression of wild populations through NCEs [10]. Modelling provides a useful framework for estimating the scalability of individual-level NCEs to impacts on population growth [7]. For example, models showed that changes in individual physiology among porcupines at risk of predation could lead to reduced birth rates and subsequently a reduction in population size [7].

Recent research has extended the concept of NCEs to describe interactions between hosts and parasites, as well as other natural enemies and their victims (e.g. parasitoids) [11, 12, 13]. Parasites have consumptive effects on hosts during infection when they feed on host tissues/energy [14, 15]. Laboratory studies have found there are physiological, behavioural, and fitness impacts of exposure to parasites on individual hosts even when infection does not occur [16; 17; 18]. To date it is unclear if these individual-level NCEs impact hosts on a population level. Parasite infection has smaller effects than predation, and this disparity may explain the smaller individual-level NCEs observed in tadpole-parasite interactions than tadpole-predator interactions in a recent meta-analysis [13]. However, almost all free-living organisms face the risk of parasitism, and small individual effects may scale up into substantial effects on host populations [14; 19]. Thus, there is a need to study NCEs of parasites as they may be widespread yet underestimated [14, 15]. In this study, we simulated host populations that experience either 1) fear (NCEs) only, 2) fear and infection (i.e., NCEs and consumptive effects), or 3) neither.

We used published data on a *Drosophila-Macrocheles* association (reviewed in [20]) to model the consumptive and non-consumptive effects of parasitism. The mite *Macrocheles subbadius* (Berlese) (Mesostigmata: Macrochelidae) is a naturally occurring ectoparasite of *Drosophila nigrospiracula* Patterson and Wheeler (Diptera: Drosophilidae) [21]. *D. nigrospiracula* feed and reproduce on rotting cacti, and migrate to new sites as the decaying cactus desiccates [22]. Mites use their chelicerae to attach to flies and feed on the hemolymph of adult flies [21]. Unlike most host-parasite systems, empirical data exists on both the consumptive and non-consumptive effects of *M. subbadius* on *D. nigrospiracula* fitness (measured in longevity and lifetime fecundity) under laboratory conditions [17; 21]. Infection with mites reduces the survival and fecundity of adult flies by up to ~60% [21; 23]. Exposure to mites, without infection, induces costly defensive behaviours in flies [24, 25], reduces glycogen and lipid stores [18], and ultimately shortens fly lifespans as well as lowering fecundity [17]. Because both NCEs and consumptive effects on fly fitness (defined in terms of survival and reproduction) have been measured, this fly-mite system provides a unique opportunity to model the ecology of fear in a host-parasite system.

We hypothesized that ectoparasites negatively impact host populations (growth and final size) through NCEs on individual fitness. Specifically, we predicted that simulated fly populations experiencing NCEs will have reduced growth rates / smaller final populations relative to populations not experiencing NCEs due to individual reductions in lifetime fecundity. Alternatively, many hosts increase reproductive effort when at risk of reduced survival due to parasites/infection, i.e. compensate [26, 27, 28]. For example, snails from populations with higher rates of castrating parasites have higher egg production than snails from low prevalence populations [29]. Among female *D. nigrospiracula*, the decrease in lifespan during mite exposure (without infection) was larger than the reduction in lifetime fecundity, 22% shorter lifespans versus 13% lower fecundity, and the daily egg laying rate was higher in exposed flies even though lifetime fecundity was lower [17]. Combined, these results suggest compensation may prevent/reduce population-level impacts of parasite NCEs. To evaluate the potential for compensation to limit population-level NCEs, we made models where we varied the NCE impacts on fecundity relative to longevity (i.e. we varied the potential for compensation).

We created individual-based models to simulate populations of flies experiencing fear (NCE), fear and infection (NCE and consumptive effects), or no parasites over 100 days (~5 overlapping generations).

A “consumption + no fear” condition was not modelled as it would require infecting flies while eliminating resistance as well as cues of mite presence / infection which is not possible in seminatural fly-mite interactions. For this reason, there is no experimental data that could be used to model a no-fear with consumption condition. By modelling NCEs and consumptive effects of parasites on host populations, we also identify gaps in our current understanding of trait-mediated interactions between hosts and parasites.

Methods

We simulated fly populations under 3 scenarios: (1) in the presence of parasitic mites and their non-consumptive effect on host flies (“no consumption + fear”), (2) with both the consumptive and non-consumptive effects of parasitic mites on their fly hosts (“consumption + fear”), or (3) in the absence of parasite effects (“no consumption + no fear”). For each scenario we constructed a stochastic matrix model of fly populations. The transition matrix considered flies living over 60 days, moving through pre-reproductive stages (eggs, larvae, pupae, pre-reproductive adults) for 20 days before becoming reproductively mature adults and producing eggs (Fig. 1). Mites could infect pre-reproductive adults and mature adults. *D. nigrospiracula* have mean lifespans of ~2-4 weeks based on the environment and can live upwards of ~50 days post-adult-emergence in laboratory conditions [21, 30]. We ran each simulation 1000 times. Data on the survival and fecundity of individual adult female *D. nigrospircaula* flies infected by mites were derived from Polak [21] (parameters used for modelling are summarized in Table 1). Fly survival drops precipitously and non-linearly with increased mite infections [23]. Therefore, we did not vary the parasite load within infected individuals and assumed adult flies either had pathogenic levels of infection or were uninfected. Prevalence of infection in simulated populations was varied by changing the daily infection rate. The survival and fecundity of female adult *D. nigrospiracula* exposed to, but not infected with, mites (i.e. no consumption + fear) were derived from Horn and Luong [17] (Table 1). NCEs of mites were measured in the previous study by housing flies in vials with mites but separated by a mesh barrier preventing infection [17, 31]. We used the demogR [32], truncnorm [33] and Tidyverse packages [34] packages in addition to R Core features (Ver. 3.5.1) [35]. Code can be accessed online (doi: 10.17605/OSF.IO/Z5A4S).

We did not account for the influences of parasitism on male flies. Because female *D. nigrospiracula* can store sperm, the assumption that females were not sperm-limited is reasonable [36].

Furthermore, mites preferentially infect female *D. nigrospiracula* over male conspecifics, infecting females 71% of the time in choice assays [37]. Our model also assumes that flies are not food limited in the short term; however, these flies live on ephemeral habitats, rotting cacti [38]. Therefore, our model represents the ability of initial colonizers to exploit this food source. By not modelling the decline of the ephemeral food source we avoid the confounding effect of food limitation on our analysis of the NCE of parasitism. Nor is there currently experimental data on a food-NCE interaction to use in models.

Daily survival was simulated out of a truncated normal distribution (ranging from 0-1) with a mean of 0.96, 0.94, and 0.93 for the no consumption + no fear, no consumption + fear, and consumption + fear scenarios, respectively (see Table 1). Models using these values matched the overall survival patterns (percentage of flies alive after 30 days) in the original data sources [17, 21]. In the absence of data, we set the standard deviation of survival to 10% of the mean survival (i.e. if survival was 0.96 the standard deviation was set to 0.096). Similarly, we calculated the per day egg production from the literature for each scenario as 4.38, 4.86, and 2.85 for the no consumption + no fear, no consumption + fear, and consumption + fear scenarios, respectively, and simulated daily values out of a Poisson distribution (Polak 1996; Horn and Luong 2018). Note that lifetime fecundity was still lower among flies experiencing only fear than flies experiencing no fear and no consumption [17]. The experimental evidence for infection impacting latency to ovipositing (age at first egg laying) was mixed and weak [17]. Thus, we assumed latency to ovipositing was equal between groups. There was no experimental data for daily survival rates of eggs, larva, and pupae with and without mites. Instead, we assigned a single value to all of them, tuned to reflect observations of fly population sizes on natural cactus rots and observations of lab cultures [39]. Nor was there data on potential inter-generational NCEs of mites, e.g. changes in the quality of offspring from mite-exposed mothers.

We estimated the population growth rate (lambda) from the stochastic matrix by calculating the mean day-over-day growth in total fly numbers. Each simulated population was initiated with 50 competent adult flies (10 of each of 20-24 days old) who colonized a hypothetical cactus rot, dispersing from nearby populations. The simulation was run for 100 days. Only the last 50 days of each simulation were used while calculating lambda to avoid early transient dynamics. We tracked and recorded the total number of uninfected and infected adult flies to determine population size. We did this to match the field

data, which counted adult flies and not pre-adult stages. The stochastic matrices of the three scenarios were simulated 1000 times and the adult fly populations plotted for each run. Likewise, the distribution of lambda for each scenario was plotted as a histogram across all simulations.

In a sensitivity analysis, we varied the consumptive effect of parasites by altering the daily probability of infection (ranging from 0-1). We set this value to 0 in mite-free scenarios. We tested the effect of a daily probability of infection on population growth by simulating 1000 populations for 100 days starting with 50 female dispersers, 20% of whom were infected with parasites (a prevalence reasonable to expect in nature, [40]). We assumed that all subsequent adults were born uninfected but became infected at some daily probability, which we varied between 0 and 1. Uninfected females survived and reproduced using the parameters from the no consumption + fear scenario, while infected females survived and reproduced using the parameters from the consumption + fear scenario. We recorded the final average population size and the proportion of the adult population that was parasitised.

Empirical data showed that the parasite-exposed females produced ~10% more eggs per day than unexposed females (4.86 vs. 4.38 eggs per day on average respectively; [17]), despite having lower lifetime fecundity, suggesting compensatory egg production may be occurring that offsets the survival detriment caused by NCEs. We investigated this possibility by running simulations of the effect of fear on egg production across a range of daily survival rates to determine compensatory egg production's impact on population growth. In other words, we simulated populations with varying or no ability to increase egg production per day to compensate for shortened lifespan. As before, simulations were run over 100 days and 1000 populations were simulated at each combination of egg production and survival; the average adult population size at the end of the simulation was recorded and compared to the "no fear + no consumption" scenario. We used these models to calculate the daily egg production required to compensate for reductions in longevity.

Results

Baseline scenarios

In order to elucidate the relative impacts of NCEs on host populations, we simulated 1000 populations over 100 days in each of 3 scenarios: reflecting (1) the presence of parasitic mites and their non-consumptive effect on host flies (no consumption + fear), (2) both the consumptive and

non-consumptive effects of parasitic mites on their fly hosts (consumption + fear), or (3) the absence of parasites (no consumption + no fear). We found that the estimated growth rate, lambda, was similar for the scenarios with no consumption (i.e., infection) with fear or without fear (=1.051, and =1.050 respectively). The mean final population size was larger in the simulations with NCEs than in groups absent parasitism: 4103 versus 3556 respectively (~15% increase) (Fig. 2). On the other hand, population growth rates (=1.030) and final average population sizes were far lower in simulations including consumptive effects (Fig. 2).

Variation in fecundity within a sample simulation

There is substantial variation in lifetime fecundity among flies, especially in simulations with no consumption. This is illustrated in the results of a single simulated population (Fig. 3). In the simulation without any impacts of parasites, flies produced 71 (sd=72.6) eggs over their lifespan and lived 16 (sd=16.4) days. Simulated flies subject to only the non-consumptive effects of parasites produced 53 (sd=67.3) eggs and lived 11 (sd=13.8) days. Finally, in a simulated population where flies were exposed to both the consumptive and non-consumptive effects of parasitism, they produced 26 (sd=36.8) eggs and lived 9 (sd=12.7) days.

Sensitivity to daily probability of infection

We investigated how variation in the daily probability of infection affected population growth. The simulations showed that a daily probability of infection of approximately 0.05 resulted in a population size half that of the simulations without mites (Fig. 4). Any daily probability of infection above 0.3 results in >75% of the population being parasitised and little difference in the overall average population size relative to a fully parasitised population (Fig. 4).

Compensation in egg laying could influence population growth

We varied the mite-mediated change in fecundity to consider the potential impacts of compensation (i.e., how/if flies compensate for early death with increased egg laying) for population growth across a range of daily survival rates. We empirically solved for the combination of egg production and survival rates that resulted in final adult population sizes that were equal to the baseline scenario of no fear + no consumption (contour line in Fig. 5). A daily egg production of ~4.6 eggs/day was required to compensate for reduced survival. Below this line (in the cooler colours) daily female egg production was

not able to compensate for the reduction in survival, while above this line (in the hotter colour) egg production more than compensated for the reduction in survival associated with the fear of being parasitised. For comparison, the three original scenarios are plotted (symbols given in Fig 5). We found that when survival was unchanged from the baseline scenarios (0.96 and 0.94 daily survival rate for uninfected and infected females, respectively), that population which compensated with 110% of the per day egg production of the baseline scenario (no fear and no infection) resulted in a population size of approximately 1000 additional adult flies, an increase of approximately 41% (Fig. 5).

Discussion

We tested the hypothesis that NCEs at the individual level scale up and impact population growth rates independent of infection by simulating 1000 fly populations experiencing no effects of parasites, just NCEs, or NCEs and consumptive effects (infection). The mean growth rates of populations experiencing both consumptive and non-consumptive effects were substantially lower (when mite prevalence=100%, =1.030). The higher the simulated prevalence of infection, the larger the impact on population growth rate (Fig. 4). In our study ~25% final infection prevalence (daily infection chance =0.05) corresponded to an average ~50% lower final simulated population size compared to mite-free populations (Fig. 4). The prevalence of *M. subbadius* infection among wild *D. nigrospiracula* generally ranges from ~10-40%, increasing as habitats age [40]. Our simulations suggest these rates of infection would have mild to moderate effects on population growth (Fig. 3). The growth rates (λ) of simulated populations experiencing only NCEs, however, were slightly higher than in the populations without mite effects, λ=1.051 and λ=1.050 respectively (Fig. 2), and the average final population size was higher in the group experiencing only fear by ~550 flies (15% higher) (Fig. 3).

Although lab studies showed reductions in survival and lifetime fecundity among individual female flies exposed to mites without infection [17], when these effects were scaled up to the population level in our simulations population growth rates were not reduced (Fig. 2). Examination of an individual simulated population suggests the variation in fecundity among flies experiencing fear was large relative to the reduction in the mean number of eggs produced over the fly lifespan (Fig. 3). Substantial variation in fecundity among flies experiencing fear may, therefore, limit the effects of NCEs on populations. One potential explanation for the simulated results is that flies compensate for the presence of parasites and

associated mortality, e.g. through earlier maturation/maximal reproduction or terminal reproductive output [28, 29, 41]. We modelled populations where flies were unable to compensate for reduced survival with increased daily fecundity (by setting fecundity in the fear group to match the fecundity of the uninfected female), on average these populations declined by approximately 650 flies or 19% relative to the mite-free scenario (Fig. 4). In our sensitivity analysis of the potential for compensation, we were able to derive the daily egg production required to offset the NCE of parasitism for survival in terms of the final adult population (~4.6 eggs/day, Fig. 5).

In our models we assumed that flies exposed to and infected with mites would lay eggs at similar the same age as mite-free flies (i.e. have the same latency to ovipositing), based on observations of flies exposed to mites for 48 hours [21]. It is possible that latency would have been affected by chronic exposure to mites or by exposure as larvae, as occurs at natural habitats. However, the long-term exposure experiment did not measure latency-to-ovipositing [17]. Alternatively, flies may vary in their daily fecundity over the course of their lifespan, although we did not account for that possibility in this model [30, 42]. Exposure and/or infection may alter the time of peak reproduction without changing the time of first ovipositing if there are constraints on reproductive maturation. Field studies are needed to test if early first/peak reproduction is a mechanism of compensation by examining the latency to ovipositing and peak reproductive age in fly populations with mites and without mites. Physiological mechanisms enabling compensatory egg production is a direction for future research. Furthermore, the ability of organisms to compensate for stress can be environmentally dependent (e.g. ubiquity of food) [26]. Since we took data from lab organisms which had sufficient and reliable food, we may observe more compensation in these simulations than if data came from wild populations facing severe caloric restriction. Experimental manipulation of resources in future experiments may provide insight into the feasibility of natural host populations ability to compensate for mite exposure.

While reviewing previous studies on the fitness effects of infection, we incidentally found additional evidence for individual-level NCEs of parasites. When measuring the effect of infection on fly longevity, Polak [21] reported flies that resisted infection and those that were never exposed. Unexposed flies lived 29.3 days, whereas the resisted group lived 24.4 days on average, an 18% difference (Polak 1996). Although this difference was insignificant in the analyses, the magnitude of the reduction was

comparable to the ~23% difference in longevity between flies chronically exposed to mites and unexposed flies reported in Horn and Luong [17]. Earlier studies of parasite infection may have incidentally detected NCEs which were not identified as such. A thorough review of the literature may find further examples of parasitic NCEs in studies not explicitly designed to test for them and may be a direction for future meta-analyses.

By building models of parasite-mediated NCEs, we identified gaps in our understanding of these trait-mediated interactions. For example, exposure to predators as larvae is known to affect the physiological and behavioural traits of adult flies, as well as other vertebrates and invertebrates [43, 44, 45]. Female *D. melanogaster* exposed to spiders as larva have lower masses and reduced fat reserves relative to unexposed conspecifics as adults [45]. Given the positive relationship between female body size and fecundity in *Drosophila*, deleterious NCEs on body size are likely to have deleterious effects on future reproduction [45, 46]. Additionally, NCEs may directly impact larval survival. For example, larval dragonflies exposed to restrained fish during growth were then less likely to survive adult emergence [47]. NCEs can also impact future generations in the form of maternal effects. In a vertebrate system the survival of offspring from mothers exposed to a sham-predator can be reduced even if the source of fear is removed post-birth [5]. Further research is needed to determine if parasites have intergenerational or interstitial NCEs on *Drosophila*.

Our results here are consistent with a recent meta-analysis that found, relative to predators, the individual-level NCEs of parasites on amphibian hosts tend to be smaller and mixed [13]. However, data on only a small number of amphibians and their parasites were available [13]. Our results extend this observation to an insect host. Furthermore, the magnitude of parasitic NCEs may vary between parasite taxa. When there are few cues of parasite presence (e.g. with small, immobile infectious stages), hosts may be under less selection to have strong pre-infection defences. In turn, the potential for costly pre-infection defenses to drive NCEs is reduced. In host-parasite systems with limited pre-infection cues of parasites, consumptive effects may be present with few to no NCEs [13; 15]. Comparative research across host and parasite taxa is an avenue for future research, in particular testing if transmission mode influences the magnitude of parasite NCEs.

Taken together, our results suggest that host compensation may reduce the impacts of individual-level NCEs on host population growth. NCEs may even have positive impacts on host population size, at least in the short-term. Future studies should investigate biological mechanisms allowing host populations to compensate for NCEs, and when this compensation could potentially have positive impacts on host population size. We also identify the need for future research on interstitial and inter-generational NCEs of parasites to improve future models and fully account for parasitic NCEs. Hosts live in an infectious world, but how this risk impacts host populations has implications for the ecology and coevolution of host-parasite symbioses.

Table 4.2.1. Daily survival and egg production used to produce the transition matrix for each of the three baseline scenarios. The standard deviations of survival (set to 10% of the mean) are given in parentheses and are drawn from a truncated (0-1) normal distribution. Egg production was modelled from a Poisson distribution. Consumption data is based on (Polak 1996); Fear data is based on Horn and Luong (2018).

Fly Stage	Days	No Consumption + No Fear		No Consumption + Fear		Consumption + Fear	
		Survival	Eggs	Survival	Eggs	Survival	Eggs
Eggs	5	0.90 (0.09)	-	0.90 (0.09)	-	0.90 (0.09)	-
Larva	8	0.90 (0.09)	-	0.90 (0.09)	-	0.90 (0.09)	-
Pupae	2	0.90 (0.09)	-	0.90 (0.09)	-	0.90 (0.09)	-
Preadult	5	0.95 (0.095)	-	0.95 (0.095)	-	0.95 (0.095)	-
Adult	40	0.96 (0.096)	4.38	0.94 (0.094)	4.86	0.93 (0.093)	2.85

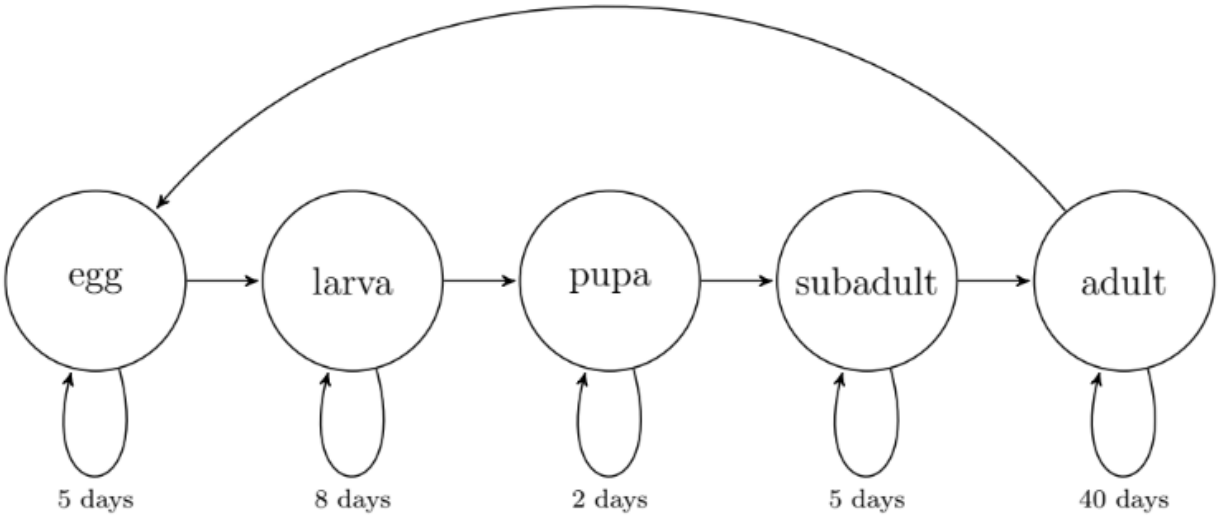


Fig. 4.2.1: Life Stages of *Drosophila* and maximum number of associated days in model.

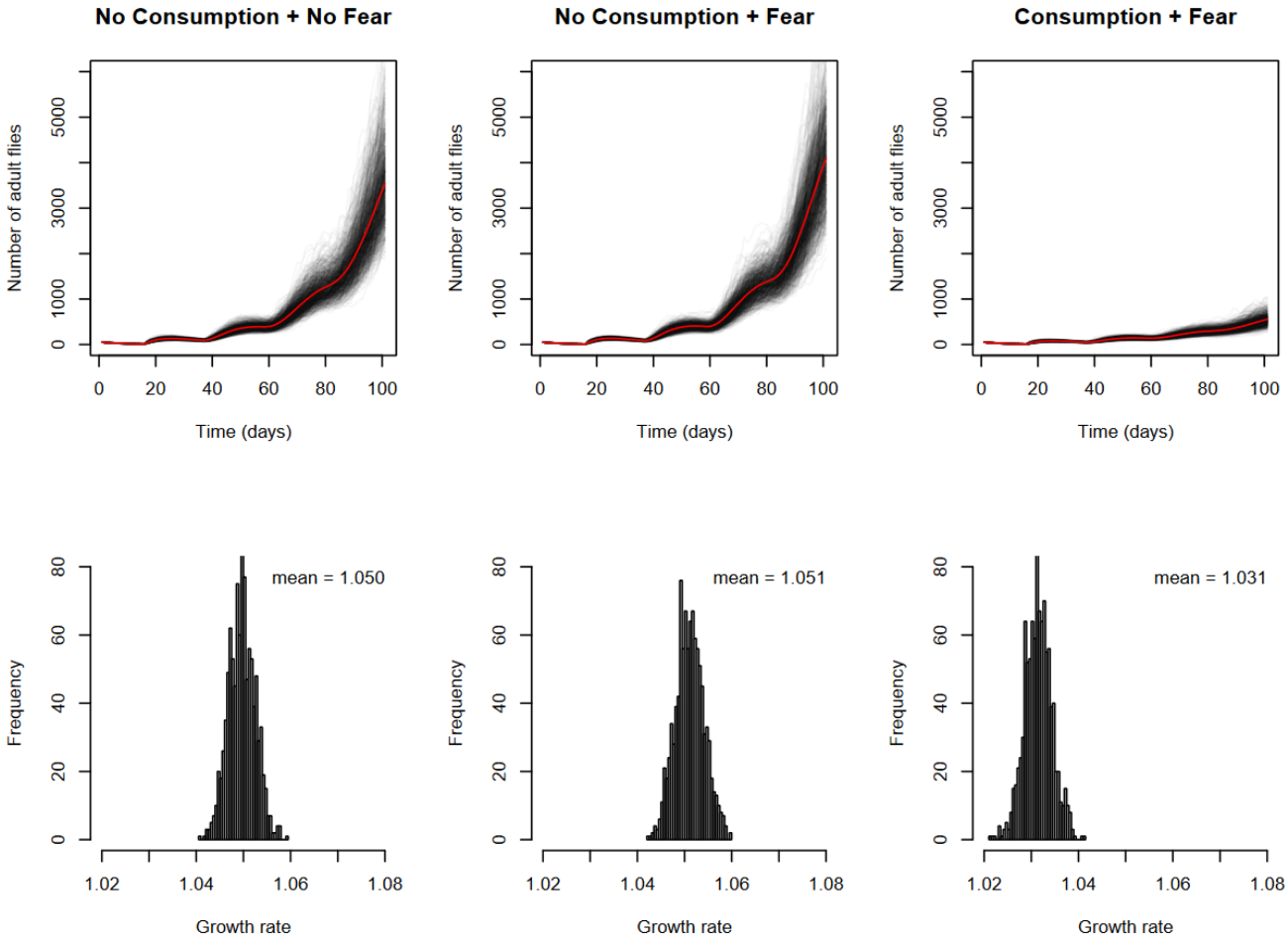


Figure 4.2.2: Population trajectories (top panels) and growth rate, lambda (bottom panels) for each of the 3 simulated baseline scenarios: (1) the absence of parasites (no consumption + no fear), (2) the presence of parasitic mites and their non-consumptive effect on host flies (no consumption + fear), (3) both the consumptive and non-consumptive effects of parasitic mites on their fly hosts (consumption + fear) . The trajectories represent 1000 simulations, with the shading indicative of where more of the simulations overlap and the red line is the average of all the simulations. Similarly, the histograms represent the estimated growth rate (lambda) from each of the 1000 simulations above them, the mean lambda from the last 50 days is recorded to avoid transient dynamics that may exist early in the simulation.

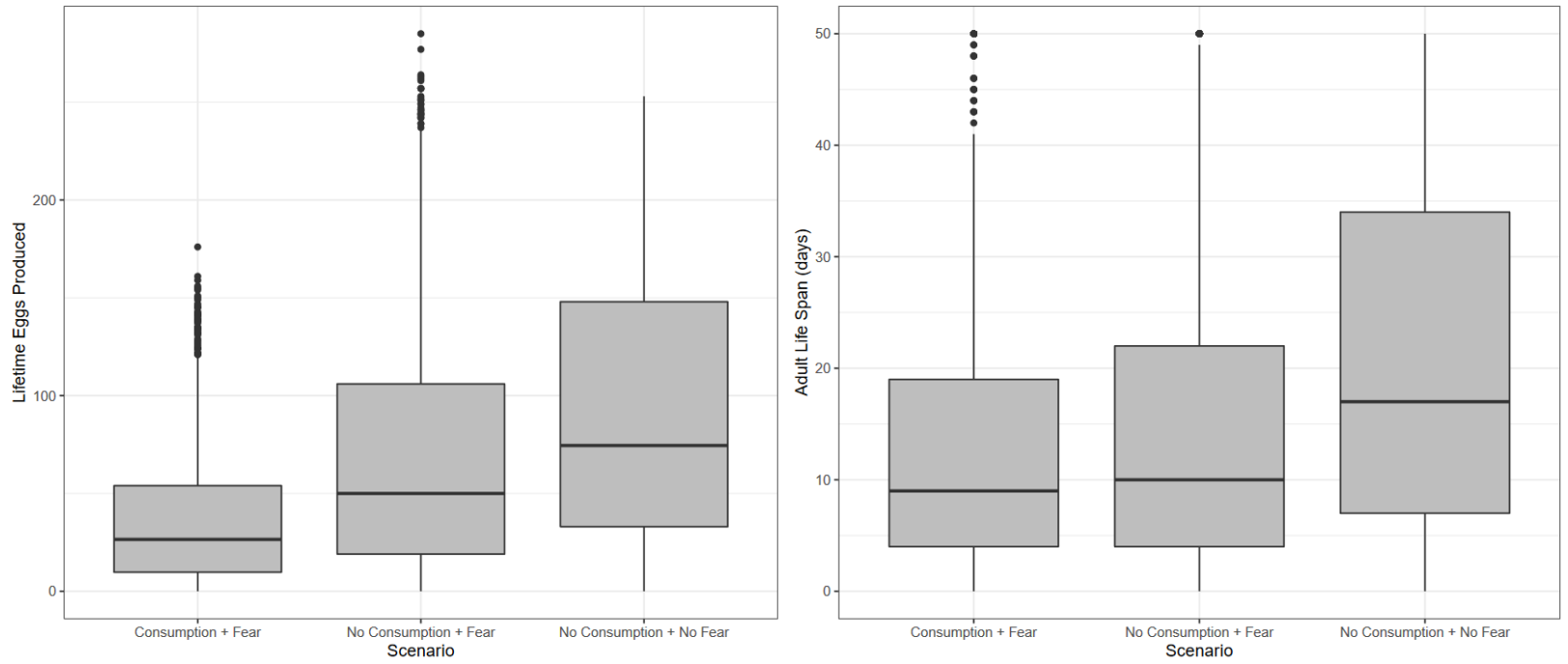


Figure 4.2.3: Example of the variation in fly lifetime reproductive success with or without parasite consumptive and non-consumptive effects in a single simulated population. Reproductive success was measured as the total number of eggs produced and the number of days lived, for a simulation run for a single population of 1000 flies in one of the three scenarios: (1) no consumption + fear, (2) consumption + no fear or (3) no consumption + no fear. Box plots represent (from inside out): mean, 25/75th percentiles, 2 SD, and individual flies beyond 2 SD.

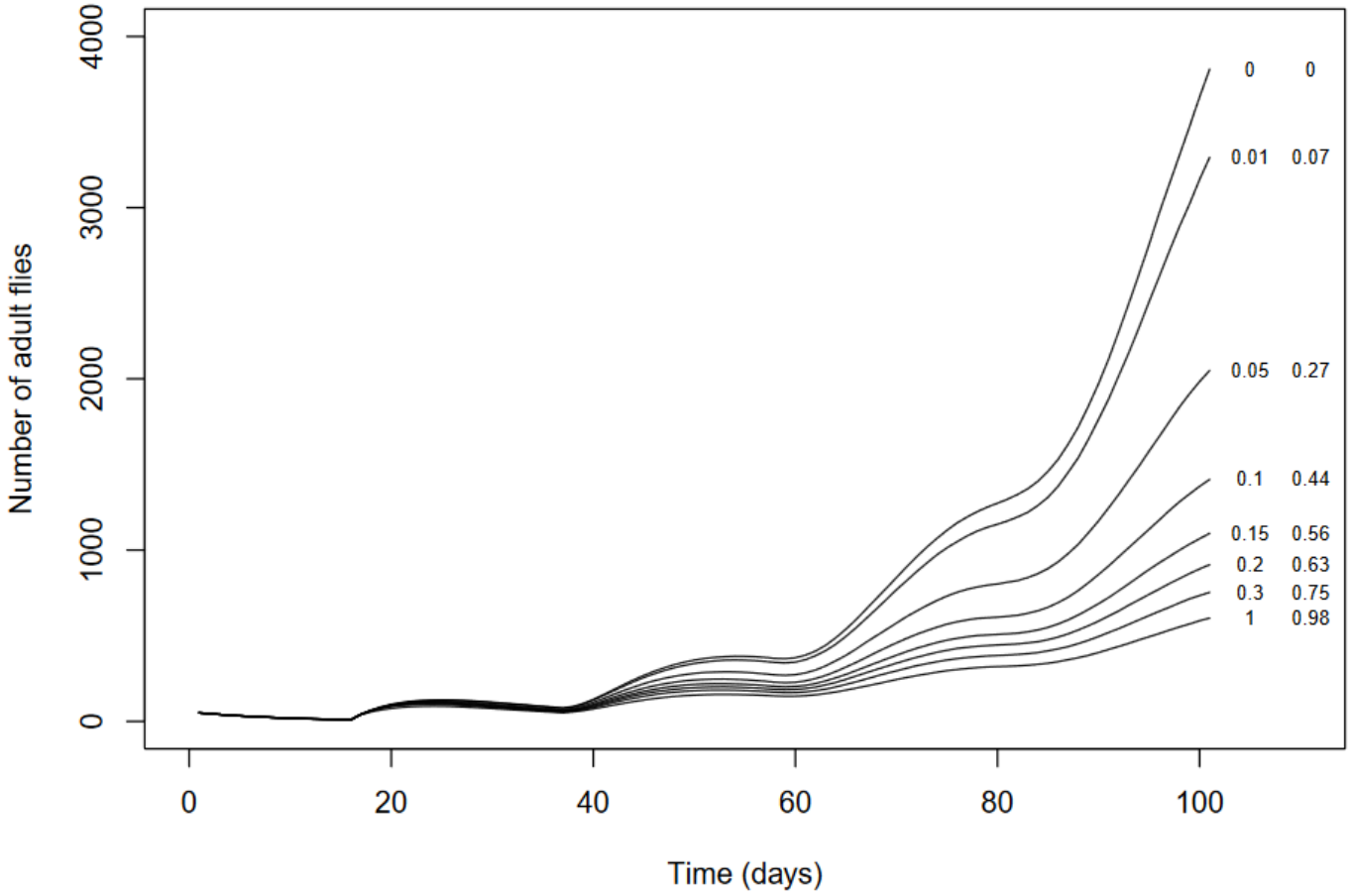


Figure 4.2.4: Sensitivity results for the effect of a daily probability of infection on population growth by simulating 1000 populations for 100 days starting with 50 female dispersers, 20% of whom were infected with parasites. We assumed that all subsequent adults born started out uninfected but became infected at a daily probability between 0 and 1 (left number); the right number shows the final proportion of flies infected with parasites (i.e. prevalence). Uninfected females survived and reproduced using the parameters from the “no consumption + fear scenario” while infected females survived and reproduced using the parameters from the “consumption + fear scenario”. The average of the 1000 populations are given by each line.

