The Evolutionary Ecology of Parasitic Strategies: Experimental Evolution with a Facultatively Ectoparasitic Mite

by

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Abstract

Although parasitism is a ubiquitous lifestyle, little empirical evidence exists for how and why parasitism evolves from free-living lineages. Analysis of phylogenetic relationships among taxa that exhibit a range of lifestyles has aided in the development of a commonly proposed hypothesis for the evolution of parasitism: that transient host-associations served as evolutionary stepping-stones towards more obligate parasitism. Using the facultatively parasitic mite, *Macrocheles muscaedomesticae*, I performed experiments to generate empirical evidence for how free-living organisms evolve parasitic strategies. I successfully artificially selected mites for increased infectious tendency to attach to (infect) *Drosophila hydei* hosts and estimated the additive genetic variation in the trait to be 16.6%. To learn more about how variation in infectious behaviour is maintained in these mites, I investigated the presence of evolutionary trade-offs (i.e. costs) associated with infectious behaviour. I compared the fecundity, longevity, motility and morphology of mite selected for increased infectious behaviour to unselected control mites. Mite fecundity and longevity were assayed in the presence or absence of a fly host to test for context-dependent trade-offs. I found that regardless of the selection treatment, female mites that attached to a fly produced significantly more nymphs, which provided evidence for the benefits of parasitic behaviour. However, I did not find any evidence for negative trade-offs (i.e. costs) associated with increased infectious behaviour. I then measured the plasticity of infection in selected and control mites to determine whether my directed selection on infectious behaviour caused genetic assimilation (i.e. a loss in plasticity). Contrary to my prediction, the selected mites maintained their infection plasticity despite my direct selection. Lastly, I investigated the biology and life history of *M. muscaedomesticae* in the context of other published studies, and compared the weights of mites that had and had not previously attached to

fly hosts to learn more about whether these mites are merely phoretic on their *Drosophila hydei* hosts. The mites used in my experiments exhibited similar life-histories to those from other published studies. The female mites that attached to fly hosts indeed weighed significantly more than the mites that did not, but definitive evidence of fluid transfer from fly to mite is necessary to confirm the parasitic nature of *M. muscaedomesticae*. My research provides experimental evidence for one of the assumptions of the hypothesis that facultative parasitism serves as an evolutionary stepping stone to obligate parasitism. However, it also illustrates the importance of variation in infectious strategies in these mites, which was maintained even in the selected populations. I propose future research to investigate the presence of phenotypic plasticity across multiple parasitic taxa to determine its importance in the evolution of parasitism from free-living ancestors. A better understanding for how parasitisms evolve is critical to developing effective control programs for medically and economically relevant parasites in our changing world.

Preface

Chapter 2 of this thesis has been published as “Durkin, E. S. and L. T. Luong. 2018. Experimental evolution of infectious behaviour in a facultative ectoparasite. Journal of Evolutionary Biology. 31: 362-370.” I was responsible for experimental design, data collection and analysis, as well as the manuscript composition. L. T. Luong was the supervisory author and was involved with experimental design and manuscript composition.

Chapters 1 & 3-6 of this thesis are original work done by Emily S. Durkin. No part of these chapters has previously been published.

Dedication

I dedicate this thesis to everyone that encouraged and supported me; especially my parents who nurtured my curiosity and creativity since day one.

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Chapter 1. General introduction

1.1 Overview

Historically, parasites were treated as an insular group of organisms reserved for those interested in their medical and agricultural impact. Not until the last 30 years have scientists formed a deeper appreciation for parasites themselves as well as the large role they play in the ecology and evolution of free-living organisms (Poulin 2007; Jackson 2015). Parasites are everywhere in nature and some researchers argue that they represent the majority of life on earth (Windsor 1998). Given the ubiquity of parasites and the likelihood that they evolved from free-living ancestors, transitions to parasitic life may outnumber any other major evolutionary shift in life history strategy (Price 1980; Poulin and Morand 2000; Poulin and Randhawa 2015).

Although this transition is prevalent, there is little direct evidence about how parasitism evolved from free-living ancestors. Many researchers have hypothesized on its evolution using comparative and phylogenetic analyses (see Maslov and Simpson 1995; Littlewood 1999; Mironov et al. 2005; Westwood et al. 2010a; Weinstein and Kuris 2016). However, direct evidence is needed to predict emerging diseases in changing environments, and to understand the ecology of medically and economically relevant parasites. Yet, experimental research on parasite evolution is relatively sparse (but see Crossan et al. 2007; Paterson and Barber 2007; Warburton and Zelmer 2010; Stasiuk et al. 2012).

A common hypothesis for the evolutionary transition to parasitism is that transient host associations, such as phoresy and facultative parasitism, serve as evolutionary stepping-stones towards obligate parasitism (Rothschild and Clay 1952; Waage 1979; Anderson 1984; Athias-Binche and Morand 1993; Poulin 2007; Dowling 2015). In my thesis research, I used the facultatively parasitic mite *Macrocheles muscaedomesticae* to experimentally investigate the evolutionary shift to parasitism. First, to provide empirical evidence for the evolution of

parasitism via transient host associations, I experimentally selected facultatively parasitic *M. muscaedomesticae* mites for increased infectious behaviour. I then investigated potential evolutionary trade-offs associated with increased infectiousness. Trade-offs may contribute to the maintenance of variation in infectiousness and restrict evolution towards obligate parasitism in *M. muscaedomesticae*. Specifically, I measured and compared the fecundity, longevity, motility and morphology of mites selected for increased infectivity to unselected control mites. Furthermore, I measured and compared infection plasticity in mites selected for increased attachment propensity to unselected control mites to determine whether direct selection on infection results in a loss in infection plasticity. I also synthesize data and observations from my own experiments with those from other researchers to create a comprehensive understanding of the biology of *M. muscaedomesticae* in laboratory culture.

1.2 Background

Parasitism is a symbiotic relationship in which one organism, the parasite, benefits at the expense of its host (Lewin 1982; Roberts and Janovy 2009).Parasites clearly evolved from free-living ancestors but to do this. They must have had: 1) the physical opportunity to form a relationship with another organism and 2) pre-adaptations that allowed resource-exploitation while in close contact (Rothschild and Clay 1952; Osche 1956; Combes 2005; Poulin 2007; Dieterich and Sommer 2009; Dowling 2015). This second requirement is essential because individuals that express parasitism need increased fitness relative to free-living individuals within a population. Without a fitness gain, natural selection cannot select for parasitism over other strategies (Waage 1979; Combes 2005; Poulin 2007).

Because most parasites are soft-bodied, the fossil record does not help to elucidate a lineage's transition to parasitism. For this reason, taxa that contain extant species with a range of resource-exploitation strategies are invaluable (Poulin 2007). One well-studied group is the

Nematoda, which includes both parasitic and free-living members. The dauer larval stage (a resistant dormant stage) is thought to be a pre-adaptation that allowed nematodes to survive being ingested by other animals: a stepping-stone in their parasitic evolution (Anderson 1984). Stasiuk et al. (2012) later provided empirical support for this hypothesis with their artificial selection experiments on dauer formation.

The mite superorder Parasitiformes, shows a complete spectrum of resource exploitation strategies ranging from free-living soil mites to obligate parasites like ticks (Walter and Proctor 2013). Members of the genus *Macrocheles* are primarily free-living predators, but some species engage in phoresy (Krantz 1998). A history of phoresy's definition is outlined by Farish and Axtell (1971) and I use their refined definition:

> "Phoresy is a phenomenon in which one animal actively seeks out and attaches to the outer surface of another animal for a limited time during which the attached animal (termed the phoretic) ceases both feeding and ontogenesis, such attachment presumably resulting in dispersal from areas unsuited for further development, either of the individual or its progeny."

Macrocheles subbadius (Berlese), can complete their lifecycle as a free-living organism, but also feed on the hemolymph of its insect host, making it a facultative parasite (Polak 1996). Unlike obligate parasites, facultative parasites are capable of parasitic activity, yet they do not rely on parasitism to complete their lifecycle (Roberts and Janovy 2009). The phoretic stage of a mite's life allows for a close relationship with another organism and may be an important stepping stone in the evolution of parasitism (Poulin 2007; Dowling 2015).

Macrocheles muscaedomesticae (Scopoli) often inhabit ephemeral habitats like dung pats and patches of decaying organic material where it preys on nematodes and other small invertebrates (Wade and Rodriguez 1961; see Chapter 5 for more on *M. muscaedomesticae*

biology). When the opportunity arises, adult female mites may attach to a fly host. Males do not attach to flies. Host-attachment behaviour is plastic (differential expression in the context of the environment) and females become more likely to attach to flies as their habitat deteriorates (Jalil and Rodriguez 1970; Krantz 1998). Similarly, Farish and Axtell (1971) observed the prevalence of phoresy in *M. muscaedomesticae* to increase as their habitat aged. Furthermore, they reported the highest levels of phoresy in manure that was aged at high temperatures. The manure habitats in the highest temperatures dried out faster than the others. Thus, the authors suggested that humidity was the limiting factor to mite survival (Farish and Axtell 1971).

The intrinsic state of a mite can also affect its propensity for attachment behaviour. Luong et al. (2017) demonstrated that adult female *Macrocheles subbadius* showed increased levels of host-attachment behaviour after mating and after periods of starvation. Genetics also plays a role in the attachment behaviour of *M. muscaedomesticae*, as demonstrated by Durkin and Luong (2018).

Macrocheles muscaedomesticae females have been suspected of feeding on their host's hemolymph like *M. subbadius*, but this has not been definitively confirmed (Jalil and Rodriguez 1970; Farish and Axtell 1971). There is evidence for negative effects imposed upon fly hosts by *M. muscaedomesticae* (Jalil and Rodriguez 1970; Luong et al. 2015), and this thesis provides some additional data to support the hypothesis that *M. muscaedomesticae* feed on fly hemolymph while attached (see Chapters 3 and 5). However, not every female will attach to a host in her lifetime. Because host-attachment is not necessary to complete their life cycle, host-attachment behaviour is facultative. Furthermore, we consider *M. muscaedomesticae* to be facultative parasites because of the evidence for the negative effects they impose on their hosts combined with the evidence for the benefits they gain from attachment (see Chapters 3 and 5).

The variation that *M. muscaedomesticae* expresses in host-attachment (also called infection) behaviour makes them an excellent study species for generating direct, experimental evidence for parasite evolution; information that is necessary to learn more about how parasitic traits might evolve.

1.3 Specific Objectives

1) Is infectious behaviour heritable in facultative parasites? Some authors have proposed that phenotypic variation in infectious behaviour is primarily determined by environmental variation (i.e. phenotypic plasticity) (Cross and Kaliszewski 1988; Athias-Binche 1993). However, according to evolutionary theory, for obligate parasitism to evolve from transient host associations, heritable genetic variation in the tendency to be infective must also exist (Futuyma 2009). I wanted to determine whether additive genetic variation contributes to the variation in infection shown by *Macrocheles muscaedomesticae*. I used artificial selection experiments to estimate the realized heritability for infectivity in the selected populations.

2) If infectious behaviour is heritable, what prevents facultative parasites from evolving obligate infection strategies (i.e. what maintains infection variation in facultative parasites)? Theoretically, for facultative parasitism to evolve by natural selection, parasitic activity must be associated with a fitness gain (Darwin and Wallace 1858; Futuyma 2009). What then prevents obligate parasitism from evolving and reaching fixation of infectious behaviour in *M. muscaedomesticae*? I investigated the potential presence of evolutionary trade-offs associated with increased infectivity. A negative genetic correlation between infectivity and other life history traits could constrain the evolution of a fixed, infection strategy. Specifically, I investigated potential costs in terms of mite fecundity and longevity. Furthermore, I investgated

potential changes in morphology, motility and infection plasticity associated with increased infectivity.

3) How parasitic are *Macrocheles muscaedomesticae* mites? Does *Macrocheles muscaedomesticae* feed on fly hemolymph? I provide a comprehensive overview of the biology and life history of *M. muscaedomesticae* and compare the weights of mites that have and have not previously attached to fly hosts to collect more evidence on whether they feed on fly hemolymph while attached.

1.4 Outline of thesis

In chapter 2, I investigate the presence of heritable variation in infectious behaviour using experimental evolution. Experimental evolution is research in which populations are observed and measured over multiple generations under defined conditions using multiple techniques, including laboratory natural selection, laboratory culling and artificial selection (Garland 2003; Rose and Garland 2009). Laboratory natural selection describes experiments by which laboratory populations are subjected to natural selection, but the environmental conditions are chosen and controlled by the researcher (Garland 2003; Futuyma and Bennett 2009; Rose and Garland 2009). Laboratory culling describes experiments that expose populations to potentially fatal conditions. A proportion of the survivors are then used to seed subsequent generations (Futuyma and Bennett 2009; Rose and Garland 2009). In both of the previous techniques, populations are subject to the selection imposed by their environment and there is no control over what traits undergo change. In artificial selection experiments, the researcher imposes selection on populations (Garland 2003; Rose and Garland 2009). Although artificial selection often requires more hands-on work, it provides more precision in what traits experience selection. Some argue that experimental evolution is an underutilized tool and this is

likely due to the assumed logistical constraints associated with such work (Fry 2003; Garland 2003; Futuyma and Bennett 2009). Regardless, experimental evolution is a powerful tool that provides the unique opportunity to observe evolutionary events in real time.

I chose to artificially select *M. muscaedomesticae* mites for a few reasons. First, *M. muscaedomesticae* are small, easy to maintain and have a quick generation time (~1 generation per week) making them excellent candidates for experimental evolution. Although more time consuming, I chose artificial selection because it would allow me to control the traits being selected. It also provided me the opportunity to calculate the additive genetic variation in my trait of interest, infection behaviour. My second thesis chapter describes my artificial selection experiments for and against infectious behaviour in *M. muscaedomesticae* and my calculation of additive genetic variation in the trait. The third chapter examines potential trade-offs associated with selection for increased infectious behaviour in the mites. I measure and compare the fecundity, longevity, morphology and motility of mites selected for increased infectiousness against unselected control mites. In chapter 4, I compare the infection plasticities of selected mites to those of the unselected control mites to determine whether a loss in infection plasticity accompanies the direct selection for infectious behaviour in *M. muscaedomesticae*. Chapter 5 provides a description of the biology and life history of *M. muscaedomesticae* used in my experiments, and places this new body of knowledge in the context of previously published works on *M. muscaedomesticae.* Debate exists over whether *M. muscaedomesticae* feed on the hemolymph of their fly hosts while they are attached. Thus, I also compare the weights of mites that had previously infected flies to mites that did not as a test of whether *M. muscaedomesticae* feed on their fly hosts while attached. The final chapter summarizes the conclusions attained in each of the previous chapters and presents some areas of future study.

Chapter 2. Experimental evolution of infectious behaviour in a facultative ectoparasite

2.1 Introduction

Parasites represent a substantial proportion of biological diversity, yet our understanding of the evolution of parasitism itself is limited (Price 1980; Poulin 2007). Comparative and phylogenetic studies have shed light on the patterns of parasite evolution (Maslov and Simpson 1995; Littlewood 1999; Mironov et al. 2005; Westwood et al. 2010, Weinstein and Kuris 2016), but the evolutionary processes involved remain largely unknown. Commonly proposed hypotheses for the evolutionary shift to parasitism include the idea that parasitic ex-aptations (traits co-opted for parasitic activity) facilitate the formation of symbiotic relationships, and the associated fitness benefits allow symbioses to then evolve towards more permanent associations like obligate parasitism by means of natural selection (Osche 1956; Poulin 2007; Dieterich and Sommer 2009; Dowling 2015). Stemming from that is the hypothesis that intermediate strategies with transient associations between symbiont and host (e.g. facultative parasitism, phoresy etc.) may serve as evolutionary stepping-stones towards obligate parasitism (Waage 1979; Anderson 1984; Anthias-Binche and Morand 1993; Poulin 2007; Dowling 2015).

Facultative parasites are parasitic under certain conditions but otherwise free-living and capable of completing their lifecycles in the absence of hosts. Indeed, environmental signals appear to play a key role in initiating symbiosis in many phoretic and facultatively parasitic organisms (Cross and Kaliszewski 1988; Stasiuk et al. 2012) and some authors have proposed

187 that the phenotypic variation they observed in transient symbiotic associations are primarily
188 determined by environmental variation (Cross and Kaliszewski 1988; Athias-Binche 1993). Few
189 data exist on the genetic basis for infectivity in facultative parasites. Yet, in order to predict the
190 evolutionary potential of facultative parasites, we need to estimate the magnitude of heritable
191 genetic variation underlying the "infectivity" trait (West-Eberhard 2005; Reece et al. 2009).

192 Crossan et al. (2007) and Paterson and Barber (2007) both demonstrate a genetic basis
193 for infection variation in two parasitic nematode species. However, these nematode species were
194 obligate parasites (i.e. infectivity is fixed) and heritability estimates were not reported for
195 comparison. Stasiuk et al. (2012) investigated the transition to parasitism by experimenting with
196 a facultatively parasitic nematode *Parastrongyloides trichosuri* (Mackerras). After the first
197 larval stage, the nematode can develop into a free-living organism or a parasite via an infective-
198 juvenile (IJ) stage in response to environmental cues (Stasiuk et al. 2012). After three
199 generations of artificial selection, the authors generated *P. trichosuri* populations with high and
200 low propensities for IJ-development, demonstrating heritable genetic variation in the trait.
201 However, in just three generations of selection, it was not possible for the authors to obtain a
202 robust estimate of additive genetic variation in IJ-formation. To our knowledge, no study has
203 experimentally evolved infectious behaviour in a facultatively parasitic animal system and
204 provided an estimate of the realized heritability for the trait.

