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Metabolic and Behavioural Response of *Drosophila nigrospiracula* to Ectoparasite Infection

by

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A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science
in
Ecology

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Abstract

Parasite aggregation is a population-based metric in which many hosts harbour few parasites but some hosts are infected by a large number of parasites. The causes of aggregation are primarily attributed to heterogeneity in host exposure and susceptibility. However, parasites can exert numerous effects upon their hosts, including physiological and metabolic changes that can in turn influence various aspects of host life history. I hypothesized that the parasites themselves can potentially generate aggregation within host populations. Host behavioural defences can vary depending on intrinsic and extrinsic factors, such as current infection status, yet few researchers have examined the impact of current infection on the efficacy of host defences against future parasite attack. To test my hypothesis, I used the *Drosophila nigrospiracula-Macrocheles subbadius* host-ectoparasite study system. I predicted that increasing mite load would increase susceptibility to future mite attachment. I also predicted that the increase in susceptibility would be mediated by a parasite-induced reduction in host defensive behaviours. I used laboratory experiments and an activity monitor to: (1) determine the relationship between parasitic infection intensity and host susceptibility and (2) examine the effect of infection intensity on a host's overall level of activity when exposed to another parasite. Results indicate that host susceptibility to future infection increased with higher current infection intensity. Activity of infected hosts change, though not in the expected direction, based on infection intensity and host sex. Parasites may also be able to affect other host traits such as host respiration that in turn may influence mite selection for certain hosts. Using flow-through respirometry, I investigated how attachment by parasites and infection intensity of the mite affects the respiratory rate of the host. In a before-and-after mite

attachment experiment, the mean respiratory rate (CO_2 production) of flies increased after infection by mites. I also found that mean fly respiratory rate increased with infection intensity, with the strongest effect occurring with 3 mites. Changes in host metabolism did not appear to be mediated wholly through increased activity among infected flies. These results show that infection by ectoparasites carry metabolic cost for hosts in an intensity-dependent manner. All together, I identify a mechanism by which a parasite alters host susceptibility and parasite load, indicating the importance of examining parasite-driven effects on aggregation within a host-parasite system.

Preface

Chapters 1-4 of this thesis are original work done by Taylor R. Brophy. No part of these chapters has previously been published.

Dedication

To all those who have helped me up to where I am now with unwavering support, especially my family including my late grandmother, Edith Lappi

Acknowledgements

This thesis research was funded in part by the Natural Sciences and Engineering Research Council.

I would like to thank my advisor, Dr. Lien Luong, for all her help. Her knowledge, guidance, and advice were invaluable throughout my degree, especially with my bull-headed approach to writing.

I would also like to thank my supervisory committee member Dr. Maya Evenden for her support and input along the way.

I thank Dr. Clement Lagrue, for his time in serving on my examination committee.

I would also like to thank my lab mates, office mates, friends and colleagues that helped me during my time.

Dr. Emily Durkin: You have been a huge help and mentor throughout this whole process. I can't say just how helpful you were over the years but you are plain fantastic. Thank you for absolutely everything!

Collin, Monika, Maesha, Kieran, and Taekwan: Without your hard work, many of my experiments would not have been possible or completed. Thank you for the time you devoted to the lab.

Courtney Ramsay and Zola: All your love and support got me through many of the dark periods during the past year when I didn't know if I would make it.

Courtney MacInnis and Kyle: Thank you for the many interesting discussions and forputting up with me in the office much longer than I thought you would.

Andrew: Thank you for the impromptu R sessions and the discussions on many different topics.

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Chapter 1. General introduction

1.1 Overview

Parasite aggregation is a well known and studied phenomenon within host-parasite systems (Shaw and Dobson, 1995; Shaw *et al.*, 1998; Poulin, 2013), and considered a general rule for macroparasites (Poulin, 2007). In short, aggregation is the pattern of many hosts harbouring few or no parasites while a few hosts are infected by many parasites. Aggregation is classically attributed to system and individual dependent variation in rates of acquisition and loss of parasites (Shaw *et al.*, 1998). Acquisition rates can be further broken down into heterogeneity in exposure or heterogeneity in susceptibility to infection, which can arise from intrinsic host traits such as age (Raffel *et al.*, 2011), sex (Zuk and McKean, 1996), behaviour, or immunity (Poulin, 2013).

Attempts to unravel the factors that produce the observed patterns of aggregation would benefit from more experimental studies (Shaw and Dobson, 1995), and explicit analysis should be conducted on potential sources of heterogeneities within a sample host population (Shaw *et al.*, 1998). A large portion of the variation in aggregation is attributed to the mean parasite burden. (Shaw and Dobson, 1995). Among the remaining 12-13% of variation, a substantial portion was attributed to the parasites themselves (Poulin, 2013). Parasitic lifestyle, in particular, is important in measuring aggregation, as ectoparasites and the majority of parasitic helminths were found to be more aggregated than cestode infections (Shaw and Dobson, 1995).Yet, most studies on aggregation have focused on host-driven heterogeneities (Shaw and Dobson, 1995; Poulin, 2013). Here, I investigate the role that parasites themselves play in generating aggregation.

Using the facultative ectoparasite, *Macrocheles subbadius* (Mesostigmata:Macrochelidae) Berlese I investigated how parasites themselves contribute directly and indirectly to patterns of aggregation in a host-parasite system. I examined host traits that current infections might alter, which could in turn influence the probability of that host acquiring more parasites (Luong *et al.*, 2017a). As mites are more likely to infect hosts with a higher current infection and higher relative metabolism, I examined how infection and infection intensity influenced host respiratory rates as a potential explanation behind host selection (Luong *et al.*, 2017a; Horn *et al.*, 2018). I also

investigated infection and intensity based variation in behavioural defences of hosts along with their susceptibility to infection. Together these experiments determined if parasite infection acted as a source of heterogeneity in host susceptibility.

1.2 Background

The detrimental effects of macroparasitic infection are not just dependent on parasite prevalence, but can depend on the intensity of infection. As infection intensity increases, hosts experience increased mortality (Shaw & Dobson, 1995). The intensity of parasitic infection can also influence a host's abilities to defend itself against other natural enemies such as predators (Luong *et al.*, 2011) Higher intensities of infection can even have negative effects on host secondary sexual traits such as host plumage (Thompson *et al.*, 1997).

Parasites themselves have been suggested as a potentially important factor in parasite aggregation (Duerr *et al.*, 2003). However the current debate centers on whether parasite aggregation is a cause or consequence, particularly in some systems in which parasites manipulate host behaviour. Some studies suggest that parasites influence aggregation via parasite-induced host mortality (Wilber *et al.*, 2016). Aggregation driven by ectoparasites themselves was suggested long ago (Bull, 1978). Pheromone cues in some ectoparasite species are capable of influencing the spatial heterogeneity of parasites, producing aggregation in the host system (My yen *et al.*, 1980; Petney and Bull, 1981; Leahy *et al.*, 1983). For instance, parasites can change host exposure to and infection by other parasites, thereby influencing aggregation dynamics. Ultimately, parasite-induced aggregation could have fitness benefits for individual parasites due to conspecifics overwhelming host defences, reducing heterospecific competition, mate finding (for itself or offspring), and protection from natural enemies/host defences (Wertheim *et al.*, 2005; Morrill and Forbes, 2015; Morrill *et al.*, 2017).