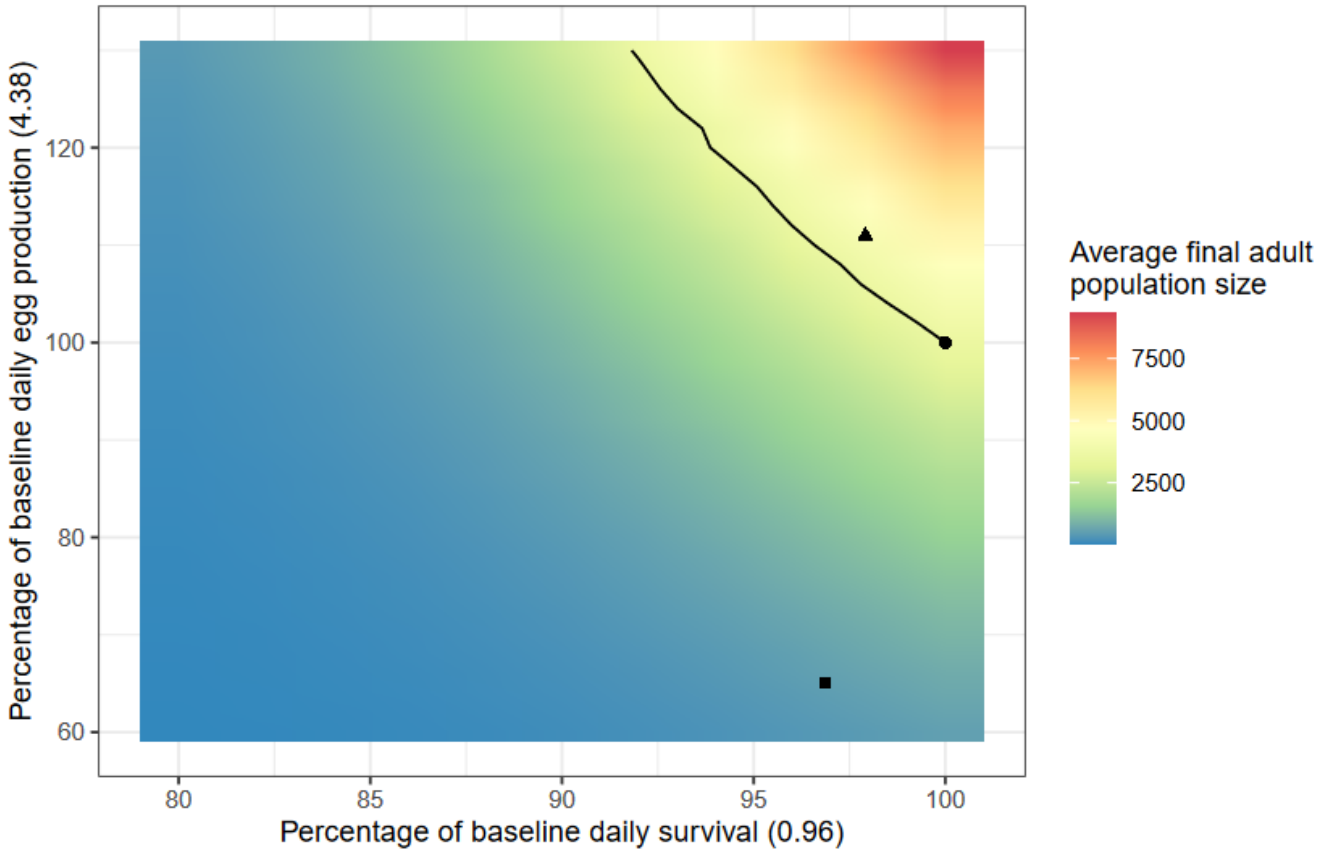


Figure 4.2.5: Simulated final adult population size when host compensation (egg production) was varied relative to the baseline egg production from experimentation (4.38 eggs per day = 100%) along with daily survival rate relative to the baseline survival rate from experimentation (0.96 = 100%). The baseline scenarios are given for reference, where the circle is the no fear + no consumption scenario, the triangle is the fear + no consumption scenario, and the square is the fear + consumption scenario. The black line represents the contour of the final adult population size for the no fear + no compensation scenario and indicates the daily egg production required to offset the reduction in survival that occurs as a result of NCE of parasitism.

4.2 References

1. Peacor, S.D., Werner, E.E., 2008. Nonconsumptive effects of predators and trait-mediated indirect effects, Encyclopedia of Life Sciences (ELS). John Wiley & Sons, Ltd, Chichester, United Kingdom, pp. 1-8.
2. Peckarsky, B.L., Abrams, P.A., Bolnick, D.I., Dill, L.M., Grabowski, J.H., Luttbeg, B., Orrock, J.L., Peacor, S.D., Preisser, E.L., Schmitz, O.J., Trussell, G.C., 2008. Revisiting the classics: Considering nonconsumptive effects in textbook examples of predator-prey interactions. Ecology 89, 2416-2425.
3. Peckarsky, B., Cowan, C., Penton, M., Anderson, C., 1993. Sublethal consequences of stream-dwelling predatory stoneflies on mayfly growth and fecundity. Ecology 74, 1836-1846.
4. Murray, R.L., Tah, S., Koprivnikar, J., Rowe, L., McCauley, S.J., 2020. Exposure to potentially cannibalistic conspecifics induces an increased immune response. Ecological Entomology 45, 355-363.
5. MacLeod, K.J., Krebs, C.J., Boonstra, R., Sheriff, M.J., 2018. Fear and lethality in snowshoe hares: the deadly effects of non-consumptive predation risk. Oikos 127, 375-380.
6. Belgrad, B.A., Griffen, B.D., 2016. Predator-prey interactions mediated by prey personality and predator hunting mode. Proceedings of the Royal Society B-Biological Sciences 283.
7. DeWitt, P.D., Visscher, D.R., Schuler, M.S., Thiel, R.P., 2019. Predation risks suppress lifetime fitness in a wild mammal. Oikos 128, 790-797.
8. Preisser, E.L., Bolnick, D.I., 2008. Themany faces of fear: comparing the pathways and impacts of non-consumptive predator effects on prey populations. Plos One 3, 1-8.
9. Kindinger, T.L., Albins, M.A., 2017. Consumptive and non-consumptive effects of an invasive marine predator on native coral-reef herbivores. Biological Invasions 19, 131-146.
10. Sheriff, M.J., Peacor, S.D., Hawlena, D., Thaker, M., 2020. Non-consumptive predator effects on prey population size: A dearth of evidence. Journal of Animal Ecology 89, 1302-1316.
11. Fill, A., Long, E.Y., Finke, D.L., 2012. Non-consumptive effects of a natural enemy on a non-prey herbivore population. Ecological Entomology 37:43-50. doi: 10.1111/j.1365-2311.2011.01333.x
12. Abram, P.K., Brodeur, J., Urbaneja, A., Tena, A., 2019. Nonreproductive Effects of Insect Parasitoids on Their Hosts. Annual Review of Entomology, Vol 64, 259-276.

13. Daversa, D.R., Hechinger, R.F., Madin, E., Fenton, A., Dell, A.I., Ritchie, E.G., Rohr, J., Rudolf, V.H.W., Lafferty, K.D., 2021. Broadening the ecology of fear: non-lethal effects arise from diverse responses to predation and parasitism. Proceedings of the Royal Society B-Biological Sciences 288.
14. Buck, J.C., 2019. Indirect Effects Explain the Role of Parasites in Ecosystems. Trends in Parasitology 35, 835-847.
15. Koprivnikar, J., Rochette, A., Forbes, M.R., 2021. Risk-Induced Trait Responses and Non-consumptive Effects in Plants and Animals in Response to Their Invertebrate Herbivore and Parasite Natural Enemies. Frontiers in Ecology and Evolution 9. doi: 10.3389/fevo.2021.667030.
16. Koprivnikar, J., Penalva, L., 2015. Lesser of Two Evils? Foraging Choices in Response to Threats of Predation and Parasitism. Plos One 10. doi: 10.1371/journal.pone.0116569.
17. Horn, C.J., Luong, L.T., 2018. Proximity to parasites reduces host fitness independent of infection in a *Drosophila-Macrocheles* system. Parasitology 145, 1564-1569.
18. 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, 1196-1205.
19. Poulin, R., Morand, S., 2000. The diversity of parasites. Quarterly Review of Biology 75, 277-293.
20. Perez-Leanos, A., Loustalot-Laclette, M.R., Nazario-Yepiz, N., Markow, T.A., 2017. Ectoparasitic mites and their *Drosophila* hosts. Fly 11, 10-18.
21. Polak, M., 1996. Ectoparasitic effects on host survival and reproduction: The *Drosophila-Macrocheles* association. Ecology 77, 1379-1389.
22. Markow, T.A., 1988. Reproductive behavior of *Drosophila melanogaster* and *Drosophila nigrospiracula* in the field and in the laboratory. Journal of Comparative Psychology 102: 169-173.
23. Polak, M., Starmer, W.T., 1998. Parasite-induced risk of mortality elevates reproductive effort in male *Drosophila*. Proceedings of the Royal Society B-Biological Sciences 265, 2197-2201.
24. Luong, L.T., Horn, C.J., Brophy, T., 2017. Mitey costly: energetic costs of parasite avoidance and infection. Physiological and Biochemical Zoology 90, 471-477.
25. Horn, C.J., Luong, L.T., 2019. Current parasite resistance trades off with future defenses and flight performance. Behavioral Ecology and Sociobiology 73, 1-10.
26. Forbes, M.R.L., 1993. Parasitism and host reproductive effort. Oikos 3: 444-450.
27. Adamo, S.A., 1999. Evidence for adaptive changes in egg laying in crickets exposed to bacteria and parasites. Animal Behaviour 57:117-124. doi: 10.1006/anbe.1998.0999.

28. Parietti, M., Merlo, M.J., Natal, M., Mendez Casariego, M. A., 2020. Reproductive compensation in female *Palaemonetes argentinu*s (Decapoda: Natantia) due to *Microphallus szidati* (Trematoda) infection. Journal of Helminthology 94: E204.
29. Krist, A.C., 2001. Variation in fecundity among populations of snails is predicted by prevalence of castrating parasites. Evolutionary Ecology Research 3: 191-197.
30. Luong, L.T., Polak, M., 2007. Costs of resistance in the *Drosophila-Macrocheles* system: a negative genetic correlation between ectoparasite resistance and reproduction. Evolution 61, 1391-1402.
31. Horn, C.J., Luong, L.T., 2021. Data from: Proximity to parasites reduces host fitness independent of infection in a *Drosophila-Macrocheles* system (Data set). Dryad. doi: 10.5061/dryad.gmsbcc2pn
32. Jones, J.H., 2007. demogR: A Package for the Construction and Analysis of Age-structured Demographic Models in R. Journal of Statistical Software 22, 1-28. doi: 10.18637/jss.v022.i10.
33. Mersmann, O., Trautmann, H., Steuer, D., Bornkamp, B., 2018. truncnorm: Truncated Normal Distribution. R package version 1.0-8. CRAN.R-project.org/package=truncnorm
34. Wickhamn, H., Averick, M., Bryan, J., Chang, W., McGowan, L.D., François, R., Grolemund, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Pedersen, T.L., Miller, E., Bache, S.M., Müller, K., Ooms, J., Robinson D., Seidel, D.P., Spinu, V., Takahashi K., Vaughan, D., Wilke, C., Woo, K., Yutani, K., 2019. Welcome to the Tidyverse. The Journal of Open Source Software, 4, 1686.
35. R Core Team (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL www.R-project.org/
36. Pitnick, S., Markow, T., Spicer, G.S., 1999. Evolution of multiple kinds of female sperm-storage organs in *Drosophila*. Evolution 53, 1804-1822.
37. Horn, C.J., Mierzejewski, M.K., Elahi, M.E., Luong, L.T., 2020. Extending the ecology of fear: parasite-mediated sexual selection drives host response to parasites. Physiology & Behavior 224, 1-7. doi: 10.1016/j.physbeh.2020.113041.
38. Markow, T.A., 1988. Reproductive behavior of *Drosophila melanogaster* and *Drosophila nigrospiracula* in the field and in the laboratory. Journal of Comparative Psychology 102: 169-173.
39. Breitmeyer, C.M., Markow, T.A., 1998. Resource availability and population size in cactophilic Drosophila. Functional Ecology 12, 14-21.
40. Polak, M., Markow, T.A., 1995. Effect of ectoparasitic mites on sexual selection in a Sonoran desert fruit-fly. Evolution 49:660-669. doi: 10.2307/2410319.
41. Duffield, K.R., Bowers E.K., Sakaluk, S.K., Sadd, B.M., 2018. A dynamic threshold model for terminal investment. Behavioral Ecology and Sociobiology 71, 185.
42. Miller PB, Obrik-Uloho OT, Phan MH, Medrano CL, Renier JS, Thayer JL, Wiessner G, Bloch Qazi MC. 2014. The song of the old mother: reproductive senescence in female *Drosophila*. Fly (Austin) 8:127–139.

43. Davenport, J.M., Hossack, B.R., Lowe, W.H., 2014. Partitioning the non-consumptive effects of predators on prey with complex life histories. Oecologia 176, 149-155.
44. Xiong, X.F., Michaud, J.P., Li, Z., Wu, P.X., Chu, Y.N., Zhang, Q.W., Liu, X.X., 2015. Chronic, predator-induced stress alters development and reproductive performance of the cotton bollworm, *Helicoverpa armigera*. Biocontrol 60, 827-837.
45. Krams, I., Inwood, S.E., Trakimas, G., Krams, R., Burghardt, G.M., Bulter, D.M., Luoto, S., Krama, T., 2016. Short-term exposure to predation affects body elemental composition, climbing speed and survival ability in *Drosophila melanogaster*. PeerJ 4.
46. Lefranc, A., Bundgaard, J., 2000. The influence of male and female body size on copulation duration and fecundity in Drosophila melanogaster. Hereditas 132, 243-247.
47. McCauley, S.J., Rowe, L., Fortin, M.J., 2011. The deadly effects of "nonlethal" predators. Ecology 92, 2043-2048.

Chapter 5: Preliminary investigation of a Fly-Endosymbiont-Mite association

Chapter 5 Introduction

In this section, I investigated the impact of a bacterial endosymbiont, *Spiroplasma poulsonii* MSRO, on the interactions between *Macrocheles subbadius* and the fruit fly *Drosophila melanogaster*. The *D. melanogaster* - *S. poulsonii* symbiosis was used because the effects of bacterial infection have been well described, and infected populations have long been successfully lab cultured. This study considered if 1) mites have preferences for flies with or without the bacterial endosymbionts and 2) if flies harbouring *S. poulsonii* have poorer anti-mite resistance (measured as endurance in geotaxis assays, see section 2.2). Flies generally had poorer endurance as they aged, and this effect was larger in flies harbouring *S. poulsonii*. However, the mite preference for infected or uninfected flies was dependent on fly age. We discuss these results in the context of a known mite preference for high metabolic rate flies and fly senescence. The effects of endosymbiont infection may alter the cost-benefit ratios of mite resistance, suggesting a future avenue for investigating NCEs.

5.1: A male-killing endosymbiotic bacterium impacts *Drosophila-Macrocheles* interactions

Introduction

Endosymbiotic bacteria are associated with nearly all forms of eukaryotic life [1; 2; 3; 4]. In particular, insects and other arthropods commonly harbour bacterial endosymbionts, acting as both primary hosts and vectors [5; 6; 7; 8]. These arthropod-bacteria symbioses are complex and may be mutualistic, commensal, and/or pathogenic in nature depending on the constituent species and conditions [9; 10; 11; 12]. Male-killing endosymbionts, frequently of the genus *Spiroplasma*, can have major impacts on host population growth, structure, and evolution [13; 14; 15].

Many bacterial endosymbionts of insects are vertically transmitted. In particular, male-killing *Spiroplasma* strains, e.g., *Spiroplasma poulsonii* MSRO (*Melanogaster* sex ratio organism), are transmitted from mother to daughter and typically kill male offspring before maturity [16; 17; 18]. Despite the generally close coevolutionary relationships between host insects and their *Spiroplasma* endosymbionts, phylogenetic research has shown *Spiroplasma spp*. were introduced into the host genus *Drosophila* multiple times [19; 20; 21]. However, the mechanism by which these introductions occurred is not clear.

Infection with ectoparasitic mites is a frequently hypothesised mechanism behind these introductions [22; 23; 24]. Under laboratory conditions, *Macrocheles subbadius* can take up male-killing *S. poulsonii* while feeding on host hemolymph and transmit it intra- and inter- specifically to naive fly hosts [25]. Circumstantial natural observations also support the hypothesis that mites transfer *Spiroplasma* horizontally between flies. For example, wild-caught *Macrocheles* infecting *Drosophila hydei* harbour *S. poulsonii* that is genetically similar to the *S. poulsonii* found in their fly hosts [21]. *Macrocheles* show consistent attachment behaviour, i.e. mites that infect one fly are more likely to attach to additional flies,

creating an opportunity for mites to mechanically vector hemolymph-dwelling endosymbionts [26].

The prevalence of *Spiroplasma* infection among female flies can be low, typically below 50% and often below 10%, across multiple *Drosophila-Spiroplasma* associations [27; 28]. In turn, mites may be unlikely to encounter and infect flies carrying *Spiroplasma*. However, infection with some strains, especially male-killing lines that also impact female health (such as MSRO), may increase the chance of secondary infection with mite vectors [29]. Here, we test three non mutually exclusive hypotheses: (i) mites preferentially infect *S. poulsonii* MSRO-infected flies over uninfected flies, (ii) mite preferences for MSRO+ or MSRO- flies are explained by mite preferences for higher-metabolic-rate (MR) hosts, and (iii) MSRO-infected flies are more susceptible to mite attack (measured as the ability to maintain energetically demanding behavioural defences).

We test these hypotheses using *Drosophila melanogaster* Oregon-R infected with MSRO. MSRO is male killing in *Drosophila melanogaster* Oregon-R [17; 29]. Transovarial transmission of MSRO occurs during yolk deposition [30], and a bacterial protein (SpAID) lethally disrupts development in male offspring [17; 31]. Bacterial titers as well as the physiological and behavioural effects of MSRO on *Drosophila melanogaster* Oregon-R have been measured across the fly life span [29]. Infected females experience multiple changes due to MSRO infection, including losing lipid/energy stores, reduced climbing ability impaired microbial immunity, and, ultimately, shorter lifespans [32]. These deleterious effects get stronger as flies age and MSRO titers increase [29]. Although *D. melanogaster* Oregon-R flies regularly live up to 60 days, MSRO+ flies are often in poor condition by approximately 4 weeks and experience ~50% mortality by 35 to 40 days [29; 33; 34]. MSRO titers in *D. melanogaster* Oregon-R are low when flies first emerge, high but still increasing at ~2 weeks old, and plateau by ~26 days posteclosion [29].

The hemolymph-feeding mite *Macrocheles subbadius* is a cosmopolitan ectoparasite that infects several insect taxa, including *Drosophila* [35; 36; 37]. Mites are known to distinguish between host species as well as between members of the same species based on chemical cues [38; 39; 40]. *M. subbadius* can distinguish between fly hosts based on size, body condition, metabolic rate, and chemical cues such as hemolymph [41; 42]. We hypothesise that mites would preferentially infect MSRO+ hosts due to increased metabolic rates in MSRO+ flies, and ostensibly this preference should increase with fly age as MSRO titers increase. Thus, we tested if *M. subbadius* selectively infects MSRO+ flies over MSRO- flies in pairwise choice tests at 2, 14, and 26 days posteclosion.

Previous studies have shown that, when controlling for fly sex, mass, age, and prior infection, *M. subbadius* preferentially infects flies with higher metabolic rates in pairwise tests [42]. We anticipated MSRO infection would increase the metabolic rates of host flies due to energetic requirements of bacterial proliferation and/or fly compensation. Therefore, we also hypothesised that increased metabolic rates among MSRO+ flies would explain mite preferences. To test this hypothesis, we conducted flowthrough respirometry measurements on age-matched flies. Bacterial titer increases with fly age, so we also predicted that metabolic differences would be greater in older flies.

Since MSRO is male killing, we also considered whether *M. subbadius* prefers to infect male or

female *D. melanogaster*. The congeneric mite *Macrocheles muscaedomesticae* has size-mediated preferences for female *D. hydei* flies [43]. Since *D. melanogaster* females are larger than males on average, we hypothesise *M. subbadius* will preferentially infect female *D. melanogaster*.

Drosophila resistance against *M. subbadius* is primarily behavioural and limited by host endurance [44; 45; 46]. Therefore, we hypothesised MSRO infection reduces the capacity of flies to resist mite infection. Furthermore, we anticipated this deleterious effect would increase with fly age as bacterial titer increased [29]. Host endurance assays are a proxy measure of resistance that remove the confounding variable of mite preference [46; 47; 48]. We used geotaxic endurance assays to measure fly endurance at 2, 14, and 26 days posteclosion, and we predicted that endurance would be lower among MSRO+ flies than MSRO- flies.

Methods

Cultures

Drosophila melanogaster Meigen (Oregon-R) (Diptera: Drosophilidae) cultures, both MSRO+ and MSRO- lines, were maintained on agar media at 25°C on a 12-h:12-h light-dark cycle. The culture temperature ensured reliable vertical transmission of MSRO [83; 94]. Fly fecundity is highest ~1 week posteclosion and decreases with age before dropping substantially ~3 to 3.5 weeks posteclosion [78; 79]. MSRO infection is not known to significantly affect the number of eggs laid by *D. melanogaster* but may shift flies toward earlier reproduction [29]. Since MSRO kills males before the adult stage, 3 to 5 males from MSRO- stocks were added to each MSRO vial for mating. Age-matched female flies for experiments were collected within 24 h of eclosion and stored in vials with 3 to 5 males (total population density, 23 to 25 flies/vial). When flies were transferred to new vials, males were replaced as necessary to ensure all experimental flies were mated. To confirm this method led to reliable mating, we conducted an additional test where we mated female flies, as in the experiments above, and monitored them for egg production. We informally tracked survival of flies as they aged. When flies were aged beyond; 26 days, mortality sometimes required reducing vial density, so MSRO+ and MSRO- vials were split as needed to keep fly populations at equal densities. Approximately 95% of flies lived to ~3 weeks posteclosion regardless of MSRO infection status. However, ~3.5 to 4 weeks posteclosion, MSRO+ fly survival dropped precipitously, and only 40 to 50% of MSRO+ flies lived to 34 days, while >80% of MSRO- flies did (personal observation). Fly survival was overall consistent with previously reported survival [29].

The MSRO+ culture was initially generated by microinjecting the MSRO- line with hemolymph from infected flies. Lines have been cultured since at least 2015, eliminating potential direct and maternal effects of initial MSRO establishment. Periodically, we inspected MSRO+ vials for the presence of male flies (examining all the offspring of a vial), which would indicate MSRO was lost or contamination occurred. Any MSRO+ vials that produced male offspring were destroyed, and all MSRO+ flies used in experiments came from vials that produced only female offspring. Previous research shows MSRO titer is low in 2-day-old *Drosophila melanogaster* Oregon-R, high but still in log growth among 14-day-old flies,

and highest but plateaued among flies by ~26 days posteclosion [29]. Thus, preference and endurance experiments were conducted with 2-day-old, 14-day-old, and 26-day-old flies.

Macrocheles subbadius (Berlese) (Mesostigmata: Macrochilidae) cultures were founded from 200 to 300 adult female mites collected in the Sonoran desert (Arizona, USA) from wild flies [35] and were maintained on a 2:1 mix of wheat bran to wood chips. Free-living nematodes (Rhabditida) were cocultured as a food source for mites, and nutritional yeast was added to support nematode and mite growth. Adult female mites were collected for experiments using Berlese funnels. These experimental mites were held overnight on moist plaster of Paris prior to experiments, as *M. subbadius* is more likely to infect flies if they are held without food [41].

Effect of fly MSRO infection on mite preference.

Y-maze experiments tested if mites have a preference for MSRO+ or MSRO- flies at 2, 14, or 26 days posteclosion. We made size-matched pairs, within <5% difference in body mass, of adult female flies (1 MSRO+ and 1 MSRO- fly) to account for the preference of *M. subbadius* for larger flies [55]. Experimental flies were glued to a small piece of cotton (Elmer's rubber cement) to eliminate differences in resistance/mobility [43]. Size-matched pairs were transferred to the ends of Y-shaped mazes (Y polypropylene connectors; Fisherbrand Tubing), alternating MSRO+ and MSRO- on the left/right, and then a single adult female mite was introduced to each maze and the ends were sealed with cotton. The Y-mazes were transferred to an opaque box to exclude light, as mites are more likely to infect in the dark (personal observation). Y-mazes were left undisturbed for 2 h at ambient temperature and then scored. A Y-maze trial was considered successful if the mite infected one of the two available flies, and in successful trials we recorded if the MSRO+ or MSRO- fly was infected. We tested if mites infected MSRO+ and MSRO- flies in each age category at the same rate using binomial tests (binom.test function [49]). Effect of fly sex on mite preference. The Y-maze preference experiment tested if *M. subbadius* preferentially infects male or female *D. melanogaster*. Y-maze trials were conducted as described for the MSRO preference experiments, except flies were not size matched (allowing natural sexual dimorphisms in mass to persist). As MSRO is male killing, both the male and female flies came from the MSRO- line. This experiment was also analysed with a binomial test (binom.test [49]).

Effect of MSRO infection on fly metabolic rate

This experiment tested if MSRO impacts the metabolic rates of infected flies and, by extension, if mite preferences for MSRO+ or MSRO- flies are potentially mediated by mite preferences to infect flies with high metabolic rates [42]. In this experiment, we measured a wider range of ages, primarily to test if metabolic rate at the plateau phase of MSRO remains constant. We measured the metabolic rates of age-matched MSRO+ and MSRO- female fly cohorts at 2, 6, 11, 15, 20, 26, and 34 days posteclosion using flowthrough respirometry. Flies were frozen post-assay, and mass was weighed when body condition allowed. Metabolic rate was measured as the rate of carbon dioxide production (microliters per

minute) [95]. A MAVEn-FT unit (Sable Systems International, NV, USA) was used to set the flow rate at 30 ml/min and to control which of 15 respirometer positions was active (up to 14 containing flies and 1 empty chamber). MSRO+ and MSRO- flies were placed in alternating respirometer positions to reduce the effects of acclimatisation/respirometer stress. A Li-7000 CO_2 sensor (Li-COR Environmental, NE, USA) was then used to measure CO_2 in air that had flown through chambers that contained a MSRO+ fly, a MSRO- fly, or a baseline (empty) chamber. To improve sensitivity, incoming air was purged of CO_2 and water vapour (FT-IR purge gas generator 75-45; Parker Canada Division, Milton, Ontario, Canada), and excurrent gas was purged of water vapour again using a magnesium perchlorate column. Between uses, the glass respirometry chambers were cleaned with detergent, sterilised with 70% ethanol, and rinsed with distilled water. Glass chambers were checked periodically for damage. Real-time flow rates were examined to detect leaks, and flies where there was evidence of leakage (either unexpectedly low flow or erratic flow) were removed prior to analysis. Data were analysed using the Expedata software (Sable Systems International, NV, USA), and the real-time flow rate reported by the MAVEn-Ft was used to calculate the mean CO_2 production rate for each fly [calculation in reference 96]. Fly metabolic rate was standardised before modelling: $[(X_{MR} - u_{MR})/\sigma_{MR}]$.

Standardised metabolic rate was modelled using linear mixed-effect models (lmer function [50.]), with fly infection status, fly age, the infection-age interaction, and mass as fixed effects and replicate block (a single run of the respirometer) and respirometer position as random effects. The mass-infection and mass-age interactions were also included. Starting with the least significant fixed effect, fixed variables were removed from the model individually and the original and new models were compared with the R function anova (old model, new model) [49]. Model reduction was also performed for each fly age with the same fixed and random effects.

Effect of fly metabolic rate on mite behaviour

In the experiment on the effect of fly metabolic rate on mite behaviour, we considered if mites are attracted to and/or prefer to infect *D. melanogaster* females with higher metabolic rates over flies with lower metabolic rates. We measured the metabolic rates of MSRO- females (2 to 5 days old) using respirometry (as described above, minus the empty chamber). We paired the highest and lowest (approximately the top and bottom thirds) metabolic rate flies, tethered them to cotton, and placed them in the ends of a Y-maze (minimum MR difference, 31%). A single adult female mite was introduced to the Y-maze. The Y-mazes were covered for 1 h, after which we recorded (i) the final position of the mite (high-MR arm, low-MR arm, or the arm with no fly) and (ii) which fly (if any) was infected. We used the glm function with a binomial distribution to test if mites were attracted to the high- or low-MR fly.

Effect of MSRO infection on fly endurance in geotaxic assays.

We used negative geotaxis endurance assays to measure the resistance capacity of MSRO+ and MSRO- flies. Behavioural defence acts as the primary line of resistance against mite infection and is limited by fly endurance. As such, the ability to maintain energetically demanding activity is a proxy

measure of resistance that eliminates the effects of mite preferences [46]. We marked empty, transparent vials at 5 cm above the base and then aspirated a single MSRO+ or MSRO- fly into a vial and allowed 15 min for acclimation. At the outset of the experiment (t = 0), the experimenter tapped the vial, causing the fly to fall to the bottom of the vial and inducing the fly to climb. This knockdown procedure was repeated whenever the fly reached the 5-cm line. A fly was considered exhausted if it failed to ascend the vial after 10 s.

We recorded two metrics of endurance: the number of knockdowns before the fly was exhausted (cycles, c) and the time until exhaustion (seconds, t); if a fly failed to climb following the initial knock down, both values were recorded as 0. Time until exhaustion and number of cycles until infection were modelled separately using linear mixed-effect models (lmer, glmer.nb [50]), with fixed effects of infection status (MSRO+ or MSRO-), fly age, and fly mass as well as replicate block (experiment date) as a random effect. The interactions between infection status and mass, fly age and mass, and infection status and fly age were also included in the initial models. Starting with the least significant fixed effect, independent variables were removed from the model and the original and new models were compared with the R function anova (old model, new model). Model reduction was also performed for each fly age with the same fixed and random effects.

The time it took for the fly to climb to the mark after the first knockdown was also recorded (first cycle time, f). If a fly never ascended the vial, f was set to an arbitrarily high value for nonparametric analysis (f = 11 s). We examined the first cycle time using nonparametric tests (wilcox_test function [51]).

Data availability.

Data is available at Dryad (https://doi.org/10.5061/dryad.t1g1jwt3f).