205 The aim of our study was to experimentally evolve infectious behaviour and estimate the
206 additive genetic variation in a facultatively parasitic mite *Macrocheles muscaedomesticae*
207 (Scopoli). Using artificial selection, we selected for either increased or decreased host-
208 attachment rates in *M. muscaedomesticae* in the presence of a susceptible host, *Drosophila hydei*
209 (Sturtevant). In natural populations of *M. muscaedomesticae*, a continuum of lifestyles is

observed, including free-living, phoretic and putative parasitic individuals. Free-living mites consume small invertebrates; but if an opportunity arises, adult female mites can attach to a fly and feed on the host's hemolymph, using it for dispersal and as a source of nutrition (Jalil and Rodriguez 1970; Farish and Axtell 1971; Luong et al. 2015). Two replicate experiments were conducted, each consisting of two replicate lines per selection regime. We monitored the response to selection as well as the stability of infectious traits post selection. By quantifying the heritable genetic variation underlying the infectivity trait in a facultative ectoparasite, we can predict the evolutionary potential of facultative strategies, i.e. as evolutionary stepping-stones towards obligate parasitism.

2.2 Material and methods

Fly and Mite Culture

Drosophila hydei (~100 per sex) were collected from residential compost bins in Edmonton, Alberta Canada in September 2013 (approx. coordinates 53.52 °N, 113.48 °W) and used to establish a laboratory culture. Flies were mass cultured in six to eight 200 mL bottles on standard agar-molasses-yeast-based fly media at 24 °C, 70% relative humidity and a 12 D: 12 L light cycle. Flies were maintained in the lab for two years before selection experiments took place. Female *D. hydei* flies were used for all experiments. The mean age of adult flies used in experiment A was 8.7 ± 5.25 SD days post-eclosion and the mean age of adult flies used in experiment B was 7.7 ± 5.6 SD days post-eclosion.

Approximately 700 female *M. muscaedomesticae* collected from field-caught *D. hydei* were used to initiate a mass culture in the laboratory. Mites were maintained for two years before selection experiments commenced. Mites were cultured in three panmictic 4 L plastic containers containing organic medium: sterilized organic wheat bran, sterilized aspen wood

233 shavings, deactivated yeast and distilled water. The organic medium contained bacteriophagic
234 nematodes as a food source for the mites. Cultures were kept at 24 °C, 70% relative humidity
235 and a 12D: 12L light cycle. New media (wheat bran, wood shavings and 15 cm^3 of deactivated
236 yeast) were added to cultures every 7-10 days.

237 *Selection Protocol*

238 Our selection for infectious behaviour in *Macrocheles muscaedomesticae* was
239 constrained by logistical challenges associated with the selection regime that limited the number
240 of simultaneous replicate lines we could maintain. Hence, we chose to repeat the selection
241 experiment on the same source population of mites yielding a total of four replicate high
242 response lines and two low response lines. The first experiment was performed in in fall of 2015
243 (experiment A) and the second in spring of 2016 (experiment B). The source mite populations
244 for each experiment were well into the thousands. Experiment A consisted of three selection
245 regimes or treatments: 'infectious', 'free-living' and control. Experiment B consisted of only
246 'infectious' and control lines because the 'free-living' treatment did not respond to selection in
247 experiment A (see Results). Two replicate lines were generated per selection regime per
248 experiment.

249 See supplemental Fig. S2.1 for an illustrated guide to our selection regime. In both
250 experiments, selection began by individually exposing 40-130 adult female mites to single
251 female *D. hydei* flies for 60 minutes in an experimental infection chamber, constructed from 200
252 µL pipette tips reduced to half their length (~1.5 cm) and stoppered with cotton. These small
253 chambers restricted fly movement, which reduced heterogeneity in behaviourally-mediated host
254 resistance or encounter possibilities (Polak 2003).

Infection was defined as a mite's attachment to a fly host. Following 60 minutes of exposure, mites were scored as attached or unattached to the fly and left for an additional 30 minutes. Attached mites that remained attached after the additional 30-minute exposure seeded the 'infectious' treatment line. In experiment A, unattached mites that remained unattached after the additional 30-minute exposure seeded the 'free-living' treatment line. This selection protocol was repeated at each generation in each replicate line.

Control lines were maintained in parallel with the selected lines during both experiments. Control mites were randomly selected to seed the next generation regardless of attachment status. In experiment A, control mites were not exposed to flies for the first six generations; seeder females were randomly selected from mass culture. To achieve similar environmental conditions experienced by the selected lines, control mites were exposed to flies in infection chambers during subsequent generations. In experiment B, control mites were exposed to flies every generation.

In experiment A, 26 to 50 female mites seeded each new generation (mean = 43.8 ± 7.2 SD). In experiment B, 13 to 50 female mites seeded each new generation (mean = 41.8 ± 10.7 SD). For a given replicate line, each treatment and control line had the same number of seeder mites every generation. To prevent overcrowding and accidental loss of an entire generation, the total number of seeder females used each generation was divided equally across 3-5 replicate 909 mL ventilated containers (6-10 seeder mites per container) in both experiments. Each container held roughly 50 mL of organic media and was sealed with Parafilm® (Neenah, WI) to prevent contamination by other mites. All mites were placed into the containers with their paired fly regardless of attachment status. The fly was crushed at the thorax, taking care not to injure an

attached mite. Offspring from each container were subsequently mixed every generation to prevent genetic isolation.

Seeder mites remained in their containers for three days to lay eggs and were then discarded. Offspring were left in their containers for an additional four days to mature and mate. At this point, roughly 20 females were removed from each replicate container (~100 total) and subjected to the selection protocol described above. In experiment A, selection continued for 15 and 16 generations in replicate lines 1 and 2, respectively. In experiment B, selection continued for 10 and 11 generations in replicate lines 1 and 2 respectively.

Response to Selection

In our experiments, mite infectiousness was treated as a threshold character with only two phenotypic classes (infectious or free-living) and a single threshold separating them. According to Falconer and Mackay (1996), the inheritance of such characters is based on an underlying continuity (the liability). When the liability is below the threshold level, the individual has one form of phenotypic expression (e.g. free-living) and the other phenotypic expression when the liability is above the threshold (e.g. infectious) (Falconer and Mackay 1996). To estimate realized heritability for a given replicate line, infection prevalence at each generation was first converted into a measure of mean liability following Falconer and Mackay (1996). Liability variance was assumed similar for the treatment lines. The difference in mean liabilities between 'infectious' and control (or 'free-living' in experiment A) treatment lines was taken as the difference in their level of infectivity, i.e. the 'divergence' between the treatment groups. The strength of the response to selection was estimated from the regression of infection divergence on generation number (Muir 1986). The significance of the regression coefficients was calculated using linear modeling (R Core Team 2017). Because selection was applied to

only one sex (female), the realized heritability of infectiousness was calculated as twice the slope of each regression relating infection divergence to generation number (Polak 2003).

In both experiments, additive genetic variation of infectious behaviour was calculated from the divergence in infection prevalence between the 'infectious' and control lines over time. In experiment A, infection prevalence was not explicitly recorded every generation in the control lines. Thus, divergence was calculated from the 'infectious' and 'free-living' lines. The 'free-living' line served as a close proxy for a control because infection prevalence never significantly diverged from the control group at generations 5, 10, and 15 (see Results). Infection prevalence was assayed in the 'infectious', 'free-living', and control lines at generations 5, 10 and 15. Thirty mites from each treatment line were exposed to flies using the procedure in the selection protocol. Mite infection prevalence was recorded after 60 minutes of exposure. We recognize that our heritability calculations in experiment A could be overestimated as a consequence, though the results do not seem to indicate so (Table 1).

Estimating Density Effects

Selection was terminated at generations 15 and 16 for replicate lines 1 and 2 respectively in experiment A. At this point, mites from replicate containers were combined into 4 L tubs (one per line per treatment) and maintained (without fly exposure) as described in the fly and mite culture methods (above). Mite population density and infection prevalence were monitored for 16 generations post selection and assayed at generations 19, 23, 25, 27 and 31. To estimate population density, each container was first thoroughly mixed by hand. Then, 15 mL of culture medium was removed and examined under a dissecting scope. All living mites in the sample were counted twice by the same individual throughout the entire experiment. To control for mite age, infection assays began by removing 75 female nymphs and 40 adult males from their

respective mass cultures and dividing them equally across five 90 mL ventilated containers with 15 mL of organic media. Nymphs were given three days to develop and mate. Adult females (34-60) were then removed from the containers and individually exposed to *D. hydei* hosts in infection chambers for 60 minutes; infection prevalence was recorded. Because of suspected out-crossing contamination (mites from stock populations accidentally mixing and breeding with experimental lines), we were unable to examine the temporal effects of replicate line and selection treatment on population density and attachment prevalence. However, we did assess the general relationships between population density and attachment prevalence over time. This information would prove pertinent in interpreting the post-selection environmental effects in experiment B.

Trait Stability

In experiment B, we monitored infection prevalence for 20 generations post-selection to assess the stability of the infectivity trait once selection was relaxed. At this stage, mites from each selection treatment within each replicate line (four cultures total) were combined into their respective 4 L container and maintained (without fly exposure) as described above. Infection prevalence was assayed in each culture at generations 15, 20, 21, 24, 27 and 30 using the same protocol described above.

Data Analyses

We utilized the statistical program R and generalized linear models (GLMs) to test the response to selection over the course of the experiments (R Core Team 2017). The response variable was mite infection prevalence. Independent explanatory variables were selection treatment, replicate line and generation. A quasibinomial error distribution was used to account

for overdispersion. The infection prevalence at generations 5, 10 and 15 in experiment A were analyzed using the 'prop.test' (R Core Team 2017).

In experiment A, the change in population density over time and its effect on the prevalence of infection was estimated using GLM with a quasi-binomial error distribution (R Core Team 2017). Temporal stability of the infectivity trait post-selection (experiment B) was determined using a GLM with a quasi-binomial error distribution (R Core Team 2017). For all GLMs, we report the results of the minimal model (using backwards model selection based on F test) presented with deviance (~sums of squares) and *p*-values.

2.3 Results

Response to selection

Experiment A

Due to significant two-way interactions of generation x replicate (deviance = -51.70, $p < 0.001$) and selection treatment x replicate (deviance = -43.56, $p = 0.001$), replicate lines were analyzed separately. In both lines, the interaction between treatment and generation was a significant predictor of infection prevalence (line 1: deviance = -81.79, $p < 0.001$; line 2: deviance = -40.53, $p = 0.006$). Thus, the level of infectivity changed over time, but the direction of change depended upon the selection treatment. In replicate line 1, infection prevalence increased in the 'infectious' line from 45.4 ± 5% SE to 64.4 ± 5% over 15 generations. Infection prevalence in the 'free-living' line decreased slightly before settling around 20% attachment (Fig. 2.1 a). Both treatments in replicate line 2 were subject to accidental out-crossing contamination by mites from the mass cultures at generations 3 and 5; as such, infection prevalence fluctuated widely early on. Although both treatments exhibited an overall decrease in

infection prevalence (55.0 ± 5% SE to 33.6 ± 5% SE in the 'infectious' line and 55.0 ± 5% SE to 19.7 ± 5% SE in the 'free-living' line), infection prevalence in the 'infectious' line remained consistently higher than the 'free-living' line (Fig. 2.1b).

The infection assays performed at generations 5, 10 and 15 yielded similar results. In replicate line 1 (Fig. 2.2a), infection prevalence in the 'infectious' line was significantly higher compared to the control line at generations 5 (prop.test $p = 0.004$) and 10 ($p = 0.002$), but only marginally higher at generation 15 ($p = 0.073$). Infection prevalence in the 'free-living' line was never significantly different from the control line ($p = 0.604$; $p = 0.170$; $p = 0.350$ generations 5, 10 and 15 respectively). In replicate line 2 (Fig. 2.2b), infection prevalence in the 'infectious' and free-living' lines was the same and not significantly different from the control population at generation 5 ($p = 0.421$). These results are likely due to out-crossing contamination (see above). After generation 5, replicate line 2 began to diverge as expected. The 'infectious' population showed significantly higher infection prevalence compared to the control population at generations 10 ($p = 0.042$) and 15 ($p < 0.001$). Similar to line 1, infection prevalence of the 'free-living' line was never significantly different from the control ($p = 1$; $p = 1$ at generations 10 and 15 respectively).

Experiment B

At generation 8, the control line in replicate line 1 exhibited an unexplainably high level of infectivity. Based on Cook's distance (R Core Team 2017), this data point was identified as an outlier and excluded from subsequent analyses. The three-way interaction between selection treatment, replication line and generation was not significant (deviance = -0.040, $p = 0.94$). The two-way interaction terms, generation x line (deviance = -7.04, $p = 0.28$) and treatment x line (deviance = -21.13, $p = 0.063$), were not significant either. Therefore, replicate lines 1 and 2

were analyzed together. Line (deviance = -43.97, $p = 0.011$) and the interaction between treatment and generation (deviance = -40.03, $p = 0.015$) were significant predictors of infection prevalence. Thus, the propensity to infect changed over time, but the direction of change depended upon the selection treatment applied: infection prevalence increased in the 'infectious' lines and decreased in the control lines (Figs. 2.4a and b).

Estimate of realized heritability

In experiment A, we detected a significantly positive response to selection in both replicate lines (Line 1: slope = 0.102 ± 0.014 SE, $p < 0.001$; Line 2: slope = 0.061 ± 0.016 SE, $p = 0.001$; Fig 2.3a). The mean realized heritability in experiment A was 0.163 ± 0.030. In experiment B, the regression of divergence in mean liability on generation was significantly different from zero in both replicates (Line 1: slope = 0.076 ± 0.033 SE, $p = 0.052$, Line 2: slope = 0.092 ± 0.020 SE, $p < 0.001$; Fig. 2.3b). The mean realized heritability in experiment B was 0.169 ± 0.058. Across both selection experiments, the mean realized heritability was 0.166 ± 0.058 (Table 2.1). The reported standard error is the empirical standard error (Falconer and Mackay 1996), estimated directly from the variance of the replicate heritability estimates (Hill 1971).

Density effects

We found that population density increased over time (slope = 2.09 ± 0.841 SE, $p = 0.018$) and infection prevalence was positively correlated with population density (slope = 0.019 ± 0.008 SE, $p = 0.031$)

Trait stability

411 After selection was terminated in experiment B, infection prevalence gradually increased
412 over time, but continued to be higher overall in 'infectious' lines compared to the control (Fig.
413 2.5). Selection treatment (deviance = -124.7, $p < 0.001$) and generation (deviance = -32.2, $p =$
414 0.004) were significant predictors of infection prevalence in both replicate lines, suggesting the
415 trait was maintained in the population even after selection and host exposure had ceased.

416 **2.4 Discussion**

417 Our results indicate that the facultative parasite, *M. muscaedomesticae* exhibits
418 significant additive genetic variation in infectious behaviour (defined as host-attachment). In
419 other words, these results suggest that the behaviour of initiating a symbiotic relationship is
420 heritable. Thus, intermediate symbiotic strategies, such as phoresy and facultative parasitism,
421 can potentially serve as candidate stepping-stones in the evolution of more permanent symbioses
422 like obligate parasitism.

423 Given that all four 'infectious' treatments responded positively to selection, our results
424 are unlikely a product of drift alone, although it likely played a minor role since estimates of
425 realized heritability ranged from 12% and 20% across both experiments. Environmental
426 variation likely contributed as well, as illustrated by the parallel peaks and dips in the infection
427 rates in both selected and control lines (Figs. 2.1 and 2.4). These synchronized changes in
428 infection prevalence likely reflect phenotypically plastic responses to unmeasured microhabitat
429 changes. A condition-dependent response also accounts for the continued rise in infection
430 prevalence post-selection (Fig. 2.5). This pattern is likely due to a gradual increase in population
431 density under the conditions of the mass culture indicating a potential role for population density
432 on the propensity to infect (Lindquist and Walter 1989).

In experiment A, the ‘free-living’ lines never diverged from the control lines. Our selection method was relatively strong for infectiousness, but possibly weaker for ‘free-living’ behaviour. In the ‘infectious’ lines, only successfully attached mites were used to seed subsequent generations. In generating the ‘free-living’ lines, we included mites that may have been infective but were unsuccessful in their attempt to attach to a host. However, it is also possible that there is no heritable basis for free-living behaviour. In experiment B, the ‘infectious’ lines exhibited higher infection prevalence relative to the control lines even 20 generations post-selection which indicates a degree of genetic stability in infectious traits. It could also suggest that no or little costs are associated with increased infectivity, such that the traits are maintained even in the absence of selection. However, ‘infectious lines’ were maintained in a single environment and trade-offs are commonly environment-dependent and manifest only under particular conditions (Sgrò and Hoffmann 2004).

Nachappa et al. (2010) successfully selected for increased dispersal behaviour in a predatory mite (*Phytoseiulus persimilis*) and estimated realized heritability to be 28%, higher than the mean heritability estimate in this study (16.6%). It is possible that infectivity in *M. muscaedomesticae* is more closely linked with life-history traits, which typically exhibit a lower heritability index compared to other traits (Mousseau and Roff 1987; Hoffmann et al. 2016). Interestingly, our heritability measures for infectivity in the mites were similar to heritability measures reported for behaviourally-mediated mite resistance in *Drosophila nigrospiracula* (15.2% and 12.3%; Polak 2003; Luong and Polak 2007 respectively). Because these antagonistic traits (infection and resistance) both exhibit additive genetic variation, the potential for coevolution exists and warrants further exploration.