The ectoparastic mite, *M. subbadius* infects a range of insect hosts, including *Drosophila nigrospiracula* (Diptera:Drosophilidae) Patterson & Wheeler. The level of parasite aggregation is environment-dependent; for instance, infection intensity increases with the age of rot in the host plant, the Saguaro cactus (Polak and Markow, 1995). Importantly, mites are more likely to infect hosts with higher infection loads and higher

relative respiratory rates (Luong *et al.*, 2017a; Horn *et al.*, 2018). These factors could be generating parasite-mediated aggregation. Flies display twitching and bursts of movement as pre-attachment behavioural defence (Polak, 2003). If mites physically interfere with these behaviours and/or infection imposes energetic constraints that reduce anti-parasite defences, it could generate further aggregation.

1.3 Specific Questions and Objectives

1) Does infection alter host metabolism and is the response intensity-dependent? Some authors indicate that there is no universal trend in changes in host metabolic rates due to parasite infection (Robar *et al.*, 2011). However mites preferentially select heavily infected individuals (Luong *et al.*, 2017a) and flies with higher relative respiratory rate (Horn *et al.*, 2018). Therefore, in Chapter 2, I examined whether higher infection intensities cause an increase in host respiration, and whether this could be the mechanistic link between the two previous findings. Changes in host activity and mite respiration were also examined as possible explanations for increased rates of respiration among infected flies. I used flow-through respirometry to measure the rate of CO_2 production (a proxy for metabolic rate) of individual flies. Host activity was collected using infrared proximity sensor-based activity monitors.

2) Can parasites influence their host's susceptibility to future infection by more parasites? While parasite manipulation of host susceptibility to predation is a well-documented and studied phenomenon (Luong *et al.*, 2011; Poulin and Maure, 2015; Schutgens *et al.*, 2015), the hypothesis that current infection can change susceptibility to future infection by conspecific is less well studied (Welicky and Sikkel, 2015). In Chapter 3, I examined how infection intensity influences susceptibility to further infection, and whether changes in host activity mediate this relationship.

Chapter 2. Ectoparasite attachment induces higher metabolic rate in hosts

2.1 Introduction

Hosts can experience significant fitness losses due to parasitism (Minias, 2015; Chakraborty *et al.*, 2017; Welicky *et al.*, 2017). Parasites can cause damage through several direct actions: obtaining nutrients from the host (Seguel and Gottdenker, 2017), eliciting an immune response (Colditz, 2008), releasing toxins (Starkl Renar *et al.*, 2016), or inducing morphological alterations in the host (Johnson *et al.*, 2002). In addition, long-term energy imbalances in the host (Walkey and Meakins, 1970) may be arise if energy is diverted towards anti-parasitic behavioural defences (Slavík *et al.*, 2017) and/or cellular and tissue repair (Goetz *et al.*, 2016). Perturbations in energy allocation can reduce host fitness by reducing reproductive success (Vollset *et al.*, 2014; Bui *et al.*, 2016) and/or longevity (Morand and Harvey, 2000).

Energetic perturbations can be detected by monitoring changes in whole body metabolism (Scantlebury *et al.*, 2007). A previous meta-analysis, however, suggested that there is no universal trend in how parasite infections affect host metabolism (Robar *et al.*, 2011). Robar et al. (2011) indicated that differences in parasite infection intensity might explain some variation in metabolic responses. Among some mammalian hosts such as bats, chipmunks, and moles, there appears to be an intensity-dependent increase in respiration rate when infected by ectoparasites (Giorgi *et al.*, 2001; Careau *et al.*, 2010; Novikov *et al.*, 2015). However, not all host species display intensity-dependent increases in respiration after infection. Molluscs have higher metabolic rates when infected with the external cysts of *Macravestibulum obtusicaudum* but the effect was not intensity-dependent (Chodkowski and Bernot, 2017). Still, some fish and amphibian hosts display lower standard metabolic rates even as infection intensity increases (Filipsson *et al.*, 2017; Moretti *et al.*, 2017).

While numerous studies have examined the respiration of insects (Contreras and Bradley, 2009; Karise *et al.*, 2010; Basson and Terblanche, 2011; Snelling *et al.*, 2011), few have examined the impact of parasites on insect respiration and metabolism (see

review by Matthews 2018). Those that have examined the influence of parasites on host respiration have found varying effects. For instance, honeybees infected with tracheal mites exhibit a reduction in metabolic rate while flying in hypoxic conditions (Harrison *et al.*, 2001). Similarly, *Plutella xylostella* (diamondback moth) larvae show decreased respiratory rates during infection by two different parasites (Fang *et al.*, 2008). Still, parasitized *Carabus* spp. and *Aedes aegyptii* larvae display no difference in respiratory rates compared to uninfected individuals (Rivero *et al.*, 2007; Gudowska *et al.*, 2016). The magnitude and direction in which parasite infection affects host metabolic rate remains equivocal, in part because some studies only consider infection status (presence/absence) rather than infection intensity. In this study, I experimentally investigated how variation in infection intensity impacts host metabolic rate, measured as the rate of respiration (Weis, 2014).

An organism's level of activity can also have a significant impact on their energy budget (Halsey *et al.*, 2015). High locomotion speeds among the harvestmen, *Paranemastoma quadripunctatum* and *Lophopilio palpinalis*, are energetically costly with metabolic rates increasing as much as five-fold over resting metabolic rates (Schmitz, 2005). Among ants, an increase in colony respiratory rate occurs at a population level with increasing worker activity (Mason *et al.*, 2015). Moreover, exposure to parasites can also impact activity in the form of anti-parasitic behavioural defences (Sears *et al.*, 2015). Given that parasitic infection may increase host activity and hence the level of respiration (Taylor *et al.*, 2004), measurements of parasite-induced changes in host metabolism may be confounded. My aim is to disentangle the effects of infection from potential parasite-mediated changes in activity on host metabolic rate.

In this study, I used the fruit fly *Drosophila nigrospiracula* (Diptera: Drosophilidae) Patterson & Wheeler and a facultative parasitic mesostigmatid mite, *Macrocheles subbadius* (Mesostigmata: Macrochelidae) Berlese, to determine the relationship between intensity of infection and the metabolic rate of the host. These mites feed on the haemolymph of their host, causing a reduction in host longevity and fecundity (Polak, 1996). High infection intensities (3-5 mites) reduce male testes and thorax size, and body: thorax ratios, indicating a shift in energy budgets away from growth (Polak, 1998). In a similar study system, Luong et al. (2017b) showed no significant difference in

the respiration rate of uninfected *D. hydei* and those infected with a single *Macrocheles muscaedomesticae* mite. However, under natural conditions *D. nigrospiracula* can be infected with 1-11 mites per fly (Polak and Markow, 1995). Here, I test the hypothesis that infection by the ectoparasite *M. subbadius* results in intensity-dependent metabolic costs that manifest in the form of increasing rates of respiration with mite load.