Results

MSRO infection status affects mite preference.

This experiment tested if mites prefer to infect flies harbouring MSRO or flies without MSRO at different ages (2, 14, and 26 days old). On average, 41% of Y mazes were successful: 65/182 with 2-day-old flies, 42/124 with 14-day-old flies, and 61/108 with 26-day-old flies. When flies were 2 days old, mites selected the MSRO+ fly in 51% (95% confidence interval [CI], 38 to 64%; N = 65) of successful pairwise choice tests (binom.test [49]). When flies were 14 days old, mites were significantly less likely to infect MSRO+ flies and only selected the MSRO+ fly in 31% (95% CI, 18 to 47%; N = 42) of successful trials. Lastly, when flies were 26 days old, mites were significantly more likely to infect MSRO+ flies and selected the MSRO+ fly in 64% (95% CI, 51 to 76%; N = 61) of successful trials. There was an age-dependent effect of MSRO on mite likelihood to infect: mites were equally likely to infect the MSRO+ and MSRO- flies at 2 days old, less likely to infect MSRO+ hosts when flies were 14 days old, and more likely to infect MSRO+ hosts once flies were 26 days old (Fig. 5.1.1).

Mite preference for female flies.

We tested if mites prefer to infect male or female *D. melanogaster* flies. In 24 of 39 (62%) successful Y-mazes, the mite infected the female fly. The confidence interval of this marginally significant preference overlaps 0.5 (95% CI, 45 to 77%) (binom.test [49]).

MSRO infection affects fly metabolic rate.

This experiment tested whether MSRO infection impacted fly metabolic rate across fly ages. The standardised metabolic rate was modelled using the lmer function with infection status, fly age, their interaction, and mass (and mass-infection and mass-age interactions) as well as random-effects respirometer position and replicate block (a single run of the respirometer) [50]. The interaction between fly age and infection status was a significant predictor of metabolic rate (X^2 = 5.5, P = 0.019). MSRO+ and MSRO- flies produced comparable amounts of CO_2 from 2 to 11 days posteclosion, MSRO+ flies produced more CO_2 than MSRO^2 flies at 15 days posteclosion, and then, after 26 days posteclosion, MSRO- flies had higher respiration rates (Fig. 5.1.2a). Fly mass was only a marginal predictor of metabolic rate (X^2 = 3.412, P = 0.065), and the correlation between mass and metabolic rate was low (R^2 = 0.068; cor.test) (Fig. 5.1.2b). Interactions between mass and infection status (X^2 = 1.6, P = 0.2) and fly age and mass (X^2 = 3.0, P = 0.09) were not significant. There was no significant difference in metabolic rate between MSRO+ and MSRO- flies at 2 days old (X^2 = 0.11, P = 0.74), and there was no significant difference at 6 days (X^2 = 0.54, P = 0.46) or 11 days (X^2 = 0.010, P = 0.92). At 15 days old, MSRO+ flies had higher metabolic rates (X^2 = 6.7, P = 0.0097). At 20 days there was no significant difference (X^2 = 0.55, P = 0.46). At 26 days (X^2 = 7.4, P = 0.0065) and 34 days, MSRO+ flies had significantly lower metabolic rates (X^2 = 4.4, P = 0.036).

Effect of fly metabolic rate on mite behaviour.

In 25 out of 32 (78%) Y-mazes, the mite was located in an arm containing a fly. In 17/25 (68%) of trials with a mite choice the mite was in the arm with the high-metabolic-rate fly. Mites were marginally more likely to be in the arm with the high-metabolic-rate fly (z = 1.76, P = 0.079). The confidence interval of this marginal preference overlapped 0.5 (95% CI, 46 to 85%; binom.test). Due to the small number of infections, we did not statistically test if more high metabolic-rate flies were infected. However, in the 6 trials where the mite infected a fly the mite infected the high-metabolic-rate fly 5 times. This experiment suggests (i) mites show a marginal attraction to higher-metabolic-rate flies and (ii) there is little evidence mites prefer to infect low-metabolic-rate flies.

MSRO impacts fly endurance in geotaxis assays.

These experiments tested fly resistance while eliminating the confounding effects of mite proclivity to infect. We recorded the number of cycles before flies were exhausted (c) as well as the time

until exhaustion (t). We modelled dependent variables c and t with infection status, fly mass, fly age, infection-age interaction, age-mass interaction, infection-mass interaction, and replicate block as a random effect.

The number of cycles to exhaustion (c) was best described with a negative binomial distribution and was modelled using the glmer.nb function (50). The interaction between fly age and infection status was a significant predictor of c (X^2 = 10.1, P = 0.0015). Mass was not a significant predictor of c (X^2 = 1.14, P = 0.29). The interactions between infection status and mass (X^2 = 1.28, P = 0.26) and fly age and mass (X^2= 1.37, P = 0.24) were also not significant. At 2 days posteclosion, there was no significant difference in c between infected and uninfected flies; MSRO- flies had a mean endurance of 132.0 ± 6.6 (mean ± standard error of the mean [SEM]) cycles, and MSRO+ flies had an endurance of 128.0 ± 6.7 cycles (X^2= 1.4, P = 0.25). There was a general decrease in the number of cycles climbed as flies aged, but this effect was larger among MSRO+ flies. At 14 days posteclosion, the MSRO+ flies had lower endurance than MSRO- flies, 43.3 ± 4.9 cycles and 68.4 ± 3.4 cycles, respectively (X^2 > 100, P < 0.0001). At 26 days posteclosion, the MSRO+ flies had lower endurance than MSRO- flies as well, 4.6 ± 1.3 cycles and 16.1 ± 1.9 cycles, respectively (X^2 >100, P < 0.0001).

The time to exhaustion (t) was modelled with the lmer function (50). The interaction between fly age and infection status was a significant predictor of t (X^2 = 7.3, P = 0.0069). Mass was not a significant predictor of t (X^2 = 0.024, P = 0.88). The interactions between infection status and mass (X^2 = 2.748, P = 0.097) and fly age and mass (X^2 = 2.12, P = 0.15) were also not significant. At 2 days posteclosion, t was also comparable between MSRO-, 492.4 ± 12.2 s, and MSRO+, 514.7 ± 13.9 s, flies (X^2 = 1.37, P = 0.24). At 14 days posteclosion, MSRO+ flies had substantially lower average t than MSRO- flies: 269.3 ± 28.8 s and 423.3 ± 19.4 s, respectively (X^2 = 8.2, P = 0.0041). Similarly, at 26 days posteclosion, MSRO+ flies had lower t than MSRO- flies; MSRO+ flies had a mean t of 43.2 ± 12.7 s, whereas MSRO- flies had a mean t of 149.8 ± 16.6 s (X^2= 15.0, P = 0.00011). Fly endurance (c and t) generally decreased with age, but the magnitude of decrease was larger among flies harbouring MSRO (Fig. 5.1.3a and b).

We also recorded the time the fly took to ascend the vial following the first knock down (i.e., first cycle time, or f). All flies from both the MSRO+ and MSRO- groups climbed the vial at least once at 2 days and 14 days posteclosion. At 26 days posteclosion, 94% of $MSRO^2$ flies climbed all the way to the mark, whereas only 47% of $MSRO^1$ flies climbed successfully. If a fly failed to ascend the vial once, the first cycle time was arbitrarily set to f = 11 s for nonparametric analysis (wilcox_test [51]).

The median first cycle time of the MSRO+ and MSRO- groups at 2 days posteclosion were nearly indistinguishable at 3.3 s (2.9 to 4.1, 10th to 90th percentile) and 3.3 s (2.6 to 3.9), respectively; this was not a significant difference (wilcox_test, z = 1.1, P = 0.25). At 14 days posteclosion, the median first cycle time of MSRO+ flies was 4.1 s (3.1 to 6.2) and 6.4 s (3.6 to 8.5) for MSRO- flies (z = 2.9, P = 0.0041). At 26 days posteclosion, the median first cycle time was 7.0 (4.9 to 9.3) for MSRO- flies and 11 s (7.9 to 11) for $MSRO^1$ flies (z = 4.9, P = 0.0001). The difference between MSRO- and MSRO+ flies at 26 days posteclosion remained significant even when only considering flies that successfully climbed (z = 3.0, P =

0.0025), for which the median time was 6.9 s (4.7 to 8.9) and 8.8 s (6.3 to 9.7), respectively. Older flies were slower to ascend the vial on the first cycle, and this was more pronounced among MSRO+ flies (Fig. 5.1.3d).

Across all fly ages, the mean mass of the MSRO+ flies was 1.14 ± 0.01 mg (N = 95), and the mean mass of the MSRO- flies was 1.10 ± 0.01 mg (N = 93). This difference was statistically significant (t test, t = 3.16, P = 0.015) but relatively small: 4.12%. Among 2-day-old flies there was no significant difference in mass (<1%) between MSRO+ and MSRO- flies, 1.07 ± 0.01 mg and 1.06 ± 0.01 mg, respectively (t = 0.24, P = 0.81). Among 14-day-old flies there was a significant difference in mass between MSRO+ and MSRO- flies, 1.14 ± 0.02 mg and 1.07 ± 0.01 mg, respectively: 6.3% (t = 3.16, P = 0.002). Similarly, there was a significant 6.8% difference in mass between 26-day-old MSRO+ and MSRO- flies, 1.22 ± 0.02 mg and 1.14 ± 0.01 mg, respectively (t = 2.73, P = 0.008). Among the older age categories, MSRO+ flies were significantly heavier than MSRO- flies.

Discussion

We set out to test three non-mutually exclusive hypotheses: (i) mites preferentially infect *S. poulsonii* MSRO+ flies over uninfected flies, (ii) mite preferences for MSRO+ or MSRO- flies are explained by mite preferences for high-metabolic-rate hosts, and (iii) MSRO-infected flies are more susceptible (measured as the ability to maintain energetically demanding behavioural defences) to mite attack. The latter hypothesis predicted that flies infected with MSRO would have reduced endurance in negative geotaxis assays (a proxy measure of Drosophila mite resistance [46]). This prediction was supported by our data (Fig. 5.1.3a, b, and d). The MSRO infection-fly age interaction was a significant predictor of endurance; specifically, 14- and 26-day-old MSRO+ flies had weaker endurance than MSRO- flies of the same age (Fig. 5.1.3a and b). These results show MSRO infection impairs fly endurance, and this deleterious effect increases as flies age and/or MSRO titer increases.

Our geotaxis results are compatible with previous observations that *Spiroplasma* infection can reduce the percentage of flies climbing in group geotaxis assays at older ages [29; 31]. One hundred percent of MSRO+ and MSRO- flies in our trials climbed at 2 and 14 days posteclosion, whereas at 26 days posteclosion, 94% of MSRO- flies climbed and only 47% of MSRO+ flies successfully climbed. However, by measuring the endurance of individual flies, we detected adverse effects of *Spiroplasma* infection earlier in the fly life span (Fig. 5.1.3a and b). We observed substantially reduced cycles to exhaustion and time to exhaustion among MSRO+ flies 2 weeks posteclosion, earlier than studies that only measured the proportion of flies climbing [29; 31; 48]. Our results suggest it may be useful in future studies to consider individual organism assays when subtler effects of endosymbionts on fly performance are important, e.g., during interactions with a secondary parasite.

Hastened senescence among MSRO+ flies could explain endurance decreasing faster among MSRO+ flies. Shortened fly lifespans, earlier reductions in metabolic rate, and declines in age-specific mobility all suggest MSRO hastens senescence [29; 52] (Fig. 5.1.2a and 5.1.3a and b). MSRO could

influence fly senescence through changes in metabolism, energy reserves, and/or immunity [reviewed in reference 53]. Activation of the insect immune system can speed senescence [53; 54]. However, MSRO generally evades *D. melanogaster* immunity [32], suggesting that if MSRO impacts fly senescence it is more likely through effects on metabolism or energy reserves.

We also hypothesised that mites would preferentially attack *D. melanogaster* infected with MSRO. However, results showed that *M. subbadius* infection bias depended on fly age: mites were less likely to infect MSRO+ 14-day-old flies but were more likely to infect MSRO+ flies at 26 days (Fig. 5.1.1). Mites did not show a bias toward MSRO+ or MSRO- hosts when flies were 2 days old (Fig. 5.1.1). Both 14-day-old and 26-day-old *D. melanogaster* Oregon-R flies have high levels of MSRO, but MSRO titers are higher and plateau by ~26 days [29]. Whether replication-phase or plateau-phase MSRO has a higher potential for horizontal transmission is currently unknown [25]. Future research should compare the ability of MSRO collected from hosts of various ages to establish persistent infection following horizontal transmission.

M. subbadius discriminates between potential hosts based on many traits. For example, they prefer larger flies and females [55]. However, in our preference experiments, we size-matched MSRO+ and MSRO- females to within 5% body mass, meaning fly sex and potential mass differences were not driving the preferences observed here (Fig. 5.1.1 and 5.1.3c). Flies were also restrained, eliminating differences in mobility between groups (Fig. 5.1.3a and b). A previous study demonstrated *M. subbadius* preferentially infects *D. nigrospiracula* with higher metabolic rates [42]. Thus, we hypothesised that fly metabolic rate would explain the preferences of mites for/against MSRO+ flies. If metabolic rate explained preferences/aversions for MSRO+ flies, we would anticipate MSRO+ flies having lower metabolic rates at 2 weeks old and higher metabolic rates at 26 days old relative to MSRO- flies. Contrary to our expectations, MSRO+ flies had higher metabolic rates than MSRO- flies15 days posteclosion and lower metabolic rates than MSRO- flies at 26 and 34 days posteclosion (i.e., the opposite of observed pairwise choices) (Fig. 5.1.2a, Table 1).

The inverted relationship between metabolic rate and mite preference observed here indicates (i) mites preferred to infect low-metabolic-rate flies or (ii) mite preferences for other infection-associated fly traits overpowered/suppressed the preference for high-metabolic-rate flies (Table 1). However, the mite species used in these experiments is known to prefer hosts with higher metabolic rates [42]. This trend is consistent within this line of *M. subbadius*. It also prefers mated female flies over unmated females, and mated females have higher metabolic rates than unmated females [55; 56; 57]. In this study, mites had a marginal attraction toward higher-metabolic-rate *D. melanogaster*. Taken together with prior results, this attraction, albeit weaker than the preference for higher-metabolic-rate *D. nigrospiracula*, suggests it is unlikely mites were merely selecting flies based on low metabolic rates [42].

It is not currently known what specific cues mites were responding to when infecting MSRO+ or MSRO- flies. Mites could conceivably directly sense MSRO-derived compounds and/or fly-derived signals of health. Furthermore, the cues influencing mite infectious behavior may change over the fly life span.

Mites may prefer MSRO+ flies at 26 days old due to a preference for flies in poor body condition, and MSRO infection appears to speed aging and declining health [41]. We expected 14-day-old MSRO+ flies to be attractive to mites (i.e. mites would preferentially infect MSRO+ flies) due to high metabolic rates and relatively high endosymbiont titers. However, mites counterintuitively avoided these flies (Fig. 5.1.1). *D. melanogaster* produces pheromones that indicate physical health that are sensed by conspecifics and potentially ectoparasites [58; 59; 60]. Since flies may partially compensate for MSRO infection in the short term, they may exhibit normal cues or even exhibit cues that mites find repellent [29]. Alternatively, rapidly proliferating MSRO may pose a risk to mites, although the effects of consumed MSRO on mites are not known [25]. Regardless of the specific mechanism(s), the fact remains that mites were disproportionately likely to infect either MSRO+ or MSRO- hosts depending on fly age.

Mites use contact and olfactory chemoreception to find and distinguish between potential hosts [59; 61; 62]. *Poecilochirus* species choose larger host beetles, potentially based on chemical cues [63]. *Dermanyssus gallinae* is attracted to volatile cues associated with the aged feathers, but not fresh feathers, of host birds [64]. Another mesostigmatid mite, *Varroa destructor*, discriminates between nurse and forager bees based on differences in cuticular hydrocarbons [65]. MSRO has significant impacts on host physiology, metabolism, and body composition [29] (Fig. 5.1.2a). All these changes could have knock-on effects on host cues [66; 67; 68]. Since mites in our preference experiments could contact flies before infection, it is possible that mites were using nonvolatile hydrocarbons on the surface of flies or volatile kairomones, i.e. fly cues that benefit host-seeking mites, to discriminate between hosts [69]. At present, it is not known how MSRO, directly or through knock-on effects on fly condition/physiology, impacts the composition of cuticular waxes or volatiles. Nor is it known if MSRO infection affects the melanization response, which could impact mite success. Because mite preferences changed across the fly life span, it may be fruitful to examine these cues in $MSRO^1$ and $MSRO^2$ flies at the ages identified here (Fig. 5.1.1).

Previous studies showed Macrocheles muscadomesticae mites preferentially infect D*. hydei* females over conspecific males in a size-mediated manner [43]. *Drosophila melanogaster* flies are also sexually dimorphic in body mass, with female flies being larger on average than male flies [70; 71]. However, the average size of *D. melanogaster* is substantially smaller than that of *D. hydei* (approximately half or less by mass), and resultantly the absolute size difference between male and female *D. melanogaster* flies is smaller [43]. Smaller absolute size differences may explain the weaker preference for females observed here.

The impact of MSRO on fly metabolic rate varied over the fly life span. At 14 days old, MSRO+ flies had higher metabolic rates, whereas at 26+ days MSRO+ flies had lower metabolic rates (Fig. 5.1.2a). We did not detect a significant effect of frozen mass on female fly metabolic rate (Fig. 5.1.2b), which is consistent with many lines of lab *D. melanogaster* [72], nor did we detect a significant interaction between MSRO infection status and mass. One potential explanation for why older MSRO+ flies had lower metabolic rates than age-matched MSRO- flies is hastened senescence, since *D. melanogaster*

metabolic rates tend to decrease with age [73; 74; 75]. Likewise, mobility also decreases with fly age [76; 77] (Fig. 5.1.3). However, rapid senescence alone does not explain elevated metabolic rates among 14-day-old MSRO+ flies. Fourteen days old corresponds to mid-late log growth and may correspond to periods of high endosymbiont energy use [29]. Alternatively, flies may compensate for infection, e.g., by mobilising stored energy or increasing feeding, leading to higher metabolic rates.

Compensating for early proliferation may preserve fly health during periods of high fly fecundity, before reproduction significantly decreases ~3 weeks posteclosion [78; 79]. MSRO+ *D. melanogaster* may lay eggs slightly sooner than MSRO- flies, suggesting they may partially compensate for early death/poor health with earlier ovipositing [29]. Organisms have a finite capacity to compensate for infection, and compensation could come with a trade-off of earlier senescence due to effects of metabolism on *Drosophila* aging [80]. Future studies could also test if MSRO+ flies engage in compensatory feeding across the fly life span. Immuno-mutant and metabolic-mutant *D. melanogaster* may also provide a method evaluating the relative contributions of the fly and bacterium to metabolic changes observed here [81; 82].

Endosymbiotic bacteria vary substantially in their fitness level effects and impacts on host insect health. MSRO has substantial negative effects on fly life span and body condition [29] (Fig. 5.1.3a and b). However, other *Spiroplasma* species have smaller effects or even are beneficial, for example contributing to parasitoid resistance [83; 84; 85]. *Spiroplasma* HapI has little to no effect on fly survival but protects *D. hydei* hosts against parasitoid attack [86; 87]. Other endosymbionts show similar variability. *Wolbachia pipientis* wMelPop is pathogenic and reduces fly longevity [88; 89], whereas other *Wolbachia* strains have relatively mild effects, such as minor changes in mobility, or are beneficial [85; 90]. Additionally, the impacts of endosymbionts on hosts can be environment dependent [91; 92]. Thus, it is important for future research to compare the impacts of different endosymbiont bacteria on host-ectoparasite interactions across environments. It may be possible that some endosymbionts improve ectoparasite resistance due to the role activity (grooming, moving, dispersal, etc) plays in resisting ectoparasite infection [90].

In this study, MSRO+ flies had reduced endurance (a proxy of mite resistance), but mites preferred MSRO+ or MSRO- flies at different fly ages. Further research is required to determine if differential susceptibility leads to mites disproportionately infecting MSRO+ flies on a population level and should test if wild-caught flies harbouring male killing *Spiroplasma* are more likely to be infected with natural ectoparasites. Given their fitness and population effects [14; 15; 93], the impacts of male-killing *Spiroplasma* on the interactions between hosts and potential vectors have implications for the ecology and evolution of these symbioses.

Table 5.1.1: Summary of mite preferences and corresponding fly metabolic rates.

Fly Age	Mite Preference	Higher-MR Fly
2	No preference	No difference
14/15	MSRO-	MSRO+
26	MSRO+	MSRO-

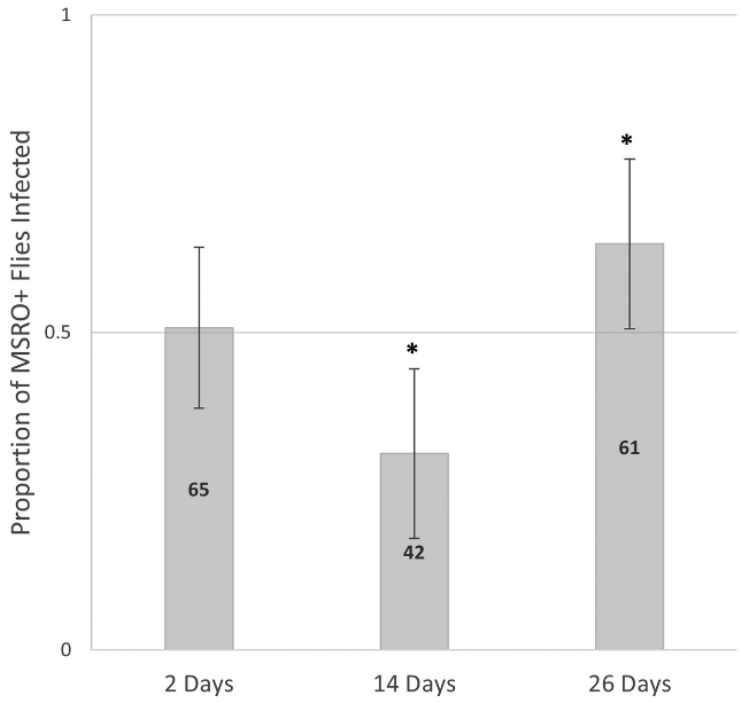


Figure 5.1.1: Proportion of mites that infected the MSRO+ fly in pairwise Y-maze tests at different fly ages. MSRO+ and MSRO- flies were age matched at 2, 14, or 26 days posteclosion. Data labels (in bar) show sample size (number of Y-mazes). Error bars represent 95% confidence intervals (using binom.test).

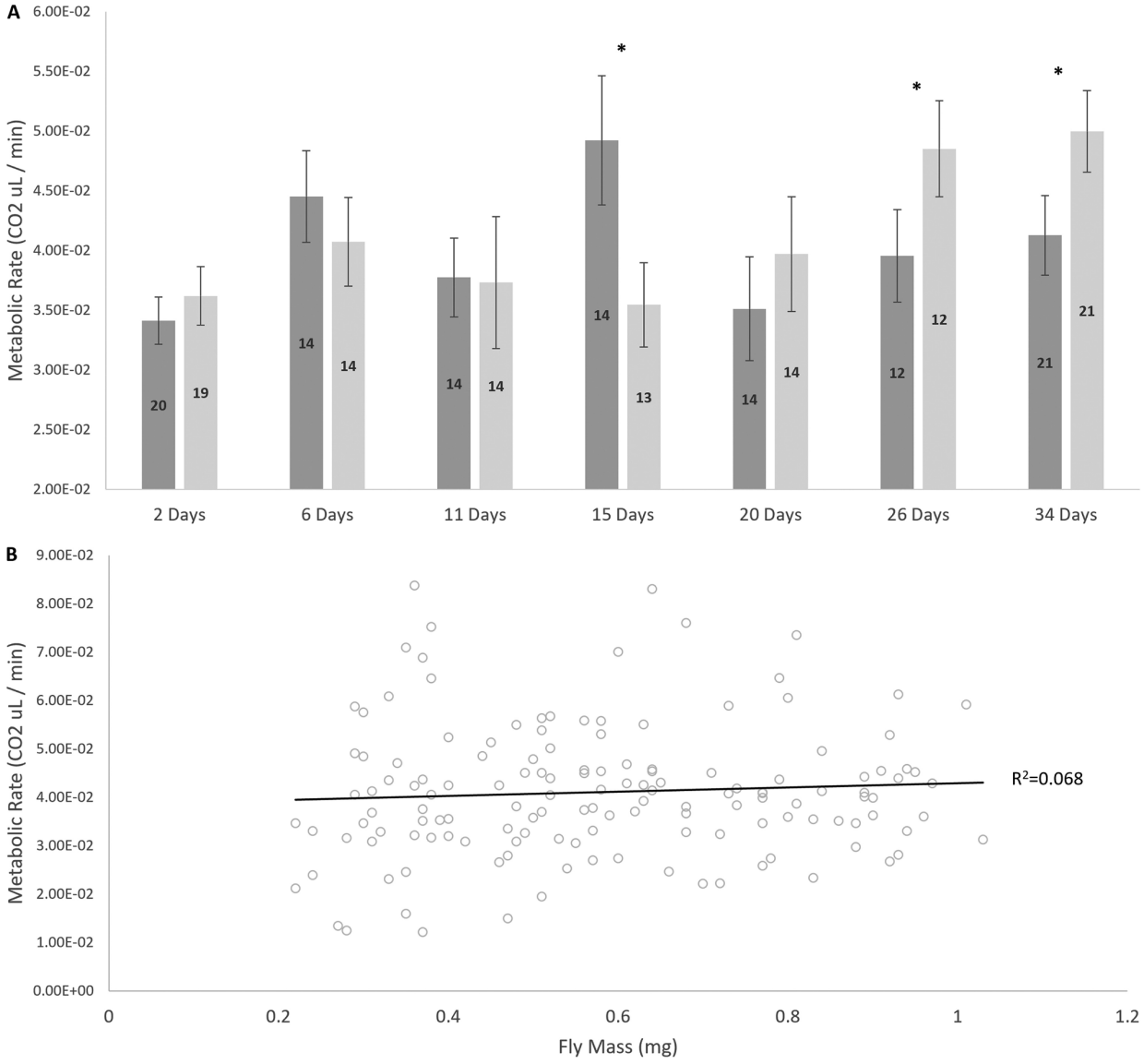


Figure 5.1.2: (A) Metabolic rates (measured as rate of carbon dioxide production) of MSRO+ (dark bars) and MSRO- (light bars) flies at different fly ages. Cohorts of MSRO+ and MSRO- flies were age matched, and CO_2 production was measured using flowthrough respirometry. Data labels (in bar) show sample size (number of files). Error bars represent 1 SEM. An asterisk indicates significant difference within an age category ($P < 0.05$). (B) Metabolic rate versus fly frozen mass. Mass was not a significant predictor of metabolic rate ($R^2 = 0.068$), and neither were the mass-infection and mass-age interactions.

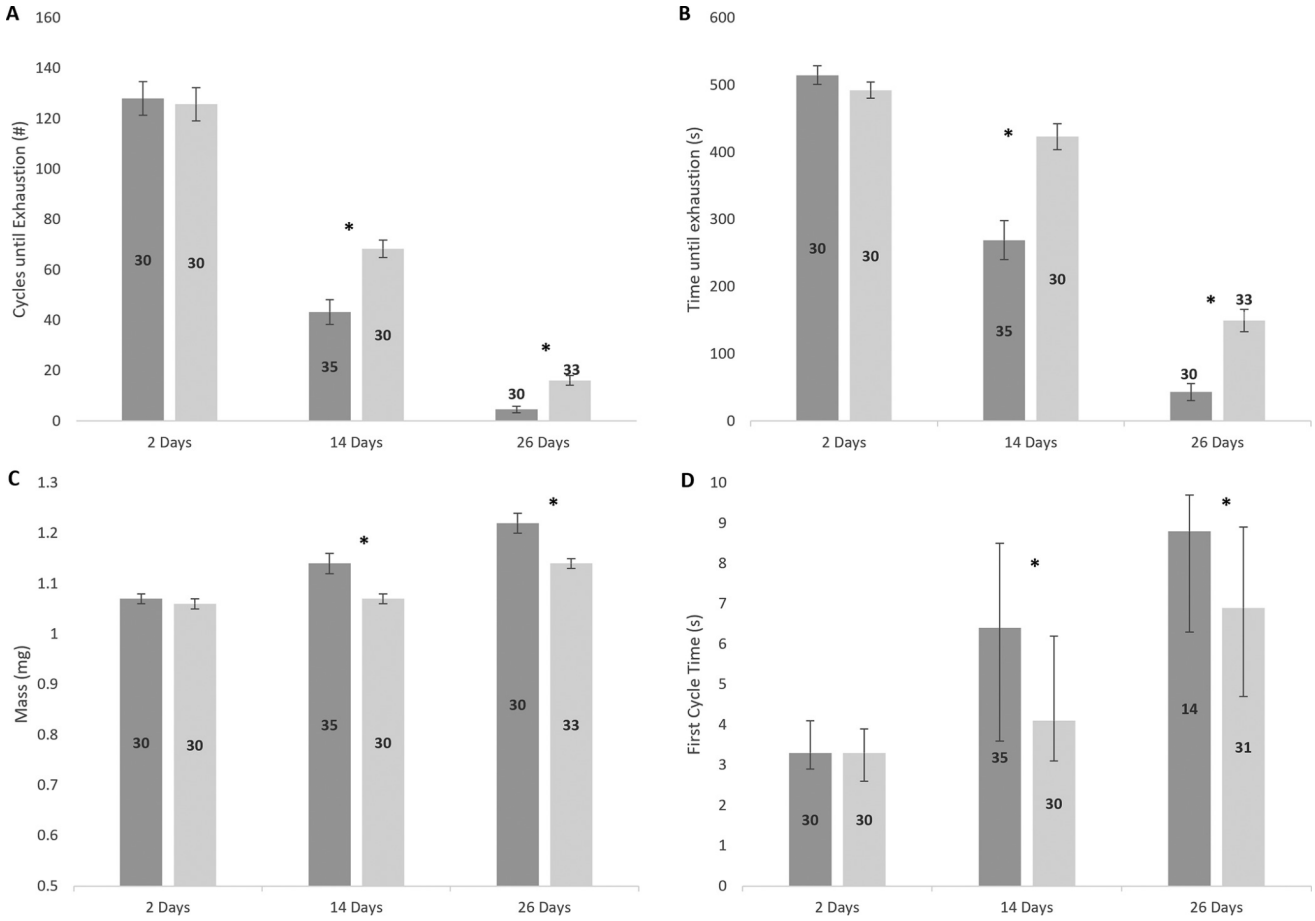


Figure 5.1.3: Endurance of MSRO+ and MSRO- flies in negative geotaxis endurance assays. (A) The number of ascents flies made following knockdown. (B) The duration of time (seconds) climbed before exhaustion. (C) Mass of flies (milligrams). (D) Time to ascend the vial in the first cycle, excluding flies that failed to climb (seconds). (All) Data labels show sample size (number of flies). Error bars represent 1 SEM (A to C) or 10th to 90th percentiles (D). An asterisk indicates a significant difference within an age category ($P < 0.05$). Data labels (in bar) show sample size (number of files).