In our system, infectivity was treated as a threshold trait, and we assumed that when the underlying variable was below the threshold level, mites exhibited a 'free-living' strategy and an 'infectious' strategy above the threshold. Although the underlying continuous variable was unknown there are two possible mechanisms by which increased infectivity was selected. First, we may have lowered the threshold for infection by increasing mite sensitivity to environmental cues (Gilbert and Epel 2015). This was the mechanism Stasiuk et al. (2012) used when selecting for infective-juvenile (IJ) formation. The authors lowered the IJ-formation threshold by increasing *P. trichosuri*'s sensitivity to IJ-formation chemicals. Alternatively, we may have selected to increase the underlying population mean, increasing the proportion of mites capable of reaching the 'infectious' threshold. Further investigation is necessary to identify the suite of traits and genetic mechanism(s) underlying infectious behaviour in *M. muscaedomesticae*, and other facultative parasites generally

We have experimentally shown that infectious behaviour can evolve to moderately high levels under laboratory conditions. So how is facultative parasitism maintained in nature? Facultative parasitism can be viewed as a phenotypically plastic strategy whereby the expression of infectious traits depends on the environmental condition (Farish and Axtell 1971; Reece et al. 2009; Luong et al. 2017). Yet, the fixation of any infectious strategy could mean a reduction in infection plasticity; which could limit an organism's ability to respond rapidly to changes in highly ephemeral and variable habitats. Trade-offs associated with increased infectiousness (e.g. fecundity and longevity) could also constrain the evolution of a fixed strategy and contribute to the persistence of facultative parasitism (Stearns 1992; Paterson and Barber 2007). Currently, we are investigating potential evolutionary trade-offs between increased infection in mites and their longevity, lifetime fecundity, and attachment plasticity.

Our study provides empirical evidence for additive genetic variation in the infectious behaviour of a facultative parasite. By estimating the magnitude of heritable genetic variation underlying infectious traits, we can make predictions about the evolutionary potential of facultative parasites and begin to test the hypothesis that intermediate parasite strategies serve as potential stepping-stones in the evolution of obligate parasitism. Mites, including *M. muscaedomesticae,* have been considered for biological control of fly pests (Azevedo et al. 2015) and we show that they have the potential to become even more effective control agents using artificial selection. More broadly, our study highlights the evolutionary potential of medically important facultative and opportunistic parasites, which is critical for the development of successful and sustainable control measures.

Table 2.1 Calculations for the estimate of realized heritability in infectious behaviour of the facultatively parasitic mite *Macrocheles muscaedomesticae*.

Slopes and intercepts of regression functions relating divergence in infection prevalence (in SD units) to generation number in four replicate selection lines. Realized heritability (h^2) of infectious behaviour values are calculated as twice the slope of each regression function.

Experiment	Line	Generations	Slope (SE)	*P*-value	Intercept (SE)	*P*-value	h^2 (SE)
A	1	15	0.102(0.013)	<0.001	0.093(0.112)	n.s. (0.421)	0.206(0.028)
	2	17	0.060(0.015)	0.001	-0.064(0.145)	n.s. (0.662)	0.121(0.032)
B	1	10	0.076(0.033)	0.052	0.207(0.190)	n.s. (0.309)	0.153(0.076)
	2	11	0.092(0.020)	<0.001	-0.179(0.130)	n.s. (0.199)	0.186(0.040)
Mean							0.166(0.044)

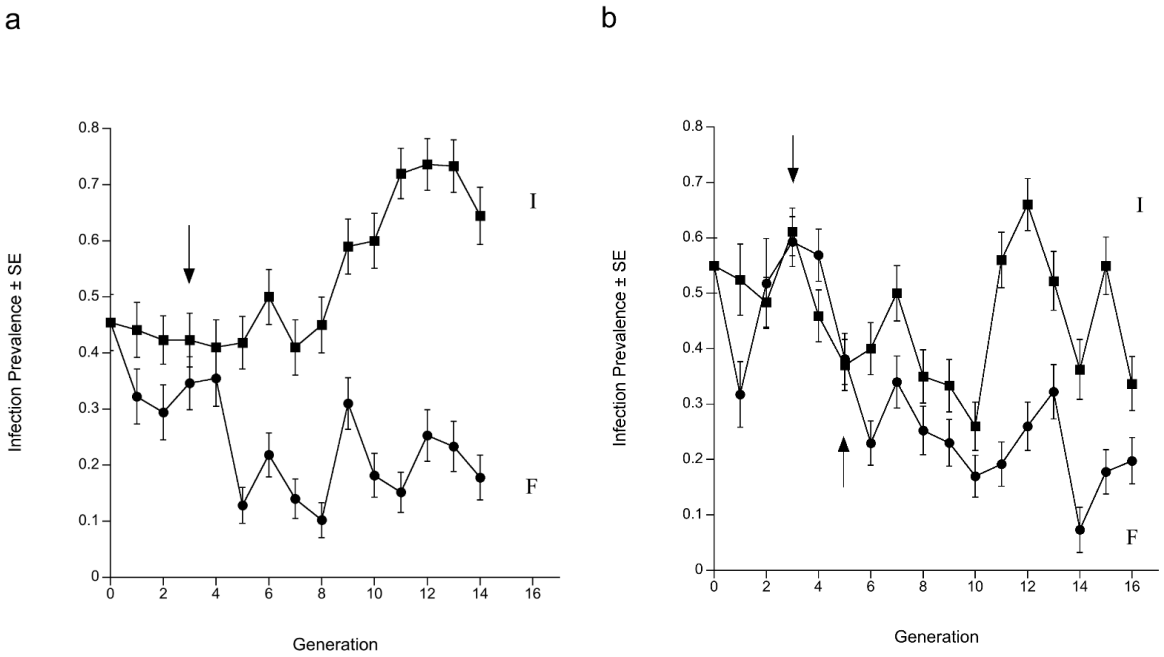


Figure 2.1 Response to selection in experiment A.

Experiment A (Fall 2015), response to selection on *Macrocheles muscaedomesticae* for 'infectious' (squares, I) and 'free-living' behaviour (circles, F), measured as the proportion of host attachment in replicate lines 1 (a) and 2 (b). Arrows indicate generations that experienced outcrossing contamination.

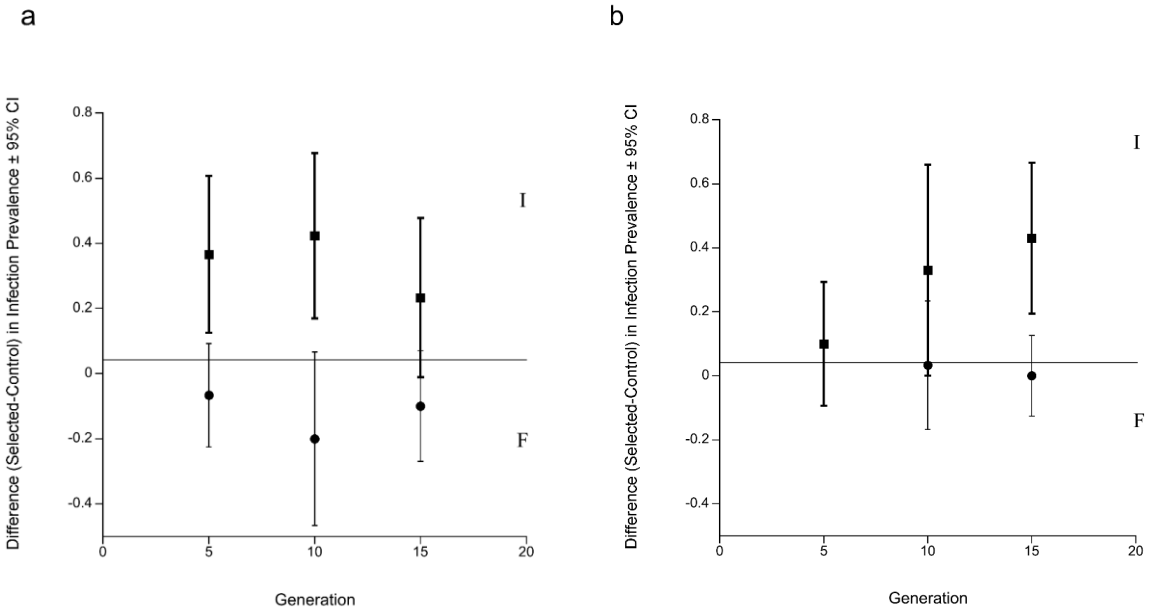


Figure 2.2 Response to selection in relation to the control group in experiment A

Experiment A (Fall 2015), prevalence of *Macrocheles muscaedomesticae* attachment in selected lines in relation to the control line at generations 5, 10 and 15 from replicate lines 1 (a) and 2 (b). Difference in infection prevalence is the difference in proportion of attached mites between the selected lines: infectious (squares, **I**) or free-living (circles, **F**) and the control line (solid line).

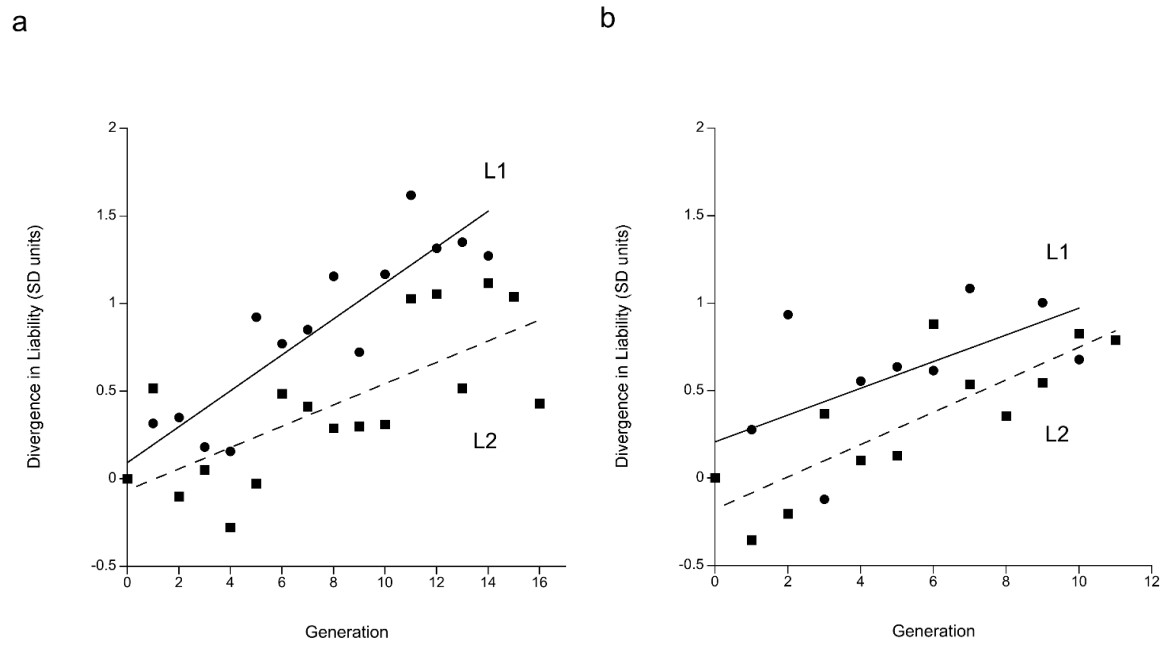


Figure 2.3 Divergence in attachment prevalence in experiments A and B.

Divergence among *Macrocheles muscaedomesticae* in response to selection for infectious behaviour in experiment A (a) and experiment B (b). Divergence is the difference in mean liability between selected and control lines expressed in standard deviation (SD) units. Circles and solid line correspond to divergence in replicate line 1 (L1). Squares and dashed line correspond to divergence in replicate line 2 (L2).

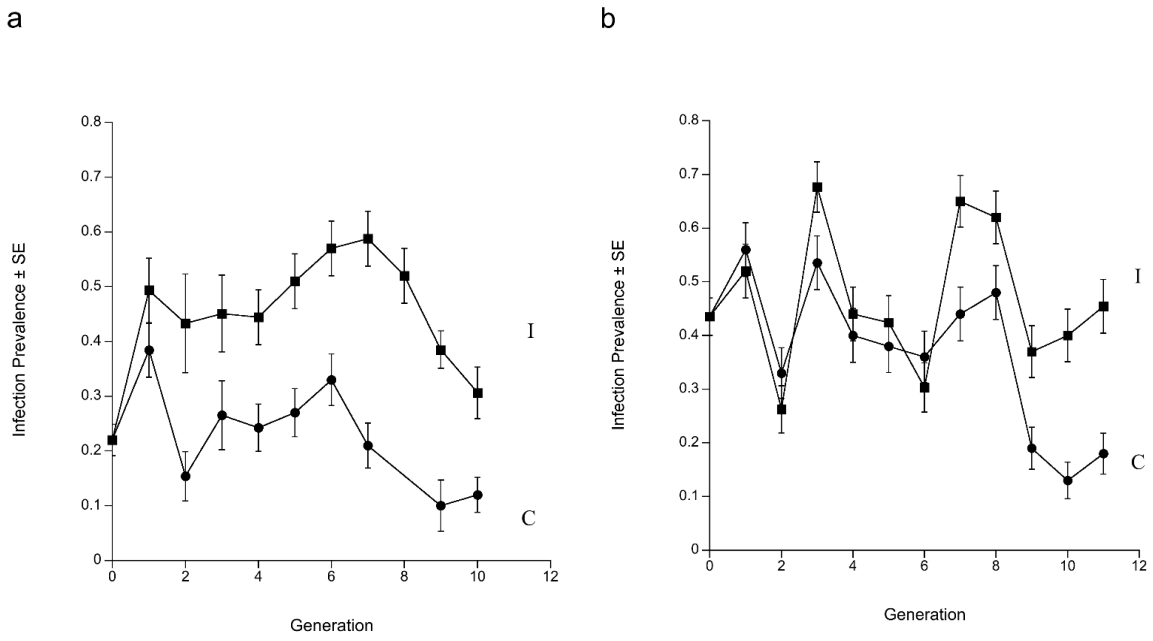


Figure 2.4 Response to selection in experiment B.

Experiment B (Spring 2016), responses to selection on *Macrocheles muscaedomesticae* for infectious behaviour in 'infectious' (squares, I) and control (circles, C) lines from replicate lines 1 (a) and 2 (b). The control group did not undergo any selection. Infectious behaviour was measured as the proportion of host attachment.

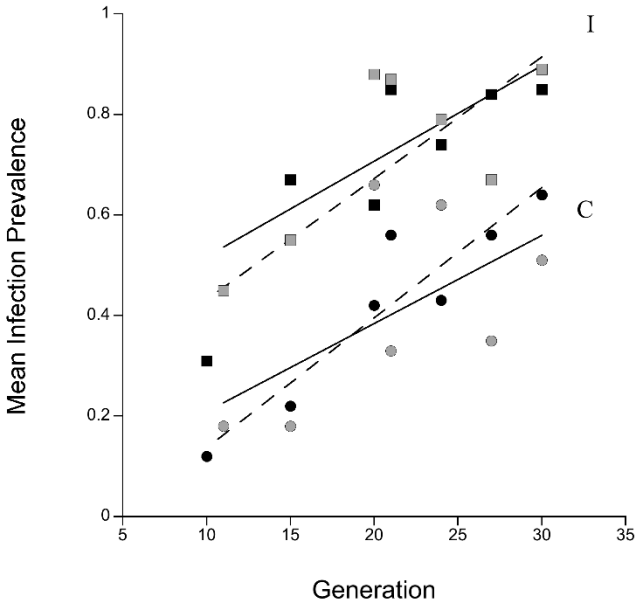


Figure 2.5 Stability of infectious behaviour post selection in experiment B.

Temporal stability of infectious behaviour in *Macrocheles muscaedomesticae* post selection in replicate lines 1 (black) and 2 (grey) in experiment B. Circles correspond to control lines (C) and squares correspond to 'infectious' lines (I). Regression lines show the relationship between infection prevalence and generation in replicate line 1 (solid line) and line 2 (dashed line).

Chapter 3. Traits associated with increased infectious behaviour in a facultatively ectoparasitic mite: potential lifehistory costs and changes in morphology

3.1 Introduction

Parasitism is a symbiotic relationship in which the parasite benefits at the cost of the host (Price 1980; Combes 2005; Roberts and Janovy 2009). Although parasitism is ubiquitous in nature, how and why this lifestyle evolved remains largely unknown. A commonly proposed hypothesis for the evolution of parasitism posits that parasitic ex-aptations facilitated the evolution of host-associations (i.e. symbioses). For instance, strategies such as phoresy and facultative parasitism may have served as stepping-stones during the evolutionary transition to obligate parasitism from free-living ancestors because they provide host exposure and adaptive strategies for host-seeking (Athias-Binche 1991). Facultative parasites exhibit varying degrees of parasitism at the individual and population level, and, although capable of parasitic activity, they can complete their lifecycle without a host (Roberts and Janovy 2009). In some lineages the fitness benefits gained from those associations likely led to the fixation of parasitic strategies by means of natural selection (Rothschild and Clay 1952; Osche 1956; Poulin 2007; Dieterich and Sommer 2009; Dowling 2015). This raises a conundrum; if host associations (i.e. infection) provide a fitness benefit to symbiotic organisms (such that facultative parasitism evolved), what prevents natural selection from driving facultative parasites to fixed, obligate parasitism? Facultative parasitism could potentially be an intermediate stage in the evolutionary transition to obligate parasitism. In other words, facultative parasitism is a necessary, but transient strategy in the evolution of obligate parasitism. Alternatively, facultative parasitism, and hence variation in

A version of this chapter will be submitted to Evolutionary Biology as "Durkin, E. S. and L. T. Luong. Evolutionary trade-offs do not constrain the evolution of infectivity in a facultative ectoparasite."

infection strategies, may be maintained by evolutionary constraints that prevent the fixation of an obligate infection strategy.