Since these mites were previously found to preferentially infect flies with higher respiratory rate (Horn *et al.*, 2018), it was necessary to measure individual metabolic rates before and after exposure to mites to verify a causal relationship between infection and changes in host metabolic rate. In a second experiment, I examined how variation in infection intensity influences host metabolic rate. In a third experiment, I tested whether parasites can indirectly affect host metabolism by elevating host activity, which could in turn increase energy requirements and hence rate of respiration. To that end, a two-by-two factorial experiment was designed in which flies were either restrained or free to move, and infected or uninfected to parse out the contribution of activity and infection on respiratory rate. I expected an additive effect of infection and parasite-mediated activity on host respiratory rate. A fourth experiment measured the respiratory rate of *M. subbadius* to ensure that changes in respiratory rates of the host were due primarily to the energetic cost of infection, and not just the addition of mites.

2.2 Methods

Study system

The ectoparasitic mite *M. subbadius* and its dipteran host *D. nigrospiracula* were originally collected from the Sonoran Desert, Arizona, USA in 2015. Mites were maintained in mass culture under standard laboratory conditions (12 h light: 12 h dark photoperiod, 24 °C, 60% RH). Mite media consisted of moist wheat bran, wood shavings, and Rhabditida bacteriophagic nematodes as food. Flies were cultured in media containing instant potato flakes, *Drosophila* medium (Formula 4–24 Instant Drosophila Medium, Carolina Biological Supply Company, Burlington, NC, USA), active yeast, and ~ 4-6 cc of autoclaved necrotic cactus. Fly cultures were maintained in a separate incubator (12 h light: 12 h dark, 25 °C, 70% RH).

Host metabolism before and after infection

Newly eclosed female flies were collected from the base laboratory culture and aged 7-14 days to sexual maturity without exposure to mates or mites. Adult female mites were collected from mass culture the day of the experiment. Following an initial ('before') respirometry measurement (see below), individual flies were exposed to five adult female mites for one hour in an infection tube (made from 100µL pipette tips cut in half, with both sides stoppered with cotton). The number of mites (range 1-5) that attached was recorded and flies were returned to the respirometer for a second ('after') reading. Hence for each fly two readings were obtained: one before exposure to mites and again following the onset of infection. Note that variation in mite load per fly was generated naturally by fly-mite interactions. Control flies were not exposed to mites, but still put into infection tubes for an hour.

Infection intensity and host metabolic rate

Infected and control flies were generated as described above. All fly respiratory rates were measured as indicated in the Respirometry setup (below). Respiration rates were measured over two temporal blocks; in the interim the respirometer tubing was modified to create a more stable flow through the SS-4 Sub-Sampler Pump (Sable Systems International, Las Vegas, NV). Fly dry mass (to nearest 0.01g) was weighed using the XP105 balance (Mettler Toledo, Missisauga, ON).

Infection and host activity

Female flies were collected from the base culture and allowed to mature for 10 to 21 days, and randomly assigned to one of four treatment groups (infected-restrained, uninfected-restrained, infected-unrestrained, and uninfected-unrestrained). Flies assigned to the "infected" group were exposed to 3 mites for 1 hour while flies in the uninfected group were not exposed to mites. Only flies infected by all 3 mites were retained in the experiment. Individual flies were placed in respirometry chambers (46 mm by 7mm diameter) and the rate of CO_2 production was measured as described below. Fly activity was directly measured using the Multiple Animal Versatile Energetics Flow Through (MAVEn-FT) system (Sable Systems International, Las Vegas, NV) and the first 10

minutes of exposure was analyzed. The MAVEn activity board monitors activity uses a proximity infrared sensor with activity measured as the relative change of reflected light. In order to prevent flies from moving in the restrained group; each fly was individually placed in Pharmed tubing (9mm x 2.5mm) capped with mesh on either end. Flies in the unrestrained group were free to move about the chamber. A second set of experiments was conducted in which only the activity alone was measured from a replicate group of unrestrained flies (infected vs. control) in order to increase sample size All flies were frozen and later weighed to determine dry body mass.

Mite respiration

To account for the possible contribution of mite respiration, female mites were collected from mass culture and their rate of CO_2 production was measured using the MAVEn respirometry system. Mites were place in respirometry chambers in replicated groups ranging from 1-6 individuals. Every assay was conducted with one empty respirometry chamber (baseline) and one containing an adult female *D. nigrospiracula* (aged 2-19 days) for comparison. Data was recorded as indicated in the Respirometry setup (below).

Respirometry setup

I measured the rate of respiration in the form of carbon dioxide (CO_2) production in the before-after and infection-intensity experiments using an infrared analyzer (LI-7000, Li-COR Biosciences, Lincoln, NE) connected to a BL-2 baseline unit (Sable Systems International, Las Vegas, NV); for a detailed description of the system, see Horn et al. (2018). Briefly, an ascarite-drierite column was used to scrub incurrent air. Dry, CO_2 –free air was then pumped into an experimental respirometry chamber (83 mm, i.d. 20 mm, Sable Systems International, Las Vegas, NV) that held a 2-mL mini-arena (40mm x 10mm microcentrifuge tube with mesh covering both ends). In each trial, a single fly (control or infected) was placed inside the mini arena. The excurrent air (flowing out of the experimental chamber) was scrubbed of water vapour with a magnesium perchlorate column to simplify calculations. A separate empty chamber served as the baseline with air passing through one chamber at a time. Baseline values

accounted for drift in the CO_2 measurement during the experiments and established a zero baseline for calculating fly CO_2 output. Data was recorded for 2 min from the baseline chamber, 8 min from the experimental chamber, followed by another 2 min of baseline; throughout the flow rate was 100 mL/sec.

Fly activity and respiratory rate in the factorial experiment and mite respiration rate were measured with the MAVEn-FT System. A FT-IR Purge Gas Generator 75-45 (Parker Balston Corporation, Milton, ON) was used to generate dry, CO_2-free air. The MAVEn system allowed multiple smaller chambers to be used for CO_2 collection, with a built in board for activity monitoring. Otherwise, the overall set up, data acquisition and analysis were similar to the setup above. Data collection on the MAVEn-FT consisted of a 3 min baseline, 5 min experimental reading, 2 min of baseline interleave (experimental chambers between baseline readings), and a flow rate of 30 mL/sec.

All respirometry calculations were performed in the Expedata Software (V1.9.14, Sable Systems International, Las Vegas, NV). The rate of CO_2 production (parts per million) was calculated using the formula $\dot{V}CO_2 = FR_i\,(F'_eCO_2 - F_iCO_2)$, where $\dot{V}CO_2$ stands for the rate of carbon dioxide production (Lighton, 2008), FR_i is the flow rate of incoming air, and F'_eCO_2 and F_iCO_2 are excurrent fractional concentration and incurrent fractional concentration of CO_2, respectively. The excurrent fractional concentration of CO_2 is equal to the experimental chamber measurement minus the baseline measurement. Water vapour was dropped from calculations as inflow and outflow water vapour was scrubbed hence = 0. Since the incoming air was scrubbed of CO_2 ($F_iCO_2 = 0$), the formula was further simplified to $\dot{V}CO_2 = FR_i \times F'_eCO_2$ (Lighton, 2008).

Data analyses

Data were analysed using generalized linear models (GLM) in the R statistical program (R Development Core Team, 2015). Backwards model selection was implemented to arrive at the minimal model in which non-significant variables (χ^2 test, $p>0.05$) were removed from subsequent models. The rate of CO_2 production from the before-after infection experiment was analyzed with generalized linear mixed-effects model (lme4 package) examining the effects of period, infection, and mass along with a random fly variable using a Gamma family error distribution. A posthoc multiple

comparison of means was performed to compare the 'before' respiratory rates between all groups, and the before-and-after changes within each group (glht, R package multcomp).