5.1 References

1. Schmitz-Esser S, Toenshoff ER, Haider S, Heinz E, Hoenninger VM, Wagner M, Horn M. 2008. Diversity of bacterial endosymbionts of environmental *Acanthamoeba* isolates. Appl Environ Microbiol 74:5822–5831. https://doi.org/10.1128/AEM.01093-08.
2. Kondorosi E, Mergaert P, Kereszt A. 2013. A paradigm for endosymbiotic life: cell differentiation of *Rhizobium* bacteria provoked by host plant factors. Annu Rev Microbiol 67:611–628. https://doi.org/10.1146/annurev-micro-092412-155630.
3. Marubayashi JM, Kliot A, Yuki VA, Rezende JAM, Krause-Sakate R, Pavan MA, Ghanim M. 2014. Diversity and localization of bacterial endosymbionts from whitefly species collected in Brazil. PLoS One 9:e108363. https://doi.org/10.1371/journal.pone.0108363.
4. Pawlowska TE, Gaspar ML, Lastovetsky OA, Mondo SJ, Real-Ramirez I, Shakya E, Bonfante P. 2018. Biology of fungi and their bacterial endosymbionts. Annu Rev Phytopathol 56:289–309. https://doi.org/10.1146/annurev-phyto-080417-045914.
5. Kikuchi Y. 2009. Endosymbiotic bacteria in insects: their diversity and culturability. Microbes Environ 24:195–204. https://doi.org/10.1264/jsme2.me09140s.
6. Ishii Y, Matsuura Y, Kakizawa S, Nikoh N, Fukatsu T. 2013. Diversity of bacterial endosymbionts associated with *Macrosteles* leafhoppers vectoring phytopathogenic phytoplasmas. Appl Environ Microbiol 79:5013–5022. https://doi.org/10.1128/AEM.01527-13.
7. Jiggins FM. 2017. The spread of *Wolbachia* through mosquito populations. PLoS Biol 15:e2002780. https://doi.org/10.1371/journal.pbio.2002780.
8. White JA, Styer A, Rosenwald LC, Curry MM, Welch KD, Athey KJ, Chapman EG. 2020. Endosymbiotic bacteria are prevalent and diverse in agricultural spiders. Microb Ecol 79:472–481. https://doi.org/10.1007/s00248-019-01411-w.
9. Ryder JJ, Hoare MJ, Pastok D, Bottery M, Boots M, Fenton A, Atkinson D, Knell RJ, Hurst GDD. 2014. Disease epidemiology in arthropods is altered by the presence of nonprotective symbionts. Am Nat 183:E89–E104.https://doi.org/10.1086/674827.
10. Douglas AE. 2015. Multiorganismal insects: diversity and function of resident microorganisms. Annu Rev Entomol 60:17–34. https://doi.org/10.1146/annurev-ento-010814-020822.
11. Jones JE, Hurst GD. 2020. Symbiont-mediated fly survival is independent of defensive symbiont genotype in the *Drosophila melanogaster*-*Spiroplasma*-wasp interaction. J Evol Biol 33:1625–1633. https://doi.org/10.1111/jeb.13702.
12. Xie K, Lu YJ, Yang K, Huo SM, Hong XY. 2020. Co-infection of *Wolbachia* and *Spiroplasma* in spider mite *Tetranychus truncatus* increases male fitness. Insect Sci 27:921–937. https://doi.org/10.1111/1744-7917.12696.
13. Anbutsu H, Fukatsu T. 2003. Population dynamics of male-killing and non-male-killing *Spiroplasmas* in *Drosophila melanogaster*. Appl Environ Microbiol 69:1428–1434. https://doi.org/10.1128/AEM.69.3.1428-1434.2003.

14. Engelstädter J, Hurst GDD. 2007. The impact of male-killing bacteria on host evolutionary processes. Genetics 175:245–254. https://doi.org/10.1534/genetics.106.060921.
15. Kwiatkowski M, Vorburger C. 2012. Modelling the ecology of symbiont-mediated protection against parasites. Am Nat 179:595–605. https://doi.org/10.1086/665003.
16. Guo Y, Hoffmann AA, Xu XQ, Mo PW, Huang HJ, Gong JT, Ju JF, Hong XY. 2018. Vertical transmission of *Wolbachia* is associated with host vitellogenin in *Laodelphax striatellus*. Front Microbiol 9:2016. https://doi.org/10.3389/fmicb.2018.02016.
17. Harumoto T, Lemaitre B. 2018. Male-killing toxin in a bacterial symbiont of *Drosophila*. Nature 557:252–255. https://doi.org/10.1038/s41586-018-0086-2.
18. Gerth M, Martinez-Montoya H, Ramirez P, Masson F, Griffin JS, Aramayo R, Siozios S, Lemaitre B, Mateos M, Hurst GDD. 2021. Rapid molecular evolution of *Spiroplasma* symbionts of *Drosophila*. Microb Genom 7:000503. https://doi.org/10.1099/mgen.0.000503.
19. Haselkorn TS, Markow TA, Moran NA. 2009. Multiple introductions of the *Spiroplasma* bacterial endosymbiont into *Drosophila*. Mol Ecol 18:1294–1305. https://doi.org/10.1111/j.1365-294X.2009.04085.x.
20. Hosokawa T, Nikoh N, Koga R, Sato M, Tanahashi M, Meng XY, Fukatsu T. 2012. Reductive genome evolution, host-symbiont co-speciation and uterine transmission of endosymbiotic bacteria in bat flies. ISME J 6:577–587. https://doi.org/10.1038/ismej.2011.125.
21. 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. https://doi.org/10.1007/s13199-013-0241-3.
22. DiBlasi E, Morse S, Mayberry JR, Avila LJ, Morando M, Dittmar K. 2011. New *Spiroplasma* in parasitic Leptus mites and their *Agathemera* walking stick hosts from Argentina. J Invertebr Pathol 107:225–228. https://doi.org/10.1016/j.jip.2011.05.013.
23. Haselkorn TS, Cockburn SN, Hamilton PT, Perlman SJ, Jaenike J. 2013. Infectious adaptation: potential host range of a defensive endosymbiont in drosophila. Evolution 67:934–945. https://doi.org/10.1111/evo.12020.
24. Xie JL, Winter C, Winter L, Mateos M. 2015. Rapid spread of the defensive endosymbiont *Spiroplasma* in *Drosophila hydei* under high parasitoid wasp pressure. FEMS Microbiol Ecol 91:1–11. https://doi.org/10.1093/femsec/fiu017.
25. Jaenike J, Polak M, Fiskin A, Helou M, Minhas M. 2007. Interspecific transmission of endosymbiotic *Spiroplasma* by mites. Biol Lett 3:23–25. https://doi.org/10.1098/rsbl.2006.0577.
26. Durkin ES, Roth AM, Keiser CN. 2020. Parasitic personalities: consistent individual differences in behavior in a facultatively parasitic mite. J Insect Behav 33:14–19. https://doi.org/10.1007/s10905-020-09741-1.
27. Kageyama D, Anbutsu H, Watada M, Hosokawa T, Shimada M, Fukatsu T. 2006. Prevalence of a

non-male-killing *Spiroplasma* in natural populations of *Drosophila hydei*. Appl Environ Microbiol 72:6667–6673. https://doi.org/10.1128/AEM.00803-06.

28. Watts T, Haselkorn TS, Moran NA, Markow TA. 2009. Variable incidence of *Spiroplasma* infections in natural populations of *Drosophila* species. PLoS One 4:e5703. https://doi.org/10.1371/journal.pone.0005703.
29. Herren JK, Paredes JC, Schupfer F, Arafah K, Bulet P, Lemaitre B. 2014. Insect endosymbiont proliferation is limited by lipid availability. Elife 3: e02964. https://doi.org/10.7554/eLife.02964.
30. Herren JK, Paredes JC, Schupfer F, Lemaitre B. 2013. Vertical transmission of a *Drosophila* endosymbiont via cooption of the yolk transport and internalization machinery. mBio 4:e00532-12. https://doi.org/10.1128/mBio.00532-12.
31. Masson F, Calderon-Copete S, Schupfer F, Vigneron A, Rommelaere S, Garcia-Arraez MG, Paredes JC, Lemaitre B. 2020. Blind killing of both male and female *Drosophila* embryos by a natural variant of the endosymbiotic bacterium *Spiroplasma poulsonii.* Cell Microbiol 22:e13156. https://doi.org/10.1111/cmi.13156.
32. Herren JK, Lemaitre B. 2011. *Spiroplasma* and host immunity: activation of humoral immune responses increases endosymbiont load and susceptibility to certain Gram-negative bacterial pathogens in *Drosophila melanogaster*. Cell Microbiol 13:1385–1396. https://doi.org/10.1111/j.1462-5822.2011.01627.x.
33. Bushey D, Hughes KA, Tononi G, Cirelli C. 2010. Sleep, aging, and lifespan in *Drosophila*. BMC Neurosci 11:56–18. https://doi.org/10.1186/1471-2202-11-56.
34. Uysal H, Genç S, Ayar A. 2017. Toxic effects of chronic feeding with food azo dyes on *Drosophila melanogaster* Oregon-R. Scientia Iranica C 24:3081–3086. https://doi.org/10.24200/sci.2017.4523.
35. Polak M. 1996. Ectoparasitic effects on host survival and reproduction: the *Drosophila-Macrocheles* association. Ecology 77:1379–1389. https://doi.org/10.2307/2265535.
36. Beresford DV, Sutcliffe JF. 2009. The effect of *Macrocheles muscaedomesticae* and *M. subbadius* (Acarina: Macrochelidae) phoresy on the dispersal of *Stomoxys calcitrans* (Diptera: Muscidae). Syst Appl Acarol Special Pub 23:1–30. https://doi.org/10.11158/saasp.23.1.1.
37. Perez-Leanos A, Loustalot-Laclette MR, Nazario-Yepiz N, Markow TA. 2017. Ectoparasitic mites and their *Drosophila* hosts. Fly (Austin) 11:10–18. https://doi.org/10.1080/19336934.2016.1222998.
38. Brown JM, Wilson DS. 1992. Local specialization of phoretic mites on Sympatric carrion beetle hosts. Ecology 73:463–478. https://doi.org/10.2307/1940753.
39. Schwarz HH, Starrach M, Koulianos S. 1998. Host specificity and permanence of associations between mesostigmatic mites (Acari: Anactinotrichida) and burying beetles (Coleoptera: Silphidae: Nicrophorus). J Nat Hist 32:159–172. https://doi.org/10.1080/00222939800770101.
40. Xie X, Huang ZY, Zeng Z. 2016. Why do *Varroa* mites prefer nurse bees? Sci Rep 6:28228.

https://doi.org/10.1038/srep28228.
41. Luong LT, Brophy T, Stolz E, Chan SJ. 2017. State-dependent parasitism by a facultative parasite of fruit flies. Parasitology 144:1468–1475. https://doi.org/10.1017/S0031182017000890.
42. Horn CJ, Mierzejewski MK, Luong LT. 2018. Host respiration rate and injury-derived cues drive host preferences by an ectoparasite of fruit flies. Physiol Biochem Zool 91:896–903. https://doi.org/10.1086/697466.
43. Campbell E, Luong LT. 2016. Mite choice generates sex and size-biased infection in *Drosophila hydei*. Parasitology 143:787–783. https://doi.org/10.1017/S0031182016000305.
44. Li JF, Zhang W, Guo ZH, Wu S, Jan LY, Jan YN. 2016. A defensive kicking behavior in response to mechanical stimuli mediated by *Drosophila* wing margin bristles. J Neurosci 36:11275–11282. https://doi.org/10.1523/JNEUROSCI.1416-16.2016.
45. Horn CJ, Luong LT. 2019. Current parasite resistance trades off with future defenses and flight performance. Behav Ecol Sociobiol 73:1–10. https://doi.org/10.1007/s00265-019-2697-5.
46. Horn CJ, Luong LT. 2021. Trade-offs between reproduction and behavioural resistance against ectoparasite infection. Physiol Behav 239:113524–113527. https://doi.org/10.1016/j.physbeh.2021.113524.
47. Tinkerhess MJ, Healy L, Morgan M, Sujkowski A, Matthys E, Zheng L, Wessells RJ. 2012. The *Drosophila* PGC-1 alpha homolog spargel modulates the physiological effects of endurance exercise. PLoS One 7:e31633. https://doi.org/10.1371/journal.pone.0031633.
48. Barone MC, Bohmann D. 2013. Assessing neurodegenerative phenotypes in *Drosophila* dopaminergic neurons by climbing assays and whole brain immunostaining. J Vis Exp 2013:e50339. https://doi.org/10.3791/50339.
49. R Core Team. 2019. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.
50. Bates D, Mächler M, Bolker B, Walker S. 2015. Fitting linear mixed-effects models using lme4. J Stat Soft 67:1–48. https://doi.org/10.18637/jss.v067.i01.
51. Hothorn T, Hornik K, van de Wiel MA, Zeileis A. 2006. A Lego system for conditional inference. Am Stat 60:257–263. https://doi.org/10.1198/000313006X118430.
52. Rhodenizer D, Martin I, Bhandari P, Pletcher SD, Grotewiel M. 2008. Genetic and environmental factors impact age-related impairment of negative geotaxis in *Drosophila* by altering age-dependent climbing speed. Exp Gerontol 43:739–748. https://doi.org/10.1016/j.exger.2008.04.011.
53. Garschall K, Flatt T. 2018. The interplay between immunity and aging in *Drosophila*. F1000Res 7:160–160. https://doi.org/10.12688/f1000research .13117.1.
54. Mackenzie DK, Bussière LF, Tinsley MC. 2011. Senescence of the cellular immune response in *Drosophila melanogaster*. Exp Gerontol 46:853–859. https://doi.org/10.1016/j.exger.2011.07.004.
55. Horn CJ, Mierzejewski MK, Elahi ME, Luong LT. 2020. Extending the ecology of fear:

parasite-mediated sexual selection drives host response to parasites. Physiol Behav 224:113041–113047. https://doi.org/10.1016/j.physbeh.2020.113041.

56. Giesel JT, Lanciani CA, Anderson JF. 1989. Metabolic rate and sexual activity in *Drosophila simulans*. J Insect Physiol 35:893–895. https://doi.org/10.1016/0022-1910(89)90106-6.
57. Videlier M, Rundle HD, Careau V. 2019. Sex-specific among-individual covariation in locomotor activity and resting metabolic rate in *Drosophila melanogaster*. Am Nat 194:E164–E176. https://doi.org/10.1086/705678.
58. Pernal SF, Baird DS, Birmingham AL, Higo HA, Slessor KN, Winston ML. 2005. Semiochemicals influencing the host-finding behaviour of *Varroa destructor*. Exp Appl Acarol 37:1–26. https://doi.org/10.1007/s10493-005-1117-x.
59. Carr AL, Roe M. 2016. Acarine attractants: chemoreception, bioassay, chemistry and control. Pestic Biochem Physiol 131:60–79. https://doi.org/10.1016/j.pestbp.2015.12.009.
60. Schultzhaus JA, Bennet CJ, Iftikhar H, Yew JY, Mallet J, Carney GE. 2018. High fat diet alters *Drosophila melanogaster* sexual behavior and traits: decreased attractiveness and changes in pheromone profiles. Sci Rep 8:1–13. https://doi.org/10.1038/s41598-018-23662-2.
61. Egan ME, Barth RH, Hanson FE. 1975. Chemically-mediated host selection in a parasitic mite. Nature 257:788–790. https://doi.org/10.1038/257788a0.
62. Kraus B. 1994. Factors influencing host choice of the honey-bee parasite *Varroa jacobsoni* oud. Exp Appl Acarol 18:435–443. https://doi.org/10.1007/BF00051525.
63. Grossman JD, Smith RJ. 2008. Phoretic mite discrimination among male burying beetle (*Nicrophorus investigator*) hosts. Ann Entomol Soc Am 101:266–271. https://doi.org/10.1603/0013-8746(2008)101
64. Koenraadt CJM, Dicke M. 2010. The role of volatiles in aggregation and host-seeking of the haematophagous poultry red mite *Dermanyssus gallinae* (Acari: Dermanyssidae). Exp Appl Acarol 50:191–199. https://doi.org/10.1007/s10493-009-9305-8.
65. Cervo R, Bruschini C, Cappa F, Meconcelli S, Pieraccini G, Pradella D, Turillazzi S. 2014. High *Varroa* mite abundance influences chemical profiles of worker bees and mite-host preferences. J Exp Biol 217:2998–3001. https://doi.org/10.1242/jeb.099978.
66. Fedina TY, Kuo TH, Dreisewerd K, Dierick HA, Yew JY, Pletcher SD. 2012. Dietary effects on cuticular hydrocarbons and sexual attractiveness in *Drosophila*. PLoS One 7:e49799-11. https://doi.org/10.1371/journal.pone .0049799.
67. Kuo TH, Yew JW, Fedina TY, Dreisewerd K, Dierick HA, Pletcher SD. 2012. Aging modulates cuticular hydrocarbons and sexual attractiveness in *Drosophila melanogaster*. J Exp Biol 215:814–821. https://doi.org/10.1242/jeb.064980.
68. Zhang W, Liu L, He S, Lu BY, Luo YB. 2020. The production and evolution pattern of “fruity smell” aggregation pheromones in genus *Drosophila*. J Syst Evol https://doi.org/10.1111/jse.12648.
69. Gay M, Lempereur L, Francis F, Megido RC. 2020. Control of *Dermanyssus gallinae* (De Geer

1778) and other mites with volatile organic compounds, a review. Parasitology 147:731–739. https://doi.org/10.1017/S0031182020000530.

70. Stillwell RC, Blanckenhorn WU, Teder T, Davidowitz G, Fox CW. 2010. Sex differences in phenotypic plasticity affect variation in sexual size dimorphism in insects: from physiology to evolution. Annu Rev Entomol 55: 227–245. https://doi.org/10.1146/annurev-ento-112408-085500.
71. Mathews KW, Cavegn M, Zwicky M. 2017. Sexual dimorphism of body size is controlled by dosage of the X-chromosomal gene *myc* and by the sex-determining gene *tra* in *Drosophila*. Genetics 205:1215–1228. https://doi.org/10.1534/genetics.116.192260.
72. Van Voorhies WA, Khazaeli AA, Curtsinger JW. 2004. Lack of correlation between body mass and metabolic rate in *Drosophila melanogaster*. J Insect Physiol 50:445–453. https://doi.org/10.1016/j.jinsphys.2004.03.002.
73. Lints FA, Lints CV. 1968. Respiration in *Drosophila*. II. Respiration in relation to age by wild inbred and hybrid *Drosophila melanogaster* imagos. Exp Gerontol 3:341–349. https://doi.org/10.1016/0531-5565(68)90047-8.
74. Van Voorhies WA, Khazaeli AA, Curtsinger JW. 2003. Selected contribution: long-lived *Drosophila melanogaster* lines exhibit normal metabolic rates. J Appl Physiol 95:2605–2613. https://doi.org/10.1152/japplphysiol.00448.2003.
75. Khazaeli AA, Van Voorhies W, Curtsinger JW. 2005. Longevity and metabolism in *Drosophila melanogaster*: genetic correlations between life span and age-specific metabolic rate in populations artificially selected for long life. Genetics 169:231–242. https://doi.org/10.1534/genetics.104.030403.
76. Gargano JW, Martin I, Bhandari P, Grotewiel MS. 2005. Rapid iterative negative geotaxis (RING): a new method for assessing age-related locomotor decline in *Drosophila*. Exp Gerontol 40:386–395. https://doi.org/10.1016/j.exger.2005.02.005.
77. Piazza N, Gosangi B, Devilla S, Arking R, Wessells R. 2009. Exercise-training in young *Drosophila melanogaster* reduces age-related decline in mobility and cardiac performance. PLoS One 4:e5886. https://doi.org/10.1371/journal.pone.0005886.
78. Sgrò CM, Partridge L, Travis J. 2000. Evolutionary responses of the life history of wild-caught *Drosophila melanogaster* to two standard methods of laboratory culture. Am Nat 156:341–353. https://doi.org/10.1086/303394.
79. Miller PB, Obrik-Uloho OT, Phan MH, Medrano CL, Renier JS, Thayer JL, Wiessner G, Bloch Qazi MC. 2014. The song of the old mother: reproductive senescence in female *Drosophila*. Fly (Austin) 8:127–139. https://doi.org/10.4161/19336934.2014.969144.
80. Fleming JE, Reveillaud I, Niedzwiecki A. 1992. Role of oxidative stress in *Drosophila* aging. Mutat Res 275:267–279. https://doi.org/10.1016/0921-8734(92)90031-j.
81. Dushay MS, Eldon ED. 1998. Insights from model systems: *Drosophila* immune responses as models for human immunity. Am J Hum Genet 62:10–14. https://doi.org/10.1086/301694.

82. Smith WW, Thomas J, Liu J, Li T, Moran TH. 2014. From fat fruitfly to human obesity. Physiol Behav 136:15–21. https://doi.org/10.1016/j.physbeh.2014.01.017.
83. Corbin C, Jones JE, Chrostek E, Fenton A, Hurst GDD. 2021. Thermal sensitivity of the *Spiroplasma-Drosophila hydei* protective symbiosis: the best of climes, the worst of climes. Mol Ecol 30:1336–1344. https://doi.org/10.1111/mec.15799.
84. Ballinger MJ, Perlman SJ. 2017. Generality of toxins in defensive symbiosis: ribosome-inactivating proteins and defense against parasitic wasps in *Drosophila*. PLoS Pathog 13:e1006431. https://doi.org/10.1371/journal.ppat.1006431.
85. Yadav S, Frazer J, Banga A, Pruitt K, Harsh S, Jaenike J, Eleftherianos I. 2018. Endosymbiont-based immunity in *Drosophila melanogaster* against parasitic nematode infection. PLoS One 13:e0192183. https://doi.org/10.1371/journal.pone.0192183.
86. Xie JL, Vilchez I, Mateos M. 2010. *Spiroplasma* bacteria enhance survival of *Drosophila hydei* attacked by the parasitic wasp *Leptopilina heterotoma*. PLoS One 5:e12149. https://doi.org/10.1371/journal.pone.0012149.
87. Xie J, Butler S, Sanchez G, Mateos M. 2014. Male killing *Spiroplasma* protects *Drosophila melanogaster* against two parasitoid wasps. Heredity (Edinb) 112:399–408. https://doi.org/10.1038/hdy.2013.118.
88. Min KT, Benzer S. 1997. *Wolbachia*, normally a symbiont of *Drosophila*, can be virulent, causing degeneration and early death. Proc Natl Acad Sci U S A 94:10792–10796. https://doi.org/10.1073/pnas.94.20.10792.
89. Carrington LB, Leslie J, Weeks AR, Hoffman AA. 2009. The popcorn *Wolbachia* infection of *Drosophila melanogaster*: can selection alter *Wolbachia* longevity effects? Evolution 63:2648–2657. https://doi.org/10.1111/j.1558-5646.2009.00745.x.
90. Hague MTJ, Woods HA, Cooper BS. 2021. Pervasive effects of *Wolbachia* on host activity. Biol Lett 17:20210052. https://doi.org/10.1098/rsbl.2021.0052.
91. De Vries EJ, Jacobs G, Sabelis MW, Menken SBJ, Breeuwer JAJ. 2004. Diet-dependent effects of gut bacteria on their insect host: the symbiosis of *Erwinia* sp. and western flower thrips. Proc Biol Sci 271:2171–2178. https://doi.org/10.1098/rspb.2004.2817.
92. Cass BN, Himler AG, Bondy EC, Bergen JE, Fung SK, Kelly SE, Hunter MS. 2016. Conditional fitness benefits of the *Rickettsia* bacterial symbiont in an insect pest. Oecologia 180:169–179. https://doi.org/10.1007/s00442-015-3436-x.
93. Montenegro H, Souza WN, Leite DD, Klaczko LB. 2000. Male-killing selfish cytoplasmic element causes sex-ratio distortion in *Drosophila melanogaster*. Heredity 85:465–470. https://doi.org/10.1046/j.1365-2540.2000.00785.x.
94. Anbutsu H, Goto S, Fukatsu T. 2008. High and low temperatures differently affect infection density and vertical transmission of male-killing *Spiroplasma* symbionts in *Drosophila* hosts. Appl Environ Microbiol 74:6053–6059. https://doi.org/10.1128/AEM.01503-08.

95. Lighton JRB, Halsey LG. 2011. Flow-through respirometry applied to chamber systems: pros and cons, hints and tips. Comp Biochem Physiol Mol Integrative Physiol 158:265–275. https://doi.org/10.1016/j.cbpa.2010.11.026.
96. Luong LT, Horn CJ, Brophy T. 2017. Mitey costly: energetic costs of parasite avoidance and infection. Physiol Biochem Zool 90:471–477. https://doi.org/10.1086/691704.

Chapter 6. Synthesis and Future Directions

Summary

My thesis research investigated the NCEs of an ectoparasitic mite (*Macrocheles subbadius*) on host *Drosophila nigrospiracula*, including short-term trade-offs and fitness-level effects. My work builds upon previous observations that mites have strong consumptive effects on flies, and found that flies experience trade-offs between grooming and dispersal, as well as between mating and mite-resistance (2.1, 2.2). Flies varied in NCEs (physiological and behavioural) based on sex and mating status, at least in some short-term traits (3.1, 3.2). However, not all intraspecific host variation in NCEs was observed on lifetime scales, or with all behaviours (namely, low energy like mite-mediated phototaxis did not vary with mating status). Moreover, individual female flies had reduced fecundity and survival during chronic mite exposure (4.1); however these changes may not scale up to population level effects based on current simulations (4.2). We also tested how *Spiroplasma poulsonii* impacts the resistance of *D. melanogaster* against mites, and found endosymbiont infection impacted fly endurance, a proxy measure of mite resistance (5.1). Future research should study how individual hosts and host populations compensate for NCEs and how this varies among different host groups. Additional research on parasite NCEs across different scales and fly lifespan may show the lifetime impacts of NCEs are larger than suggested here (4.2).

Considering Integrated Defence Systems

Recent work has highlighted that an organism's response to threats such as predators, pathogens/parasites, and environmental stressors (e.g., toxins and starvation) share similar physiological and behavioural pathways [1; 2; 3]. For example, phenoloxidase pathways are involved in both wound healing from attempted predation and parasite encapsulation [4; 5]. Likewise, starvation and bacterial challenge can alter gene regulation in similar ways [1]. Shared stress-response pathways confound interpretation and prediction of trade-offs, as they create the possibility of synergistic responses but also for responses to compete for shared resources [6]. Based on the substantial overlap between toxin, infection, and predation stress response systems, a recent review argues they should be interpreted as a single irreducible "integrated defence system (IDS)" [7].

The IDS framework offers potential challenges and opportunities in research on the ecology of fear using *Drosophila-Macrocheles* associations. In our study of the fitness impacts of mite exposure on female flies' fitness (4.1), flies were separated from mites using mesh barriers held in place with super glue. Adhesives produce fumes that could be toxic to insects. Engagement of both toxin and parasite stress responses may result in different outcomes than parasite stress alone, and could impact different hosts unevenly (e.g. reproducing and non-reproducing females) [6]. Future studies could avoid this limitation by using barriers that do not require additional chemicals (e.g. glue-less mite traps described in [8]). Larger scale replications without confounding laboratory (e.g. toxins) may yield better estimates of the relative size of parasite NCEs on host fitness (sections 4.1, 4.2). Similarly, better methods to induce

grooming separate of mite exposure are needed; volcanic ash had moderate effects on fly respiration even when grooming was restricted, potentially over estimating the effect of grooming *per se*. Furthermore, the relative effects of parasites in the lab and in nature may be affected by different secondary stressors in those environments. These differences complicate applying simulations based on lab data to natural systems (4.2). Lab experiments that include more of the stressors found in natural environments (e.g. predation risk and/or temperature stress) may yield parameters that better represent natural populations.

Studies that consider integrated stress responses provide a potentially insightful avenue for future research in the ecology of fear. In addition to *Macrocheles* and *Drosophila* (4.1), many predators of flies, such as jumping spiders, are also tractable for lab culturing and co-habitation experiments [9]. Thus, the opportunity exists to test combined effects of predator and parasite exposure on hosts in the lab. The IDS model may make predictions about the magnitude of NCEs experienced by potential hosts/prey experiencing multiple threats. When there is high overlap in the responses to potential threats, the IDS suggests that the combined NCEs will be smaller than predicted by the sum of the individual NCEs. Contrarily, when the responses are dissimilar, the NCEs may be equal or larger than the sum of the individual NCEs.

Variation in the defence systems of hosts and NCEs

We observed differences in metabolic responses to mites between males and females, as well as mated and unmated females (3.1). However, we did not see a difference in mite-mediated phototaxis between mated and unmated female flies (3.2). Metabolic responses were likely driven by increased energetically demanding changes in activity (anti-mite grooming and escape behaviours; sections 2.1, 3.1). By contrast, short-term positive phototaxis may not be energetically costly, requiring only walking short distances in our assays (3.2). Future assays could also manipulate the food (cactus) and water (cactus exudate) available in light and dark conditions, as these likely covary with light. Based on these results, I hypothesise variation in responses to mite exposure based on mating status likely depends on the type of response being observed. In particular, the energetic costs of mating are more likely to also affect other energetically demanding activities. It may be fruitful for future research to test for trade-offs between mating and energetically demanding activities generally (such as dispersal, [10]). In the long-term mite exposure, we did not detect a survival difference between mated and unmated females during chronic mite exposure (4.1). Differences in survival between mated and unmated female *Drosophila* may be confounded by environment and lab adaptation [11]. Wild-type *D. melanogaster* females may benefit from mating, in the form of longer survival, unlike lab cultured lines [11]. If this difference is present in *D. nigropsiracula* is not currently known. Future research could manipulate environmental factors and fly strain to test if the mite-mediated reduction in survival is influenced by an environment-strain interaction.

Further research should also consider other host traits that could influence the NCEs they experience. One ecologically significant source of variation are the endosymbionts potential hosts harbour (5.1). In our study of *Drosophila melanogaster* infected with *Spiroplasma poulsonii*, flies harbouring the male-killing endosymbiont had poorer endurance (a proxy measure of mite resistance). Differing costs of resistance may influence investment in anti-mite behaviour via altered cost:benefit ratios; by extension the relative NCEs experienced by potential hosts may be influenced by their microbiome. I hypothesise that flies harbouring *S. poulsonii* would experience fewer NCEs from mite exposure than uninfected flies due to reduced investment in resistance. One method to test this hypothesis is considering the degree of mite avoidance between infected and uninfected flies using a behavioural tracking system (e.g. ethovision, [12]). Although mites are attracted to higher metabolic rate flies all else being equal, MSRO+ flies were repulsive to flies at 14-days old despite having higher metabolic rates (5.1). This suggests mites have hierarchical preferences for flies, and whether this manifests in hierarchical NCEs is a direction for future research. For example, since both fly size and mating status may affect the NCEs experienced by flies (3.1 & Appendix 2), do larger mated or smaller unmated flies have larger increases in MR during short-term mite exposure?

A need to study higher order interactions

In this thesis, I studied the effects of *M. subbadius* on potential *D. nigrospiracula* hosts outside of infection. We found evidence that the presence of parasites affects the behaviour, physiology, and fitness of potential host individuals. These changes were analogous to non-consumptive effects (NCEs) as defined by Peacor and Werner [13]: effects of predator presence on the plastic traits of potential prey. Trait-mediated indirect effects (TMIEs) are an example of the broader effects the presence of predators can have on their communities beyond consumption. TMIEs occur when trait changes in potential prey due to predator presence have impacts on a third species [13]. These are often contrasted with Density-Mediated Indirect Effects (DMIEs), which occur when changes in prey population size due to predation impact a third species [13]. Considering overlapping and competing responses by organisms (such as through an IDS) experiencing multiple threats may help elucidate when TMIEs occur and the direction of the effects [7].

I observed multiple behavioural responses in flies exposed to parasitic mites, including increased movement and grooming at the expense of time spent resting (1.1). Others have observed similar changes in fly behaviour during predator exposure based on the type of predator (e.g. ambush or active searcher) [14]. For example, *D. melanogaster* increased movement in the presence of active-hunting jumping spiders, but did not show significant changes in mobility during exposure to ambush hunters [14]. These changes may minimise predation risk by reducing the odds of encountering different styles of predator [14]. If the presence of parasites increases movement, this may synergize with evasion of some active hunting predators, but increase vulnerability to ambush hunters (1.1, 3.1, [14]). Thus, mites may exert TMIEs on fly predators via altered behaviour in flies. Although infection is known to influence

predation risk (both positively and negatively across systems), there is little evidence that the NCEs of parasites alone can influence predation risk [reviewed in 5]. Alternatively, altered behaviour in the presence of predators may increase susceptibility to mite infection [16]. Relative investment in anti-parasite and anti-predator behaviours is likely dependent on the rarity of the types of natural enemy [17]. Despite the lower costs of parasitism than predation, flies may invest more in anti-parasite behaviour than in behaviour to avoid rare predation events if parasites are common. Further research could co-expose flies to mites and predators to assess if flies have hierarchical responses to these natural enemies [18]. Future studies could test if fly predators are more successful at hunting when mites are also present and exerting NCEs on potential prey, and/or if mites are more successful at infecting hosts when predators are also present.