Generally speaking, the infection phenotype of a facultative parasite can be considered a threshold trait, which is characterized by two phenotypes (i.e. infective and non-infective) with an underlying continuous variable and a single threshold that delineates the two phenotypes. When the underlying continuous variable is below the threshold, one phenotype (e.g. non-infective) is expressed, and the alternative phenotype (e.g. infective) is expressed when the continuous variable is above the threshold. Although the phenotypes vary discontinuously, the underlying variable exhibits continuous variation, and that variation can be both genetic and environmental in origin (Falconer and Mackay 1996). In this study, we hypothesize that the genetic variation for infectivity among facultative parasites is partially maintained by costs associated with selection on the infectivity trait (i.e. the threshold).

Negative correlations between traits can occur from trade-offs, which occur in two primary forms: physiological and evolutionary. Physiological trade-offs typically result from resource allocations for two or more processes that compete directly with one another for limited resources and occur at the individual level (Stearns 1992). Evolutionary trade-offs can result from antagonistic pleiotropy or linkage disequilibrium between traits that can simultaneously increase and decrease fitness (Stearns 1992). Evolutionary trade-offs in host-parasite interactions have been identified, however, costs associated with host resistance have been the primary focus (Kraaijeveld and Godfray 1997; Luong and Polak 2007a,b).

In this study, we determined whether evolutionary trade-offs play a role in maintaining infection variation in the facultative parasite, *Macrocheles muscaedomesticae* (Scopoli) (Mesostigmata: Macrochelidae). These mites typically inhabit rotting plant tissue and are

564 commonly found in compost and dung, where they feed upon small invertebrates, mate and lay
565 eggs (Rodriguez and Wade 1961; Jalil and Rodriguez 1970; Krantz 1998). As conditions
566 deteriorate, adult female mites can attach to a fly host (initiating infection) for dispersal to a new
567 habitat as well as a potential meal (Jalil and Rodriguez 1970; Krantz 1998).

568 Within a population of *M. muscaedomesticae* the propensity to infect varies widely;
569 some females attach immediately to an available host while others postpone or ignore the
570 opportunity altogether. Previous studies suggest that their infection behaviour is plastic and
571 influenced by their own internal state (age, Jalil and Rodriguez 1970; mating status and
572 starvation, Luong et al. 2017), the state of their potential hosts (sex and size, Campbell and
573 Luong 2016; infection status, Luong et al. 2017) as well as the external environment (Farish and
574 Axtell 1971; Durkin and Luong 2018). Infection plasticity could act alone to maintain existing
575 variation in a population; however, this does not preclude the importance of genotypic variation.
576 In a previous study, we showed that *M. muscaedomesticae* exhibited additive genetic variation
577 for infectious behaviour towards fly hosts (Durkin and Luong 2018) and generated populations,
578 using artificial selection, with significantly increased infection propensity. Artificial selection
579 provides a powerful tool for investigating evolutionary trade-offs because we can compare traits
580 of the selected populations to those of the controls (Muir 1986).

581 Trade-offs are often context-dependent, changing in direction and severity depending on
582 the environment, thereby amplifying their ability to maintain genetic variation (Stearns et al.
583 1991; Kassen 2002; Sgrò and Hoffmann 2004; Chamberlain et al. 2014). Here, we examined the
584 longevity and fecundity of selected and control mites with and without access to a fly host (Fig.
585 S3.1). In the presence of a host, selected mites can reap the benefits of increased infectivity.
586 However, in the absence of hosts, the benefits of increased infectivity cannot be realized, and the

cost (if any) of maintaining increased infectivity should manifest. After infecting a fly, we expect selected mites to exhibit higher fecundity and longevity compared to control mites (regardless of host availability) overall. Conversely, when a fly is not available, we expect selected mites to exhibit lower fecundity and longevity compared to control mites overall.

Organisms that utilize patchily distributed ephemeral habitats often face difficulties dispersing to other suitable habitats. When infecting a fly, not only do mites receive a potential meal, they also gain a mode of transportation. Thus, given this passive mode of transport, infectious mites may evolve decreased investment in their own motility. When comparing the off-host motility of a phoretic louse species to a non-phoretic louse species, Bartlow et al. (2016) found that phoretic lice were significantly less motile. Accordingly, we predict a negative correlation between mite infectivity and motility, i.e. a reduction in the active motility of selected mites compared to control mites.

Selection for increased infectivity may also affect other traits such as body size and chelicerae size. Poulin and Morand (1997) reported that among ticks, the distribution of scutum size was log right-skewed, suggesting a trend towards smaller body sizes. They hypothesized that this pattern resulted from host grooming which selected for smaller body sizes. Given that fly hosts respond to the presence of ectoparasitic mites by grooming (Polak 2003), we predict a negative correlation between increased infectivity and mite body size. Lastly, because *M. muscaedomesticae* use their chelicerae for host attachment (Farish and Axtell 1971; Dowling 2015), we expect that selection for increased infectivity will lead to positive selection on chelicerae size. Furthermore, we expect positive selection on cheliceral grip strength.

3.2 Material and methods

Fly and Mite Culture

610 *Drosophila hydei* (~100 per sex) were collected from residential compost bins in
611 Edmonton, Alberta Canada in September 2013 (approx. coordinates 53.52 °N, 113.48 °W) and
612 used to establish a laboratory culture. Flies were mass cultured in six to eight 200 mL bottles on
613 standard agar-molasses-yeast-based fly media at 24 °C, 70% relative humidity and a 12D: 12L
614 photoperiod. Flies were maintained in the lab for two years before selection experiments took
615 place. Female *D. hydei* flies were used for all experiments.

616 Approximately 700 female *M. muscaedomesticae* collected from field-caught *D. hydei*
617 were used to initiate a mass culture in the laboratory. Mites were maintained for two years
618 before selection experiments commenced. Mites were cultured in three panmictic 4 L plastic
619 containers containing organic medium: sterilized organic wheat bran, sterilized aspen wood
620 shavings, deactivated yeast and distilled water. The organic medium contained bacteriophagic
621 nematodes as a food source for the mites. Cultures were kept at 24 °C, 70% relative humidity
622 and a 12 D: 12 L photoperiod.

623 *Artificial Selection*

624 The selection protocol consisted of individually exposing 60-130 adult female mites of
625 each generation and replicate line to a single female *D. hydei* fly for 60 minutes in experimental
626 infection chambers. Infection chambers were 200 µL pipette tips reduced to half their length by
627 cutting off the narrow end (~1.5 cm); both ends were stoppered with cotton. These small
628 chambers restricted fly movement which allowed control of heterogeneity in behaviourally-
629 mediated host resistance or encounter possibilities.

630 A single mite was placed into an infection chamber followed by a single fly. Following
631 the 60-minute exposure, mites were scored as attached or unattached to their fly and then

exposed for an additional 30 minutes. Based on unpublished observations, mites that attach often do so within 60 minutes and then steadily drop off over the next 48 hours. By eliminating the mites that "switched" after 60 minutes, we were able to select mites more consistent in their behaviour. Mites that were attached after both 60 and 30 minutes of exposure were used to seed the infectious mite line. A total of 13 to 50 mites was used to seed each new generation of each line (mean = 41.8 ± 2.3 SE). The same number of seed mites was used in each selection group, each generation within each replicate line. The total number of seed females used during each generation was divided equally across 2-5 replicate containers (the number of replicate containers depended on upon the number of seeder females). Offspring from replicate containers were mixed each generation; replicate containers were not genetically isolated. Each container held roughly 50 mL of organic media and was sealed with Parafilm® (Neenah, WI) to prevent contamination by other mites. Control lines were maintained in parallel to selected lines within each replication line. At each generation, the control line was seeded with the same number of mites as the selected line. Unlike the selected lines, control mites were randomly selected to seed generations regardless of attachment.

In both the selected and control groups, mites were placed into the containers with their fly host regardless of attachment. In all cases, the fly host was crushed at the thorax using forceps, taking care not to injure an attached mite.

Mites remained in the containers for 3 days to lay eggs and were then removed from the media. Three days was an important number because the offspring are still easily distinguished from the adults, making adult removal easier. Offspring then remained in their containers for four days to mature and mate with siblings. Once developed, a roughly equal number of females was removed from each of the replicate containers and subjected to the selection protocol

655 described above. For logistical reasons, replicate line 2 experienced an additional generation of
656 selection, which allowed me to separate the life history measurements of each replicate line by
657 one week. Measuring the replicate lines separately provided a more manageable workload.
658 Selection lasted 10 and 11 generations in replicate lines 1 and 2 respectively.

659 Control lines were maintained in parallel to selected lines within each replication line. At
660 each generation, the control line was seeded with the same number of mites as the selected line.
661 Unlike the selected lines, control mites were randomly selected to seed generations regardless of
662 attachment.

663 *Fecundity and Longevity*

664 To determine whether selection for increased infectivity yielded a trade-off with
665 fecundity or longevity, we measured the lifetime fecundity of selected and control mites from
666 experiment A following the final generations of selection. To determine if the nature of the
667 trade-off is context-dependent (i.e. affected by access to a suitable host), half of the mites from
668 each of the control and selection treatments parasitized a fly, while the other half only had access
669 to nematodes, as a food source (Fig. S3.1).

670 After 15 (replicate line 1) and 17 generations (replicate line 2) of selection, individual
671 adult female mites were extracted from the selected and control cultures and allowed 72 hours to
672 lay eggs in 90 mL ventilated, plastic containers filled with 50 mL of organic medium containing
673 nematodes (see Durkin and Luong 2018). The adult females were then discarded, and their
674 offspring were allowed 96 hours to mature and mate. Hence, we performed the fitness assays on
675 the F1 generation (post selection). Mites from each replicate line within the selected and control
676 lines were further split into two groups: with and without access to a host. Thirty newly matured

677 adult female mites were individually placed into an experimental infection chamber with a single
678 female *D. hydei* host (14.63 ± 0.37 SE days post-eclosion) for 60 minutes. The infection
679 chamber was constructed from a 200 µL pipette tip reduced to half its length (~1.5 cm) and
680 stoppered with cotton. Mites that attached remained in their respective infection chambers with
681 their host for an additional 20 hours to feed. During this time infection chambers were housed in
682 an incubator (25 °C, 70% RH and a 12 D: 12 L photoperiod). Of the mites that remained
683 attached to the fly, ten were randomly selected from each of the treatment and replicate lines to
684 measure longevity and fecundity (Fig. S3.1). These mites were then transferred individually to
685 fresh mite media. First, the fly's thorax was crushed with forceps. The dead fly and mite were
686 then placed into a new 90 mL ventilated plastic container filled with 50 mL of organic medium.
687 At the same time, mites without access to hosts were prepared by individually transferring ten
688 newly matured adult female mites from each of the treatment and replicate lines directly into a
689 90 mL ventilated plastic container with 50 mL of organic medium. Containers were sealed with
690 Parafilm® (Neenah, WI) to prevent contamination by other mites. All containers were kept at 25
691 °C, 70% relative humidity and a 12 D: 12 L photoperiod.

692 Each container was inspected under a dissecting scope every 48 hours, and once located,
693 the female was transferred to a new container with fresh organic media. The media that each
694 female was recovered from was placed into a plastic container and preserved in 95% ethanol to
695 later count offspring from. Eggs hatch roughly seven hours after they are laid (Wade and
696 Rodriguez 1961; Farahi et al. 2018). Thus, most of the offspring in the preserved media samples
697 were in their nymphal stage of development. Female recovery ceased when the female was
698 found dead or was assumed dead if the female could not be located after two complete rounds of

699 inspection. Any dead females recovered were preserved in 95% ethanol, and the number of days
700 alive since they were a newly matured adult was recorded (i.e. longevity).

701 The preserved media samples were then examined for nymphs. Taking advantage of the
702 mite's hydrophobic exoskeleton, preserved media samples were transferred to 150 mL plastic
703 containers and filled with 75 mL of tap water. The containers were covered with parafilm® and
704 thoroughly mixed by inversion. Once the substrate settled, the supernatant was poured off into a
705 14 mm diameter petri dish and examined under a dissecting microscope. All recovered nymphs
706 were counted and recorded (eggs were not included in the counts). Each sample of media was
707 washed and examined for nymphs three times to ensure full recovery.

708 *Motility*

709 To determine whether increased infectivity is negatively correlated with motility, we
710 measured the time elapsed for selected and control mites to travel an 8 cm "racetrack". The
711 motility experiments were performed on mites from selection experiment B. At the time of the
712 motility experiments, mites had been mass-cultured without selection for five generations.
713 However, the selected lines exhibited significantly higher infection prevalence compared to the
714 control lines at this time and continued for 20 generations post-selection (see Durkin and Luong
715 2018).

716 Adult female mites were haphazardly retrieved from both selected and control replicate
717 lines. The mite racetrack was constructed from two 2.6 x 7.6 cm (1 mm thick) glass microscope
718 slides that sandwiched three wooden applicator sticks that ran the length of the slides. The
719 applicator sticks were placed 0.9 cm apart from each other to create two lanes. The applicator
720 sticks were adhered between the slides using a silicon sealant. Two racetracks were combined

lengthwise to create a single racetrack 15.2 cm long. A horizontal line was drawn one cm in from the edge of the racetrack to indicate the start line and at every cm up to the last to indicate the finish line (Fig. S3.2).

For logistical reasons, only one mite was assayed at a time. A single mite was placed into the starting end of the racetrack facing the finish line. Once the mite was in the racetrack, both ends of the racing-lane were stoppered with cotton to prevent airflow. Timing began when the mite's entire body crossed the start line and continued until the mite's entire body crossed the 8 cm finish line. If a mite stopped or turned around during the trial, the mite was discarded from the study. Once the race was completed (10.81-37.97 sec), the mite was discarded, and the racetrack was cleaned using distilled water (mites reacted adversely to tracks rinsed with ethanol) to wash away residual sensory cues from the previous mite. All races were run during the day under ambient conditions (20°C, fluorescent lights).

Morphological Measurements

To determine whether increased infectivity had a correlated effect on body size, we measured the body size of adult female mites from selection experiment B. Mites were taken from selected and control treatment mass cultures ten generations after selection ceased. Although selection had ended at this point, the selected mites continued to exhibit significantly higher infection prevalence compared to the control (Durkin and Luong 2018).

Fifty adult female mites were collected from each of the replicate lines of selected and control treatments and stored in 70% ethanol. To prepare mites for slide mounting, each specimen was placed in distilled water for 48 hours to soften the cuticle. Mites were then

742 individually slide-mounted in 90% polyvinyl alcohol mounting medium (PVA). Care was taken
743 to separate the chelicerae from the mite during the mounting process.

744 To estimate body size we measured the dorsal and ventrianal shields of the preserved
745 specimens (Newton and Proctor 2013). Images of the dorsal shield and the ventrianal shield
746 were captured using a Leica MC 120 HD camera at 10X and 20X, respectively, on a compound
747 microscope. Shield measurements were made using the Leica application suite (v4.6). Dorsal
748 shield length was measured from the anterior edge of the shield between the j1 setae, to the
749 dorsal edge of the shield midway through the J5 setae (Fig. S3.3a; see Özbek et al. 2015 for *M.*
750 *muscaedomesticae* setae identification). Ventrianal shield length was measured from the anterior
751 edge of the shield midway between the anterior-most pair of setae to the beginning of the
752 cribiform plate, midway through the most-posterior pair of setae (Fig. S3.3b). Dorsal and
753 ventrianal shields were measured only if they were not damaged during the mounting process.
754 The cheliceral moveable digit and first and second segments were captured at 40X and 20X
755 magnification, respectively, using the same microscope and camera. We measured the length of
756 the chelicera's moveable digit if the entire chelicera could be brought into focus in a single
757 viewing plane (Fig. S3.3c). We measured the length of the first and second cheliceral digit, from
758 the apodeme of moveable digit to the end of the sclerotization of the first digit as a proxy for
759 cheliceral strength (Fig. S3.3d). There is a tendon attached to the apodeme which runs the entire
760 length of the chelicerae and into the gnathosoma (Alberti and Coons 1999; Krantz and Walter
761 2009). Muscles attach to the tendon from the sclerotized wall of the chelicerae (Alberti and
762 Coons 1999; Krantz and Walter 2009). When the muscles contract, the tendon is pulled and the
763 chelicera closes (Alberti and Coons 1999; Krantz and Walter 2009). During the mounting
764 process, all of the muscle tissue was cleared. Thus, we measured the length of the sclerotized

765 segments. Longer segments would allow for more muscle attachment which could translate into
766 stronger cheliceral grip strength. The dorsal shields, ventrianal shields and moveable cheliceral
767 digits were measured by a single researcher that was blind to the identity of the mites. A
768 different researcher that was blind to mite identity made all of the cheliceral segment
769 measurements.