In the infection-intensity experiment, I used generalized linear mixed-effects model (lme4 package) with Gamma family error distribution to analyze the effect of mite load and body mass on the mean rate of carbon dioxide production. Block was put into the models as a random factor.

Activity of individual hosts was analyzed using GLMs with the Gaussian family error distribution. The first ten minutes of exposure was analyzed. Only the effect of infection on fly activity was analyzed in this dataset.

2.3 Results

Host metabolism before and after infection

The interaction between period (before and after infection) and infection status was statistically significant (Fig.2.1, χ^2=8.75, P=0.01). However, the relationship between mite load and rate of CO_2 production among infected individuals, either before (P=0.62) or after (P=0.47) infection was not significant. Therefore infected flies, regardless of mite load were pooled into one group, while flies that were exposed but uninfected were categorized as "exposed". Posthoc analysis showed no significant difference in respirometry rates between treatment groups prior to exposure to mites ($z >$ 0.05). The mean respirometry rate before and after exposure did not change significantly among flies in the control (unexposed, z= 0.77) and exposed but uninfected group (z= 0.95). However, flies in the infected group increased CO_2 production by 11% following mite attachment (z= 0.06). Together these data suggest that the host metabolic rate changed as a consequence of infection, and not simply because mites were predisposed to attaching to flies with higher respiratory rates.

Infection intensity

Infection intensity was a significant predictor of the mean rate of CO_2 production ($P < 0.001$), as was body weight ($P < 0.01$). Compared to unexposed individuals, the rate of CO_2 production among flies infected with one or two mites increased by15% and 16%, respectively.,Flies infected with 3 mites showed a 40% increase in CO_2 production

compated to control flies (Fig. 2.2). Body mass was positively correlated ($P < 0.01$) with respiratory rate. Two outliers were removed due to high leverage and residuals (Cooke's distance <0.5).

Infection and host activity

Again, infection status was a significant predictor of CO_2 production (deviance=-0.85, P <0.01; Fig. 2.3A), however being restrained did not affect respiratory rate overall (deviance=-0.09, P =0.27). The interaction between restraint status and infection was not significant (deviance=-0.06, P =0.31). However, body weight was not statistically significant in this particular experiment (deviance=-0.08, P =0.29), likely due to the narrow range of available fly sizes. The respiratory rate of infected flies increased by an average of 31% compared to uninfected flies overall. Infection had a statistically significant effect on host activity (P =0.002; Fig 2.3B) increasing overall activity 1.5 times over uninfected individuals.

Mite respiration

The MAVEn system was able to detect the respiratory rate of the mite *M subbadius*. On average, the rate of CO_2 production of a single mite was roughly equivalent to 1.8% of the respiratory rate of an adult female *D. nigrospiracula* (0.087 μL/hr and 4.75 μL/hr, Fig 2.4). This value is much less than the 40% increase observed in the infection experiment; therefore the respiration of mites had a negligible effect on measurements of fly CO_2 output.

2.4 Discussion

The results show that infection by the ectoparasitic mite, *M. subbadius,* causes significant metabolic changes in the host that scale with infection intensity. However, this increase did not occur in a consistently linear fashion, there was a threshold (2 mites) above which mites exerted a substantial impact, suggesting an equivalent threshold of physiological tolerance to mite infection. Parasite-induced changes in host metabolic rate may be attributed to an up-regulation of immune responses (Lochmiller and Deerenberg, 2000) and/or increased energy demands of elevated (mite) load, somatic maintenance and

tissue repair (Kristan and Hammond, 2000; Talloen *et al.*, 2004). Body weight was positively correlated with the production of CO_2 in the infection intensity experiment, which fits with previous studies (Promislow and Haselkorn, 2002; Luong *et al.*, 2017b). While not statistically significant, the results from the before-and-after experiment were taken as biologically significant, which is due to: the 11% increase in respiration after 1 hour of infection being higher than the highest rate of the age-dependent increase per day seen in the system (Horn *et al.*, 2018). The failure to detect an intensity-dependent effect in the before-and-after experiment was likely because most of the flies only acquired 1-2 mites. In general, the energetic cost of parasitism is likely to impact host energy allocation towards survival and reproduction (Lettini and Sukhdeo, 2010; Careau *et al.*, 2013).

Other studies have also found increased respiratory rate following parasite infection (Booth *et al.*, 1993; Khokhlova *et al.*, 2002; Chodkowski and Bernot, 2017). For example, the bridled monocle bream (*Scolopsis bilineatus*) showed an increase in metabolic rate while infected with an isopod (*Anilocra nemipteri*) (Binning *et al.*, 2012). This relationship may in part be due to the effects a relatively large parasite living on or in a small host, as is the case in the *Drosophila-Macrocheles* system. However, other studies have also failed to detect an significant effect or the full magnitude of parasitism on host respiration; and this may be because they only considered parasite presence/absence or a single level of infection (Careau *et al.*, 2010; Garrido *et al.*, 2016; Gudowska *et al.*, 2016; Filipsson *et al.*, 2017; Luong *et al.*, 2017b). Therefore, future studies should examine the consequences of a range of infection intensities on host metabolism, as done here and in other studies (Giorgi *et al.*, 2001; Careau *et al.*, 2010; Moretti *et al.*, 2017). Also, another current limitation that needs to be remedied is the convention of measuring respiratory rates at a single time point, usually once an infection has established (Khokhlova *et al.*, 2002; Garrido *et al.*, 2016; Chodkowski and Bernot, 2017; Luong *et al.*, 2017b). These studies only provide a snapshot in time, whereas this study highlights the value of using a before-and-after design as it controls for pre-existing biases, and offers clear evidence of a causal relationship between parasitism and increased host metabolism.

Parasite-mediated changes in host activity can also potentially contribute to elevated levels of respiration. For example, oxygen consumption by adult rodents significantly increased due to flea infestation, which is most pronounced at night but was in part due to changes in activity (Garrido *et al.*, 2016). Since flies were able to move in the first two experiments, it was important to rule out the effect of locomotion, especially flight as it is energetically costly (Mattila and Hanski, 2014). However, despite the increase in activity during infection, the change in respiratory rate among infected flies in this study cannot be wholly attributed to increased activity. Indeed, the respiration rate of infected flies was still significantly lower than control flies even when restrained (Fig. 2.3). These findings also indicate that the physical burden of carrying a parasite is not wholly responsible for the increased respiratory rate with higher infection intensities, as these hosts were unable to move.

Parasite aggregation in host populations is often attributed to heterogeneities in host exposure and susceptibility (Anderson and Gordon, 1982; Shaw *et al.*, 1998; Warburton and Vonhof, 2018). This study suggests another potential driver of parasite aggregation in the fly-mite system, and possible other systems in which the parasite actively seeks out the host (Polak and Markow, 1995). Since infection leads to an increase in respiratory rates, flies already harbouring mites may attract even more mites. A previous study showed that *M. subbadius* mites preferentially infected hosts with higher basal metabolic rates (Horn *et al.*, 2018). An intensity-dependent increase in host respiration (Fig. 2.2) could generate a small-scale positive feedback loop. However, this loop could only occur up to a point where the benefits of aggregation (e.g., overcoming host resistance, efficiency in obtaining resources, etc.) (Wertheim *et al.*, 2005) are outweighed by increased competition, reduced dispersal capabilities by the host (Luong *et al.*, 2015; Terui *et al.*, 2017) or even parasite-induced host mortality (Polak, 1996; Polak and Starmer, 1998). More research is needed to understand the ecological implication of this positive-feedback loop.