Mite presence also impacted fly physiology (elevated metabolic rates indicative of energy use), and induced-grooming lowered the future endurance of flies. These changes may reduce the energy hosts have available for anti-predator defences, and reduce the ability to sustain flight/bolting behaviours used against some predators [14; 19]. Chronic exposure to mites, *sans,* infection could increase host vulnerability to predation by exhausting resistance behaviours that share energy demands. In turn, parasites may exert a positive TMIE on predators by increasing the success of predator hunting efforts. Alternatively, some defensive mechanisms support both immune function (parasite resistance) and predator-survival (wound healing) such as the melanization response [20]. Exposure to cannibalistic conspecifics upregulates the phenoloxidase response in dragon fly larvae, and this has a knock-on effect of also improving encapsulation during immune challenge [20]. Thus predation could exert negative TMIEs on parasites when melanization is a component of the anti-parasite response. In predator-prey systems the TMIEs can be large relative to the DMIEs, and the potential for parasites to influence their communities via TMIEs could have wide-spread ecological implications [13]. Thus there is a need for studying potential TMIEs between parasites and predators

Lifetime and Intergenerational NCEs

My research only considered the NCEs of mites on adult *Drosophila nigrospiracula*. However, studies on predator-prey systems show larval flies can sense predators and other natural enemies, and show physiological and behavioural responses [21; 22]. These responses can also impact future adult body condition even after the predator is removed [22]. Fly larvae could benefit from sensing and responding to parasites both 1) directly by avoiding attack and 2) by pupating and emerging in less infectious environments. However, responding to the presence of mites may be costly for larvae. For example, larvae may have reduced feeding opportunities if they invest in avoiding parasites [23; 24].

Trade-offs between responses to natural enemies and larval feeding could impact juvenile survival (e.g. failing to successfully pupate) or future adult fitness (e.g. through reduced body size) [22; 25]. Exposure to predator cues as larvae reduced the pupation success rate of dragonfly larvae (i.e. increased mortality) [25]. *Drosophila melanogaster* exposed to predators or their cues as larvae had

reduced masses as adults [22]. Similar effects are observed in aquatic insect systems as well [26]. There is also growing evidence that insect eggs can take in chemical cues from the environment, and have physiological responses that affect development rate and post-hatch embryo phenotype [27; 28]. These changes can also come with the trade-off of decreased egg survival [29]. However, it is currently unknown if/how pre-adult *D. nigrospiracula* respond to *M. subbadius*. One experiment did not find a significant difference in egg hatch rates between dishes with mites and without mites, but there was limited sample size due to mould/desiccation ($N \leq 13$, [8]). However, the average hatch rates were 0.12 (mites) versus 0.18 (no mites), a 40% difference, and repeating this experiment with greater power could elucidate a NCE of mites on egg survival.

Drosophila larvae have visual, chemical, and mechanical sensory organs and exhibit plasticity in their behaviour in response to the environment [30; 31; 32]. Fly larvae show negative chemotaxis away from the cues associated with parasitoid wasps [33]. Analogous studies could test if flies show similar avoidance behaviours against mites or mite cues. The height and location of pupation can also be affected by factors in the larval environment including food quality, moisture, and light [34; 35; 36]. I predict that flies will pupate further from mites, and future experiments can test the pupation height of *Drosophila* in vials with and without mite presence. These responses may require developmental and nutritional trade-offs, and I also hypothesise that flies exposed to mites as larvae will have poorer body condition as adults (measured in body size) and lower fitness (lifetime fecundity). NCEs on larvae could have ecologically relevant impacts on hosts by reducing survival and reducing future adult body size.

The NCEs of natural enemies may occur across generations. In a hare system maternal exposure to sham predators can reduce the survival of offspring [37]. Predator-exposure induced changes can persist two generations post exposure in *Daphnia* [38]. Among mammals, increases in stress hormones (e.g. cortisol) may impact maternal and subsequently juvenile health [37]. Analogous stress hormones are upregulated in insects exposed to predators [39]. Furthermore, previous injection with octopamine increased cricket survival during exposure to a bearded dragon [40]. The octopamine system also helps regulate egg development in insects [41; 42]. Octopamine added as a dietary supplement reduced the rate of egg laying and oocytes per ovary in *Drosophila* [41]. If NCEs due to predators and parasitism share similar stress responses, chronic exposure to mites may also affect the stress hormones of flies [7]. Given potential connections between reproduction and octopamine in *Drosophila*, future research could test if chronic mite exposure upregulates octopamine and may drive reductions in fecundity among mite-exposed female flies (4.1). Flies with upregulated octopamine also have higher metabolic rates in respirometry studies [43], suggesting similar physiological mechanisms may drive both altered reproduction (4.1) and elevated metabolic rates (3.1) among flies exposed to mites. Currently, if fewer eggs from parasite or predator exposed female flies survive, analogous to hare-predator interactions [37], is unknown. Testing the hatch rate, larval survival, and pupation success of the offspring of mite-exposed female flies may help elucidate the role of stress hormones in the NCEs of parasites.

Evolution of fly responses and NCEs

The NCEs of predators can vary over multiple generations [37; 38; 44]. Maternal effects may drive some of these inter-generational effects; however, across multiple generations prey/hosts may adapt to NCEs [37]. Traditionally, host populations are expected to increase investment in resistance in environments with parasites due to the selective pressure of infection [45; 46]. However, NCEs may exert a selective pressure even when infection does not occur (4.1). A potential avenue for investigation is culturing flies for several generations with parasites present but unable to infect. A recent study on *Daphnia* found that predator NCEs can lead to selection for reduced body size, faster maturation, and altered fecundity [47]. NCEs could be having evolutionary consequences for potential prey and hosts that are only beginning to be understood.

Previous studies have found predators and parasitoids can suppress the populations of herbivores (Fill et al. 2012; Dewitt et

Conclusion

Parasite ecology has increasingly emphasised the roles of parasites outside of infection. Recent work by others and my research suggests parasites' NCEs could influence their communities outside of infection. Parasites are present in nearly all environments, and understanding their NCEs may be necessary to fully understand their total impact. Organisms experience variable and overlapping threats in ever changing environments, and this thesis fits into our growing understanding of how organisms manage these challenges.

6 References

1. Stucki, D., Freitak, D., Bos, N., Sundstrom, L., 2019. Stress responses upon starvation and exposure to bacteria in the ant *Formica exsecta*. Peerj 7. doi: 10.7717/peerj.6428
2. Adamo, S.A., 2020. Animals have a Plan B: how insects deal with the dual challenge of predators and pathogens. Journal of Comparative Physiology B-Biochemical Systems and Environmental Physiology 190, 381-390. doi: 10.1007/s00360-020-01282-5
3. Cinel, S.D., Hahn, D.A., Kawahara, A.Y., 2020. Predator-induced stress responses in insects: A review. Journal of Insect Physiology 122. Doi: 10.1016/j.jinsphys.2020.104039
4. Srygley, R.B., 2012. Age- and Density-Dependent Prophylaxis in the Migratory, Cannibalistic Mormon Cricket *Anabrus simplex* (Orthoptera: Tettigoniidae). Environmental Entomology 41, 166-171. doi: 10.1603/en11020
5. Murray, R.L., Tah, S., Koprivnikar, J., Rowe, L., McCauley, S.J., 2020. Exposure to potentially cannibalistic conspecifics induces an increased immune response. Ecological Entomology 45, 355-363. doi: 10.1111/een.12806
6. Adamo, S.A., 2021. How insects protect themselves against combined starvation and pathogen challenges, and the implications for reductionism. Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology 255. doi: 10.1016/j.cbpb.2021.110564
7. Adamo, S.A., 2022. The Integrated Defense System: Optimizing Defense against Predators, Pathogens, and Poisons. I*ntegrative and Comparative Biology*, icac024 (Early access).
8. Mierzejewski, M.K., Horn, C.J., Luong, L.L. 2019. Ecology of fear: environment-dependent parasite avoidance among ovipositing *Drosophila*. Parasitology 146: 1564-1570.
9. Jackson, R.R., Cross, F.R., 2011. Olfaction-based mate-odor identification by jumping spiders from the genus *Portia*. Journal of Arachnology 39, 439-443. doi: 10.1636/Ha11-32.1
10. Luong, L.T., Penoni, L.R., Horn, C.J., Polak, M., 2015. Physical and physiological costs of ectoparasitic mites on host flight endurance. Ecological Entomology 40, 518-524. doi: 10.1111/een.12218
11. Markow, T.A., 2011. "Cost" of virginity in wild *Drosophila melanogaster* females. Ecology and Evolution 1. doi: 10.1002/ece3.54
12. Noldus, L., Spink, A.J., Tegelenbosch, R.A.J., 2002. Computerised video tracking, movement analysis and behaviour recognition in insects. Computers and Electronics in Agriculture 35, 201-227. doi: 10.1016/s0168-1699(02)00019-4
13. Peacor, S.D., Werner, E.E., 2008. The contribution of trait-mediated indirect effects to the net effects of a predator. Proceedings of the National Academy of Sciences of the United States of America 98, 3904-3908. doi: 10.1073/pnas.071061998
14. Parigi, A., Porter, C., Cermak, M., Pitchers, W.R., Dworkin, I., 2019. The behavioral repertoire of *Drosophila melanogaster* in the presence of two predator species that differ in hunting mode. Plos One 14. doi: 10.1371/journal.pone.0216860

15. Gutierrez, S.O., Minchella, D.J., Bernal, X.E., Survival of the sickest: selective predation differentially modulates ecological and evolutionary disease dynamics. Oikos. doi: 10.1111/oik.09126
16. Zukowski, N., Kirk, D., Wadhawan, K., Shea, D., Start, D., Krkosek, M., 2020. Predators can influence the host-parasite dynamics of their prey via nonconsumptive effects. Ecology and Evolution 10, 6714-6722. doi: 10.1002/ece3.6401
17. Broom, M., Higginson, A.D., Ruxton, G.D., 2010. Optimal investment across different aspects of anti-predator defences. Journal of Theoretical Biology 263, 579-586. doi: 10.1016/j.jtbi.2010.01.002
18. Koprivnikar, J., Penalva, L., 2015. Lesser of Two Evils? Foraging Choices in Response to Threats of Predation and Parasitism. Plos One 10. doi: 10.1371/journal.pone.0116569
19. Cinel, S.D., Hahn, D.A., Kawahara, A.Y., 2020. Predator-induced stress responses in insects: A review. Journal of Insect Physiology 122. doi: 10.1016/j.jinsphys.2020.104039
20. Murray, R.L., Tah, S., Koprivnikar, J., Rowe, L., McCauley, S.J., 2020. Exposure to potentially cannibalistic conspecifics induces an increased immune response. Ecological Entomology 45, 355-363. doi: 10.1111/een.12806
21. Robertson, J.L., Tsubouchi, A., Tracey, W.D., 2013. Larval Defense against Attack from Parasitoid Wasps Requires Nociceptive Neurons. Plos One 8. doi: 10.1371/journal.pone.0078704
22. Krams, R., Krama, T., Munkevics, M., Eichler, S., Butler, D.M., Dobkevica, L., Joers, P., Contreras-Garduno, J., Daukste, J., Krams, I.A., 2021. Spider odors induce stoichiometric changes in fruit fly *Drosophila melanogaster*. Current Zoology 67, 127-129. doi: 10.1093/cz/zoaa070
23. Heads, P.A., 1986. The costs of reduced feeding due to predator avoidance - potential effects on growth and fitness in *Ischnura elegans* larvae (odonata, zygoptera). Ecological Entomology 11, 369-377. doi: 10.1111/j.1365-2311.1986.tb00315.x
24. Roberts, D., 2014. Mosquito Larvae Change Their Feeding Behavior in Response to Kairomones From Some Predators. Journal of Medical Entomology 51, 368-374. doi: 10.1603/me13129
25. McCauley, S.J., Rowe, L., Fortin, M.J., 2011. The deadly effects of "nonlethal" predators. Ecology 92, 2043-2048. doi: 10.1890/11-0455.1
26. Fontana-Bria, L., Selfa, J., Tur, C., Frago, E., 2017. Early exposure to predation risk carries over metamorphosis in two distantly related freshwater insects. Ecological Entomology 42, 255-262. doi: 10.1111/een.12382
27. Sniegula, S., Raczynski, M., Golab, M.J., Johansson, F., 2020. Effects of predator cues carry over from egg and larval stage to adult life-history traits in a damselfly. Freshwater Science 39, 804-811. doi: 10.1086/711374

28. Antol, A., Sniegula, S., 2021. Damselfly eggs alter their development rate in the presence of an invasive alien cue but not a native predator cue. Ecology and Evolution 11, 9361-9369. doi: 10.1002/ece3.7729
29. Sniegula, S., Nsanzimana, J.D., Johansson, F., 2019. Predation risk affects egg mortality and carry over effects in the larval stages in damselflies. Freshwater Biology 64, 778-786. doi: 10.1111/fwb.13261
30. Gomez-Marin, A., Louis, M., 2014. Multilevel control of run orientation in *Drosophila* larval chemotaxis. Frontiers in Behavioral Neuroscience 8. doi: 10.3389/fnbeh.2014.00038
31. Dombrovski, M., Kim, A., Poussard, L., Vaccari, A., Acton, S., Spillman, E., Condron, B., Yuan, Q., 2019. A Plastic Visual Pathway Regulates Cooperative Behavior in *Drosophila* Larvae. Current Biology 29, 1866-+. doi: 10.1016/j.cub.2019.04.060
32. Chen, M., Sokolowski, M.B., 2022. How Social Experience and Environment Impacts Behavioural Plasticity in *Drosophila*. Fly 16, 68-84. doi: 10.1080/19336934.2021.1989248
33. Ebrahim, S.A.M., Dweck, H.K.M., Stoekl, J., Hofferberth, J.E., Trona, F., Weniger, K., Rybak, J., Seki, Y., Stensmyr, M.C., Sachse, S., Hansson, B.S., Knaden, M., 2015. Drosophila Avoids Parasitoids by Sensing Their Semiochemicals via a Dedicated Olfactory Circuit. Plos Biology 13. doi: 10.1371/journal.pbio.1002318
34. Markow, T.A., 1979. Survey of intraspecific and interspecific variation for pupation height in *Drosophila*. Behavior Genetics 9, 209-217. doi: 10.1007/bf01071301
35. Casares, P., Carracedo, M.C., Garcia-Florez, L., 1997. Analysis of larval behaviours underlying the pupation height phenotype in *Drosophila simulans* and *D. melanogaster*. Genetics Selection Evolution 29, 589-600. doi: 10.1051/gse:19970504
36. Krittika, S., Lenka, A., Yadav, P., 2019. Evidence of dietary protein restriction regulating pupation height, development time and lifespan in *Drosophila melanogaster*. Biology Open 8. doi: 10.1242/bio.042952
37. MacLeod, K.J., Krebs, C.J., Boonstra, R., Sheriff, M.J., 2017. Fear and lethality in snowshoe hares: the deadly effects of non-consumptive predation risk. Oikos 127, 375-380. doi: 10.1111/oik.04890
38. Walsh, M.R., Cooley, F., Biles, K., Munch, S.B., 2015. Predator-induced phenotypic plasticity within- and across-generations: a challenge for theory? Proceedings of the Royal Society B-Biological Sciences 282. doi: 10.1098/rspb.2014.2205
39. Adamo, S.A., Baker, J.L., 2011. Conserved features of chronic stress across phyla: The effects of long-term stress on behavior and the concentration of the neurohormone octopamine in the cricket, *Gryllus texensis*. Hormones and Behavior 60, 478-483. doi: 10.1016/j.yhbeh.2011.07.015
40. Adamo, S.A., Kovalko, I., Mosher, B., 2013. The behavioural effects of predator-induced stress responses in the cricket (*Gryllus texensis*): the upside of the stress response. Journal of Experimental Biology 216, 4608-4614. doi: 10.1242/jeb.094482

41. Gruntenko, N.E., Karpova, E.K., Alekseev, A.A., Chentsova, N.A., Bogomolova, E.V., Bownes, M., Rauschenbach, I.Y., 2007. Effects of octopamine on reproduction, juvenile hormone metabolism, dopamine, and 20-hydroxyecdysone contents in *Drosophila*. Archives of Insect Biochemistry and Physiology 65, 85-94. doi: 10.1002/arch.20187
42. Lim, J., Sabandal, P.R., Fernandez, A., Sabandal, J.M., Lee, H.-G., Evans, P., Han, K.-A., 2014. The Octopamine Receptor Oct beta 2R Regulates Ovulation in *Drosophila melanogaster*. Plos One 9. doi: 10.1371/journal.pone.0104441
43. Li, Y., Hoffmann, J., Stephano, F., Bruchhaus, I., Fink, C., Roeder, T., 2016. Octopamine controls starvation resistance, life span and metabolic traits in *Drosophila*. Scientific Reports 6. doi: 10.1038/srep35359
44. Ingerslew, K.S., Finke, D.L., 2020. Non-consumptive effects stabilize herbivore control over multiple generations. Plos One 15. doi: 10.1371/journal.pone.0241870
45. Wielgoss, S., Bergmiller, T., Bischofberger, A.M., Hall, A.R., 2016. Adaptation to Parasites and Costs of Parasite Resistance in Mutator and Nonmutator Bacteria. Molecular Biology and Evolution 33, 770-782. doi: 10.1093/molbev/msv270
46. Koskella, B., 2018. Resistance gained, resistance lost: An explanation for host-parasite coexistence. PLoS biology 16, e3000013-e3000013. doi: 10.1371/journal.pbio.3000013
47. Zhang, C., Goitom, E., Brans, K., De Meester, L., Stoks, R., 2022. Scared to evolve? Non-consumptive effects drive rapid adaptive evolution in a natural prey population. Proceedings. Biological sciences 289, 20220188-20220188. doi: 10.1098/rspb.2022.0188

Bibliography

Abram, P.K., Brodeur, J., Urbaneja, A., Tena, A., 2019. Nonreproductive Effects of Insect Parasitoids on Their Hosts. Annual Review of Entomology, Vol 64 64, 259-+. doi: 10.1146/annurev-ento-011118-111753

Acharya, L., 1995. Sex-biased predation on moths by insectivorous bats. Animal Behaviour 49, 1461-1468. doi: 10.1016/0003-3472(95)90067-5

Adamo, S.A., 1999. Evidence for adaptive changes in egg laying in crickets exposed to bacteria and parasites. Animal Behaviour 57:117-124. doi: 10.1006/anbe.1998.0999.

Adamo, S.A., 2020. Animals have a Plan B: how insects deal with the dual challenge of predators and pathogens. Journal of Comparative Physiology B-Biochemical Systems and Environmental Physiology 190, 381-390. doi: 10.1007/s00360-020-01282-5

Adamo, S.A., 2021. How insects protect themselves against combined starvation and pathogen challenges, and the implications for reductionism. Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology 255. doi: 10.1016/j.cbpb.2021.110564

Adamo, S.A., 2022. The Integrated Defense System: Optimizing Defense against Predators, Pathogens, and Poisons. Integrative and Comparative Biology. doi: 10.1093/icb/icac024

Adamo, S.A., Baker, J.L., 2011. Conserved features of chronic stress across phyla: The effects of long-term stress on behavior and the concentration of the neurohormone octopamine in the cricket, Gryllus texensis. Hormones and Behavior 60, 478-483. doi: 10.1016/j.yhbeh.2011.07.015

Adamo, S.A., Easy, R.H., Kovalko, I., MacDonald, J., McKeen, A., Swanburg, T., Turnbull, K.F., Reeve, C., 2017. Predator exposure-induced immunosuppression: trade-off, immune redistribution or immune reconfiguration? Journal of Experimental Biology 220, 868-875. doi: 10.1242/jeb.153320

Adamo, S.A., Kovalko, I., Mosher, B., 2013b. The behavioural effects of predator-induced stress responses in the cricket (*Gryllus texensis*): the upside of the stress response. Journal of Experimental Biology 216, 4608-4614. doi: 10.1242/jeb.094482

Adelman, J.S., Hawley D.M., 2017. Tolerance of infection: a role for animal behavior, potential immune mechanisms, and consequences for parasite transmission. Hormones and Behavior 88, 79–86.

Allan, B.F., Varns, T.S., Chase, J.M., 2010. Fear of parasites: lone star ticks increase giving-up densities in white-tailed deer. Israel Journal of Ecology & Evolution 56, 313-324. doi: 10.1560/ijee.56.3-4.313

Alsaiyah, M.A.M., Ebssa, L., Zenner, A., O'Callaghan, K.M., Griffin, C.T., 2009. Sex ratios and sex-biased infection behaviour in the entomopathogenic nematode genus *Steinernema*. International Journal for Parasitology 39, 725-734. doi: 10.1016/j.ijpara.2008.11.003

Anbutsu, H., Fukatsu, T., 2003. Population dynamics of male-killing and non-male-killing Spiroplasmas in *Drosophila melanogaster*. Applied and Environmental Microbiology 69, 1428-1434. doi: 10.1128/aem.69.3.1428-1434.2003

Anbutsu, H., Goto, S., Fukatsu, T., 2008. High and low temperatures differently affect infection density and vertical transmission of male-killing *Spiroplasma* symbionts in *Drosophila* hosts. Applied and Environmental Microbiology 74, 6053-6059. doi: 10.1128/aem.01503-08

Antol, A., Sniegula, S., 2021. Damselfly eggs alter their development rate in the presence of an invasive alien cue but not a native predator cue. Ecology and Evolution 11, 9361-9369. doi: 10.1002/ece3.7729

Ardia, D.R., Schat, K.A., Winkler, D.W., 2003. Reproductive effort reduces long-term immune function in breeding tree swallows (*Tachycineta bicolor*). Proceedings of the Royal Society B-Biological Sciences 270, 1679-1683. doi: 10.1098/rspb.2003.2424

Arnold, P.A., White, C.R., Johnson, K.N., 2015. *Drosophila melanogaster* does not exhibit a behavioural fever response when infected with Drosophila C virus. Journal of General Virology 96, 3667-3671. doi: 10.1099/jgv.0.000296

Auld, S.K.J.R., Penczykowski, R.M., Ochs, J.H., Grippi, D.C., Hall, S.R., Duffy, M.A., 2013. Variation in costs of parasite resistance among natural host populations. Journal of Evolutionary Biology 26, 2479-2486. doi: 10.1111/jeb.12243

Baker, R.L., Smith, B.P., 1997. Conflict between antipredator and antiparasite behaviour in larval damselflies. Oecologia 109, 622-628. doi: 10.1007/s004420050125

Baleba, S.B.S., Torto, B., Masiga, D., Getahun, M.N., Weldon, C.W., 2020. Stable Flies, *Stomoxys calcitrans* L. (Diptera: muscidae), improve offspring fitness by avoiding oviposition substrates with competitors or parasites. Frontiers in Ecology and Evolution 28, 1-12. doi:

Ball, S.L., Baker, R.L., 1996., Predator-induced life history changes: antipredator behavior costs or facultative life history shifts? Ecology 77, 1116–1124, https://doi. org/10.2307/2265580.

Ballinger, M.J., Perlman, S.J., 2017. Generality of toxins in defensive symbiosis: ribosome-inactivating proteins and defense against parasitic wasps in *Drosophila*. Plos Pathogens 13. doi: 10.1371/journal.ppat.1006431

Barber, I., Dingemanse, N.J., 2010. Parasitism and the evolutionary ecology of animal personality. Philosophical Transactions of the Royal Society B-Biological Sciences 365, 4077-4088. doi: 10.1098/rstb.2010.0182

Barone, M.C., Bohmann, D., 2013. Assessing neurodegenerative phenotypes in *Drosophila* dopaminergic neurons by climbing assays and whole brain immunostaining. Jove-Journal of Visualized Experiments. doi: 10.3791/50339

Barradale, F., Sinha, K., Lebestky, T., 2017. Quantification of *Drosophila* grooming behavior. Jove-Journal of Visualized Experiments. doi: 10.3791/55231

Bates, D., Maechler, M., Bolker, B.M., Walker, S.C., 2015. Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software 67, 1-48. doi: 10.18637/jss.v067.i01

Beckerman, A.P., de Roij, J., Dennis, S.R., Little, T.J., 2013. A shared mechanism of defense against predators and parasites: chitin regulation and its implications for life-history theory. Ecology and Evolution 3, 5119-5126. doi: 10.1002/ece3.766

Beenakke, B.M.T, 1969. Carbohydrate and fat as a fuel for insect flight. A comparative study. Journal of Insect Physiology 15, 353-&. doi: 10.1016/0022-1910(69)90281-9

Belgrad, B.A., Griffen, B.D., 2016. Predator - prey interactions mediated by prey personality and predator hunting mode. Proceedings of the Royal Society B-Biological Sciences 283. doi: 10.1098/rspb.2016.0408

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, 1196-1205. doi: 10.1017/s0031182020000918

Beresford, D.V., Sutcliffe, J.F., 2009. The effect of *Macrocheles muscaedomesticae* and *M. subbadius* (Acarina: Macrochelidae) phoresy on the dispersal of *Stomoxys calcitrans* (Diptera: Muscidae). Systematic and Applied Acarology Special Publications, 1-30.

Berger, D., Olofsson, M., Friberg, M., Karlsson, B., Wiklund, C., Gotthard, K., 2012. Intraspecific variation in body size and the rate of reproduction in female insects - adaptive allometry or biophysical constraint? Journal of Animal Ecology 81, 1244-1258. doi: 10.1111/j.1365-2656.2012.02010.x

Bergeron, P., Careau, V., Humphries, M.M., Reale, D., Speakman, J.R., Garant, D., 2011. The energetic and oxidative costs of reproduction in a free-ranging rodent. Functional Ecology 25, 1063-1071. doi: 10.1111/j.1365-2435.2011.01868.x

Bergland, A.O., Genissel, A., Nuzhdin, S.V., Tatar, M., 2008. Quantitative trait loci affecting phenotypic plasticity and the allometric relationship of ovariole number and thorax length in *Drosophila melanogaster*. Genetics 180, 567-582. doi: 10.1534/genetics.108.088906

Betini, G.S., McAdam, A.G., Griswold, C.K., Norris, D.R., 2017. A fitness trade-off between seasons causes multigenerational cycles in phenotype and population size. Elife 6. doi: 10.7554/eLife.18770

Boltana, S., Rey, S., Roher, N., Vargas, R., Huerta, M., Huntingford, F.A., Goetz, F.W., Moore, J., Garcia-Valtanen, P., Estepa, A., MacKenzie, S., 2013. Behavioural fever is a synergic signal amplifying the innate immune response. Proceedings of the Royal Society B-Biological Sciences 280. doi: 10.1098/rspb.2013.1381

Boots, M., Haraguchi, Y., 1999. The evolution of costly resistance in host-parasite systems. American Naturalist 153, 359-370. doi: 10.1086/303181

Boroczky, K., Wada-Katsumataa, A., Batchelor, D., Zhukovskaya, M., Schal, C., 2013. Insects groom their antennae to enhance olfactory acuity. Proceedings of the National Academy of Sciences of the United States of America 110, 3615-3620. doi: 10.1073/pnas.1212466110

Bradley, C.A., Altizer, S., 2005. Parasites hinder monarch butterfly flight: implications for disease spread in migratory hosts. Ecology Letters 8, 290-300. doi: 10.1111/j.1461-0248.2005.00722.x

Breitmeyer, C.M., Markow, T.A., 1998. Resource availability and population size in cactophilic *Drosophila*. Functional Ecology 12, 14-21. doi: 10.1046/j.1365-2435.1998.00152.x

Broom, M., Higginson, A.D., Ruxton, G.D., 2010. Optimal investment across different aspects of anti-predator defences. Journal of Theoretical Biology 263, 579-586. doi: 10.1016/j.jtbi.2010.01.002

Brophy, T., Luong, L.T., 2021. Ectoparasite-induced increase in *Drosophila* host metabolic rate. Physiological Entomology 46, 1-7. doi: 10.1111/phen.12334

Brown, J.H., Gillooly, J.F., Allen, A.P., Savage, V.M., West, G.B., 2004. Toward a metabolic theory of ecology. Ecology 85, 1771-1789. doi: 10.1890/03-9000

Brown, J.M., Wilson, D.S., 1992. Local specialization of phoretic mites on sympatric carrion beetle hosts. Ecology 73, 463-478. doi: 10.2307/1940753

Buchanan, A.L., Hermann, S.L., Lund, M., Szendrei, Z., 2017. A meta-analysis of non-consumptive predator effects in arthropods: the influence of organismal and environmental characteristics. Oikos 126, 1233-1240. doi: 10.1111/oik.04384

Buck, J.C., 2019. Indirect Effects Explain the Role of Parasites in Ecosystems. Trends in Parasitology 35, 835-847. doi: 10.1016/j.pt.2019.07.007

Buck, J.C., Weinstein, S.B., Young, H.S., 2018. Ecological and evolutionary consequences of parasite avoidance. Trends in Ecology & Evolution 33, 619-632. doi: 10.1016/j.tree.2018.05.001

Bushey, D., Hughes, K.A., Tononi, G., Cirelli, C., 2010. Sleep, aging, and lifespan in *Drosophila*. BMC Neuroscience 11. doi: 10.1186/1471-2202-11-56

Caballero, I.C., Sakla, A.J., Detwiler, J.T., Le Gall, M., Behmer, S.T., Criscione, C.D., 2015. Physiological status drives metabolic rate in mediterranean geckos infected with pentastomes. Plos One 10. doi: 10.1371/journal.pone.0144477

Campbell, E.O., Luong, L.T., 2016. Mite choice generates sex- and size-biased infection in *Drosophila hydei*. Parasitology 143, 787-793. doi: 10.1017/s0031182016000305

Careau, V., Thomas, D.W., Humphries, M.M., 2010. Energetic cost of bot fly parasitism in free-ranging eastern chipmunks. Oecologia 162, 303-312. doi: 10.1007/s00442-009-1466-y

Carr, A.L., Roe, M., 2016. Acarine attractants: Chemoreception, bioassay, chemistry and control. Pesticide Biochemistry and Physiology 131, 60-79. doi: 10.1016/j.pestbp.2015.12.009

Carrington, L.B., Leslie, J., Weeks, A.R., Hoffmann, A.A., 2009. The popcorn *Wolbachia* infection of *Drosophila melanogaster*: can selection alter *Wolbachia* longevity effects? Evolution 63, 2648-2657. doi: 10.1111/j.1558-5646.2009.00745.x

Casares, P., Carracedo, M.C., Garcia-Florez, L., 1997. Analysis of larval behaviours underlying the pupation height phenotype in *Drosophila simulans* and *D. melanogaster*. Genetics Selection Evolution 29, 589-600. doi: 10.1051/gse:19970504

Cass, B.N., Himler, A.G., Bondy, E.C., Bergen, J.E., Fung, S.K., Kelly, S.E., Hunter, M.S., 2016. Conditional fitness benefits of the *Rickettsia* bacterial symbiont in an insect pest. Oecologia 180, 169-179. doi: 10.1007/s00442-015-3436-x

Cervo, R., Bruschini, C., Cappa, F., Meconcelli, S., Pieraccini, G., Pradella, D., Turillazzi, S., 2014. High *Varroa* mite abundance influences chemical profiles of worker bees and mite-host preferences. Journal of Experimental Biology 217, 2998-3001. doi: 10.1242/jeb.099978

Chapman, T., Bangham, J., Vinti, G., Seifried, B., Lung, O., Wolfner, M.F., Smith, H.K., Partridge, L., 2003. The sex peptide of *Drosophila melanogaster*: Female post-mating responses analyzed by using RNA interference. Proceedings of the National Academy of Sciences of the United States of America 100, 9923-9928. doi: 10.1073/pnas.1631635100

Chapman, T., Hutchings, J., Partridge, L., 1993. No reduction in the cost of mating for *Drosophila melanogaster* females mating with spermless males. Proceedings of the Royal Society B-Biological Sciences 253, 211-217. doi: 10.1098/rspb.1993.0105

Chen, M., Sokolowski, M.B., 2022. How social experience and environment impacts behavioural plasticity in *Drosophila*. Fly 16, 68-84. doi: 10.1080/19336934.2021.1989248

Chen, P.S., Stummzollinger, E., Aigaki, T., Balmer, J., Bienz, M., Bohlen, P., 1988. A male accessory-gland peptide that regulates reproductive-behavior of female *Drosophila melanogaster*. Cell 54, 291-298. doi: 10.1016/0092-8674(88)90192-4

Christe, P., Glaizot, O., Evanno, G., Bruyndonckx, N., Devevey, G., Yannic, G., Patthey, P., Maeder, A., Vogel, P., Arlettaz, R., 2007. Host sex and ectoparasites choice: preference for, and higher survival on female hosts. Journal of Animal Ecology 76, 703-710. doi: 10.1111/j.1365-2656.2007.01255.x

Cinel, S.D., Hahn, D.A., Kawahara, A.Y., 2020. Predator-induced stress responses in insects: A review. Journal of Insect Physiology 122. doi: 10.1016/j.jinsphys.2020.104039

Clinchy, M., Schulkin, J., Zanette, L.Y., Sheriff, M.J., McGowan, P.O., Boonstra, R., 2011. The neurological ecology of fear: insights neuroscientists and ecologists have to offer one another. Frontiers in Behavioral Neuroscience 5, 1-6. doi: 10.3389/fnbeh.2011.00021

Clinchy, M., Sheriff, M.J., Zanette, L.Y., 2013. Predator-induced stress and the ecology of fear. Functional Ecology 27, 56-65. doi: 10.1111/1365-2435.12007

Cloutier, C.J., Kavaliers, M., Ossenkopp, K.P., 2017. Rodent sex differences in disgust behaviors (anticipatory nausea) conditioned to a context associated with the effects of the toxin LiCl: inhibition of conditioning following immune stimulation with lipo-polysaccharide. Pharmacol. Biochem. Behav. 152, 4-12. doi: 10.1016/j.bb.2016.08.006

Corbin, C., Jones, J.E., Chrostek, E., Fenton, A., Hurst, G.D.D., 2021. Thermal sensitivity of the *Spiroplasma-Drosophila hydei* protective symbiosis: The best of climes, the worst of climes. Molecular Ecology 30, 1336-1344. doi: 10.1111/mec.15799

Cox, R.M., Parker, E.U., Cheney, D.M., Liebl, A.L., Martin, L.B., Calsbeek, R., 2010. Experimental evidence for physiological costs underlying the trade-off between reproduction and survival. Functional Ecology 24, 1262-1269. doi: 10.1111/j.1365-2435.2010.01756.x

Dadda, M., 2015. Female social response to male sexual harassment in Poeciliid fish: a comparison of six species. Frontiers in Psychology 6. doi: 10.3389/fpsyg.2015.01453

Daly, E.W., Johnson, P.T.J., 2011. Beyond immunity: quantifying the effects of host anti-parasite behavior on parasite transmission. Oecologia 165, 1043-1050. doi: 10.1007/s00442-010-1778-y

Davenport, J.M., Hossack, B.R., Lowe, W.H., 2014. Partitioning the non-consumptive effects of predators on

prey with complex life histories. Oecologia 176, 149-155.