770 *Data Analyses*

771 We used generalized linear modeling (GLM) to analyze the data with R statistical
772 software (R Core Team 2017). The minimal model was determined using backwards model
773 selection; significant variables (chi-square test, $p < 0.05$) were retained in the models. The
774 selection criterion ($p < 0.05$) was based on an F-test for models that required a quasi-likelihood
775 error distribution to account for over-dispersion. Final models were validated by checking the
776 homogeneity, normality and independence of the residuals. We report the deviance (~sums of
777 squares) and *p*-value of variables.

778 *Fecundity and Longevity*

779 To investigate the evolutionary trade-off between increased infectivity and fecundity, we
780 compared the fecundity of selected and control mites. Five different researchers collected the
781 fecundity data; however, each researcher examined media samples from all treatment groups.
782 Using a GLM with a quasi-poisson (log link) error distribution, we found the identity of the
783 washer did not have a significant effect on the number of nymphs collected from a sample
784 (deviance = -22.41, $p = 0.16$). Thus, the fecundity data were pooled for analysis.

785 Generalized linear models with quasi-poisson (log link) error distributions were used to
786 determine the effects of selection treatment and host availability on lifetime fecundity (total

number of nymphs produced during a single mite's lifetime). The full model contained replicate line, selection treatment and fly attachment as well as their interactions. Mite longevity was included in the model as a covariate because mite lifetime fecundity and longevity were positively correlated (slope = 3.00 ± 0.38, $p < 0.001$). Differences between replicate lines are most likely due to genetic drift, however, any effects due to drift are potentially informative so replicate line was treated as a fixed factor. The lifetime fecundity from one selected mite in replicate line 2 was discarded because one of the samples was contaminated with at least one other female mite. Two individual mites were missing a single nymph count for a given 48-hour period and were not included in the lifetime fecundity analyses.

In addition to lifetime fecundity, we also calculated weighted fecundity ($l_x m_x$) at age x (average nymph production at age x, weighted by the probability of surviving to that age, see Connell et al. 1970; see supplementary material for the complete life table calculations for each replicate line's treatment groups). The weighted fecundity was plotted against age to visualize the pattern of nymph production (i.e. the fecundity schedule) and was analyzed using a GLM with a gaussian (identity link) error distribution. The response variable ($l_x m_x$) and age were both log(x+1) transformed to normalize the errors. The full model included age, selection treatment, fly attachment and replicate line along with their interactions. A polynomial age term was also included in the model to account for the possible non-linearity.

A GLM with a gamma (inverse link) error distribution was used to determine the effects of selection treatment and fly attachment on mite longevity. Replication line, selection treatment and fly attachment were included in the full model.

Motility

Our motility data violated homogeneity of variance; race-time variances between the two replicate lines were significantly different (Bartlett's K squared = 8.09; $p = 0.004$), whereas variances between the selection treatments were similar (Bartlett's K squared = 0.064; $p = 0.80$). We compared the race times of the selected and control mites across replicate line to determine whether we could pool the selected and control mites using a two-way t-test. The selected (t (50.57) = -0.95; $p = 0.35$) and control (t (52.24) = -0.71; $p = 0.48$) groups were similar across replicate line. Thus, we pooled the selected and control race times and compared them using a GLM with a gaussian (identity link) error distribution to determine whether selection treatment affected mite motility.

Morphological Measurements

The consistency of morphological measurements was determined by estimating the correlation between the first and second repeated measurements of 10 randomly selected mites. A separate R^2 value for repeated measures was calculated for each of the morphological measurements.

GLM models with a gaussian (identity link) error distribution were used to determine whether selection treatment affected the size of the dorsal and ventrianal shields. GLM models with a gamma (inverse link) error distribution were used to determine whether selection treatment affected the size and strength of the chelicerae. Two outliers in the cheliceral moveable digit measurements were identified using Tukey's method for outlier identification and removed before analysis. Three outliers in the cheliceral strength measurements were identified using Tukey's method for outlier identification and removed before analysis. For each body measurement, the initial models contained selection treatment, replicate line as well as their interaction.

3.3 Results

Lifetime Fecundity

The 3-way interaction (deviance = -11.29, $p = 0.13$) and the interaction between selection treatment and fly attachment were not significant (deviance = -3.39, $p = 0.41$). However, replicate line significantly interacted with selection treatment (deviance = -33.69, $p = 0.011$) and fly attachment (deviance = -30.02, $p = 0.016$). For this reason, we analyzed the replicate lines separately.

We predicted that mites selected for increased fly attachment, in the absence of hosts, would show lower fecundity compared to control mites. Conversely, we predicted that selected mites with access to a host would show higher fecundity compared to control mites overall. In replicate line 1, mean lifetime fecundity was similar across all treatment groups (Fig. 3.1a). There was one outlier in replicate line 1, which was removed before statistical analysis. Model selection confirmed that neither fly attachment (deviance = -0.37, $p = 0.75$), selection treatment (deviance = -4.63, $p = 0.26$) nor their interaction (deviance = -1.83, $p = 0.49$) were significant predictors for lifetime fecundity. Contrary to our predictions, lifetime fecundity was similar across all mites in replicate line 1 regardless of selection treatment or fly attachment.

In replicate line 2, both selection treatment (deviance = -35.79, $p = 0.023$) and fly attachment (deviance = -77.60, $p = 0.001$) had a significant affect on lifetime fecundity, but their interaction was not significant (deviance = -14.77, $p = 0.12$). Selection treatment had a positive effect on mean lifetime fecundity for mites regardless of host availability. Overall, the lifetime fecundity of selected mites (mean = 55.6 ± 7.04 SE) was significantly higher than the control mites' (mean = 46.1 ± 5.85 SE). Host availability also had a positive effect on lifetime fecundity for both selected and control mites (Fig. 3.1b). Mites that attached to flies produced significantly

855 more offspring in their lifetime (mean = 64.4 ± 6.94 SE) than the mites without access to hosts
856 (mean = 37.7 ± 4.51 SE) regardless of the selection treatment. In other words, mites that infected
857 flies had higher reproductive success, which implies a fitness advantage to parasitism.

858 *Weighted Fecundity*

859 The minimal model for the analysis of weighted fecundity over time included fly
860 attachment (deviance = -0.75, $p = 0.007$), mite age (deviance = -58.21, $p < 0.001$) and the
861 quadratic term of mite age (deviance = -74.13, $p < 0.001$). Selection treatment, replicate line, as
862 well as all the possible interaction terms were not significant and eliminated from the model ($p >$
863 0.05; see supplementary table S3.9 for the deviances and p-values of all variables and interaction
864 terms). The only significant experimental factor was fly attachment; mites that attached to flies
865 produced more nymphs over time compared to mites without access to hosts (Fig. 3.2).

866 *Longevity*

867 We predicted that selected mites would exhibit greater longevity compared to control
868 mites when they had access to a host and vice versa when hosts were not available. However, the
869 mean longevity among the treatment groups was not significantly different (Fig. 3.3). Selection
870 treatment, host attachment, replicate line and their interactions were not significant predictors of
871 mite longevity ($p > 0.05$; See supplementary table S3.10 for the deviances and p-values of all
872 variables and interaction terms).

873 *Motility*

874 We predicted selected mites to exhibit a decrease in their motility. However, the time it
875 took selected mites to travel 8 cm (16.59 ± 0.52 SE) was similar to that of the control mites

(16.05 ± 0.50 SE). Selection treatment did not have a statistically significant effect on mite motility (deviance = -8.69, $p = 0.46$).

Morphological Measurements

Contrary to our predictions, the dorsal shield length of selected mites (mean = 949.90 ± 3.13 µm SE) was similar to that of the control mites (mean = 949.25 ± 3.15 µm SE; deviance = -53.79, $p = 0.81$). Since the interaction between selection treatment and replicate line (deviance = -1098.98, $p < 0.001$) was significant, the ventri-anal shield length was analyzed separately for each replicate line. In line 1, the ventri-anal shield length of selected mites (mean = 319.40 ± 1.75µm SE) was significantly larger (deviance = -585.82, $p = 0.039$) than that of the control mites (mean = 314.32 ± 1.68µm SE). Conversely, in line 2, the ventri-anal shield of the selected mites (mean = 313.91 ± 1.78µm SE) was marginally smaller (deviance = -513.71, $p = 0.058$) than that of the control mites (mean = 318.58 ± 1.66µm SE). The size of the moveable chelicera digit was similar across selected (mean = 83.63 ± 0.20µm SE) and control (mean = 83.88 ± 0.34µm SE; deviance = -0.17, $p = 0.83$) mites. Similarly, the strength (i.e. length of the first and second cheliceral segments) of the chelicerae was similar between selected (mean = 251.16 ± 1.30µm SE) and control (mean = 249.01 ± 1.59µm SE; deviance = $-7.19e^{-06}$, $p = 0.93$) mites.

We also examined the relationship between chelicerae size and dorsal shield size (i.e. body size) in the mites to determine whether chelicerae size differed relative to body size between selected and control mites. The relationship between dorsal shield and chelicera size was linear, indicating isometric growth. In other words, chelicerae grew proportionally with the body, and the chelicerae and body grew at similar rates. Selection treatment had no effect on the growth rate (deviance = 0.14, $p = 0.81$). However, growth rate differed significantly between the replicate lines (deviance = -11.47, $p = 0.032$; figure 3.4). The relationship between dorsal shield

size and chelicerae size was linear and steeper in line 1 (slope = 0.042 ± 0.006, $p < 0.001$) compared to line 2 (slope = 0.023 ± 0.006, $p < 0.001$).

3.4 Discussion

We hypothesized that evolutionary trade-offs between infectivity and other life-history traits help maintain the facultative parasitic strategy in *M. muscaedomesticae*. Because many trade-offs are context-dependent (Chamberlain et al. 2014), we predicted costs associated with infectivity to manifest in the absence of hosts. Our results did not indicate the presence of trade-offs between increased infectivity and fecundity, longevity, motility, body size or chelicerae morphology. Increased infectivity therefore does not appear to be costly. In our previous selection experiments, selected mites continued to exhibit significantly increased attachment prevalence 20 generations post-selection without host access, which suggests that increased infectivity is maintained with little or no cost in *M. muscaedomesticae*. Castagnone-Sereno et al. (2015) also suggested costs were minimal when they selected for increased virulence in nematodes and failed to detect trade-offs. Alternatively, trade-offs may manifest in traits that we did not measure. Direct selection for a single infection strategy may result in a loss of infection plasticity, a potentially critical trait for parasites (Reece et al. 2009). Future research should examine potential negative correlations between infectivity and infection plasticity.

Expected trade-offs can also go undetected because of genotype x environment interactions (Stearns 1992; Sgrò and Hoffmann 2004). Environmental conditions can have a large effect on the direction and magnitude of a trade-off (Stearns 1992). For example, nematodes selected for a fast-infection strategy exhibited increased fecundity, but only in low-density populations (Paterson and Barber 2007). The costs associated with increased parasite resistance in *Drosophila nigrospiracula* were influenced by temperature and conspecific density

(Luong and Polak 2007b,a). Furthermore, increased parasite resistance in *Plodia interpunctella* was associated with a trade-off with growth rate, but the magnitude of the trade-off depended upon resource availability; the cost of parasite resistance was lower with unlimited resources (Boots 2011). Similarly, in their experimental evolution of *Pseudomonas fluorescens* bacteria, Hall and Colegrave (2008) observed trade-offs between motility and fitness to subside in the presence of high resource availability. In our experiments, selected and control mites were maintained with large amounts of food, low population densities and under ideal abiotic conditions. Further research on trade-offs under variable environmental conditions, such as increased temperatures, conspecific-competition or decreased resources might reveal trade-offs which we were unable to detect here.

We found that the motility of mites selected for increased infection behaviour was similar to that of the unselected control mites. Our results contrast with results for lice (Bartlow et al. 2016). Unlike lice, which must feed on the keratinized tissues of their avian host, *M. muscaedomesticae* are only facultatively parasitic and rely upon predation for nutrition. Thus, motility is likely a fixed trait among mites. However, our experimental design may have limited our ability to detect a motility trade-off if it did exist. The distance the mites travelled (8 cm) may not have been long enough to detect a noticeable difference in race time. We attempted to assay mites over a longer distance, but the mites were more likely to stop or wander than they were at shorter distances.

Our study revealed no differences in the morphologies between selected and control mites, except in the ventrianal shield. However, the results depended on replicate line: selected mites exhibited larger ventrianal shields in line 1 and a marginally smaller ventrianal shields in line 2. The difference between replicate lines suggests a role of founder effect in our mites. Like

945 the other assayed traits, morphological changes may not be linked to infectivity traits in these
946 mites. Although we predicted a decrease in body size associated with parasitism in our mites,
947 predicting changes in body size may be more complicated. Poulin (1995, 2007) suggested that,
948 because so many selective pressures are at play, parasite body-size evolution is difficult to
949 predict accurately. We also predicted an increase in chelicera size and strength in the selected
950 mites because of their utility in host-attachment. However, we did not find any differences in
951 chelicera size or strength associated with infection selection. According to Krantz (1998) and
952 Manning and Halliday (1994), the size of the chelicerae themselves may not be critical for host-
953 attachment. Instead, bidentate teeth on the chelicerae may be more useful for host attachment
954 and thus a candidate trait for change associated with increased parasitism. Other morphological
955 changes associated with parasitic lifestyles in Acari include a reduction in chelicera segment
956 number and loss of the moveable digit, essentially creating a functional piercing mouthpart
957 (Dowling 2015). Due to their small size, investigation of cheliceral morphology would likely
958 require scanning electron microscopy, which was beyond the scope of this project (Manning
959 1991).

960 Finally, we examined chelicera size in relation to mite body size and our data indicate
961 that chelicerae grow proportionally with body size. Although the selected and control mites
962 exhibited similar relationships between body size and chelicera size, the replicate lines were
963 significantly different. The relationship was steeper in line 1 compared to line 2, meaning the
964 line 1 mites exhibited larger chelicerae for their body size. Again, this is likely a result of
965 founder effects among replicate lines.

966 Selected and control mites from replicate line 1 had similar lifetime fecundities
967 regardless of whether they had access to a host. However, the lifetime fecundity of line 2's mites

968 was significantly affected by host attachment and selection treatment. Mean lifetime fecundity
969 was significantly higher in mites that attached to flies suggesting a fitness benefit associated
970 with parasitism. This finding further supports the suggestion that *M. muscaedomesticae* feed off
971 the host while attached, rather than simply hitching a ride (i.e. phoresy; Jalil and Rodriguez
972 1970; Krantz 1998). The effect of selection treatment on lifetime fecundity was positive but
973 small relative to that of host availability. Also, during selection for increased infectious
974 behaviour we may have inadvertently selected simultaneously for higher reproductive output
975 (e.g. due to pleiotropy or linkage disequilibrium). The evolution of a parasitic strategy may be
976 linked to higher rates of egg production relative to free-living congenerics to ensure successful
977 transmission of infective stages (Poulin 2007).

978 Throughout our experiments, effects from replicate lines were ubiquitous. Although the
979 replicate lines underwent the same selection protocol (Durkin and Luong 2018), each line was
980 generated with a different subset of mites, which may have been a source for some of the
981 discrepancies between replicate lines. However, we are not alone in finding varying or even
982 contradictory trade-off results across replicate lines (see Velicer and Lenski 1999; Bennett and
983 Lenski 2007; Marxer et al. 2016). Velicer and Lenski (1999) proposed that the ecological history
984 experienced by each of their experimental bacterial strains played a role in their evolutionary
985 responses to the selection regime. Such variation suggests strong genetic variation/covariation in
986 the traits of interest among founder populations. However, such variation can make finding
987 evidence for trade-offs challenging (Velicer and Lenski 1999). We were constrained by
988 logistical challenges associated with the selection as well as the fecundity and longevity
989 experiments, which limited the number of simultaneous replicate lines we could maintain.
990 Ideally, our experiments would have included more replicate lines, though this shouldn't negate

991 the validity of our results. We did find evidence for trade-offs associated with increased
992 infectivity in one replicate line, which warrants further investigation for the pervasiveness of this
993 trade-off.

994 If evolutionary trade-offs between infectivity and fitness-related traits are not observed in
995 a particular population of facultative parasites, how is variation in infection strategy maintained?
996 Phenotypic plasticity, in the form of facultative parasitism, may confer a selective advantage in
997 unpredictable environments. *Macrocheles muscaedomesticae* use ephemeral and highly patchy
998 resources and therefore lead unpredictable lives (Krantz 1998). As habitat deteriorates, mites
999 have the option to stay a bit longer or to move elsewhere, and the costs and benefits of dispersal
1000 are constantly fluctuating (Bowler and Benton 2009). Eventually, all mites must move on or go
1001 down with the ship. Infectious mites may benefit from attaching to a host (dispersal, blood meal)
1002 but there is no guarantee they will successfully arrive at a suitable habitat. Furthermore,
1003 dispersing too soon could result in the loss of current reproductive opportunities in the existing
1004 habitat. We hypothesize that constantly changing and shifting environmental conditions may
1005 maintain variation in infection strategy (Sgrò and Hoffmann 2004; Reece et al. 2009).

1006 Bet-hedging may provide an alternative strategy for survival in highly stochastic
1007 environments. Bet-hedging is a reproductive strategy whereby a female produces offspring that
1008 exhibit variation in survival tactics to spread the risk of failure (Cohen 1966). For parasites, the
1009 probability of locating a susceptible host can be highly variable. According to Fenton and
1010 Hudson (2002), as probability of host availability decreases, a mixed, bet-hedging strategy
1011 becomes optimal. Pasternak et al. (2000) compared the life histories of a fish ectoparasite,
1012 *Argulus foliaceus*, found in commercial fish farms to those from natural lakes. Host availability
1013 was predictable and consistent on farms, leading to highly synchronized egg laying and

development among the ectoparasites, and a single best infection strategy. However, egg batches exhibited highly variable development times in natural lakes. In nature, host availability was unpredictable and thus, the optimal infection strategy was a mixed one. Accordingly, females spread the risk of failure to increase the chance of some of her offspring infecting a host. In nature, host availability is likely unpredictable for *M. muscaedomesticae*. Thus, variation in propensity to infect a fly host may represent a form of bet-hedging strategy in natural mite populations. Further research is needed to unravel how the degree of variation in infectivity changes in response to temporal and spatial fluctuation in host availability in nature.