One noticeable issue within our data is that the values of the fly respiratory rates are largely different between flies in Figures 2.1 and 2.2 in comparison to Figure 2.3 and 2.4. This is likely due to the changeover from the BL-2 system to the MAVEn system. The differing concentrations of the span gas in the two setups (1000 ppm and 20 ppm,

394 respectively), as well as a higher flow rate (100ml/min compared to 20ml/min), could
395 account for the difference in the absolute values. However scaling issues aside, the results
396 remain biologically relevant as consistency within experiments was assured
397 Our study shows how hosts energy demands may change depending on the extent
398 of infection and highlights the importance of using a range of infection levels when
399 examining the costs of parasitism. Under natural conditions, the consequences of
400 parasitism on host energy budgets may be more pronounced than suggested by our data.
401 In the wild, hosts are exposed to dynamic and potentially stressful environments,
402 including changing availability and quality of resources, mating conditions, fluctuating
403 temperatures, and threats from other natural enemies (e.g., parasites, pathogens,
404 predators, etc.). The energetic costs of parasitism are likely to shift host energy allocation
405 away from growth, reproduction and maintenance. The re-allocation of energy budgets
406 may provide an important mechanism for parasite-mediated reduction in host fitness.

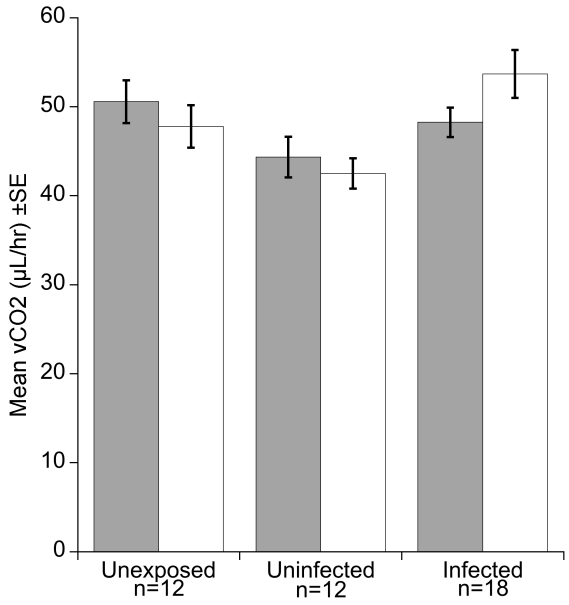
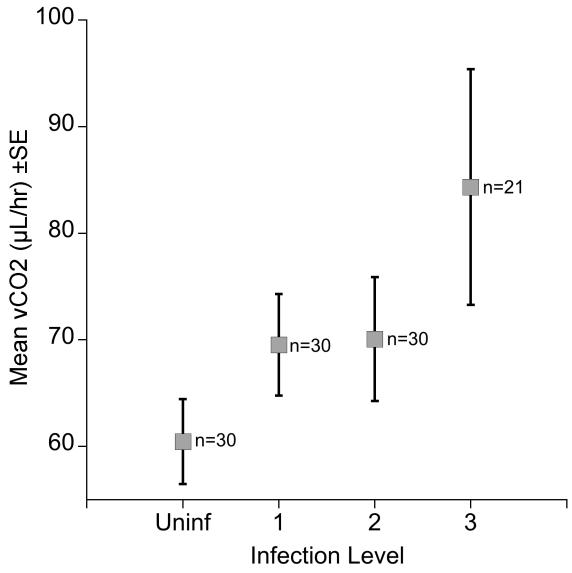


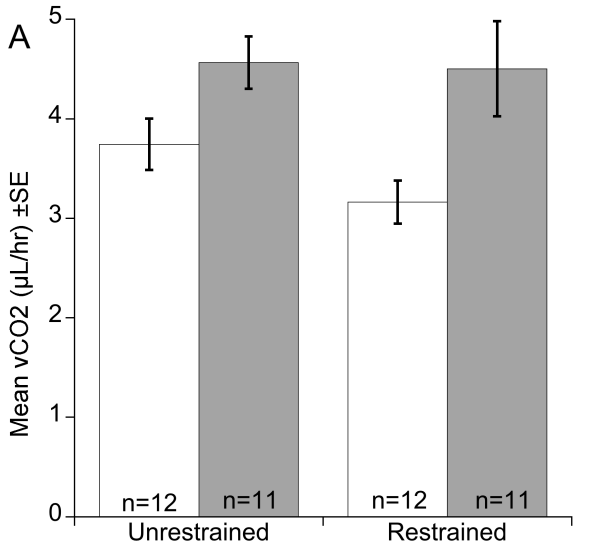
Figure 2.1 Mean respiratory rate (± S.E) of adult female *D. nigrospiracula* before (dark bars) and after (light bars) exposure to mites or an empty chamber. Sample sizes for each treatment group are indicated below each group.



411

412 Figure 2.2 Mean respiratory rate (± S.E) of adult female *D. nigrospiracula* infected with 0-3

413 mites.



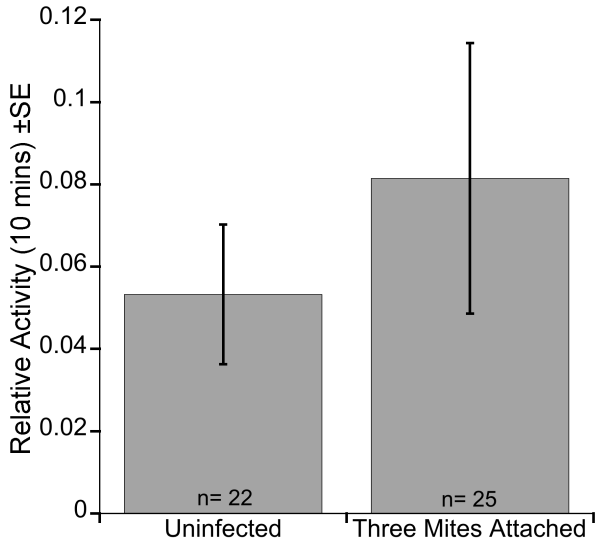


Figure 2.3 (A) Mean respiratory rate (± S.E) of *D. nigrospiracula* adult female flies in a 2-by-2 factorial experiment based on infection and restraint status. Light bars are uninfected individuals, while dark bars represent flies infected with three mites. (B) Activity levels (± S.E) of unrestrained flies for the first 10 minutes of exposure based on infection status.