Daversa, D.R., Hechinger, R.F., Madin, E., Fenton, A., Dell, A.I., Ritchie, E.G., Rohr, J., Rudolf, V.H.W., Lafferty, K.D., 2021. Broadening the ecology of fear: non-lethal effects arise from diverse responses to predation and parasitism. Proceedings of the Royal Society B-Biological Sciences 288. doi: 10.1098/rspb.2020.2966

Daversa, D.R., Manica, A., Cenis, H.B., Lopez, P., Garner, T.W.J., Bosch, J., 2021. Alpine newts (*Ichthyosaura alpestris*) avoid habitats previously used by parasite-exposed conspecifics. Frontiers in Ecology and Evolution 9. doi: 10.3389/fevo.2021.636099

de la Flor, M., Chen, L.J., Manson-Bishop, C., Chu, T.C., Zamora, K., Robbins, D., Gunaratne, G., Roman, G., 2017. *Drosophila* increase exploration after visually detecting predators. Plos One 12. doi: 10.1371/journal.pone.0180749

de Roode, J.C., Lefevre, T., 2012. Behavioral immunity in insects. Insects 3, 789-820. doi:

de Vries, E.J., Jacobs, G., Sabelis, M.W., Menken, S.B.J., Breeuwer, J.A.J., 2004. Diet-dependent effects of gut bacteria on their insect host: the symbiosis of *Erwinia* sp and western flower thrips. Proceedings of the Royal Society B-Biological Sciences 271, 2171-2178. doi: 10.1098/rspb.2004.2817

DeWitt, P.D., Visscher, D.R., Schuler, M.S., Thiel, R.P., 2019. Predation risks suppress lifetime fitness in a wild mammal. Oikos 128, 790-797. doi: 10.1111/oik.05935

DiBlasi, E., Morse, S., Mayberry, J.R., Avila, L.J., Morando, M., Dittmar, K., 2011. New *Spiroplasma* in parasitic *Leptus* mites and their *Agathemera* walking stick hosts from Argentina. Journal of Invertebrate Pathology 107, 225-228. doi: 10.1016/j.jip.2011.05.013

Dimopoulos, G., 2003. Insect immunity and its implication in mosquito-malaria interactions. Cellular Microbiology 5, 3-14. doi: 10.1046/j.1462-5822.2003.00252.x

Dobson, A., Lafferty, K.D., Kuris, A.M., Hechinger, R.F., Jetz, W., 2008. Homage to Linnaeus: How many parasites? How many hosts? Proceedings of the National Academy of Sciences of the United States of America 105, 11482-11489. doi: 10.1073/pnas.0803232105

Dobzhansky, T., Spassky, B., 1969. Artificial and natural selection for 2 behavioral traits in *Drosophila pseudoobscura*. Proceedings of the National Academy of Sciences of the United States of America 62, 75-80. doi: 10.1073/pnas.62.1.75

Doherty, J.F., Ruehle, B., 2020. An integrated landscape of fear and disgust: the evolution of avoidance behaviors amidst a myriad of natural enemies. Frontiers in Ecology and Evolution 8. doi: 10.3389/fevo.2020.564343

Dombrovski, M., Kim, A., Poussard, L., Vaccari, A., Acton, S., Spillman, E., Condron, B., Yuan, Q., 2019. a plastic visual pathway regulates cooperative behavior in *Drosophila* larvae. Current Biology 29, 1866-1876. doi: 10.1016/j.cub.2019.04.060

Donelan, S.C., Grabowski, J.H., Trussell, G.C., 2017. Refuge quality impacts the strength of nonconsumptive effects on prey. Ecology 98, 403-411. doi: 10.1002/ecy.1647

Donelan, S.C., Trussell, G.C., 2020. Sex-specific differences in the response of prey to predation risk. Functional Ecology 34, 1235-1243. doi: 10.1111/1365-2435.13569

Douglas, A.E., 2015. Multiorganismal insects: Diversity and function of resident microorganisms. Annual Review of Entomology, Vol 60 60, 17-34. doi: 10.1146/annurev-ento-010814-020822

Duffield, K.R., Bowers, E.K., Sakaluk, S.K., Sadd, B.M., 2017. A dynamic threshold model for terminal investment. Behavioral Ecology and Sociobiology 71. doi: 10.1007/s00265-017-2416-z

Duong, T.M., McCauley, S.J., 2016. Predation risk increases immune response in a larval dragonfly (*Leucorrhinia intacta*). Ecology 97, 1605-1610. doi: 10.1890/15-1964.1

Durkin, E.S., Roth, A.M., Keiser, C.N., 2020. Parasitic personalities: consistent individual differences in behavior in a facultatively parasitic mite. Journal of Insect Behavior 33, 14-19. doi: 10.1007/s10905-020-09741-1

Dushay, M.S., Eldon, E.D., 1998. *Drosophila* immune responses as models for human immunity. American Journal of Human Genetics 62, 10-14. doi: 10.1086/301694

Ebrahim, S.A.M., Dweck, H.K.M., Stoekl, J., Hofferberth, J.E., Trona, F., Weniger, K., Rybak, J., Seki, Y., Stensmyr, M.C., Sachse, S., Hansson, B.S., Knaden, M., 2015. *Drosophila* avoids parasitoids by sensing their semiochemicals via a dedicated olfactory circuit. Plos Biology 13. doi: 10.1371/journal.pbio.1002318

Egan, M.E., Barth, R.H., Hanson, F.E., 1975. Chemically-mediated host selection in a parasitic mite. Nature 257, 788-790. doi: 10.1038/257788a0

Eilam, D., 2005. Die hard: A blend of freezing and fleeing as a dynamic defense - implications for the control of defensive behavior. Neuroscience and Biobehavioral Reviews 29, 1181-1191. doi: 10.1016/j.neubiorev.2005.03.027

Ekengren, S., Tryselius, Y., Dushay, M.S., Liu, G., Steiner, H.å.k., Hultmark, D., 2001. A humoral stress response in *Drosophila*. Current Biology 11, 714-718. doi:

Ellers, J., Jervis, M., 2003. Body size and the timing of egg production in parasitoid wasps. Oikos 102, 164-172. doi: 10.1034/j.1600-0706.2003.12285.x

Engelstadter, J., Hurst, G.D.D., 2007. The impact of male-killing bacteria on host evolutionary processes. Genetics 175, 245-254. doi: 10.1534/genetics.106.060921

Fedina, T.Y., Kuo, T.-H., Dreisewerd, K., Dierick, H.A., Yew, J.Y., Pletcher, S.D., 2012. Dietary effects on cuticular hydrocarbons and sexual attractiveness in *Drosophila*. Plos One 7. doi: 10.1371/journal.pone.0049799

Fedorka, K.M., Linder, J.E., Winterhalter, W., Promislow, D., 2007. Post-mating disparity between potential and realized immune response in *Drosophila melanogaster*. Proceedings of the Royal Society B-Biological Sciences 274, 1211-1217. doi: 10.1098/rspb.2006.0394

Fedorka, K.M., Zuk, M., 2005. Sexual conflict and female immune suppression in the cricket, *Allonemobious socius*. Journal of Evolutionary Biology 18, 1515-1522. doi: 10.1111/j.1420-9101.2005.00942.x

Fellows, D.P., Heed, W.B., 1972. Factors Affecting Host Plant Selection in Desert-Adapted Cactiphilic *Drosophila*. Ecology 53: 850-858.

Fill, A., Long, E.Y., Finke, D.L., 2012. Non-consumptive effects of a natural enemy on a non-prey herbivore population. Ecological Entomology 37, 43-50. doi: 10.1111/j.1365-2311.2011.01333.x

Fleming, J.E., Reveillaud, I., Niedzwiecki, A., 1992. Role of oxidative stress in *Drosophila* aging. Mutation Research 275, 267-279. doi: 10.1016/0921-8734(92)90031-j

Fontana-Bria, L., Selfa, J., Tur, C., Frago, E., 2017. Early exposure to predation risk carries over metamorphosis in two distantly related freshwater insects. Ecological Entomology 42, 255-262. doi: 10.1111/een.12382

Forbes, M.R.L., 1993. Parasitism and host reproductive effort. Oikos 3: 444-450.

Frank, M.R., Fogleman, J.C., 1992. Involvement of cytochrome-p450 in host-plant utilization by Sonoran desert *Drosophila*. Proceedings of the National Academy of Sciences of the United States of America 89, 11998-12002. doi: 10.1073/pnas.89.24.11998

French, S.S., DeNardo, D.F., Moore, M.C., 2007. Trade-offs between the reproductive and immune systems: Facultative responses to resources or obligate responses to reproduction? American Naturalist 170, 79-89. doi: 10.1086/518569

Fritzsche, A., Allan, B.F., 2012. The ecology of fear: host foraging behavior varies with the spatio-temporal abundance of a dominant ectoparasite. Ecohealth 9, 70-74. doi: 10.1007/s10393-012-0744-z

Gallagher, A.J., Creel, S., Wilson, R.P., Cooke, S.J., 2017. Energy landscapes and the landscape of fear. Trends in Ecology & Evolution 32, 88-96. doi: 10.1016/j.tree.2016.10.010

Galvez, D., Chapuisat, M., 2014. Immune priming and pathogen resistance in ant queens. Ecology and Evolution 4, 1761-1767. doi: 10.1002/ece3.1070

Gao, K., van Wijk, M., Dang, Q.T.D., Heckel, D.G., Zalucki, M.P., Groot, A.T., 2021. How healthy is your mate? Sex-specific consequences of parasite infections in the moth *Helicoverpa armigera*. Animal Behaviour 178, 105-113. doi: 10.1016/j.anbehav.2021.06.005

Garcia-Diaz, D.F., Campion, J., Milagro, F.I., Lomba, A., Marzo, F., Martinez, J.A., 2007. Chronic mild stress induces variations in locomotive behavior and metabolic rates in high fat fed rats. Journal of Physiology and Biochemistry 63, 337-346. doi: 10.1007/bf03165765

Gargano, J.W., Martin, I., Bhandari, P., Grotewiel, M.S., 2005. Rapid iterative negative geotaxis (RING): a new method for assessing age-related locomotor decline in *Drosophila*. Experimental Gerontology 40, 386-395. doi: 10.1016/j.exger.2005.02.005

Garrido, M., Adler, V.H., Pnini, M., Abramsky, Z., Krasnov, B.R., Gutman, R., Kronfeld-Schor, N., Hawlena, H., 2016. Time budget, oxygen consumption and body mass responses to parasites in juvenile and adult wild rodents. Parasites & Vectors 9. doi: 10.1186/s13071-016-1407-7

Garschall, K., Flatt, T., 2018. The interplay between immunity and aging in *Drosophila*. F1000Research 7, 160-160. doi: 10.12688/f1000research.13117.1

Gaudry, Q., Nagel, K.I., Wilson, R.I., 2012. Smelling on the fly: sensory cues and strategies for olfactory navigation in *Drosophila*. Current Opinion in Neurobiology 22, 216-222. doi: 10.1016/j.conb.2011.12.010

Gay, M., Lempereur, L., Francis, F., Megido, R.C., 2020. Control of *Dermanyssus gallinae* (De Geer 1778) and other mites with volatile organic compounds, a review. Parasitology 147, 731-739. doi: 10.1017/s0031182020000530

Geraldi, N.R., Macreadie, P.I., 2013. Restricting prey dispersal can overestimate the importance of predation in trophic cascades. Plos One 8, 1-9. doi: 10.1371/journal.pone.0055100

Gerth, M., Martinez-Montoya, H., Ramirez, P., Masson, F., Griffin, J.S., Aramayo, R., Siozios, S., Lemaitre, B., Mateos, M., Hurst, G.D.D., 2021. Rapid molecular evolution of *Spiroplasma* symbionts of *Drosophila*. Microbial Genomics 7. doi: 10.1099/mgen.0.000503

Giesel, J.T., Lanciani, C.A., Anderson, J.F., 1989. Metabolic-rate and sexual-activity in *Drosophila simulans*. Journal of Insect Physiology 35, 893-895. doi: 10.1016/0022-1910(89)90106-6

Giorgi, M.S., Arlettaz, R., Christe, P., Vogel, P., 2001. The energetic grooming costs imposed by a parasitic mite (*Spinturnix myoti*) upon its bat host (*Myotis myotis*). Proceedings of the Royal Society B-Biological Sciences 268, 2071-2075. doi: 10.1098/rspb.2001.1686

Gomez-Marin, A., Louis, M., 2014. Multilevel control of run orientation in *Drosophila* larval chemotaxis. Frontiers in Behavioral Neuroscience 8. doi: 10.3389/fnbeh.2014.00038

Gonzalez-Santoyo, I., Cordoba-Aguilar, A., 2012. Phenoloxidase: a key component of the insect immune system. Entomologia Experimentalis Et Applicata 142, 1-16. doi: 10.1111/j.1570-7458.2011.01187.x

Gorostiza, E.A., Colomb, J., Brembs, B., 2016. A decision underlies phototaxis in an insect. Open Biology 6. doi: 10.1098/rsob.160229

Grossman, J.D., Smith, R.J., 2008. Phoretic mite discrimination among male burying beetle (*Nicrophorus investigator*) hosts. Annals of the Entomological Society of America 101, 266-271. doi: 10.1603/0013-8746(2008)101[266:pmdamb]2.0.co;2

Gruntenko, N.E., Karpova, E.K., Alekseev, A.A., Chentsova, N.A., Bogomolova, E.V., Bownes, M., Rauschenbach, I.Y., 2007. Effects of octopamine on reproduction, juvenile hormone metabolism, dopamine, and 20-hydroxyecdysone contents in *Drosophila*. Archives of Insect Biochemistry and Physiology 65, 85-94. doi: 10.1002/arch.20187

Guo, Y., Hoffmann, A.A., Xu, X.Q., Mo, P.W., Huang, H.J., Gong, J.T., Ju, J.F., Hong, X.Y., 2018. Vertical transmission of *Wolbachia* is associated with host vitellogenin in *Laodelphax striatellus*. Frontiers in Microbiology 9. doi: 10.3389/fmicb.2018.02016

Gutierrez, S.O., Minchella, D.J., Bernal, X.E., Survival of the sickest: selective predation differentially modulates ecological and evolutionary disease dynamics. Oikos. doi: 10.1111/oik.09126

Gwynn, D.M., Callaghan, A., Gorham, J., Walters, K.F.A., Fellowes, M.D.E., 2005. Resistance is costly: trade-offs between immunity, fecundity and survival in the pea aphid. Proceedings of the Royal Society B-Biological Sciences 272, 1803-1808. doi: 10.1098/rspb.2005.3089

Haase, C.G., Long, A.K., Gillooly, J.F., 2016. Energetics of stress: linking plasma cortisol levels to metabolic rate in mammals. Biology Letters 12. doi: 10.1098/rsbl.2015.0867

Hadler, N.M., 1964. Heritability and phototaxis in *Drosophila melanogaster*. Genetics 50, 1269-1277. doi: 10.1093/genetics/50.6.1269.

Hart, B.L., 2011. Behavioural defences in animals against pathogens and parasites: parallels with the pillars of medicine in humans. Philosophical Transactions of the Royal Society B-Biological Sciences 366, 3406-3417. doi: 10.1098/rstb.2011.0092

Harumoto, T., Lemaitre, B., 2018. Male-killing toxin in a bacterial symbiont of *Drosophila*. Nature 557, 252-+. doi: 10.1038/s41586-018-0086-2

Haselkorn, T.S., Cockburn, S.N., Hamilton, P.T., Perlman, S.J., Jaenike, J., 2013. Infectious adaptation: potential host range of a defensive endosymbiont in *Drosophila*. Evolution 67, 934-945. doi: 10.1111/evo.12020

Haselkorn, T.S., Markow, T.A., Moran, N.A., 2009. Multiple introductions of the *Spiroplasma* bacterial endosymbiont into *Drosophila*. Molecular Ecology 18, 1294-1305. doi: 10.1111/j.1365-294X.2009.04085.x

Hague, M.T.J., Woods, H.A., Cooper, B.S.., 2021. Pervasive effects of *Wolbachia* on host activity. Biology Letters 17. doi: doi.org/10.1098/rsbl.2021.0052

Haussmann, I.U., Hemani, Y., Wijesekera, T., Dauwalder, B., Soller, M., 2013. Multiple pathways mediate the sex-peptide-regulated switch in female *Drosophila* reproductive behaviours. Proceedings of the Royal Society B-Biological Sciences 280. doi: 10.1098/rspb.2013.1938

Hawlena, D., Schmitz, O.J., 2010. Herbivore physiological response to predation risk and implications for ecosystem nutrient dynamics. Proceedings of the National Academy of Sciences of the United States of America 107, 15503-15507. doi: 10.1073/pnas.1009300107

Hawlena, H., Bashary, D., Abramsky, Z., Krasnov, B.R., 2007. Benefits, costs and constraints of anti-parasitic grooming in adult and juvenile rodents. Ethology 113, 394-402. doi: 10.1111/j.1439-0310.2007.01332.x

Heads, P.A., 1986. The costs of reduced feeding due to predator avoidance - potential effects on growth and fitness in *Ischnura elegans* larvae (Odonata, Zygoptera). Ecological Entomology 11, 369-377. doi: 10.1111/j.1365-2311.1986.tb00315.x

Herren, J.K., Lemaitre, B., 2011. *Spiroplasma* and host immunity: activation of humoral immune responses increases endosymbiont load and susceptibility to certain Gram-negative bacterial pathogens in *Drosophila melanogaster*. Cellular Microbiology 13, 1385-1396. doi: 10.1111/j.1462-5822.2011.01627.x

Herren, J.K., Paredes, J.C., Schupfer, F., Arafah, K., Bulet, P., Lemaitre, B., 2014. Insect endosymbiont proliferation is limited by lipid availability. Elife 3. doi: 10.7554/eLife.02964

Herren, J.K., Paredes, J.C., Schupfer, F., Lemaitre, B., 2013. Vertical transmission of a *Drosophila* endosymbiont via cooption of the yolk transport and internalization machinery. Mbio 4. doi: 10.1128/mBio.00532-12

Heuschele, J., Ceballos, S., Borg, C.M.A., Bjaerke, O., Isari, S., Lasley-Rasher, R., Lindehoff, E., Souissi, A., Souissi, S., Titelman, J., 2014. Non-consumptive effects of predator presence on copepod reproduction: insights from a mesocosm experiment. Marine Biology 161, 1653-1666. doi: 10.1007/s00227-014-2449-z

Hicks, O., Burthe, S.J., Daunt, F., Newell, M., Butler, A., Ito, M., Sato, K., Green, J.A., 2018. The energetic cost of parasitism in a wild population. Proceedings of the Royal Society B-Biological Sciences 285. doi: 10.1098/rspb.2018.0489

Horn, C.J., Luong, L.T., 2018. Proximity to parasites reduces host fitness independent of infection in a *Drosophila-Macrocheles* system. Parasitology 145, 1564-1569. doi: 10.1017/s0031182018000379

Horn, C.J., Luong, L.T., 2019a. Current parasite resistance trades off with future defenses and flight performance. Behavioral Ecology and Sociobiology 73. doi: 10.1007/s00265-019-2697-5

Horn, C.J., Luong, L.T., 2021. Trade-offs between reproduction and behavioural resistance against ectoparasite infection. Physiology & Behavior 239. doi: 10.1016/j.physbeh.2021.113524

Horn, C.J., Mierzejewski, M.K., Elahi, M.E., Luong, L.T., 2020. Extending the ecology of fear: Parasite-mediated sexual selection drives host response to parasites. Physiology & Behavior 224. doi: 10.1016/j.physbeh.2020.113041

Horn, C.J., Mierzejewski, M.K., Luong, L.T., 2018. Host respiration rate and injury-derived cues drive host preference by an ectoparasite of fruit flies. Physiological and Biochemical Zoology 91, 896-903. doi: 10.1086/697466

Hosokawa, T., Nikoh, N., Koga, R., Sato, M., Tanahashi, M., Meng, X.Y., Fukatsu, T., 2012. Reductive genome evolution, host-symbiont co-speciation and uterine transmission of endosymbiotic bacteria in bat flies. Isme Journal 6, 577-587. doi: 10.1038/ismej.2011.125

Hothorn, T., Hornik, K., Van de Wiel, M.A., Zeileis, A., 2006. A Lego system for conditional inference. American Statistician 60, 257-263. doi: 10.1198/000313006x118430

Hunt, V.L., Zhong, W.H., McClure, C.D., Mlynski, D.T., Duxbury, E.M.L., Priest, N.K., 2016. Cold-seeking behaviour mitigates reproductive losses from fungal infection in *Drosophila*. Journal of Animal Ecology 85, 178-186. doi: 10.1111/1365-2656.12438

Hutchings, M.R., Judge, J., Gordon, I.J., Athanasiadou, S., Kyriazakis, I., 2006. Use of trade-off theory to advance understanding of herbivore-parasite interactions. Mammal Review 36, 1-16. doi: 10.1111/j.1365-2907.2006.00080.x

Ingerslew, K.S., Finke, D.L., 2020. Non-consumptive effects stabilize herbivore control over multiple generations. Plos One 15. doi: 10.1371/journal.pone.0241870

Isaac, R.E., 2019. The effect of mating and the male sex peptide on group behaviour of post-mated female *Drosophila melanogaster*. Neurochemical Research 44, 1508-1516. doi: 10.1007/s11064-019-02722-7

Ishii, Y., Matsuura, Y., Kakizawa, S., Nikoh, N., Fukatsu, T., 2013. Diversity of bacterial endosymbionts associated with *Macrosteles* leafhoppers vectoring phytopathogenic phytoplasmas. Applied and Environmental Microbiology 79, 5013-5022. doi: 10.1128/aem.01527-13

Jackson, R.R., Cross, F.R., 2011. Olfaction-based mate-odor identification by jumping spiders from the genus *Portia*. Journal of Arachnology 39, 439-443. doi: 10.1636/Ha11-32.1

Jaenike, J., Polak, M., Fiskin, A., Helou, M., Minhas, M., 2007. Interspecific transmission of endosymbiotic *Spiroplasma* by mites. Biology Letters 3, 23-25. doi: 10.1098/rsbl.2006.0577

Jalil, M., Rodriguez, J.G., 1970. Studies of behavior of *Macrocheles muscaedomesticae* (Acarina-Macrochelidae) with emphasis on its attraction to house fly. Annals of the Entomological Society of America 63, 738-744. doi: 10.1093/aesa/63.3.738

James, W.R., McClintock, J.B., 2017. Anti-predator responses of amphipods are more effective in the presence of conspecific chemical cues. Hydrobiologia 797, 277-288. doi: 10.1007/s10750-017-3191-6

Jermacz, L., Kobak, J., 2017. Keep calm and don't stop growing: Non-consumptive effects of a sympatric predator on two invasive Ponto-Caspian gammarids *Dikerogammarus villosus* and *Pontogammarus robustoides*. Plos One 12. doi: 10.1371/journal.pone.0182481

Jiggins, F.M., 2017. The spread of *Wolbachia* through mosquito populations. Plos Biology 15. doi: 10.1371/journal.pbio.2002780

Jigisha, Iglesias-Carrasco, M., Vincent, A., Head, M.L., 2020. Disentangling the costs of mating and harassment across different environments. Animal Behaviour 165, 79-88. doi: 10.1016/j.anbehav.2020.05.005

Johnston, J.S., Heed, W.B., 1976. Dispersal of desert-adapted *Drosophila*: the *Saguaro*-breeding *D. nigrospiracula*. American Naturalist 110, 629-651. doi: 10.1086/283095

Jones, J.E., Hurst, G.D., 2020. Symbiont-mediated fly survival is independent of defensive symbiont genotype in the *Drosophila melanogaster*-*Spiroplasma*-wasp interaction. Journal of Evolutionary Biology In Press. doi: https://doi.org/10.1111/jeb.13702

Jones, J.H., 2007. demogR: A Package for the Construction and Analysis of Age-structured Demographic Models in R. Journal of Statistical Software 22, 1-28. doi: 10.18637/jss.v022.i10.

Kageyama, D., Anbutsu, H., Watada, M., Hosokawa, T., Shimada, M., Fukatsu, T., 2006. Prevalence of a non-male-killing *Spiroplasma* in natural populations of *Drosophila hydei*. Applied and Environmental Microbiology 72, 6667-6673. doi: 10.1128/aem.00803-06

Kain, J.S., Stokes, C., de Bivort, B.L., 2012. Phototactic personality in fruit flies and its suppression by serotonin and white. Proceedings of the National Academy of Sciences of the United States of America 109, 19834-19839. doi: 10.1073/pnas.1211988109

Keiser, C.N., Rudolf, V.H.W., Luksik, M.C., Saltz, J.B., 2020. Sex differences in disease avoidance behavior vary across modes of pathogen exposure. Ethology 126, 304-312. doi: 10.1111/eth.12969

Kelly, C.D., Stoehr, A.M., Nunn, C., Smyth, K.N., Prokop, Z.M., 2018. Sexual dimorphism in immunity across animals: a meta-analysis. Ecology Letters 21, 1885-1894. doi: 10.1111/ele.13164

Kersch-Becker, M.F., Thaler, J.S., 2015. Plant resistance reduces the strength of consumptive and non-consumptive effects of predators on aphids. Journal of Animal Ecology 84, 1222-1232. doi: 10.1111/1365-2656.12371

Khazaeli, A.A., Van Voorhies, W., Curtsinger, J.W., 2005. Longevity and metabolism in *Drosophila melanogaster*: Genetic correlations between life span and age-specific metabolic rate in populations artificially selected for long life. Genetics 169, 231-242. doi: 10.1534/genetics.104.030403

Kikuchi, Y., 2009. Endosymbiotic bacteria in insects: Their diversity and culturability. Microbes and Environments 24, 195-204. doi: 10.1264/jsme2.ME09140S

Kim, K.N., Huang, Q.Y., Lei, C.L., 2019. Advances in insect phototaxis and application to pest management: a review. Pest Management Science 75, 3135-3143. doi: 10.1002/ps.5536

Kindinger, T.L., Albins, M.A., 2017. Consumptive and non-consumptive effects of an invasive marine predator on native coral-reef herbivores. Biological Invasions 19, 131-146. doi: 10.1007/s10530-016-1268-1

Kirk, D., Greischar, M., Mideo, N., Krkosek, M., 2021. Environmental variability affects optimal trade-offs in ecological immunology. Ecosphere 12. doi: 10.1002/ecs2.3654

Krist, A.C., 2001. Variation in fecundity among populations of snails is predicted by prevalence of castrating
parasites. Evolutionary Ecology Research 3: 191-197.

Klemme, I., Karvonen, A., 2017. Vertebrate defense against parasites: Interactions between avoidance, resistance, and tolerance. Ecology and Evolution 7, 561-571. doi: 10.1002/ece3.2645

Koenraadt, C.J.M., Dicke, M., 2010. The role of volatiles in aggregation and host-seeking of the haematophagous poultry red mite *Dermanyssus gallinae* (Acari: Dermanyssidae). Experimental and Applied Acarology 50, 191-199. doi: 10.1007/s10493-009-9305-8

Koliada, A., Gavrilyuk, K., Burdylyuk, N., Strilbytska, O., Storey, K.B., Kuharskii, V., Lushchak, O., Vaiserman, A., 2020. Mating status affects *Drosophila* lifespan, metabolism and antioxidant system. Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology 246. doi: 10.1016/j.cbpa.2020.110716

Kondorosi, E., Mergaert, P., Kereszt, A., 2013. A paradigm for endosymbiotic life: cell differentiation of rhizobium bacteria provoked by host plant factors. Annual Review of Microbiology, Vol 67 67, 611-628. doi: 10.1146/annurev-micro-092412-155630

Koprivnikar, J., Forbes, M.R., Baker, R.L., 2006. On the efficacy of anti-parasite behaviour: a case study of tadpole susceptibility to cercariae of *Echinostoma trivolvis*. Canadian Journal of Zoology-Revue Canadienne De Zoologie 84, 1623-1629. doi: 10.1139/z06-158

Koprivnikar, J., Penalva, L., 2015. Lesser of two evils? Foraging choices in response to threats of predation and parasitism. Plos One 10. doi: 10.1371/journal.pone.0116569

Koprivnikar, J., Urichuk, T.M.Y., 2017. Time-lagged effect of predators on tadpole behaviour and parasite infection. Biology Letters 13. doi: 10.1098/rsbl.2017.0440

Koprivnikar, J., Rochette, A., Forbes, M.R., 2021. Risk-Induced Trait Responses and Non-consumptive Effects in Plants and Animals in Response to Their Invertebrate Herbivore and Parasite Natural Enemies. Frontiers in Ecology and Evolution 9. doi: 10.3389/fevo.2021.667030.