Hosts can potentially alter the costs and benefits associated with parasitism as well, thus maintaining facultative parasitism. Huang et al. (2017) hypothesized that antagonistic coevolution can cause dynamic trade-offs: an adaptation in one species might result in significant fitness gains initially but may decrease as antagonistic species counter-adapt. In nature, fly hosts can resist mites (Polak 2003) and potentially coevolve in response to increased infection. This type of antagonistic coevolution could drive down some of the benefits of increased infectivity, changing the cost-benefit ratio and the nature of the trade-off. In our experiments, flies were not allowed to coevolve with the mites. Future research that allows the evolutionary response of the host could uncover more of the potential costs of increased infectivity experienced in nature.

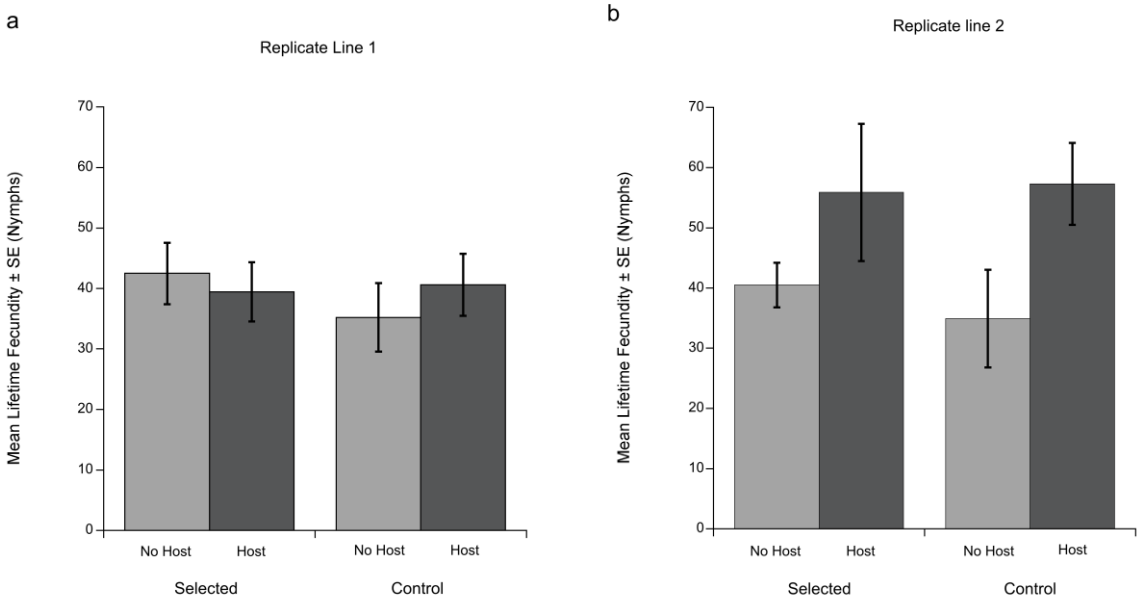


Figure 3.1 Lifetime fecundities of selected and control mites with and without host exposure

Mean lifetime fecundities of *Macrocheles muscaedomesticae* mites selected for increased infectious behaviour compared to unselected control mites in replicate lines 1(a) and 2(b). Mites were without a host (light gray bars) or allowed to attach to a fly host, *Drosophila hydei* (dark gray bars). In replicate line 1 selected and control mites exhibited similar lifetime fecundities regardless of host attachment. However, in replicate line 2, mites that attached to hosts produced significantly more offspring in their lifetimes compared to those that did not, regardless of their selection treatment.

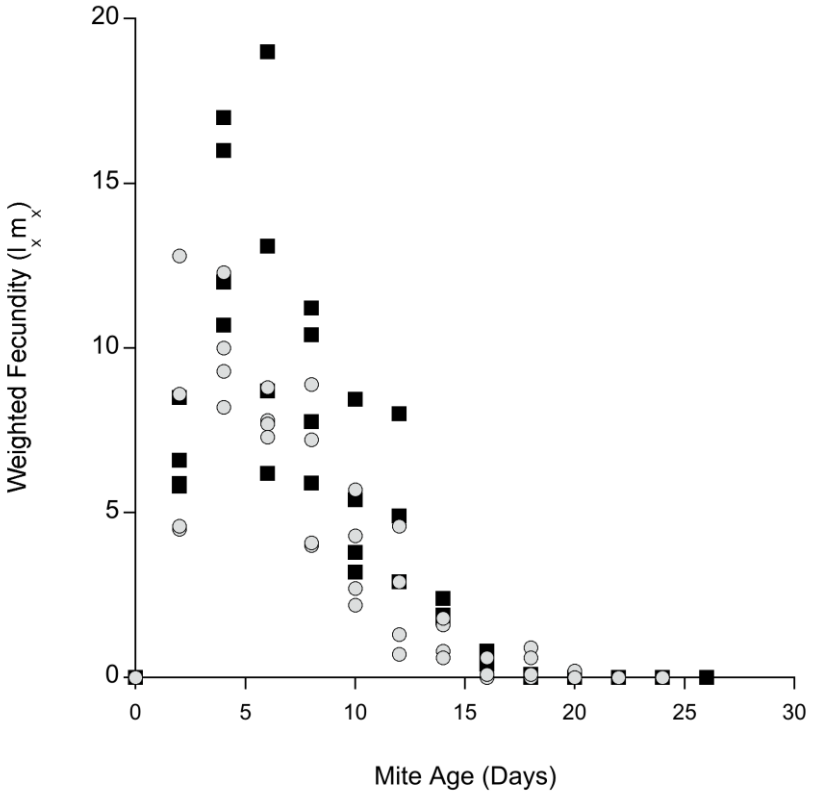
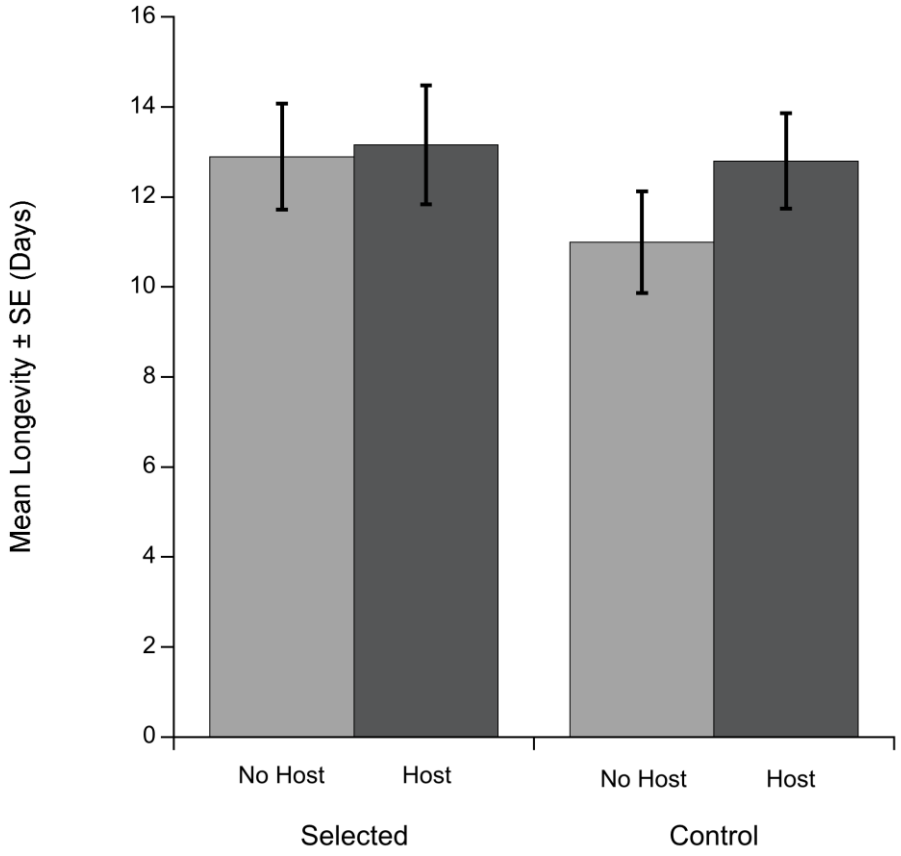


Figure 3.2 Fecundity schedules of selected and control mites with and without host exposure

Fecundity schedules of *Macrocheles muscaedomesticae* mites selected for increased infectious behaviour and unselected control mites that did (black squares) and did not (gray circles) previously attach to a *Drosophila hydei* fly host.



1044

Figure 3.3 Longevities of selected and control mites with and without host exposure

1045 Mean longevities of *Macrocheles muscaedomesticae* mites selected for increased infectious behaviour and
1046 unselected control mites. Light gray bars represent mites that did not have access to a host, gray bars represent mites
1047 that successfully attached to a fly host. Data from both replicate lines were pooled. Selected and control mites
1048 exhibited similar longeveties regardless of host attachment.

1049

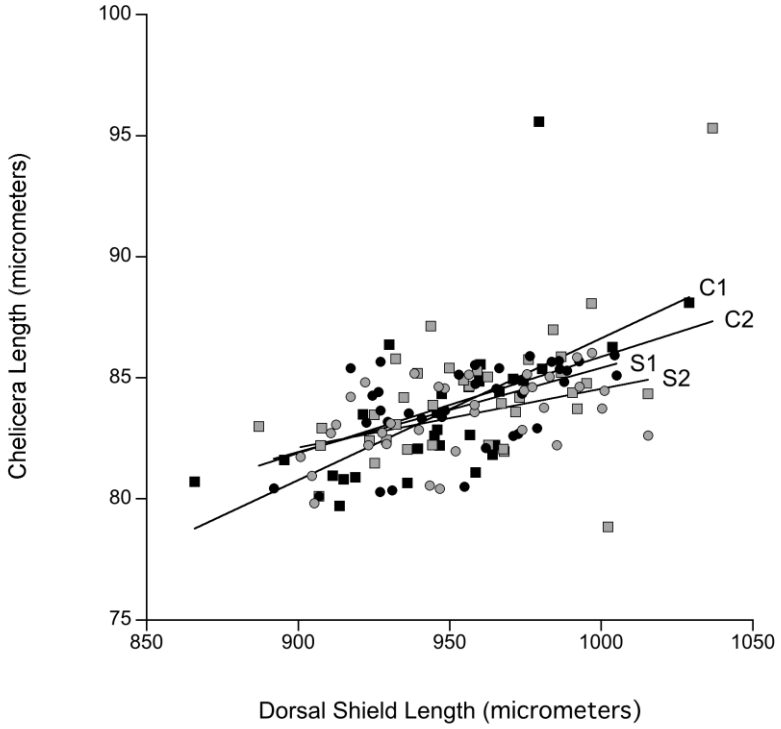


Figure 3.4 Relationship between bodysize and chelicera length of *Macrocheles muscaedomesticae* mites selected for increased infectious behaviour and unselected control mites

Relationship between body size (dorsal shield length) and chelicera length of *Macrocheles muscaedomesticae* mites selected for increased infectious behaviour (S; circles) and unselected control mites (C; squares). Selected and control mites exhibited similar relationships between body and chelicera size. However, the relationship between body and chelicera size was significantly different between mites from replicate line 1 (S1 and C1; black symbols) and replicate line 2 (S2 and C2; gray symbols).

Chapter 4. Selection for increased infectivity in a facultative ectoparasite: consequences for phenotypic plasticity

4.1 Introduction

In heterogenous environments, a single phenotype will rarely confer the greatest fitness across all scenarios (Via et al. 1995). One strategy for surviving and reproducing in variable conditions involves phenotypic plasticity: the ability of one genotype to produce multiple phenotypes across different environments (Via et al. 1995; DeWitt 1998; Garland and Kelly 2006; Pigliucci 2006). The range of phenotypes produced by a single genotype under multiple environmental conditions is termed the 'reaction norm' (Stearns 1992; Garland and Kelly 2006; Pigliucci 2006). If variation for the components of a reaction norm are heritable, then plasticity itself should respond to natural selection (Stearns 1992; Via et al. 1995; DeWitt et al. 1998; David et al. 2004; Sarkar 2004; Garland and Kelly 2006; Pigliucci 2006).

Phenotypically plastic traits can evolve to become more or less plastic over time (Crispo 2007; Gilbert and Epel 2015). The Baldwin effect describes a phenomenon whereby plastic organisms are better able to survive novel environments and thus, natural selection favors phenotypic plasticity; the result is an increase in or maintenance of phenotypic plasticity over time (Baldwin 1896; Crispo 2007). As an example, Nussey et al. (2005) observed a positive relationship between the plasticity in egg-laying time and fitness in a wild population of Great-Tits (*Parus major* Linneaus). They hypothesized that the plastic females were better able to synchronize their egg-laying time with prey availability, which increased their overall fitness and thus plasticity in egg-laying was under positive selection (Nussey et al. 2005).

A version of this chapter will be submitted to International Journal of Parasitology as "Durkin, E. S. and L. T. Luong. Selection for increased infection in a facultative ectoparasite: consequences for phenotypic plasticity in infection?"

Conversely, genetic assimilation describes events in which the range of expression in an originally phenotypically plastic trait is reduced or eliminated so that it no longer responds to environmental stimuli (Waddington 1942; Pigliucci 2006; Crispo 2007; Gilbert and Epel 2015). Suzuki and Nijhout (2006) observed evidence for genetic assimilation in their experiments with hornworms (*Manduca sexta* Linneaus). Larvae of *M. sexta* are generally green, regardless of temperature; however, black morphs will occasionally occur with heat-shock stress (Suzuki and Nijhout 2006). The authors generated a monomorphic selection line in which they continued to select for heat-shock induced black larvae. After seven generations of selection, the black larval coloration in the monomorphic line became fixed: the larvae expressed black coloration without being exposed to heat-shock (Suzuki and Nijhout 2006). In nature, genetic assimilation might occur when an environmental condition stabilizes and repeatedly induces the same adaptive phenotype in a plastic trait. Over time, natural selection acts upon the adaptive phenotype and, if the maintenance of plasticity is costly, is perhaps lost (DeWitt 1998; DeWitt et al. 1998; Relyea 2002; Pigliucci 2006; Crispo 2007; Gilbert and Epel 2015).

Evidence for phenotypic plasticity among parasites is mounting (Thomas et al. 2002; Poulin 2007). Parasites often encounter variable and unpredictable environments in their lifetimes (Poulin 2007). Adaptive plasticity in infection strategies may allow parasites to deal with heterogenous environments. For example, Birget et al. (2017) showed that malaria parasites adjust gametocyte density in response to resource availability, allowing them to adaptively balance the costs and benefits of gametocyte production. Similarly, Lagrue and Poulin (2009) demonstrated that the trematode *Coitocaecum parvum* was capable of sensing the absence of its definitive host and inducing progenesis and reproduction in the intermediate host (see also Thomas et al. 2002; Kaltz and Koella 2003; Reece et al. 2009; Leggett et al. 2013; Searle et al.

1102 2015 for more examples). However, how plastic parasitic strategies evolve in animal systems is
1103 less well understood, and the way in which infection plasticity evolves could have important
1104 implications for host-parasite interactions and their evolution.

1105 The genetic assimilation hypothesis predicts that phenotypically plastic parasites will
1106 experience a loss in plasticity when a single phenotype is selected for. Should the environment
1107 subsequently change, this loss in plasticity might be detrimental to their survival. *Macrocheles*
1108 *muscaedomesticae* is a facultatively parasitic mite with a cosmopolitan distribution found
1109 inhabiting rotting organic matter including compost and dung. Typically, *M. muscaedomesticae*
1110 feed on nematodes and fly eggs and larvae (Wade and Rodriguez 1961; Krantz and Whitaker
1111 1988). However, when given the opportunity, some adult females will attach to a fly host as a
1112 means of dispersal as well as a source of nutrition (Jalil and Rodriguez 1970; Farish and Axtell
1113 1971). As their habitats deteriorate, (e.g. increased mite population density, decreased humidity),
1114 the prevalence of fly-attachment increases (Farish and Axtell 1971; Durkin and Luong 2018).
1115 The plastic nature of *M. muscaedomesticae*'s tendency to 'infect' flies is critical to their survival
1116 in ephemeral and stochastic environments.

1117 In a previous study we successfully selected *M. muscaedomesticae* for increased
1118 infectious behaviour and found 16.6% of the variation in the "infectious" phenotype was due to
1119 additive genetic variation (Durkin and Luong 2018). So, why do *M. muscaedomesticae*
1120 populations do not exhibit higher levels of infection prevalence in nature, given the evidence for
1121 fitness benefits associated with parasitism (see Chapter 3)? We hypothesize that our direct
1122 artificial selection for a single phenotype (infectious behaviour) may result in genetic
1123 assimilation (i.e. loss in infection plasticity). Because infection plasticity is likely adaptive for
1124 *M. muscaedomesticae*, a single infection strategy and concomitant loss of plasticity could be

1125 detrimental to their survival. We predict the mite populations under strong (artificial) selection
1126 will experience genetic assimilation and consistently express similar infection prevalences
1127 across multiple environments (i.e. flat reaction norms). Control populations should respond to
1128 their environment and exhibit varying levels of infection (i.e. plasticity).