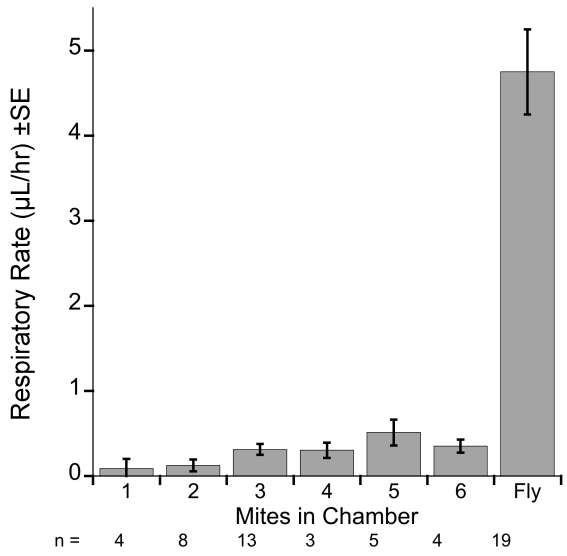


Figure 2.4 Mean respiratory rates (± S.E) of *M. subbadius* adult female mites and adult female *D. nigrospiracula*.

Chapter 3: Ectoparasites increase host susceptibility to future infections

3.1 Introduction

Parasitic infections can have adverse effects on host fecundity, longevity, development, or combinations thereof and impose strong selection on hosts to evolve adaptations to prevent or reduce the detrimental effects of infection (Anderson and May, 1982). Often, the first lines of defence are behavioural adaptions to avoid contact with the infective stages of the parasites (Hart, 1990), even before the need to mount a costly immune response (Zuk and Stoehr, 2002; Ardia *et al.*, 2012). Behavioural defences against parasites usually fall into one of two categories: 1) avoiding exposure to or contact with parasites and 2) preventing or minimizing parasite establishment. Hosts can prevent parasite encounters by avoiding infected prey, infected conspecifics, or habitats with high infection risk (Alma *et al.*, 2010; Walter and Proctor, 2013; Behringer *et al.*, 2018). Upon exposure to parasites, the use of innate or learned defensive behaviours such as grooming or self-medication becomes important (Villalba and Landau, 2012).

The impacts of anti-parasitic behaviours vary widely from beneficial and innocuous (e.g., grooming) to self-detrimental (e.g., host suicide, self-sacrifice) as seen in some social insects (Shorter and Rueppell, 2012). A single species may employ multiple behavioural defences against a single parasite. For example the honey bee (*Apis mellifera*) uses hygienic behaviour (capping infected broods), along with self- and allo-grooming to control the ectoparasitic mite *Varroa jacobsoni* (Boecking and Spivak, 1999). Heterogeneity in these host defences can be an important driver of various ecological processes and patterns, including aggregation of parasites within host populations (Poulin, 2013).

Parasite exposure and infection can influence the types and degree of activity of the host. Increased parasitic ant prevalence changes the behaviour of eusocial ants (*Temnothorax* spp.) from fending off parasitic intrusion to flight from the nest (Jongepier *et al.*, 2014). A general increase in activity has been observed among some tadpole species in response to the presence of trematode cercaria, which can prevent parasitic

infection (Koprivnikar *et al.*, 2014). Physical attachment by ectoparasites can also influence the ability of a host to mount behavioural defences. For example, attachment by large ectoparasitic isopods (*Anilocra haemuli*) reduces the overall level of activity in French grunt fish which can increase secondary infection (*Haemulon flavolineatum*) (Welicky and Sikkel, 2015).

In this study, I investigated the role of current parasite infection on host susceptibility to subsequent infection, and test whether this is mediated by changes in host behavioural defense. The facultative ectoparasitic mite *Macrocheles subbadius* (Acari: Macrochelidae) Berlese , infects the fruit fly *Drosophila nigrospiracula* (Diptera: Drosophilidae) Patterson & Wheeler (Perez-Leanos *et al.*, 2017),. These flies employ a variety of behavioural defences including: sudden reflex movements, tarsal flicking, bursts of flight, and rapid changes in direction (Polak, 2003). These pre-attachment behavioural defences are the primary form of resistance against infection. Mite infection negatively affects host fecundity and longevity (Polak, 1996), and physically interferes with male copulatory success (Polak et al., 2007).

Due to the energetic costs associated with mite infection (Luong *et al.*, 2015), I hypothesized that infected hosts will be less capable of mounting an effective behavioural defence against subsequent mite attack and be more susceptible to further infection. I predicted that the current mite load would result in an intensity-dependent increase in susceptibility to secondary mite infection. I then investigated the role of current infection status on host activity as a possible mechanism for increased susceptibility. Through the use of an activity monitor, I measured differences in the overall level of activity in hosts (naïve and infected) upon exposure to another mite.

3.2 Methods

Study system

Macrocheles subbadius is a cosmopolitan facultative ectoparasite of numerous fly species, including *D. nigrospiracula* which are primarily found in the Sonoran Desert (Perez-Leanos *et al.*, 2017). Our laboratory culture of *D. nigrospiracula* and *M. subbadius* mites were originally collected from necrotic cacti (*Carnegiea gigantea*) in Arizona, USA, 2015. Natural infection levels vary according to the age of necrosis in the

485 cactus; intensities can be as high as 8 mites per fly (mean 0.05 - 1.28) (Polak and
486 Markow, 1995).
487 Mites were originally collected from infected flies caught at necrotic saguaro cacti
488 in the Sonoran Desert (Phoenix, Arizona, USA) and have been maintained in mass
489 culture under standard laboratory conditions in incubators (12:12 L:D light cycle, 25°C,
490 70% RH). Mite media consisted of moist wheat bran, wood shavings, and bacteriophagic
491 nematodes as food. Flies were cultured in media containing instant potato flakes,
492 *Drosophila* medium (Formula 4–24 Instant Drosophila Medium, Carolina Biological
493 Supply Company, Burlington, NC, USA), active yeast, and roughly 4-6 cc of autoclaved
494 necrotic saguaro cactus. Fly cultures were maintained in a separate incubator under
495 similar conditions (12 h light, 25°C: 12 h dark, 24 °C, 70% RH). Experiments were
496 conducted between May 2017 and March 2018.
497
498 *Initial Infection and Subsequent Mite Attachment*
499 Virgin male and female adult flies were collected from mass culture and aged in
500 separate-sex vials for 10 to 20 days post-eclosion. Mites were collected from mass culture
501 the day of the experiment using a Berlese funnel (André *et al.*, 2002). Experimental flies
502 were individually exposed to 5 adult female mites in an infection chamber, constructed
503 from a 200µL pipette tip cut in half with both ends stoppered with cotton, which
504 immobilized the host and prevented behavioural resistance. Control flies were placed in
505 similar infection chambers without mites. After 1 hr of exposure, the number of mites
506 attached (hereby called initial mite or infection) was recorded. All flies were then
507 immediately exposed to a single mite (hereby called the secondary mite or infection) in a
508 1.5 mL microfuge tubes for ~1 hr. This larger tube allowed the flies to use behavioural
509 defences against the secondary mite. Infection status and placement of all mites (initial
510 and secondary infection) was recorded. Halfway through the experiments, I realized that I
511 could not distinguish between the initial and secondary mites if they had either detached
512 or changed positions on the fly. Henceforth, the secondary mite was marked on the
513 idiosoma with Archival Ink covering roughly 25% of the dorsal side (Sakura Color
514 Products Corporation, Osaka).