Koskella, B., 2018. Resistance gained, resistance lost: An explanation for host-parasite coexistence. PLoS biology 16, e3000013-e3000013. doi: 10.1371/journal.pbio.3000013

Koutroumpa, F.A., Monsempes, C., Francois, M.C., de Cian, A., Royer, C., Concordet, J.P., Jacquin-Joly, E., 2016. Heritable genome editing with CRISPR/Cas9 induces anosmia in a crop pest moth. Scientific Reports 6, 1-9. doi: 10.1038/srep29620

Krams, I., Inwood, S.E., Trakimas, G., Krams, R., Burghardt, G.M., Bulter, D.M., Luoto, S., Krama, T., 2016. Short-term exposure to predation affects body elemental composition, climbing speed and survival ability in *Drosophila melanogaster*. Peerj 4. doi: 10.7717/peerj.2314

Krams, R., Krama, T., Munkevics, M., Eichler, S., Butler, D.M., Dobkevica, L., Joers, P., Contreras-Garduno, J., Daukste, J., Krams, I.A., 2021. Spider odors induce stoichiometric changes in fruit fly *Drosophila melanogaster*. Current Zoology 67, 127-129. doi: 10.1093/cz/zoaa070

Krasnov, B.R., Khokhlova, I.S., Arakelyan, M.S., Degen, A.A., 2005. Is a Starving Host Tastier? Reproduction in Fleas Parasitizing Food-Limited Rodents. Functional Ecology 19, 625-631. doi:

Kraus, B., 1994. Factors influencing host choice of the honey-bee parasite *Varroa jacobsoni* Oud. Experimental & Applied Acarology 18, 435-443. doi: 10.1007/bf00051525

Krittika, S., Lenka, A., Yadav, P., 2019. Evidence of dietary protein restriction regulating pupation height, development time and lifespan in *Drosophila melanogaster*. Biology Open 8. doi: 10.1242/bio.042952

Krusemark, E.A., Li, W., 2011. Do all threats work the same way? Divergent effects of fear and disgust on sensory perception and attention. Journal of Neuroscience 31, 3429-3434. doi: 10.1523/jneurosci.4394-10.2011

Kubli, E., Bopp, D., 2012. Sexual behavior: how sex peptide flips the postmating switch of female flies. Current Biology 22, R520-R522. doi: 10.1016/j.cub.2012.04.058

Kubrak, O.I., Lushchak, O.V., Zandawala, M., Nassel, D.R., 2016. Systemic corazonin signalling modulates stress responses and metabolism in *Drosophila*. Open Biology 6. doi: 10.1098/rsob.160152

Kulkarni, P.S., Gramapurohit, N.P., 2017. Effect of corticosterone on larval growth, antipredator behaviour and metamorphosis of *Hylarana indica*. General and Comparative Endocrinology 251, 21-29. doi: 10.1016/j.ygcen.2016.09.001

Kuo, T.H., Yew, J.Y., Fedina, T.Y., Dreisewerd, K., Dierick, H.A., Pletcher, S.D., 2012. Aging modulates cuticular hydrocarbons and sexual attractiveness in *Drosophila melanogaster*. Journal of Experimental Biology 215, 814-821. doi: 10.1242/jeb.064980

Kurz, C.L., Charroux, B., Chaduli, D., Viallat-Lieutaud, A., Royet, J., 2017. Peptidoglycan sensing by octopaminergic neurons modulates *Drosophila* oviposition. Elife 6. doi: 10.7554/eLife.21937

Kwiatkowski, M., Vorburger, C., 2012. Modeling the ecology of symbiont-mediated protection against parasites. American Naturalist 179, 595-605. doi: 10.1086/665003

Lafferty, K.D., Dobson, A.P., Kuris, A.M., 2006. Parasites dominate food web links. Proceedings of the National Academy of Sciences of the United States of America 103, 11211-11216. doi: 10.1073/pnas.0604755103

Lafferty, K.D., Kuris, A.M., 2002. Trophic strategies, animal diversity and body size. Trends in Ecology & Evolution 17, 507-513. doi: 10.1016/s0169-5347(02)02615-0

Lagos, P.A., Herberstein, M.E., 2017. Are males more scared of predators? Differential change in metabolic rate between males and females under predation risk. Physiology & Behavior 173, 110-115. doi: 10.1016/j.physbeh.2017.02.002

Larsson, M.C., Domingos, A.I., Jones, W.D., Chiappe, M.E., Amrein, H., Vosshall, L.B., 2004. Or83b encodes a broadly expressed odorant receptor essential for *Drosophila* olfaction. Neuron 43, 703-714. doi: 10.1016/j.neuron.2004.08.019

Lazopulo, S., Lopez, J.A., Levy, P., Syed, S., 2015. A stochastic burst follows the periodic morning peak in individual *Drosophila* locomotion. Plos One 10. doi: 10.1371/journal.pone.0140481

Lazzaro, B.P., Tate, A.T., 2022. Balancing sensitivity, risk, and immunopathology in immune regulation. Current opinion in insect science 50, 100874-100874. doi: 10.1016/j.cois.2022.100874

Lefevre, T., de Roode, J.C., Kacsoh, B.Z., Schlenke, T.A., 2012. Defence strategies against a parasitoid wasp in *Drosophila*: fight or flight? Biology Letters 8, 230-233. doi: 10.1098/rsbl.2011.0725

Lefranc, A., Bundgaard, J., 2000. The influence of male and female body size on copulation duration and fecundity in *Drosophila melanogaster*. Hereditas 132, 243-247. doi: 10.1111/j.1601-5223.2000.00243.x

Li, J.F., Zhang, W., Guo, Z.H., Wu, S., Jan, L.Y., Jan, Y.N., 2016a. A defensive kicking behavior in response to mechanical stimuli mediated by *Drosophila* wing margin bristles. Journal of Neuroscience 36, 11275-11282. doi: 10.1523/jneurosci.1416-16.2016

Li, Y., Hoffmann, J., Li, Y., Stephano, F., Bruchhaus, I., Fink, C., Roeder, T., 2016b. Octopamine controls starvation resistance, life span and metabolic traits in *Drosophila*. Scientific Reports 6. doi: 10.1038/srep35359

Lighton, J.R.B., 2008. Measuring metabolic rates: a manual for scientists. Oxford University Press, New York, United States of America.

Lighton, J.R.B., Halsey, L.G., 2011. Flow-through respirometry applied to chamber systems: pros and cons, hints and tips. Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology 158, 265-275. doi: 10.1016/j.cbpa.2010.11.026

Lim, J., Sabandal, P.R., Fernandez, A., Sabandal, J.M., Lee, H.-G., Evans, P., Han, K.-A., 2014. The Octopamine Receptor Oct beta 2R regulates ovulation in *Drosophila melanogaster*. Plos One 9. doi: 10.1371/journal.pone.0104441

Lints, F.A., Lints, C.V., 1968. Respiration in *Drosophila* .2. Respiration in relation to age by wild inbred and hybrid *Drosophila melanogaster* Imagos. Experimental Gerontology 3, 341-349. doi: 10.1016/0531-5565(68)90047-8

Liu, S.-H., Li, H.-F., Yang, Y., Wei, D., Jiang, H.-B., Dou, W., Yuan, G.-R., Wang, J.-J., 2018. Antimicrobial peptide gene BdPho responds to peptidoglycan infection and mating stimulation in oriental fruit fly, *Bactrocera dorsalis* (Hendel). Amb Express 8. doi: 10.1186/s13568-017-0533-8

Lu, Z.C., Wang, Y.M., Zhu, S.G., Yu, H., Guo, J.Y., Wan, F.H., 2014. Trade-offs between survival, longevity, and reproduction, and variation of survival tolerance in Mediterranean *Bemisia tabaci* after temperature stress. Journal of Insect Science 14, 1-14. doi: 10.1093/jis/14.1.124

Lung, O., Kuo, L., Wolfner, M.F., 2001. *Drosophila* males transfer antibacterial proteins from their accessory gland and ejaculatory duct to their mates. Journal of Insect Physiology 47, 617-622. doi: 10.1016/s0022-1910(00)00151-7

Luong, L.T., Brophy, T., Stolz, E., Chan, S.J., 2017. State-dependent parasitism by a facultative parasite of fruit flies. Parasitology 144, 1468-1475. doi: 10.1017/s0031182017000890

Luong, L.T., Heath, B.D., Polak, M., 2007. Host inbreeding increases susceptibility to ectoparasitism. Journal of Evolutionary Biology 20, 79-86. doi: 10.1111/j.1420-9101.2006.01226.x

Luong, L.T., Horn, C.J., Brophy, T., 2017. Mitey costly: energetic costs of parasite avoidance and infection. Physiological and Biochemical Zoology 90, 471-477. doi: 10.1086/691704

Luong, L.T., Penoni, L.R., Horn, C.J., Polak, M., 2015. Physical and physiological costs of ectoparasitic mites on host flight endurance. Ecological Entomology 40, 518-524. doi: 10.1111/een.12218

Luong, L.T., Polak, M., 2007. Costs of resistance in the *Drosophila-Macrocheles* system: a negative genetic correlation between ectoparasite resistance and reproduction. Evolution 61, 1391-1402. doi: 10.1111/j.1558-5646.2007.00116.x

Luong, L.T., Polak, M., 2007b. Environment-dependent trade-offs between ectoparasite resistance and larval competitive ability in the *Drosophila-Macrocheles* system. Heredity 99, 632-640. doi: 10.1038/sj.hdy.6801040

Luong, L.T., Subasinghe, D., 2017. A facultative ectoparasite attains higher reproductive success as a parasite than its free-living conspecifics. Experimental and Applied Acarology 71, 63-70. doi: 10.1007/s10493-016-0098-2

Mack, P.D., Kapelnikov, A., Heifetz, Y., Bender, M., 2006. Mating-responsive genes in reproductive tissues of female *Drosophila melanogaster*. Proceedings of the National Academy of Sciences of the United States of America 103, 10358-10363. doi: 10.1073/pnas.0604046103

Mackenzie, D.K., Bussiere, L.F., Tinsley, M.C., 2011. Senescence of the cellular immune response in *Drosophila melanogaster*. Experimental Gerontology 46, 853-859. doi: 10.1016/j.exger.2011.07.004

MacLeod, K.J., Krebs, C.J., Boonstra, R., Sheriff, M.J., 2018. Fear and lethality in snowshoe hares: the deadly effects of non-consumptive predation risk. Oikos 127, 375-380. doi: 10.1111/oik.04890

Mappes, T., Koskela, E., 2004. Genetic basis of the trade-off between offspring number and quality in the bank vole. Evolution 58, 645-650. doi: 10.1111/j.0014-3820.2004.tb01686.x

Markow, T.A., 1975. Genetic-analysis of phototactic behavior in *Drosophila melanogaster* .1. Selection in presence of inversions. Genetics 79, 527-534.

Markow, T.A., 1979. Survey of intraspecific and interspecific variation for pupation height in *Drosophila*. Behavior Genetics 9, 209-217. doi: 10.1007/bf01071301

Markow, T.A., 1988. Reproductive-behavior of *Drosophila melanogaster* and *Drosophila nigrospiracula* in the field and in the laboratory. Journal of Comparative Psychology 102, 169-173. doi: 10.1037/0735-7036.102.2.169

Markow, T.A., 1996. Evolution of *Drosophila* mating systems. Evolutionary Biology, Vol 29 29, 73-106.

Markow, T.A., 2011. "Cost" of virginity in wild *Drosophila melanogaster* females. Ecology and Evolution 1. doi: 10.1002/ece3.54

Markow, T.A., Fogleman, J.C., 1981. Behavioral differentiation between 2 species of cactiphilic *Drosophila* .1. Adult geotaxis and phototaxis. Experientia 37, 145-146. doi: 10.1007/bf01963198

Marshall, J.S., Warrington, R., Watson, W., Kim, H.L., 2018. An introduction to immunology and immunopathology. Allergy Asthma and Clinical Immunology 14. doi: 10.1186/s13223-018-0278-1

Marubayashi, J.M., Kliot, A., Yuki, V.A., Marques Rezende, J.A., Krause-Sakate, R., Pavan, M.A., Ghanim, M., 2014. Diversity and localization of bacterial endosymbionts from whitefly species collected in Brazil. Plos One 9. doi: 10.1371/journal.pone.0108363

Masson, F., Calderon-Copete, S., Schuepfer, F., Vigneron, A., Rommelaere, S., Garcia-Arraez, M.G., Paredes, J.C., Lemaitre, B., 2020. Blind killing of both male and female *Drosophila* embryos by a natural variant of the endosymbiotic bacterium *Spiroplasma poulsonii*. Cellular Microbiology 22. doi: 10.1111/cmi.13156

Masuzzo, A., Maniere, G., Viallat-Lieutaud, A., Avazeri, E., Zugasti, O., Grosjean, Y., Kurz, C.L., Royet, J., 2019. Peptidoglycan-dependent NF-kappa B activation in a small subset of brain octopaminergic neurons controls female oviposition. Elife 8. doi: 10.7554/eLife.50559

Mathews, K.W., Cavegn, M., Zwicky, M., 2017. Sexual dimorphism of body size is controlled by dosage of the X-chromosomal gene myc and by the sex-determining gene tra in *Drosophila*. Genetics 205, 1215-1228. doi: 10.1534/genetics.116.192260

Mathot, K.J., Dingemanse, N.J., Nakagawa, S., 2019. The covariance between metabolic rate and behaviour varies across behaviours and thermal types: meta-analytic insights. Biological Reviews 94, 1056-1074. doi: 10.1111/brv.12491

McCauley, S.J., Rowe, L., Fortin, M.-J., 2011. The deadly effects of "nonlethal" predators. Ecology 92, 2043-2048. doi: 10.1890/11-0455.1

McCullough, E.L., Chou, C.-C., Backwell, P.R.Y., 2020. Cost of an elaborate trait: a trade-off between attracting females and maintaining a clean ornament. Behavioral Ecology 31, 1218-1223. doi: 10.1093/beheco/araa072

McGeary, M.K., Findlay, G.D., 2020. Molecular evolution of the sex peptide network in *Drosophila*. Journal of Evolutionary Biology 33, 629-641. doi: 10.1111/jeb.13597

McKee, K.M., Koprivnikar, J., Johnson, P.T.J., Arts, M.T., 2020. Parasite infectious stages provide essential fatty acids and lipid-rich resources to freshwater consumers. Oecologia 192, 477-488. doi: 10.1007/s00442-019-04572-0

Mersmann, O., Trautmann, H., Steuer, D., Bornkamp, B., 2018. truncnorm: Truncated Normal Distribution. R package version 1.0-8. CRAN.R-project.org/package=truncnorm

Mierzejewski, M.K., Horn, C.J., Lien, T.L., 2019. Ecology of fear: environment-dependent parasite avoidance among ovipositing *Drosophila*. Parasitology 146, 1564-1570. doi: 10.1017/s0031182019000854

Miller, P.B., Obrik-Uloho, O.T., Phan, M.H., Medrano, C.L., Renier, J.S., Thayer, J.L., Wiessner, G., Qazi, M.C.B., 2014. The song of the old mother: reproductive senescence in female *Drosophila*. Fly 8, 127-139. doi: 10.4161/19336934.2014.969144

Min, K.T., Benzer, S., 1997. *Wolbachia*, normally a symbiont of *Drosophila*, can be virulent, causing degeneration and early death. Proceedings of the National Academy of Sciences of the United States of America 94, 10792-10796. doi: 10.1073/pnas.94.20.10792

Montenegro, H., Souza, W.N., Leite, D.D., Klaczko, L.B., 2000. Male-killing selfish cytoplasmic element causes sex-ratio distortion in *Drosophila melanogaster*. Heredity 85, 465-470. doi: 10.1046/j.1365-2540.2000.00785.x

Morand, S., De Bellocq, J.G., Stanko, M., Miklisova, D., 2004. Is sex-biased ectoparasitism related to sexual size dimorphism in small mammals of Central Europe? Parasitology 129, 505-510. doi: 10.1017/s0031182004005840

Moret, Y., Schmid-Hempel, P., 2000. Survival for immunity: The price of immune system activation for bumblebee workers. Science 290, 1166-1168. doi: 10.1126/science.290.5494.1166

Moritz, K.K., Bjorkman, C., Parachnowitsch, A.L., Stenberg, J.A., 2016. Female *Salix viminalis* are more severely infected by *Melampsora* spp. but neither sex experiences associational effects. Ecology and Evolution 6, 1154-1162. doi: 10.1002/ece3.1923

Murillo, A.C., Mullens, B.A., 2020. Collecting and Monitoring for Northern Fowl Mite (Acari: Macronyssidae)
and Poultry Red Mite (Acari: Dermanyssidae) in Poultry Systems. Journal of Insect Science 20: 12.

Murrayl, R.L., Tah, S., Koprivnikar, J., Rowe, L., McCauley, S.J., 2020a. Exposure to potentially cannibalistic conspecifics induces an increased immune response. Ecological Entomology 45, 355-363. doi: 10.1111/een.12806

Nadler, L.E., Bengston, E., Eliason, E.J., Hassibi, C., Helland-Riise, S.H., Johansen, I.B., Kwan, G.T., Tresguerres, M., Turner, A.V., Weinersmith, K.L., Overli, O., Hechinger, R.F., 2021. A brain-infecting parasite impacts host metabolism both during exposure and after infection is established. Functional Ecology 35, 105-116. doi: 10.1111/1365-2435.13695

Nagell, B., 1977. Phototactic and thermotactic responses facilitating survival of *Cloeon dipterum* (Ephemeroptera) larvae under winter anoxia. Oikos 29, 342-347. doi: 10.2307/3543625

Nakhleh, J., Moussawi, L.E., Osta, M.A., 2017. The melanization response in insect immunity, in: Ligoxygakis, P. (Ed.), Advances in Insect Physiology, Volume 52, 1st ed. Academic Press, Cambridge, Massachusetts, United States of America, pp. 83-109.

Navarro, C., de Lope, F., Marzal, A., Moller, A.P., 2004. Predation risk, host immune response, and parasitism. Behavioral Ecology 15, 629-635. doi: 10.1093/beheco/arh054

Nehring, V., Teubner, H., Koenig, S., 2019. Dose-independent virulence in phoretic mites that parasitize burying beetles. International Journal for Parasitology 49, 759-767. doi: 10.1016/j.ijpara.2019.05.011

Nelson, E.H., Matthews, C.E., Rosenheim, J.A., 2004. Predators reduce prey population growth by inducing changes in prey behavior. Ecology 85, 1853-1858. doi: 10.1890/03-3109

Niogret, J., Lumaret, J.-P., Bertrand, M., 2006. Semiochemicals mediating host-finding behaviour in the phoretic association between *Macrocheles saceri* (Acari : Mesostigmata) and *Scarabaeus*

species (Coleoptera : Scarabaeidae). Chemoecology 16, 129-134. doi: 10.1007/s00049-006-0338-8

Niven, J.E., Scharlemann, J.P.W., 2005. Do insect metabolic rates at rest and during flight scale with body mass? Biology Letters 1, 346-349. doi: 10.1098/rsbl.2005.0311

Nolan, M.P., Delaplane, K.S., 2017. Parasite dispersal risk tolerance is mediated by its reproductive value. Animal Behaviour 132, 247-252. doi: 10.1016/j.anbehav.2017.08.016

Noldus, L., Spink, A.J., Tegelenbosch, R.A.J., 2002. Computerised video tracking, movement analysis and behaviour recognition in insects. Computers and Electronics in Agriculture 35, 201-227. doi: 10.1016/s0168-1699(02)00019-4

Nordling, D., Andersson, M., Zohari, S., Gustafsson, L., 1998. Reproductive effort reduces specific immune response and parasite resistance. Proceedings of the Royal Society B-Biological Sciences 265, 1291-1298. doi: 10.1098/rspb.1998.0432

Norrdahl, K., Korpimaki, E., 1998. Does mobility or sex of voles affect risk of predation by mammalian predators? Ecology 79, 226-232.

O'Dwyer, K., Dargent, F., Forbes, M.R., Koprivnikar, J., 2019. Parasite infection leads to widespread glucocorticoid hormone increases in vertebrate hosts: A meta-analysis. The Journal of animal ecology. doi: 10.1111/1365-2656.13123

Odiere, M.R., Koski, K.G., Weiler, H.A., Scott, M.E., 2010. Concurrent nematode infection and pregnancy induce physiological responses that impair linear growth in the murine foetus. Parasitology 137, 991-1002. doi: 10.1017/s0031182009991764

Oku, K., Price, T.A.R., Wedell, N., 2019. Does mating negatively affect female immune defences in insects? Animal Biology 69, 117-136. doi: 10.1163/15707563-20191082

Oliver, J.H., Krantz, G.W., 1963. *Macrocheles rodriguezi*, a new species of mite from Kansas (Acarina: Macrochelidae) with notes on its life cycle and behavior. Acarologia 5, 519-525. doi:

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. doi: 10.1007/s13199-013-0241-3

Paludan, S.R., Pradeu, T., Masters, S.L., Mogensen, T.H., 2021. Constitutive immune mechanisms: mediators of host defence and immune regulation. Nature Reviews Immunology 21, 137-150. doi: 10.1038/s41577-020-0391-5

Parietti, M., Merlo, M.J., Natal, M., Mendez Casariego, M. A., 2020. Reproductive compensation in female *Palaemonetes argentinu*s (Decapoda: Natantia) due to *Microphallus szidati* (Trematoda) infection. Journal of Helminthology 94: E204.

Parigi, A., Porter, C., Cermak, M., Pitchers, W.R., Dworkin, I., 2019. The behavioral repertoire of *Drosophila melanogaster* in the presence of two predator species that differ in hunting mode. Plos One 14. doi: 10.1371/journal.pone.0216860

Paukku, S., Kotiaho, J.S., 2005. Cost of reproduction in *Callosobruchus maculatus*: effects of mating on male longevity and the effect of male mating status on female longevity. Journal of Insect Physiology 51, 1220-1226. doi: 10.1016/j.jinsphys.2005.06.012

Pawlowska, T.E., Gaspar, M.L., Lastovetsky, O.A., Mondo, S.J., Real-Ramirez, I., Shakya, E., Bonfante, P., 2018. Biology of fungi and their bacterial endosymbionts. Annual Review of Phytopathology, Vol 56 56, 289-309. doi: 10.1146/annurev-phyto-080417-045914

Peacor, S.D., Werner, E.E., 2001. The contribution of trait-mediated indirect effects to the net effects of a predator. Proceedings of the National Academy of Sciences of the United States of America 98, 3904-3908. doi: 10.1073/pnas.071061998

Peacor, S.D., Werner, E.E., 2008. Nonconsumptive effects of predators and trait-mediated indirect effects, Encyclopedia of Life Sciences (ELS). John Wiley & Sons, Ltd, Chichester, United Kingdom, pp. 1-8.

Peckarsky, B.L., Abrams, P.A., Bolnick, D.I., Dill, L.M., Grabowski, J.H., Luttbeg, B., Orrock, J.L., Peacor, S.D., Preisser, E.L., Schmitz, O.J., Trussell, G.C., 2008. Revisiting the classics: Considering nonconsumptive effects in textbook examples of predator-prey interactions. Ecology 89, 2416-2425. doi: 10.1890/07-1131.1

Peckarsky, B.L., Cowan, C.A., Penton, M.A., Anderson, C., 1993. Sublethal consequences of stream-dwelling predatory stoneflies on mayfly growth and fecundity. Ecology 74, 1836-1846. doi: 10.2307/1939941

Perez-Leanos, A., Ramirez Loustalot-Laclette, M., Nazario-Yepiz, N., Ann Markow, T., 2017. Ectoparasitic mites and their *Drosophila* hosts. Fly 11, 10-18. doi: 10.1080/19336934.2016.1222998

Pernal, S.F., Baird, D.S., Birmingham, A.L., Higo, H.A., Slessor, K.N., Winston, M.L., 2005. Semiochemicals influencing the host-finding behaviour of *Varroa destructor*. Experimental and Applied Acarology 37, 1-26. doi: 10.1007/s10493-005-1117-x

Pfeiler, E., Ngo, N.M., Markow, T.A., 2005. Linking behavioral ecology with population genetics: insights from *Drosophila nigrospiracula*. Hereditas 142, 1-6. doi: 10.1111/j.1601-5223.2005.01900.x

Piazza, N., Gosangi, B., Devilla, S., Arking, R., Wessells, R., 2009. exercise-training in young *Drosophila melanogaster* reduces age-related decline in mobility and cardiac performance. Plos One 4. doi: 10.1371/journal.pone.0005886

Pinheiro J, Bates D, R Core Team (2022). *nlme: Linear and Nonlinear Mixed Effects Models*. R package version 3.1-159, https://CRAN.R-project.org/package=nlme.

Pitnick, S., Markow, T., Spicer, G.S., 1999. Evolution of multiple kinds of female sperm-storage organs in *Drosophila*. Evolution 53, 1804-1822. doi: 10.2307/2640442

Polak, M., 1996. Ectoparasitic effects on host survival and reproduction: The *Drosophila-Macrocheles* association. Ecology 77, 1379-1389. doi: 10.2307/2265535

Polak, M., 2003. Heritability of resistance against ectoparasitism in the *Drosophila-Macrocheles* system. Journal of Evolutionary Biology 16, 74-82. doi: 10.1046/j.1420-9101.2003.00500.x

Polak, M., Luong, L.T., Starmer, W.T., 2007. Parasites physically block host copulation: a potent mechanism of parasite-mediated sexual selection. Behavioral Ecology 18, 952-957. doi: 10.1093/beheco/arm066

Polak, M., Markow, T.A., 1995. Effect of ectoparasitic mites on sexual selection in a Sonoran desert fruit-fly. Evolution 49, 660-669. doi: 10.2307/2410319

Polak, M., Starmer, W.T., 1998. Parasite-induced risk of mortality elevates reproductive effort in male *Drosophila*. Proceedings of the Royal Society B-Biological Sciences 265, 2197-2201. doi: 10.1098/rspb.1998.0559

Poulin, R., 2007. Are there general laws in parasite ecology? Parasitology 134, 763-776. doi: 10.1017/s0031182006002150

Poulin, R., Morand, S., 2000. The diversity of parasites. Quarterly Review of Biology 75, 277-293. doi: 10.1086/393500

Preisser, E.L., Bolnick, D.I., 2008. The many faces of fear: comparing the pathways and impacts of nonconsumptive predator effects on prey populations. Plos One 3. doi: 10.1371/journal.pone.0002465

R Core Team (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/

Raeymaekers, J.A.M., Hablutzel, P.I., Gregoir, A.F., Bamps, J., Roose, A.K., Vanhove, M.P.M., Van Steenberge, M., Pariselle, A., Huyse, T., Snoeks, J., Volckaert, F.A.M., 2013. Contrasting parasite communities among allopatric colour morphs of the Lake Tanganyika cichlid *Tropheus*. BMC Evolutionary Biology 13. doi: 10.1186/1471-2148-13-41

Raffel, T.R., Martin, L.B., Rohr, J.R., 2008. Parasites as predators: unifying natural enemy ecology. Trends in Ecology & Evolution 23, 610-618. doi: 10.1016/j.tree.2008.06.015

Rakus, K., Ronsmans, M., Vanderplasschen, A., 2017. Behavioral fever in ectothermic vertebrates. Developmental and Comparative Immunology 66, 84-91. doi: 10.1016/j.dci.2016.06.027

Ramsey, S.D., Ochoa, R., Bauchan, G., vanEngelsdorp, D., 2019. *Varroa destructor* feeds primarily on honey bee fat body tissue and not hemolymph. PNAS 116, 1792-1801.

Rhodenizer, D., Martin, I., Bhandari, P., Pletcher, S.D., Grotewiel, M., 2008. Genetic and environmental factors impact age-related impairment of negative geotaxis in *Drosophila* by altering age-dependent climbing speed. Experimental Gerontology 43, 739-748. doi: 10.1016/j.exger.2008.04.011

Rieger, D., Fraunholz, C., Popp, J., Bichler, D., Dittmann, R., Helfrich-Foerster, C., 2007. The fruit fly *Drosophila melangaster* favors dim light and times its activity peaks to early dawn and late dusk. Journal of Biological Rhythms 22, 387-399. doi: 10.1177/0748730407306198

Rigby, M.C., Hechinger, R.F., Stevens, L., 2002. Why should parasite resistance be costly? Trends in Parasitology 18, 116-120. doi: 10.1016/s1471-4922(01)02203-6

Robar, N., Burness, G., Murray, D.L., 2010. Tropics, trophics and taxonomy: the determinants of parasite-associated host mortality. Oikos 119, 1273-1280. doi: 10.1111/j.1600-0706.2009.18292.x

Robar, N., Murray, D.L., Burness, G., 2011. Effects of parasites on host energy expenditure: the resting metabolic rate stalemate. Canadian Journal of Zoology 89, 1146-1155. doi: 10.1139/z11-084

Roberts, D., 2017. Mosquito larvae can detect water vibration patterns from a nearby predator. Bulletin of Entomological Research 107, 499-505. doi: 10.1017/s0007485316001140

Robertson, J.L., Tsubouchi, A., Tracey, W.D., 2013. Larval defense against attack from parasitoid wasps requires nociceptive neurons. Plos One 8. doi: 10.1371/journal.pone.0078704

Rodriguez-Prieto, I., Martin, J., Fernandez-Juricic, E., 2011. Individual variation in behavioural plasticity: direct and indirect effects of boldness, exploration and sociability on habituation to predators in lizards. Proceedings of the Royal Society B-Biological Sciences 278, 266-273. doi: 10.1098/rspb.2010.1194

Rohr, J.R., Raffel, T.R., Hall, C.A., 2010. Developmental variation in resistance and tolerance in a multi-host-parasite system. Functional Ecology 24, 1110-1121. doi: 10.1111/j.1365-2435.2010.01709.x

Rohr, J.R., Raffel, T.R., Halstead, N.T., McMahon, T.A., Johnson, S.A., Boughton, R.K., Martin, L.B., 2013. Early-life exposure to a herbicide has enduring effects on pathogen-induced mortality. Proceedings of the Royal Society B-Biological Sciences 280. doi: 10.1098/rspb.2013.1502

Rohr, J.R., Swan, A., Raffel, T.R., Hudson, P.J., 2009. Parasites, info-disruption, and the ecology of fear. Oecologia 159, 447-454. doi: 10.1007/s00442-008-1208-6

Ryder, J.J., Hoare, M.-J., Pastok, D., Bottery, M., Boots, M., Fenton, A., Atkinson, D., Knell, R.J., Hurst, G.D.D., 2014. Disease epidemiology in arthropods is altered by the presence of nonprotective symbionts. American Naturalist 183, 89-104. doi: 10.1086/674827

Sadd, B.M., Schmid-Hempel, P., 2009. Principles of ecological immunology. Evolutionary Applications 2, 113-121. doi: 10.1111/j.1752-4571.2008.00057.x

Sadd, B.M., Siva-Jothy, M.T., 2006. Self-harm caused by an insect's innate immunity. Proceedings of the Royal Society B-Biological Sciences 273, 2571-2574. doi: 10.1098/rspb.2006.3574

Schmitz, O.J., Rosenblatt, A.E., 2017. The temperature dependence of predation stress and prey nutritional stoichiometry. Frontiers in Ecology and Evolution 5. doi: 10.3389/fevo.2017.00073

Schmitz-Esser, S., Toenshoff, E.R., Haider, S., Heinz, E., Hoenninger, V.M., Wagner, M., Horn, M., 2008. Diversity of bacterial endosymbionts of environmental *Acanthamoeba* isolates. Applied and Environmental Microbiology 74, 5822-5831. doi: 10.1128/aem.01093-08

Schulenburg, H., Kurtz, J., Moret, Y., Siva-Jothy, M.T., 2009. Introduction. Ecological immunology. The Royal Society, Philosophical Transactions of the Royal Society B, pp. 3-14.

Schultzhaus, J.N., Bennett, C.J., Iftikhar, H., Yew, J.Y., Mallett, J., Carney, G.E., 2018. High fat diet alters *Drosophila melanogaster* sexual behavior and traits: decreased attractiveness and changes in pheromone profiles. Scientific Reports 8. doi: 10.1038/s41598-018-23662-2

Schwarz, H.H., Starrach, M., Koulianos, S., 1998. Host specificity and permanence of associations between Mesostigmatic mites (Acari : Anactinotrichida) and burying beetles (Coleoptera : Silphidae : Nicrophorus). Journal of Natural History 32, 159-172. doi: 10.1080/00222939800770101

Schwenke, R.A., Lazzaro, B.P., Wolfner, M.F., 2016. Reproduction-Immunity Trade-Offs in Insects. Annual Review of Entomology, Vol 61 61, 239-256. doi: 10.1146/annurev-ento-010715-023924

Sears, B.F., Snyder, P.W., Rohr, J.R., 2015. Host life history and host-parasite syntopy predict behavioural resistance and tolerance of parasites. Journal of Animal Ecology 84, 625-636. doi: 10.1111/1365-2656.12333

Sgro, C.M., Partridge, L., 2000. Evolutionary responses of the life history of wild-caught *Drosophila melanogaster* to two standard methods of laboratory culture. American Naturalist 156, 341-353. doi: 10.1086/303394

Shaw, P.J., Cirelli, C., Greenspan, R.J., Tononi, G., 2000. Correlates of sleep and waking in *Drosophila melanogaster*. Science 287, 1834-1837. doi: 10.1126/science.287.5459.1834

Sheldon, B.C., Verhulst, S., 1996. Ecological immunology: Costly parasite defences and trade-offs in evolutionary ecology. Trends in Ecology & Evolution 11, 317-321. doi: 10.1016/0169-5347(96)10039-2

Sheridan, L.A.D., Poulin, R., Ward, D.F., Zuk, M., 2000a. Sex differences in parasitic infections among arthropod hosts: is there a male bias? Oikos 88, 327-334. doi: 10.1034/j.1600-0706.2000.880211.x

Sheriff, M.J., Krebs, C.J., Boonstra, R., 2009. The sensitive hare: sublethal effects of predator stress on reproduction in snowshoe hares. Journal of Animal Ecology 78, 1249-1258. doi: 10.1111/j.1365-2656.2009.01552.x

Sheriff, M.J., Peacor, S.D., Hawlena, D., Thaker, M., 2020. Non-consumptive predator effects on prey population size: A dearth of evidence. Journal of Animal Ecology 89, 1302-1316. doi: 10.1111/1365-2656.13213

Short, S.M., Wolfner, M.F., Lazzaro, B.P., 2012. Female *Drosophila melanogaster* suffer reduced defense against infection due to seminal fluid components. Journal of Insect Physiology 58, 1192-1201. doi: 10.1016/j.jinsphys.2012.06.002

Simon, J.C., Dickson, W.B., Dickinson, M.H., 2011. Prior mating experience modulates the dispersal of *Drosophila* in males more than in females. Behavior Genetics 41, 754-767. doi: 10.1007/s10519-011-9470-5

Sloman, K.A., Motherwell, G., O'Connor, K.I., Taylor, A.C., 2000. The effect of social stress on the standard metabolic rate (SMR) of brown trout, *Salmo trutta*. Fish Physiology and Biochemistry 23, 49-53. doi: 10.1023/a:1007855100185

Slos, S., De Meester, L., Stoks, R., 2009. Food level and sex shape predator-induced physiological stress: immune defence and antioxidant defence. Oecologia 161, 461-467. doi: 10.1007/s00442-009-1401-2

Slos, S., Stoks, R., 2008. Predation risk induces stress proteins and reduces antioxidant defense. Functional Ecology 22, 637-642. doi: 10.1111/j.1365-2435.2008.01424.x

Smith, R.L., 1980. Ecology and Field Biology, 3rd Edition ed. Harper & Row, Publishers, New York, New York, United States of America.