1129 **4.2 Material and methods**

1130 *4.2.1 Fly and mite cultures*

1131 *Drosophila hydei* (~100 per sex) were collected from residential compost bins in
1132 Edmonton, Alberta Canada in September 2013 (approx. coordinates 53.52 °N, 113.48 °W). Fly
1133 cultures were maintained in 200 mL bottles on standard agar-molasses-yeast-based fly media at
1134 24 °C, 70% relative humidity and a 12 D: 12 L light cycle. Flies were maintained in the lab for
1135 two years before experiments took place. The mean age of flies used for selection and plasticity
1136 experiments was 7.73 ± 5.62 days post-eclosion.

1137 Approximately 700 female *Macrocheles muscaedomesticae* infecting the field-caught *D.*
1138 *hydei* were used to initiate mass cultures. Mites were maintained for two years before
1139 experiments were performed. Mites were cultured in 4 L plastic containers filled with organic
1140 medium: sterilized organic wheat bran, sterilized aspen wood shavings, deactivated yeast,
1141 distilled water. The organic medium was inoculated with bacteriophagic nematodes as a food
1142 source for the mites. Cultures were kept at 24 °C, 75% relative humidity and a 12 D: 12 L light
1143 cycle.

1144 *4.2.2 Selection protocol*

1145 Individual adult female mites (60-130 each generation and replicate line) were exposed
1146 to a single female *D. hydei* fly for 60 minutes in experimental infection chambers. Infection

chambers were 200 µL pipette tips reduced to half their length by cutting off the narrow end (~1.5 cm); both ends were stoppered with cotton. These small chambers restricted fly movement which allowed control of heterogeneity in behaviourally-mediated host resistance or encounter possibilities.

A single mite was placed into an infection chamber followed by a single fly. Following the 60-minute exposure, mites were scored as attached or unattached to their fly and then exposed for an additional 30 minutes. Based on unpublished observations, mites that attach often do so within 60 minutes and then steadily drop off over the next 48 hours. By eliminating the mites that "switched" after the additional 30 minutes, we were able to select mites more consistent in their behaviour. Mites that were attached after both 60 and 30 minutes of exposure were used to seed the parasitic mite line. A total of 13 to 50 mites was used to seed each new generation of each line (mean = 42 ± 2.3 SE). The same number of seed mites were used in each selection group, each generation within each replicate line. The total number of seeder females used during each generation was divided equally across 2-5 replicate containers (the number of replicate containers depended on upon the number of seeder females). Offspring from replicate containers were mixed each generation; replicate containers were not genetically isolated. Each container held roughly 50 mL of organic media and was sealed with Parafilm® (Neenah, WI) to prevent contamination by other mites. Control lines were maintained in parallel to selected lines within each replication line. At each generation, the control line was seeded with the same number of mites as the selected line. Unlike the selected lines, control mites were randomly selected to seed generations regardless of attachment.

In both the selected and control groups, mites were placed into the containers with their fly host regardless of attachment. In all cases, the fly host was crushed at the thorax using forceps, taking care not to injure an attached mite.

Mites remained in the containers for 3 days to lay eggs and were then removed from the media. Three days was an important number because the offspring are still easily distinguished from the adults, making adult removal easier. Offspring then remained in their containers for four days to mature and mate with siblings. Once developed, a roughly equal number of females was removed from each of the replicate containers and went through the selection protocol described above. Selection continued for 10 and 11 generations in replicate lines 1 and 2 respectively and lasted 11 weeks before plasticity measurements were made.

Control lines were maintained in parallel to selected lines within each replication line. At each generation, the control line was seeded with the same number of mites as the selected line. Unlike the selected lines, control mites were randomly selected to seed generations regardless of attachment.

4.2.3 Plasticity measurements

To determine whether selected lines experienced genetic assimilation we measured the infection plasticity of selected and control lines post selection. The prevalence of fly infection in selected and control lines was recorded over three environments, each with varying levels of food (no food, low food and high food). Populations of a closely related mite species, *Macrocheles subbadius*, exhibit increased infection prevalence when starved (Luong et al. 2017). We viewed food availability to be a key component of habitat quality and that habitat quality diminishes with lower food availability.

Fifty females from control and selected lines were randomly selected from the final generation of selection. These females were not exposed to a fly host (to prevent maternal effects that may be associated with host attachment). Mites were divided across five replicate containers filled with 50 mL of organic nematode media. Organic nematode media consisted of autoclaved wheat bran, wood shavings, and deactivated yeast moistened with distilled water. Organic nematode media was inoculated with free-living nematodes as a food source for the mites. The mites were allowed to lay eggs in the media for three days before being removed. The F1 females from these mites were then used in the plasticity experiment.

The no food treatment group was 14.8 cm^3 of aspen wood chips moistened with distilled water. The batch of low food media was made by diluting 29.6 cm^3 of organic nematode media containing nematodes with 118.3 cm^3 of moistened aspen wood chips. The high food treatment consisted of 14.8 cm^3 of undiluted organic media containing nematodes. The final volume of food media in all replicates and treatments was 14.8 cm^3 per container. Nematode density of the low and high food treatments was estimated by extracting nematodes from 14.8 cm^3 of media using a Baermann funnel. The extracted nematodes were then counted under a stereomicroscope. Replicate line one had 1.7 ± 0.27 SD nematodes / cm^3 in the low food treatments and 12.4 ± 0.66 SD nematodes/ cm^3 in the high food treatments. Replicate line two had 0.5 ± 0.15 SD nematodes /1 cm^3 in the low food treatments and 25 ± 1.66 SD nematodes/ cm^3 in the high food treatments.

Each food treatment was placed into five individual 90 mL plastic containers. Ten female deutonymphs and five adult males were added to each treatment container (n = 5). The mites were left to mature and mate in their treatment condition for five days. Individual mites (now matured) were then removed and exposed to a female *D. hydei* host in a pipette tip for 60

minutes. Each mite was then scored as attached or unattached and infection prevalence was calculated.

Data Analysis

We used a generalized linear model (glm) with a binomial error distribution (logit link) (R Core Team 2017) to determine whether selected and control lines exhibited significantly different reaction norms. Due to overdispersion, we used a quasibinomial error distribution glm and the backwards model selection criterion ($p < 0.05$) was based on an F-test for models. The prevalence of infection was the response variable, and food treatment, selection and replicate line were the fixed explanatory variables. Final models were validated by checking for homogeneity of variance, normality and independence of the residuals. We report the deviance (~sums of squares) and *p*-values. We also report an estimation of phenotypic plasticity of infection for the selected and control mites. Plasticity was calculated by dividing the standard deviation of the mean infection prevalence for the selected or control mites by the mean infection prevalence of all (selected and control) mites.

4.3 Results

Selection treatment (deviance = -32.68, $p < 0.001$) and food treatment (deviance = -63.37, $p < 0.001$) were both significant predictors of infection prevalence. Neither replicate line nor any of the possible interactions were significant ($p > 0.05$). In other words, the genetics of the mite (selection regime) and the environment (food treatment) had a significant effect on mite infection prevalence. However, there was no evidence for a genotype-by-environment interaction (deviance = 0.74, p=0.84); the infection plasticity of selected mites (0.78) was similar to that of the control mites (0.62). The similarity in infection plasticities of the selected and

control mites is illustrated by the similar infection patterns across the different environments (Fig. 4.1).

Although we predicted a negative relationship between food availability and infection prevalence, we saw the opposite relationship. Both selected and control mites showed increased infection with increased food availability. In the no food treatment, 10.2 ± 3.9% SE of the unselected control mites and 28.0 ± 5.2% SE of the selected mites attached to a fly host. Infection increased in the low food treatment to 14.9 ± 3.8% SE in the unselected control mites and to 41.1 ± 5.2% SE in the selected mites. Infection prevalence was highest in the high food treatment and increased to 45.7 ± 5.2% SE in the control mites and to 69.5 ± 4.7% SE in the selected mites.

4.4 Discussion

Contrary to our prediction, selected mites exhibited reaction norms similar to those of control mites, which does not support the hypothesis of genetic assimilation. Selected mites also exhibited significantly greater levels of infection across all environments compared to the controls, which confirms the success of our selection regime.

Perhaps infection plasticity (slope of reaction norm) is not correlated with the infection mean (intercept) and/or lacks heritable variation. In this case, selection on the mean trait value (propensity to infect) may not affect the degree of plasticity. Similar to Scheiner and Lyman (1991), our direct selection on the trait of interest did not produce a correlated response in the plasticity of the target trait. The lack of a correlated response between the mean and plasticity of infection supports an epistatic model of plasticity, in which the mean and plasticity of a trait are determined by different genes (Lynch and Gabriel 1987; Schlichting and Pigliucci 1993). However, more research is required to tease apart whether genes for infection mean and

1257 infection plasticity act independently. Experimental evolution studies that select on infection
1258 plasticity itself could reveal whether it acts independently from the infection mean.

1259 Alternatively, our selected mites instead may have experienced the Baldwin effect. The
1260 Baldwin effect describes the phenomenon whereby plastic organisms are better able to survive
1261 novel environments and thus, natural selection favors phenotypic plasticity; the result is an
1262 increase in or maintenance of phenotypic plasticity over time (Baldwin 1896; Crispo 2007).
1263 Infectious mites might also exhibited high levels of infection plasticity. Thus, our selection for
1264 the 'infectious' mites concomitantly favored infection plasticity, which would explain the
1265 maintained reaction norm exhibited by the selected mites (Fig. 4.1). Continued selection
1266 experiments that monitor infection plasticity could provide more support for these mites
1267 experiencing the Baldwin effect (Garland and Kelly 2006).

1268 Although we generated a plastic response in our mites, the relationship between infection
1269 prevalence and food availability was opposite to what we predicted. Past research found a
1270 closely related mite, *Macrocheles subbadius*, exhibited increased infection prevalence with
1271 starvation period (Luong et al. 2017). Thus, we predicted a negative relationship between food
1272 availability and infection prevalence. Conversely, we found that infection prevalence increased
1273 with nematode food availability. One potential explanation for this unexpected relationship is
1274 that we did not communicated the appropriate environmental cues to the mites. We expected the
1275 mites to perceive environments with low or no nematode availability as low-quality relative to
1276 an environment with an abundant food source (Luong et al. 2017). However, the way we
1277 generated the different environments may have sent mixed or contradicting signals. The no food
1278 environment was essentially moistened wood chips. The high food environment was nematode
1279 culture media which was composed of moistened wheat bran and woodchips seeded with

nematodes. Once moistened, microscopic organisms (e.g. bacteria, fungus etc.) begin breaking down the media, releasing compounds for the mites to sense and respond to. Compounds generated from the decomposing media in the food treatments might have signaled poor conditions and our experimental design did not account for these additional and potentially contradicting signals. Ideally, all food treatments would have been composed of moistened woodchips only with the addition of nematodes. Although the direction of the relationship between food availability and infection prevalence has no effect on our results in the context of plasticity evolution, future plasticity research using this system should be careful to control the signals communicated to the mites.

Our study contributes to growing evidence for phenotypic plasticity in infection strategy expressed by parasites. We did not find evidence for reduced phenotypic plasticity in infection subsequent to directional selection for a single infection phenotype. Although selected mites exhibited significantly greater infection prevalence, their reaction norms were similar to control mites, consistent with the predictions of the Baldwin effect. Significantly, the organisms used in our experiments are facultative parasites: capable of parasitic behaviour but not relying on it. Facultative parasitism is among the proposed evolutionary stepping-stones in the evolution of parasitism from free-living organisms (Rothschild and Clay 1952; Poulin 2007; Dowling 2015). Whether infection plasticity is lost, maintained, or even favoured by natural selection (as suggested by the Baldwin effect), our data suggest that phenotypic plasticity might be a critical trait in the evolution of parasitic organisms.

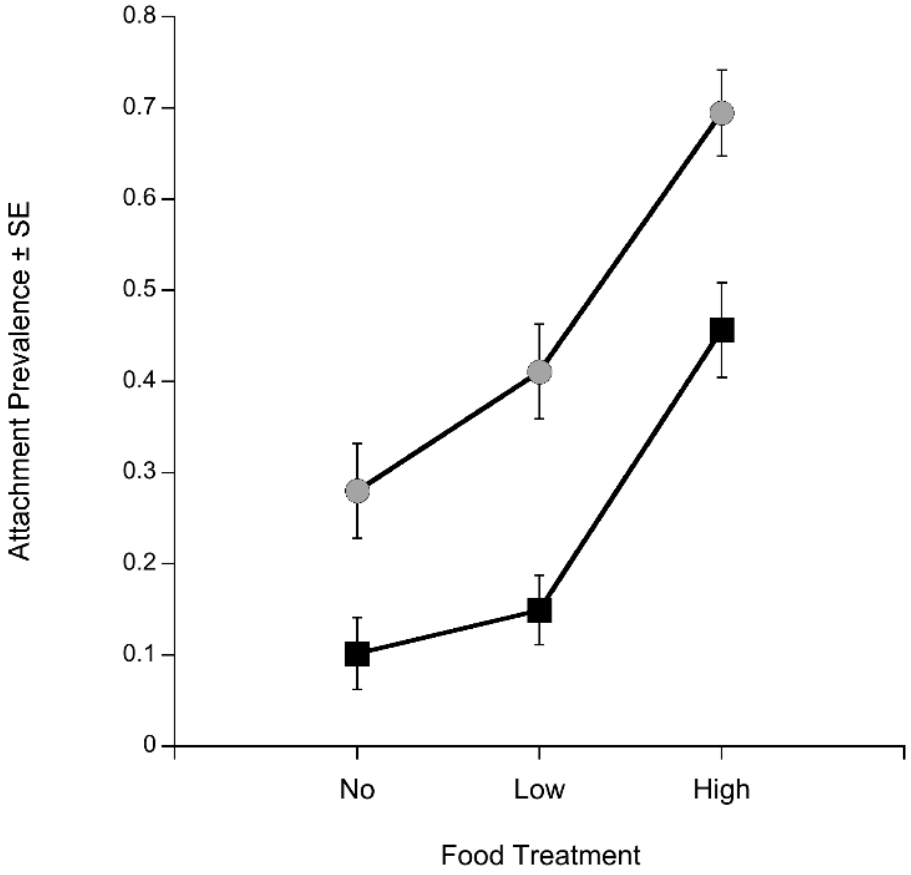


Figure 4.1 Reaction norms illustrating the phenotypic plasticity exhibited by *Macrocheles muscaedomesticae* selected for increased infectious behaviour and unselected control mites

The reaction norms of *Macrocheles muscaedomesticae* mites selected for infectious behaviour (gray circles) and control mites, which did not experience selection (black squares). Infection prevalence of mites was measured in three different environments: without nematode food (No), diluted nematode food (Low) and normal culture levels of nematode food (High). The selected mites exhibited significantly greater attachment prevalence across all environments, but their reaction norm was similar to the control.

Chapter 5. Laboratory culture of *Macrocheles muscaedomesticae* (Parasitiformes: Macrochelidae) with new insights on life history and their relationship with fly hosts

5.1 Introduction

Macrocheles muscaedomesticae (Scopoli) is a cosmopolitan macrochelid mite commonly found in rotting organic matter including dung and compost (Wade and Rodriguez 1961; Jalil and Rodriguez 1970; Yasui 1988; Halliday 2000; Krantz and Walter 2009). It posses a world-wide distribution and has been reported from all continents except Antarctica (Axtell 1961; Emberson 1973; Ho 1990; Halliday 2000; Hartini et al. 2003; Achiano and Giliomee 2006; Niogret et al. 2008; Makarova 2013; Azevedo et al. 2017). *Macrocheles muscaedomesticae* has been a focus of study for many decades as a biological control agent for agricultural fly pests because they will feed on juvenile fly stages and are phoretic, potentially parasitic, on adult flies (Wade and Rodriguez 1961; Jalil and Rodriguez 1970; Farish and Axtell 1971). Most of the work published on *M. muscaedomesticae*'s biology and life history has been in the context of their association with the house fly, *Musca domestica* Linnaeus (Diptera: Muscidae) growing in livestock manure (Filipponi 1955; Axtell 1961, 1969; Rodriguez and Wade 1961; Wade and Rodriguez 1961; Kinn 1966; Jalil and Rodriguez 1970; Farish and Axtell 1971; Wicht et al. 1971).

Given their worldwide distribution, *M. muscaedomesticae* populations have likely diverged ecologically, physiologically and behaviourally in the contexts of these diverse environments. Here, I outline the life history of a *M. muscaedomesticae* population collected from adult *Drosophila hydei* Sturtevant (Diptera: Drosophilidae) from Alberta, Canada, and

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maintained for roughly 100 generations under laboratory conditions. I compare the life history of our mite cultures to that reported for other *Macrocheles muscaedomesticae* populations. I also provide the Cytochrome c Oxidase Subunit I (COI) 'barcode' DNA sequence from this population of mites to assess the degree of divergence of Albertan populations from other populations for which COI has been sequenced. Authors sometimes disagree about whether *M. muscaedomesticae* is purely phoretic on *Musca domestica* or whether it is occasionally parasitic (Filipponi 1955; Kinn 1966; Jalil and Rodriguez 1970; Farish and Axtell 1971). A closely related mite, *Macrocheles subbadius* (Berlese), has been shown to feed occasionally on *Drosophila nigrospiracula* (Polak 1996). Here, I also compare the weights of *M. muscaedomesticae* mites allowed to attach to a host to those that did not to provide insight into whether the mites are obtaining host tissue or nutrients from *Drosophila* hosts.