Flies from the unmarked trials were excluded from the analysis if a mite detached and/or changed attachments sites during the assay. For example, I could not determine if the new site of attachment was due to movement by the initial mite or attachment by the secondary mite. Trials were also omitted from analysis if the initial mite load decreased during the secondary exposure period (mites detached).

Host Activity Level

Virgin male and female flies were collected from the mass culture and aged for 10 - 20 days post-eclosion, and mites were collected using a Berlese funnel as described above. Experimental flies were individually exposed to 0-3 mites for an hour within pipette-tip infection chambers after which the infection intensity and position of mite attachment were recorded. Each fly was placed into separate chambers in the Multiple Animal Versatile Energetics (MAVEn) Flow-Through system (Sable Systems, Las Vegas, NV) to measure activity levels. The MAVEn system consists of 16 individual chambers; each chamber is flooded with beams of infrared light that detect changes in animal movement. A single female mite was introduced into each chamber along with the fly to stimulate mite avoidance and/or defence behaviours in the flies. Activity levels were monitored and analyzed for the first 10 minutes. To account for the secondary mite's activity, I also measured the activity of a single mite in a separate chamber. I subtracted the mean value of all mite assays (n=56) from the activity of each fly exposed to a mite.

Statistical Analyses

Both the behavioural activity and infection intensity experiments were analyzed with Generalized Linear Models (glms) with Gamma and Poisson error distributions, respectively, using the R statistical program (R Development Core Team, 2015). Backwards model selection was implemented to arrive at the minimal model; non-significant variables (χ^2 test, $p>0.05$) were removed from subsequent models.

In the infection experiment, I analyzed the effect of initial mite load on the proportion of secondary mite attachment. Covariates included length of exposure to the secondary mite, fly age, and last date of media addition. The last date of media addition

546 was recorded since mites infect hosts to a higher degree as local conditions deteriorate in
547 nature (Polak, 1998)
548 Two analyses were conducted on the host activity experiment. In order to test for
549 changes in activity due to anti-parasitic defensive behaviours, I compared the activity
550 level of an uninfected fly with and without the presence of a free-roaming mite in the
551 chamber. I also analyzed the effect of increasing mite load on the level of activity in flies
552 exposed to a second round of infection. Covariates included age of flies, days since mite
553 culture change, humidity, luminosity, barometric pressure, and temperature.
554
555 **3.3 Results**

556 *Initial Infection and Subsequent Mite Attachment*
557 Initial infection intensity (model comparison, change in deviance= -33.4,
558 $P<0.0001$), sex (deviance= -9.75, $P = 0.002$), the last date of media addition (deviance= -
559 3.74, $P = 0.053$) and marked status (deviance= 5.60, $P = 0.018$) affected secondary mite
560 attachment. No interactions were statistically significant ($P > 0.05$). Therefore,
561 subsequent mite infection was more likely to occur for flies with higher initial mite load.
562 The proportion of secondary infection increased sharply as the intensity of the initial
563 infection approached five mites. The rate of secondary infection among males increased
564 roughly 12% with each additional initial mite, whereas female rate of infection increased
565 5% with each additional mite load (Fig. 3.1).
566
567 *Behavioural activity during infection*
568 Uninfected flies exposed to a single mite increase their activity relative to
569 uninfected flies left alone in the chamber ($P <0.005$, Fig. 3.2). Fly sex ($P <0.01$) was an
570 important predictors of fly activity, but the interaction between fly sex and exposure was
571 not significant (deviance=0.157, $P = 0.150$). Uninfected females increased their activity 3
572 times upon mite exposure, whereas uninfected males increased their activity 17 times
573 when exposed to a mite.
574 I then analyzed the activity of infected flies relative to uninfected flies (same
575 group as above) upon secondary exposure to mites. Since the interaction between initial
576 infection and fly sex (P=0.003) was significant, males and females were analyzed

separately. The relationship between mite load and male activity followed a polynomial function (quadratic function, deviance=-0.440, P =0.016): activity initially declined at lower infection levels, but increased at the highest infection intensity (3 mites). Female activity remained unchanged until the infection intensity reached 3 mites (linear function, deviance=-0.109, p=0.089).

3.4 Discussion

In this study, I investigated the role of current infection by an ectoparasite on host ability to resist further infection by another ectoparasite. I predicted that current levels of infection would result in an intensity-dependent reduction in behavioural defences, manifesting in the form of increasing rates of secondary infection by a new mite. Indeed, results indicate that hosts with heavy mite loads were more susceptible to secondary infection. However changes in the activity level were not correlated with increasing susceptibility. Lightly infected male hosts displayed lower activity than predicted, but those with the highest level of infection (3 mites) exhibited increased activity levels. Females only increased their activity under the heaviest level of infection. Uninfected flies of both sexes increase their activity in response to mite exposure indicating a behavioural response to the presence of a threat.

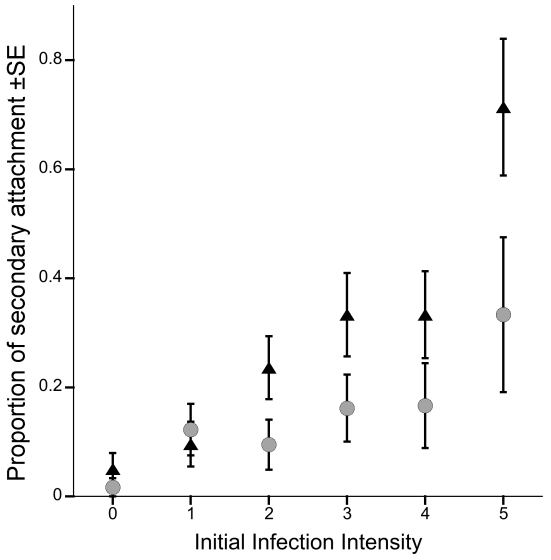
A possible mechanism exists for the initial activity differences between the sexes. The effects of accumulating higher intensity infection may be disproportionately more detrimental to males, which are on average smaller (Matzkin *et al.*, 2007). As such females are likely better able to tolerate infection, which explains that lack of compromise in female activity at low levels of infection.

Yet for both makes and females, the activity levels rose sharply with 3 mites. Subject to high infection intensities, hosts may switch from defensive behaviours (e.g., grooming, short burst of flight) to habitat-escape and dispersal (Behringer *et al.*, 2018). High parasitic infections reduce body condition in both males and females and is linked to mortality (Polak, 1996; Polak and Starmer, 1998). Therefore, the sharp increase in activity observed for male and female flies with 3 mites suggests a threshold beyond which the risk of mortality is no longer tolerable. The increased activity may be linked to habitat escape rather than defensive behaviours, and may explain the lack of correlation

between rate of secondary infections and activity level. Differentiating between behavioural defences and escape behaviour would require further experimentation.

These results clearly suggest that current parasite load can influence host susceptibility to future infections. Among some endoparasites, the ability to increase host susceptibility to further conspecific infection has been documented (Karvonen *et al.*, 2004; McPherson *et al.*, 2018). Hence, parasites themselves may be driving heterogeneity in susceptibility among hosts. A "snowball effect" whereby heavily infected individuals suffer greater susceptibility to infection may occur, which would in turn generate stronger parasite aggregation within the host population (Poulin, 2013). Ultimately, parasite-induced changes in host susceptibility to infection could benefit individual parasites by increasing infection success, but only up to a point; intraspecific competition and compromised host dispersal capabilities could lead to a negative feedback loop.