Smith, W.W., Thomas, J., Liu, J., Li, T., Moran, T.H., 2014. From fat fruit fly to human obesity. Physiology & Behavior 136, 15-21. doi: 10.1016/j.physbeh.2014.01.017

Sniegula, S., Nsanzimana, J.d.A., Johansson, F., 2019. Predation risk affects egg mortality and carry over effects in the larval stages in damselflies. Freshwater Biology 64, 778-786. doi: 10.1111/fwb.13261

Sniegula, S., Raczynski, M., Golab, M.J., Johansson, F., 2020. Effects of predator cues carry over from egg and larval stage to adult life-history traits in a damselfly. Freshwater Science 39, 804-811. doi: 10.1086/711374

Soller, M., Bownes, M., Kubli, E., 1997. Mating and sex peptide stimulate the accumulation of yolk in oocytes of *Drosophila melanogaster*. European Journal of Biochemistry 243, 732-738. doi: 10.1111/j.1432-1033.1997.00732.x

Soller, M., Bownes, M., Kubli, E., 1999. Control of oocyte maturation in sexually mature *Drosophila* females. Developmental Biology 208, 337-351. doi: 10.1006/dbio.1999.9210

Srygley, R.B., 2012. Age- and Density-Dependent Prophylaxis in the Migratory, Cannibalistic Mormon Cricket *Anabrus simplex* (Orthoptera: Tettigoniidae). Environmental Entomology 41, 166-171. doi: 10.1603/en11020

Stearns, S.C., 1989. Trade-offs in life-history evolution. Functional Ecology 3, 259-268. doi: 10.2307/2389364

Steinauer, M.L., Bonner, K.M., 2012. Host susceptibility is altered by light intensity after exposure to parasites. Journal of Parasitology 98, 1052-1054. doi: 10.1645/ge-3109.1

Steiner, U.K., Van Buskirk, J., 2009. Predator-induced changes in metabolism cannot explain the growth/predation risk tradeoff. Plos One 4. doi: 10.1371/journal.pone.0006160

Stillwell, R.C., Blanckenhorn, W.U., Teder, T., Davidowitz, G., Fox, C.W., 2010. Sex differences in phenotypic plasticity affect variation in sexual size dimorphism in insects: from physiology to evolution. Annual Review of Entomology 55, 227-245. doi: 10.1146/annurev-ento-112408-085500

Stucki, D., Freitak, D., Bos, N., Sundstrom, L., 2019. Stress responses upon starvation and exposure to bacteria in the ant *Formica exsecta.* Peerj 7. doi: 10.7717/peerj.6428

Swartz, T.E., Tseng, T.-S., Frederickson, M.A., Paris, G., Comerci, D.J., Rajashekara, G., Kim, J.-G., Mudgett, M.B., Splitter, G.A., Ugalde, R.A., Goldbaum, F.A., Briggs, W.R., Bogomolni, R.A., 2007.

Blue-light-activated histidine kinases: two-component sensors in bacteria. Science 317, 1090-1093. doi: 10.1126/science.1144306

Szentivanyi, T., Vincze, O., Estok, P., 2017. Density-dependent sex ratio and sex-specific preference for host traits in parasitic bat flies. Parasites and Vectors 10, article number 405.

Takahashi, Y., Watanabe, M., 2010. Female reproductive success is affected by selective male harassment in the damselfly by *Ischnura senegalensis*. Animal Behaviour 79, 211-216. doi: 10.1016/j.anbehav.2009.10.032

Therneau T (2022). A package for survival analysis in R. R package version 3.4-0, https://CRAN.R-project.org/package=survival.

Thornhill, R., Fincher, C.L., 2013. The parasite-driven-wedge model of parapatric speciation. Journal of Zoology 291, 23-33. doi: 10.1111/jzo.12070

Tinkerhess, M.J., Healy, L., Morgan, M., Sujkowski, A., Matthys, E., Zheng, L., Wessells, R.J., 2012. The *Drosophila* PGC-1 alpha Homolog spargel modulates the physiological effects of endurance exercise. Plos One 7. doi: 10.1371/journal.pone.0031633

Tixier, M., 2018. Predatory Mites (Acari: Phytoseiidae) in Agro-Ecosystems and Conservation Biological Control: A Review and Explorative Approach for Forecasting Plant-Predatory Mite Interactions and Mite Dispersal. Frontiers in Ecology and Evolution 14. Doi 10.3389/fevo.2018.00192

Tollrian, R., Duggen, S., Weiss, L.C., Laforsch, C., Kopp, M., 2015. Density-dependent adjustment of inducible defenses. Scientific Reports 5. doi: 10.1038/srep12736

Tsuda, Y., 1982. Reproductive strategy of insects as adaptation to temporally varying environments. Researches on Population Ecology 24, 388-404. doi: 10.1007/bf02515584

Uysal, H., Genc, S., Ayar, A., 2017. Toxic effects of chronic feeding with food azo dyes on *Drosophila melanogaster* Oregon R. Scientia Iranica 24, 3081-3086. doi: 10.24200/sci.2017.4523

Van Dievel, M., Janssens, L., Stoks, R., 2016. Short- and long-term behavioural, physiological and stoichiometric responses to predation risk indicate chronic stress and compensatory mechanisms. Oecologia 181, 347-357. doi: 10.1007/s00442-015-3440-1

Van Voorhies, W.A., Khazaeli, A.A., Curtsinger, J.W., 2003. Selected Contribution: Long-lived *Drosophila melanogaster* lines exhibit normal metabolic rates. Journal of Applied Physiology 95, 2605-2613. doi: 10.1152/japplphysiol.00448.2003

Van Voorhies, W.A., Khazaeli, A.A., Curtsinger, J.W., 2004. Lack of correlation between body mass and metabolic rate in *Drosophila melanogaster*. Journal of Insect Physiology 50, 445-453. doi: 10.1016/j.jinsphys.2004.03.002

Venables WN, Ripley BD (2002). Modern Applied Statistics with S, Fourth edition. Springer, New York. ISBN 0-387-95457-0, https://www.stats.ox.ac.uk/pub/MASS4/.

Videlier, M., Rundle, H.D., Careau, V., 2019. Sex-specific among-individual covariation in locomotor activity and resting metabolic rate in *Drosophila melanogaster*. American Naturalist 194, 164-176. doi: 10.1086/705678

Walkey, M., Meakins, R.H., 1970. An attempt to balance the energy budget of a host-parasite system. Journal of Fish Biology 2, 361-&. doi: 10.1111/j.1095-8649.1970.tb03294.x

Walsh, M.R., Cooley, F., Biles, K., Munch, S.B., 2015. Predator-induced phenotypic plasticity within- and across-generations: a challenge for theory? Proceedings of the Royal Society B-Biological Sciences 282. doi: 10.1098/rspb.2014.2205

Walter DE, Proctor H. 2013 Systemic and morphological survey. In Mites: ecology, evolution & behaviour: life at a microscale, 2nd edn, pp. 39–68. Dordrecht, The Netherlands: Springer.

Watts, T., Haselkorn, T.S., Moran, N.A., Markow, T.A., 2009. Variable incidence of *Spiroplasma* infections in natural populations of *Drosophila* species. Plos One 4. doi: 10.1371/journal.pone.0005703

Wedekind, C., Jakobsen, P.J., 1998. Male-biased susceptibility to helminth infection: an experimental test with a copepod. Oikos 81, 458-462. doi: 10.2307/3546767

Weinstein, S.B., Buck, J.C., Young, H.S., 2018. A landscape of disgust. Science 359, 1213-1214. doi: 10.1126/science.aas8694

Wheeler, D.A., Hamblencoyle, M.J., Dushay, M.S., Hall, J.C., 1993. Behavior in light dark cycles of *Drosophila* mutants that are arrhythmic, blind, or both. Journal of Biological Rhythms 8, 67-94. doi: 10.1177/074873049300800106

White, J.A., Styer, A., Rosenwald, L.C., Curry, M.M., Welch, K.D., Athey, K.J., Chapman, E.G., 2020. Endosymbiotic bacteria are prevalent and diverse in agricultural spiders. Microbial Ecology 79, 472-481. doi: 10.1007/s00248-019-01411-w

Wickhamn, H., Averick, M., Bryan, J., Chang, W., McGowan, L.D., François, R., Grolemund, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Pedersen, T.L., Miller, E., Bache, S.M., Müller, K., Ooms, J., Robinson D., Seidel, D.P., Spinu, V., Takahashi K., Vaughan, D., Wilke, C., Woo, K., Yutani, K., 2019. Welcome to the Tidyverse. The Journal of Open Source Software, 4, 1686.

Wielgoss, S., Bergmiller, T., Bischofberger, A.M., Hall, A.R., 2016. Adaptation to parasites and costs of parasite resistance in mutator and nonmutator bacteria. Molecular Biology and Evolution 33, 770-782. doi: 10.1093/molbev/msv270

Wigby, S., Chapman, T., 2005. Sex peptide causes mating costs in female *Drosophila melanogaster*. Current Biology 15, 316-321. doi: 10.1016/j.cub.2005.01.051

Wilber, M.Q., Weinstein, S.B., Briggs, C.J., 2016. Detecting and quantifying parasite-induced host mortality from intensity data: method comparisons and limitations. International Journal for Parasitology 46, 59-66. doi: 10.1016/j.ijpara.2015.08.009

Windsor, D.A., 1998. Most of the species on Earth are parasites. International Journal for Parasitology 28, 1939-1941. doi: 10.1016/s0020-7519(98)00153-2

Wone, B.W.M., Madsen, P., Donovan, E.R., Labocha, M.K., Sears, M.W., Downs, C.J., Sorensen, D.A., Hayes, J.P., 2015. A strong response to selection on mass-independent maximal metabolic rate without a correlated response in basal metabolic rate. Heredity 114, 419-427. doi: 10.1038/hdy.2014.122

Xie, J., Butler, S., Sanchez, G., Mateos, M., 2014. Male killing *Spiroplasma* protects *Drosophila melanogaster* against two parasitoid wasps. Heredity 112, 399-408. doi: 10.1038/hdy.2013.118

Xie, J.L., Vilchez, I., Mateos, M., 2010. Spiroplasma bacteria enhance survival of *Drosophila hydei* attacked by the parasitic wasp *Leptopilina heterotoma*. Plos One 5. doi: 10.1371/journal.pone.0012149

Xie, J.L., Winter, C., Winter, L., Mateos, M., 2015. Rapid spread of the defensive endosymbiont *Spiroplasma* in *Drosophila hydei* under high parasitoid wasp pressure. Fems Microbiology Ecology 91. doi: 10.1093/femsec/fiu017

Xie, K., Lu, Y.J., Yang, K., Huo, S.M., Hong, X.Y., 2020. Co-infection of *Wolbachia* and *Spiroplasma* in spider mite *Tetranychus truncatus* increases male fitness. Insect Science 27, 921-937. doi: 10.1111/1744-7917.12696

Xie, X.B., Huang, Z.Y., Zeng, Z.J., 2016. Why do *Varroa* mites prefer nurse bees? Scientific Reports 6. doi: 10.1038/srep28228

Xiong, X.F., Michaud, J.P., Li, Z., Wu, P.X., Chu, Y.N., Zhang, Q.W., Liu, X.X., 2015. Chronic, predator-induced stress alters development and reproductive performance of the cotton bollworm, *Helicoverpa armigera*. Biocontrol 60, 827-837.

Xue, L., Noll, M., 2000. *Drosophila* female sexual behavior induced by sterile males showing copulation complementation. Proceedings of the National Academy of Sciences of the United States of America 97, 3272-3275. doi: 10.1073/pnas.060018897

Yadav, S., Frazer, J., Banga, A., Pruitt, K., Harsh, S., Jaenike, J., Eleftherianos, I., 2018. Endosymbiont-based immunity in *Drosophila melanogaster* against parasitic nematode infection. Plos One 13. doi: 10.1371/journal.pone.0192183

Yanagawa, A., Guigue, A.M.A., Marion-Poll, F., 2014. Hygienic grooming is induced by contact chemicals in *Drosophila melanogaster*. Frontiers in Behavioral Neuroscience 8. doi: 10.3389/fnbeh.2014.00254

Yanagawa, A., Neyen, C., Lemaitre, B., Marion-Poll, F., 2017. The gram-negative sensing receptor PGRP-LC contributes to grooming induction in *Drosophila*. Plos One 12. doi: 10.1371/journal.pone.0185370

Yapici, N., Kim, Y.-J., Ribeiro, C., Dickson, B.J., 2008. A receptor that mediates the post-mating switch in *Drosophila* reproductive behaviour. Nature 451, 33-U31. doi: 10.1038/nature06483

Yule, K.J., Burns, K.C., 2019. Parasite - offspring competition for female resources can explain male-biased parasitism in plants. Biology Letters 15. doi: 10.1098/rsbl.2018.0761

Zhang, C., Goitom, E., Brans, K., De Meester, L., Stoks, R., 2022a. Scared to evolve? Non-consumptive effects drive rapid adaptive evolution in a natural prey population. Proceedings. Biological sciences 289, 20220188-20220188. doi: 10.1098/rspb.2022.0188

Zhang, W.-F., Liu, L.-M., He, S., Lu, B.-Y., Luo, Y.-B., 2022b. Production and evolution pattern of "fruity smell" aggregation pheromones in genus *Drosophila*. Journal of Systematics and Evolution 60, 208-219. doi: 10.1111/jse.12648

Zhukovskaya, M., Yanagawa, A., Forschler, B.T., 2013. Grooming behavior as a mechanism of insect disease defense. Insects 4, 609-630.

Zuk, M., Stoehr, A.M., 2002. Immune defense and host life history. American Naturalist 160, S9-S22. doi: 10.1086/342131

Zukowski, N., Kirk, D., Wadhawan, K., Shea, D., Start, D., Krkosek, M., 2020. Predators can influence the host-parasite dynamics of their prey via nonconsumptive effects. Ecology and Evolution 10, 6714-6722. doi: 10.1002/ece3.6401

Appendices

Appendix 1: Additional information on system and methods

Additional background on Drosophila nigrospiracula and *Macrocheles subbadius*

Drosophila nigrospriacula inhabits the Sonoran desert. In its northern range it exploits rotting *Carnegiea gigantea* as a food source and lays eggs on rotting cactus tissue [1]. The flies and mites used in this thesis were collected from *C. gigantea* in Arizona (Phoenix, USA). Flies were aspirated from the cactus directly, and mites were collected from wild caught flies. In the southern range the fly exploits rots of *Pachycereus pringlei*. Population growth of *D. nigrospiracula* populations are significantly higher on its natural host plants than when artificially seeded on non-host plants, suggesting flies are restricted in their selection of habitats [1]. In fact, >99% of wild caught *D. nigrospiracula* were found on their host cacti [1]. Eggs to adult emergence takes ~2 weeks, and flies are reproductively mature in 4-7 days post-emergence.

Macrocheles subbadius is a facultative ectoparasite of flies. Radiolabelling studies show that these mites consume internal tissues of flies [2], and the large claw-like chelicerae suggest this is the hemolymph of the fly (not the fat body, as with another Mesostigmatid mite, *Varroa destructor*, [3]). Mesostigmata is a diverse order of mites, including both free-living predators (e.g. the family Phytoseiidae, used as a biocontrol of other small invertebrate pests) and obligate parasites (e.g. the red poultry mite *Dermanyssus gallinae* order: Dermanyssidae) [4; 5]. By comparison, *M. subbadius* can successfully complete its life cycle without a host, i.e. are facultative parasites, but achieve higher fitness if they feed on a fly host [6]. The mite generation is <10 days, and many generations of mites can occur between colonisation of a new cactus and dispersal (attached to a host fly).

Y-maze / Preference experiments

These experiments tested if flies had a preference for one of two flies. Flies were glued down to cotton to eliminate resistance and placed in a y-shaped piece of tubing (Fisherbrand Tubing, Y Polypropylene Connector). A single adult female fly was placed into the third arm of the maze, and the end was sealed with cotton. Y-mazes laid flat on the surface, and were covered with an opaque box to exclude light. After the experimental period, the chamber was inspected and which fly, if either, was infected was recorded.

Across trials I alternated which arm the fly was in. For example if the pairs of flies were unmated and mated females, the mated female would be on the left 50% of the time and the right 50% of the time. Between experiments, y-mazes were washed with detergent and water, sterilized with 70% ethanol, then rinsed with distilled water.

Figure A1.1: Y Polypropylene Connector (~0.5 cm diameter) used in y-maze, i.e. preference, experiments with ruler for scale. Flies were placed in the bent arms (right side of figure), and a mite was placed in the straight arm (left side of figure). Y-maze is displayed horizontal, as it was orientated during assays.

Micro-arena resistance experiments

These experiments tested the infection outcome of flies exposed individually to a mite(s). A single fly was placed into a semi-translucent cropped polypropylene pipette tip (Axygen T-200-Y, 1-200µL Yellow Tips) with 1 or more mites (see specific chapter / experiment) and the ends were sealed with cotton. The chamber was either placed under an opaque box or left under ambient light (fluorescent) depending on the experiment (see section 3.2). After the experimental period flies were inspected and the number of infections and/or if infection occurs was recorded.

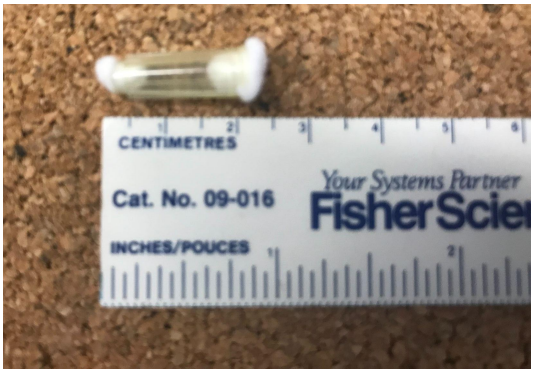


Figure A1.2: Cropped pipette tip (~0.5 cm internal diameter) used as a micro-arena. Individual flies were exposed to mite(s) either in the dark or the light. Following the exposure period, the number/presence of mite infections was recorded.

Negative geotaxis endurance assays

These experiments tested the endurance, a proxy measure of resistance that eliminates the effects of mite proclivity to infect, of flies by measuring induced climbing. Individual flies were placed into vials with a mark 5-6 cm above the base (see specific experiment). *D. nigrospiracula* require at least an hour of acclimation before climbing readily, whereas *D.melanogaster* are willing to initiate repeat climbing in <10 minutes (pers. observation). For that reason, *D.nigrospiracula* trials were conducted on fresh agar vials to minimize desiccation-stress during the acclimation period. When flies ascended to the mark, the knockdown was repeated. We defined exhaustion as when flies no longer ascended within 10-15 s of the drop (*D. melanogaster* were slightly faster to climb, and therefore given less time before being considered exhausted). We recorded the number of times the fly climbed to the line (cycles) and the length of time until exhaustion (seconds, s).

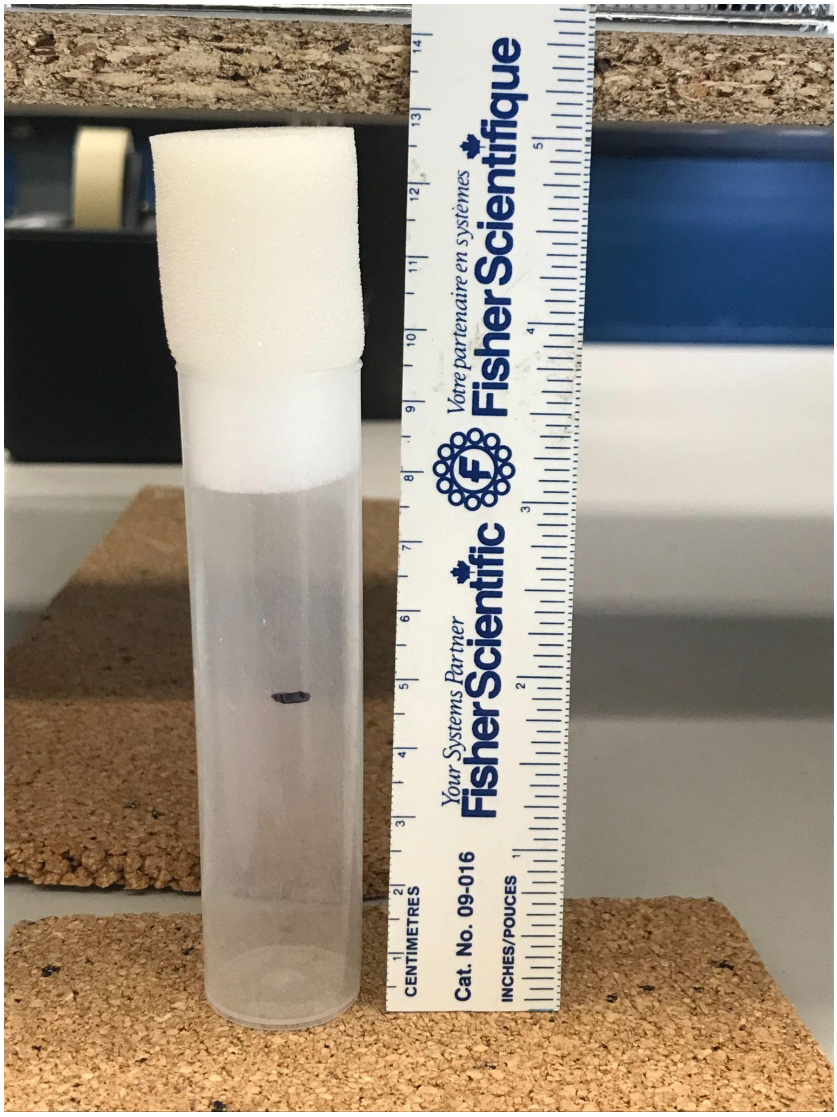
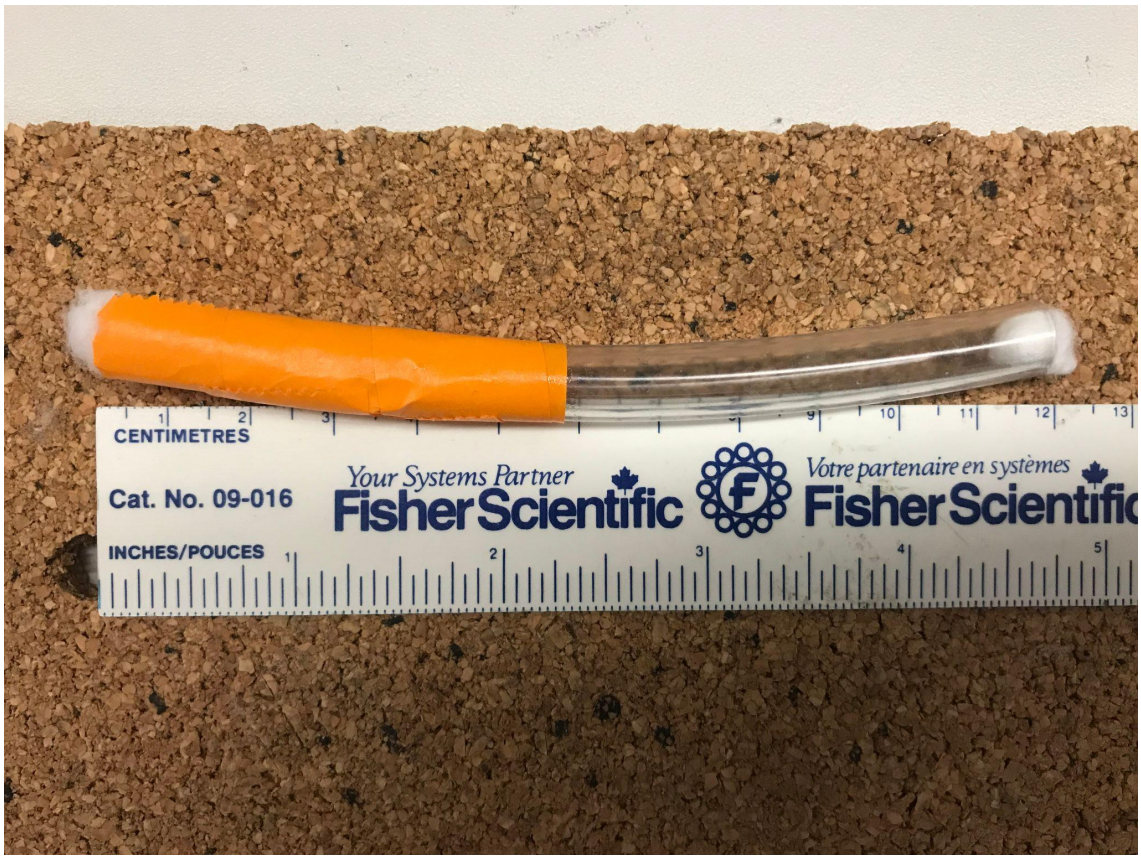


Fig A1.3: Empty plastic vial (2 cm diameter) used for geotaxis experiments. Individual flies were induced to climb until exhaustion via repeated knock-downs. Approximately 0.5 cm of cork padding, visible in photo, was the surface against which the vial was tapped. This padding mainly served to make lab mates less angry at the noise and therefore less likely to disrupt trials

Phototaxis chambers

These chambers tested if flies had a preference for a lighter or darker environment. Tubes consisted of Bev-A-Line blacked out with two layers of opaque tape and sealed with cotton. A fly was placed at the midpoint (partially in dark, partially in light) to begin the experiment. The light condition was under standard laboratory light (fluorescent). Between trials, chambers were cleaned with a detergent wash and then an ethanol (70%) wash.



FigA1.4: Phototaxis chamber (~12 cm long X ~1 cm diameter) used to test if flies prefer a light or darker environment. Chambers were scanned once a minute and the location of the organism was marked.

Appendix 2: *Macrocheles subbadius* preferentially infect larger *Drosophila nigrospiracula*.

This additional experiment tested if mites have a preference for larger (by mass) flies over smaller flies. In conjunction with the preference experiments in section 3.2, this experiment lends further support to the hypothesis that the mite preference for female over male flies is size-mediated. These data were collected in collaboration with Caroline Liang.

Method: Female *D.nigrospiracula* were weighed, and high mass flies and low mass flies were paired. Flies were tethered to cotton with Elmer's Rubber Cement, eliminating differences in resistance (Campbell and Luong 2016). Pairs of flies were placed into y-mazes. A single adult female mite was introduced to the arena and the ends were sealed with cotton. Y-mazes were placed under an opaque box to exclude light. After one hour we recorded if the mite infected the heavy fly, light fly, or neither. A binomial test (binom.test, R Stats) was used to test if flies had a preference for high or low mass flies ($H_{o:}$ proportion=0.5).

Result: In 21 of 43 (49%) pairs, the mite infected one of the flies. Among trials where infection occurred (N=21), 18 (86%) mites infected the heavier fly and 3 (14%) infected the lighter fly (Fig. A2.1). The heavy fly was significantly more likely to be infected (binom.test, P=0.0015). The mean mass of the heavy fly was 3.00±0.04 mg (Mean±SEM), and the mean mass of the light fly was 2.22±0.05 mg. In absolute terms the average difference between the heavy and light flies was 0.78±0.06 mg, and the average percent difference was 30.2%. The smallest absolute difference was 0.44 mg or 16%. The largest absolute difference was 1.36 mg or 54% difference.

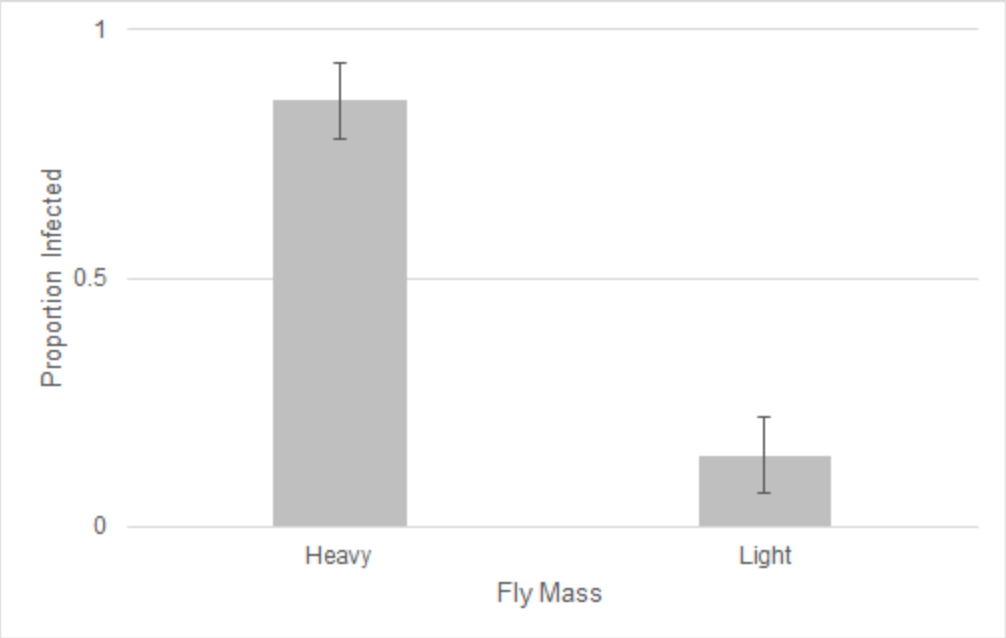


Figure A2.1: Proportion of flies infected by mites in a y-maze preference experiment. Mites had a choice between a heavy and light fly in a y-maze; flies were glued down to eliminate differential resistance. Error bars represent 1 Standard Error of the Proportion.

Additional references in appendix

1) Fellows, D.P., Heed, W.B., 1972. Factors Affecting Host Plant Selection in Desert-Adapted Cactiphilic *Drosophila*. Ecology 53: 850-858.
2) Polak, M., 1996. Ectoparasitic effects on host survival and reproduction: The *Drosophila-Macrocheles* association. Ecology 77, 1379-1389. doi: 10.2307/2265535
3) Ramsey, S.D., Ochoa, R., Bauchan, G., vanEngelsdorp, D., 2019. *Varroa destructor* feeds primarily on honey bee fat body tissue and not hemolymph. PNAS 116, 1792-1801.
4) Tixier, M., 2018. Predatory Mites (Acari: Phytoseiidae) in Agro-Ecosystems and Conservation Biological Control: A Review and Explorative Approach for Forecasting Plant-Predatory Mite Interactions and Mite Dispersal. Frontiers in Ecology and Evolution 14. Doi 10.3389/fevo.2018.00192
5) Murillo, A.C., Mullens, B.A., 2020. Collecting and Monitoring for Northern Fowl Mite (Acari: Macronyssidae) and Poultry Red Mite (Acari: Dermanyssidae) in Poultry Systems. Journal of Insect Science 20: 12.
6) Luong, L.T., Subasinghe, D., 2017. A facultative ectoparasite attains higher reproductive success as a parasite than its free-living conspecifics. Experimental and Applied Acarology 71, 63-70. doi: 10.1007/s10493-016-0098-2