620

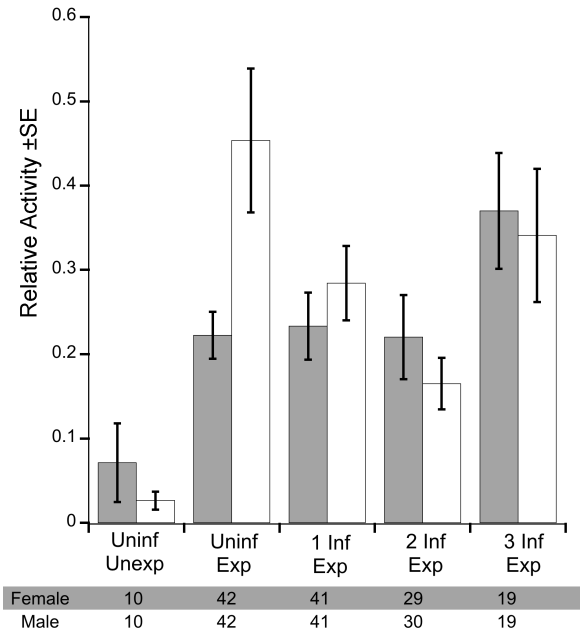


Female	60	49	42	37	24	12
Male	59	52	55	39	36	14

621

622 Figure 3.1 Proporion of secondary mite attachment (± S.E) based on original infection
623 intensity. Flies with varying mite loads were exposed to a single mite in the secondary
624 infection. Grey circles represent female flies, black triangles represent males. The table
625 below the graph indicates the number of replicates.

626



627

628 Figure 3.2 Relative fly activity (± S.E) based on initial level of infection. Infected flies were
629 exposed to a single free-roaming mite in each of the MAVEn activity chambers. X-axis
630 labels refer to infection and exposure status. Grey bars represent females, white bars
631 represent males. The table below the graph indicates the number of replicates.

Chapter 4. Conclusions

4.1 Thesis Conclusions

Aggregation is a well described and studied phenomenon in parasitology and disease ecology, often attributed to intrinsic variation in host heterogeneity in exposure and susceptibility (Shaw *et al.*, 1998; Poulin, 2013). One major shortfalling in the literature is that most work attempting to understand aggregation has been done on vertebrate systems (Shaw and Dobson, 1995; Poulin, 2013; Wilber *et al.*, 2017; Sarabeev *et al.*, 2019) with a recent shift towards modeling (Gourbière *et al.*, 2015; Morrill and Forbes, 2015; Wilber *et al.*, 2016; Morrill *et al.*, 2017; Sarabeev *et al.*, 2019). It was suggested that there was no need to further understand the causes of aggregation (Poulin, 2013). Parasites themselves, however, can drive parasite distribution in host populations (Bull, 1978), for example through the use of chemical cues (My yen *et al.*, 1980; Petney and Bull, 1981; Leahy *et al.*, 1983).

Another method by which parasites can influence aggregation in a host system is to alter susceptibility to infection. One of the most compelling results obtained from my study was that susceptibility to infection increased with increasing initial infection intensity (Fig 3.1). Since preventing fly defences generated the initial infections, hosts were equally susceptible; therefore differences in susceptibility detected upon secondary exposure were likely parasite-mediated. How exactly parasites influence susceptibility is unknown due to the inability of the MAVEn system to detect fine scale movements such as grooming behaviour, which could have been suppressed among infected flies. The increase in host activity at high infection levels may be the side-effect of habitat escape behaviours, which could limit infection by moving to a parasite-free habitat (Hart, 1990; Folstad *et al.*, 1991). Regardless our results clearly show that the proportion of secondary mite attachments increases when they were already heavily infected. Secondary attachments to males soared to 71% and females to 33%, which increased from uninfected individuals 14 and 20 times respectively. Susceptibility to future infections substantially increased as infection intensity increased, which fits with some previous findings (Karvonen *et al.*, 2004; McPherson *et al.*, 2018). A possible explanation is that

infected hosts have lower available energy to fight off infection, leading to less effective or abbreviated defences.

The other major method for a parasite to influence aggregation in host systems would be by increasing heterogeneity among host-parasite encounters. Since mites preferentially infect hosts with a higher relative respiratory rate (Horn et al., 2018), any parasite-mediated increase in host metabolic rate would alter host-parasite encounters. Herein I found that an individual's respiratory rate increases after infection (Fig 2.1) and mean respiratory rate increases with infection intensity (Fig 2.2). Taken together, this indicates a possible explanation behind the selection of more heavily infected individuals (Luong *et al.*, 2017a). Our results also confirm that the increase in respiratory rate is due to parasitism and not activity since individuals that were restrained and unable to move still had higher respiratory rates (Fig 2.3A) than uninfected conspecifics.

4.2 Future Directions

It's not clear what changes in behavioural defenses are taking place under heavy infection. In the future, direct observations of fly defensive behaviours would be useful to determine the types and frequency of defensive behaviours used by *D. nigrospiracula* under high intensity of infection. Mesocosm experiments that incorporate refuges free of parasites may reveal if parasite exposure influences habitat avoidance/escape behaviours.

As some acarid mites are known to use aggregation cues (My yen *et al.*, 1980), future research should examine whether pheromones also play a role in mite preference for more heavily infected individuals (Luong *et al.*, 2017a). Mites also preferentially infect injured hosts (Horn *et al.*, 2018) and do discriminate among hosts based on the presence of an exterior coating of host haemolymph. As such, there may be an infection-induced kairomone-based component to mites selectively infecting hosts already carrying mites.

One of the broader aspects that would be interesting for further research is whether increases in respiration universally occur with increasing intensity of parasites. While Robar *et al.* (2011) found no universal trend for infection alone on respiratory rate of hosts, there may be an effect of parasites on host metabolic rate among macroparasites acting at higher intensities of infection.

692 Through more examination, the missing variables leading to aggregation could be
693 determined (Poulin, 2013). More studies are needed to examine macroparasite
694 distribution among invertebrate host populations. By using contstraint-based modeling
695 methods, suggested by Wilber *et al.* (2017), when doing surveys researchers could
696 possibly identify if factors may be increasing or decreasing aggregation earlier, thereby
697 leading to more studies like this one examining mechanisms influencing aggregation.

Supplementary Table

Supplementary Table 1. Collection of all final minimal models with descriptive names of factors. Minimal models were obtained using backwards stepwise model selection.

Experiment	Figure	Final model	
Host metabolism before and after infection	2.1	Respiration~ TimePeriod*InfectionType+ (1 | FlyID), family=Gamma	
Infection intensity	2.2	Respiration~Infection+bodyweight|Block , family=Gamma	
Infection and host activity	2.3A	Respiration ~ Infection, family = Gamma	
Infection and host activity	2.3B	Movement(10mins) ~ Infection, family = Gaussian	
Initial Infection and Subsequent Mite Attachment	3.1	MiteAttachment~HostSex+InitialInfection +Marked+Mediaaddition, family=Poisson	
Host Activity Level	3.2	Mites induce activity	Activity~MiteExposure+Sex, family=Gamma
		Female activity	Activity~1,family=Gamma
		Male activity	Activity~Infection+I(Infection^2), family=Gamma

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