5.2 Material and methods

5.2.1 Culturing

Drosophila hydei (~100 per sex) were collected from residential compost bins in Edmonton, Alberta Canada in September 2013 (ca. 53.52 °N, 113.48 °W) and used to establish a laboratory culture. Flies were mass cultured in six to eight 200 mL bottles on standard agar-molasses-yeast-based fly media at 24 °C, 70% relative humidity and a 12L and 12D photoperiod in a Percival biological incubator.

Laboratory mite cultures were initiated from roughly 700 adult female *M. muscaedomesticae* collected from the field-caught *D. hydei* in September 2018. Separate collection of male mites was not necessary for two reasons: *M. muscaedomesticae* are haplodiploid and can produce males from unfertilized eggs, and mating occurs immediately after female eclosion and thus adult females attached to flies are likely already fertilized. Careful to

not injure the attached mites, infected flies were killed and placed (with attached mites) into 4 L plastic containers filled with 1 L of organic culture media seeded with free-living rhabditid nematodes. Organic culture media was composed of autoclaved wheat bran, aspen wood shavings, deactivated yeast and distilled water. I maintained the mite cultures at 24 °C, 70% relative humidity and a 12 D: 12 L photoperiod in a Percival biological incubator. Autoclaved wheat bran, wood shavings, deactivated yeast and just enough distilled water were added every 7-10 days. Larger-scale mite culturing is described by Royce and Krantz (1991).

Cultures were aggitated by physical shaking twice a week. Aggitation prevented excessive putrefaction and inhibited fungal growth. In our experience, fungal growth did not affect the apparent health of *M. muscaedomesticae*. However, mite cultures were sometimes susceptible to what I identified as *Serratia* sp., a gram-negative species of bacteria. *Serratia* infections may have developed when mite cultures were stressed. Lighthart et al. (1988) described *Serratia* as facultative or weak pathogens of arthropods and observed increased susceptibility in a predatory mite, *Metaseiulus occidentalis* (Acari: Phytoseiidae), to *S. marcescens* when under temperature and humidity stress (Lighthart et al. 1988). Generally, our *M. muscaedomesticae* cultures remained healthy with minimal efforts.

Drosophila hydei (~100 per sex) were collected from residential compost bins in Edmonton, Alberta Canada in September 2013 (ca. 53.52 °N, 113.48 °W) and used to establish a laboratory culture. Flies were mass cultured in six to eight 200 mL bottles on standard agar-molasses-yeast-based fly media at 24 °C, 70% relative humidity and a 12L and 12D photoperiod in a Percival biological incubator.

I maintained nematode cultures as a food source for the *M. muscaedomesticae*. Although nematodes often maintain themselves within mite cultures (Rodriguez et al. 1962), I kept pure

nematode cultures as well for experiments that required mite-free media. The nematode cultures from which our cultures began came from Michal Polak at the University of Cinncinatti. The nematode cultures were kept in ventilated 4L plastic containers. Occasionally, the nematode cultures were supplemented with *Caenorhabditis elegans* (Maupas) from the labs of David Pilgrim and Martin Srayko at the University of Alberta. As with the mite media, fresh autoclaved wheat bran and wood chips were added weekly. When nematode culture containers were full, approximately 2 L of media was discarded.

5.2.2 Life history observations

During the trade-off experiments (Chapter 3), I measured the longevity and fecundity of selected and control female *M. muscaedomesticae* in two environments. The first environment was without access to *Drosophila hydei* fly hosts; females fed on nematodes only. The second was with access to flies; females had the opportunity to feed on an adult *D. hydei* in addition to their nematode diet. I used mites from the control lines only to examine the life history traits of mites fed on a nematode only diet and mites with a *D. hydei* host in addition a nematode diet. With these data, I can assess how host access might affect *M. muscaedomesticae* life history.

To better control for age in the mites, I used females that were born within roughly 36 hours of eachother. To obtain similarly aged mites, one hundred adult female mites were haphazardly obtained from the control lines using a paintbrush and dissecting probe. Twenty were placed in each of five 90 mL ventilated, plastic containers filled with 50 mL of organic medium containing nematodes (see above). Each set of females was allowed to lay eggs for 72 hours. The adult females were then discarded, and their offspring were allowed 96 hours to mature and mate. Thirty newly matured adult female mites were individually placed into an experimental infection chamber with a single female *D. hydei* (14.63 ± 0.37 SE days post-

eclosion) for 60 minutes. The infection chamber was constructed from a 200 µL pipette tip reduced to half its length (~1.5 cm) and stoppered with cotton. Mites that attached to a fly were left in the infection chambers with their host for an additional 20 hours, providing them the opportunity to feed. During this time, infection chambers were housed in an incubator (25 °C, 70% RH and a 12 D:12 L photoperiod). Of the mites that remained attached to flies, 20 were randomly selected and transferred, individually, to fresh nematode media. To remove mites from flies, the fly's thorax was first crushed with forceps. The dead fly and attached mite were then placed into a new 90 mL ventilated plastic container filled with 50 mL of organic nematode culture medium. At the same time, 20 mature female mites without access to flies were individually transferred into a 90 mL ventilated plastic container with 50mL of organic nematode culture medium. Containers were sealed with Parafilm® (Neenah, WI) to prevent contamination by other mites. All containers were kept at 25 °C, 70% relative humidity and a 12 D:12 L photoperiod in a Percival biological incubator.

Each container was inspected using a dissecting microscope, every 48 hours. Once located, the original adult female was transferred to a new container with fresh organic media. The media that each female had previously inhabited was placed into a plastic container and preserved in 95% ethanol to later count offspring from. Eggs hatch roughly seven hours after they are laid, and the larval stage lasts about 8 hours; thus, most of the offspring in the preserved media samples were in a nymphal stage of development. Attempts to recover original females ceased when the female was found dead or was assumed dead if the female could not be located after the media was fully inspected twice. Any dead females recovered were preserved in 95% ethanol, and the number of days alive since they were a newly matured adult was recorded (= longevity).

The preserved media samples were then examined for nymphs. Preserved media samples were transferred to 150 mL plastic containers and filled with 75 mL of tap water. The containers were covered with parafilm® and thoroughly mixed by inversion. Once the substrate settled, the supernatant was poured off into a 14 cm diameter petri dish and examined under a dissecting microscope. The hydrophobic skeletons of the mites resulted in their bodies being stuck to the surface film. All recovered nymphs were counted and recorded (eggs were not included in the counts). Each media sample was washed and examined for nymphs three times to ensure full recovery.

From the counts of nymphs, I determined each female mite's lifetime fecundity as the total number of nymphs produced until the female's death. To be comparable to other published records of fecundity, daily nymph production was calculated by dividing each sample count by two because females inhabited each media sample for 48 hours. I did not include zero counts in my calculation of mean daily offspring production.

5.2.3 Sequencing

DNA was extracted from three 70% ethanol-preserved adult female *Macrocheles muscaedomesticae* using a Qiagen DNeasy® Tissue Kit with slight protocol modifications. Mites were first washed with 95% ethanol to remove any external contaminants before DNA extraction. Mites were incubated with proteinase K for 4 hours and after the initial 2 hours of incubation, mites were crushed using a micropipette tip and incubated for an additional 2 hours. For the final protocol step, mite DNA was stored in 150 µL of AE buffer rather than the suggested 300 µL to reduce the dilution of the DNA.

I then amplified a fragment of CO1 using primers LepF (5'-ATTCAACCAATCATAAAGATATTGG- 3') and LepR (5'-TAAACTTCTGGATGTCCAAAAAAT- 3'). PCRs were performed in 20 µL reaction volumes: 2 µL 10X PCR buffer, 2 µL MgCl, 0.4 µL dNTPs, 9.76 µL dd H_2O, 0.04 µL taq DNA polymerase (10X PCR buffer, MgCl and taq polymerase from Qiagen Toptaq® kit). The thermocycler program consisted of 94°C for 2 min, 35 cycles of 94°C for 30 seconds, 45°C for 30 seconds and 72°C for 2 min, and finally, 72°C for 5 minutes. The Molecular Biology Service Unit at the University of Alberta sequenced the CO1 fragment using Sanger sequencing with the amplification primers. Sequences were assembled using Geneious version 11.1.4 (http://www.geneious.com, Kearse et al. 2012) and subsequently uploaded to GenBenk (Accession numbers MH507145, MH507146, MH507147). Our mite sequences were compared to other available sequences using the Barcode of Life Database (http://www.boldsystems.org/). Voucher specimens of the cultured *M. muscaedomesticae* mites and *Drosophila hydei* fly hosts are deposited in the E. H. Strickland Entomological Museum at the University of Alberta.

5.2.4 Host-feeding experiment

The aim of this experiment was to assess whether *M. muscaedomesticae* feed on their dipteran hosts while attached, or whether they are purely phoretic. We haphazardly selected 350 adult female mites from mass culture and split them evenly across two ventilated 90 mL plastic containers. Two pieces of paper towel, cut to fit, were placed in the bottom of the containers and wet with 1 mL of distilled water. The containers were then sealed with parafilm to prevent escape and contamination, and placed into the incubator (25°C; 70% RH; 12D: 12L cycle) for 22-24 hours to starve the mites.

Containers were randomly assigned to one of two treatments: control (no fly) or fly present. For each treatment, 150 mites were individually placed into small infection chambers. The chambers were constructed from a 200 µL pipette tip reduced to half its length (~1.5 cm) and stoppered with cotton. A single adult female *Drosophila hydei* was added to the fly treatment chambers, mites remained alone for the control group. All chambers were then placed into the incubator for 60 minutes. Mites in the fly treatment that did not attach to their fly during the initial 60 minutes were discarded. The remaining attached mites and all of the control chambers were left in the incubator for two hours, at which time the fly treatment was again checked to ensure the mites were still attached. Any attached mites that had detached at this point were discarded. The attached mites and control mites were left in the incubator for an additional two-hour period. After the final two-hour period, mites that had detached were discarded; only the mites that maintained host-attachment for the entire four hours were retained in the experiment. This procedure was repeated over time to generate 11 fly treatment groups and 11 control groups, each containing 15 mites.

In the fly treatment group, flies were killed by crushing the thorax with forceps; care was taken to avoid damaging the mites. The mites were then gently removed from the host using a small paintbrush and cleaned of any host tissue or excrement using a small paintbrush under a dissecting microscope.

Mites were then placed into a microcentrifuge tube in groups of 15 mites/tube. Because the mites are so small, we weighed them in groups to get a detectable reading. The microcentrifuge tubes were then placed into the -20° C for 20 minutes to kill the mites, but not long enough to desiccate them.

Mites from the control and treatment group were then weighed to the nearest 0.1 µg using a microscale (Orion Cahn C-35, Thermo Electron Corporation), alternating the order which control and attached groups were weighed. The groups of mites were transferred from the microcentrifuge tubes into a small tared weigh-boat (tinfoil). Mites from each group were counted before and after weighing to ensure mites were not lost during the weighing process.

I used generalized linear modeling (GLM) to analyze the data with R statistical software (R Core Team 2017). The minimal model was determined using backwards model selection; significant variables (chi-square test, $p<0.05$) were retained in the models. The minimal model was validated by checking the homogeneity of variance, normality and independence of the residuals. We report the deviance (~sums of squares) and *p*-value of variables.

The response variable was the mean individual mite weight calculated from the group weight (group weight/15 mites). Since the weight data were normally distributed (Shapiro Wilks test of normality, $p = 0.51$) we used a Gaussian error distribution. The original model included fly attachment and weighing order, as well as their interaction as independent variables.

5.3 Results

5.3.1 Life history observations

Our mites exhibited typical development for macrochelid mites, passing through the following life stages: egg, larva, protonymph, deutonymph and adult (Wade and Rodriguez 1961; see Fig. 5.1a-d for life stage images). Egg to adult development for the female *Macrocheles muscaedomesticae* in our study was roughly three days, slightly longer than the development times reported elsewhere (Table 5.1). The relationship observed between development time and culture temperature was comparable to other published studies (Fig. 5.2).

Adult males mate-guarded (Fig. 5.1e) deutonymph females to be the first to mate with her upon her eclosion as an adult (Fig. 5.1f) in order to sire the majority of her female offspring (Yasui 1988). Adult females began laying eggs 2-3 days post eclosion (Wade and Rodriguez 1961). Eggs were laid one at a time and concealed within the media, likely to reduce predation by other mites (Rodriguez and Wade 1961). I did not observe time to hatch, but according to Wade and Rodriguez (1961), it takes about 7 hours. On average, the adult females in my cultures survived 11.9 ± 0.8 SE days, produced 42.18 ± 3.60 SE nymphs in their lifetimes and 4.28 ± 0.17 SE nymphs per oviposition day. However, fecundity differed in the context of their diet. Mites that had access to *D. hydei* produced more offspring in their lifetimes (48.95 ± 4.64 SE nymphs) than the mites that had access to nematodes alone (35.05 ± 5.05 SE nymphs; Table 5.2).

5.3.2 Sequencing

The final alignment of COI was 667 bp in length and the sequences were the same for all three mites. Using BOLD, I compared the mite sequences to other available sequences. The mites in this study clustered with sequences from 5 other *Macrocheles muscaedomesticae* collected in Alberta, Canada (Figure S.5.1).

5.3.3 Host-feeding

Fly treatment (attachment) was a significant predictor of mite weight (deviance = 0.25, $p < 0.001$); the order of weighing (deviance = $-5.26e^{-8}$, $p = 0.967$), and its interaction with fly attachment (deviance = $-7.21e^{-5}$, $p = 0.114$) were not. The mites that were attached to host flies for 4 hours weighed 5.64% more (0.109 ± 0.001 SE mg per mite) than the control mites (0.103 ± 0.002 SE mg; Fig. 5.3 per mite).

5.4 Discussion

Life history

On average, females in our *M. muscaedomesticae* populations produced 42.18 ± 3.60 SE offspring in their lifetimes. The mites in this study produced fewer offspring than other published observations (see Table 5.2), our mites produced fewer offspring. Temperature can play a role in fecundity of *M. muscaedomesticae*, as seen in Filipponi's (1955) observations (Table 5.2); as culture temperature increased from 29 to 34°C, female fecundity decreased. Relative to the other studies, our cultures were maintained at a lower temperature (25°C). Unfortunately, no other published study used a temperature of 25°C for the culturing temperature, so we cannot directly compare our results. Another potential explanation for differences in fecundity between mites in this study and other observations is mite diet; our cultures were sustained on a nematode-only diet as opposed to fly eggs and nematodes (see Table 5.2). However, in the life history experiments in which half of the mites had access to a fly host (in addition to nematodes), those females produced more offspring in their lifetimes (48.9 ± 4.64 SE) than females that had a nematode-only diet (35.1 ± 5.05 SE). A study comparing the lifetime fecundities of parasitic and non-parasitic mites of a related species, *Macrocheles subbadius*, reported similar results (Luong and Subasinghe 2017). Rodriguez et al. (1962) reported that *M. muscaedomesticae* females fed a diet of nematodes and *Musca domestica* eggs produced nearly twice as many eggs per day (23.1 ± 5.58 95% CI) as the females fed a diet of only nematodes (12.1 ± 1.42 95% CI). Host species may also affect fecundity: the mites in our study produced fewer offspring compared to the mites that had access to *Musca domestica* eggs from other studies (Table 5.2). Perhaps *D. hydei* are less nutritionally valuable relative to *M. domestica* eggs.

Host-feeding

The female *Macrocheles muscaedomesticae* that were attached to *Drosophila hydei* for four hours weighed significantly more than females that were not exposed to a fly, which suggests that *M. muscaedomesticae* are extracting hemolymph or other tissue from their fly hosts. However, the 5.64% weight increase in the attached mites may have occurred from fly exposure alone. For example, the mere presence of a fly in the chamber could have reduced the rate of desiccation experienced by the mites (irrespective of attachment), which could have resulted in a higher mean weight. An experiment that controlled for fly exposure would provide a better estimate of weight gain experienced from feeding. To my knowledge, there are no other studies that compare mite weight before and after host attachment.At this time, we are unsure of the biological relevance associated with a 5.64% weight gain in adult female *M. muscaedomesticae*.

So, the question remains: Are female *M. muscaedomesticae* parasitic? Multiple studies have observed costs experienced by hosts harbouring *M. muscaedomesticae*. Luong et al. (2015), found physiological costs in *D. hydei* that were subjected to mite attachment. Jalil and Rodriguez (1970) observed weight loss in *Musca domestica* flies exposed to *M. muscaedomesticae* mites; moreover, the weight loss was dependent upon duration of exposure. Furthermore, mite exposure significantly reduced fly longevity and the negative effect increased with the number of mites the fly was exposed to (Jalil and Rodriguez 1970). Although *M. muscaedomesticae* inflict costs on flies, whether the costs are due to the mites feeding on host hemolymph is more equivocal. Jalil and Rodriguez (1970) hypothesized that the costs experienced by flies were due to the mites feeding on hemolymph. Unfortunately, the authors do not clarify their definition of mite ‘exposure’, thus the duration of mite attachment is unknown in their experiments.

1577 Experiments that track the uptake of host hemolymph (see Polak 1996) are required to confirm
1578 that *M. muscaedomesticae* indeed feed while attached.
1579

Table 5.1 Life history data comparison of *Macrocheles muscaedomesticae* cultures from our studies to that of other published studies

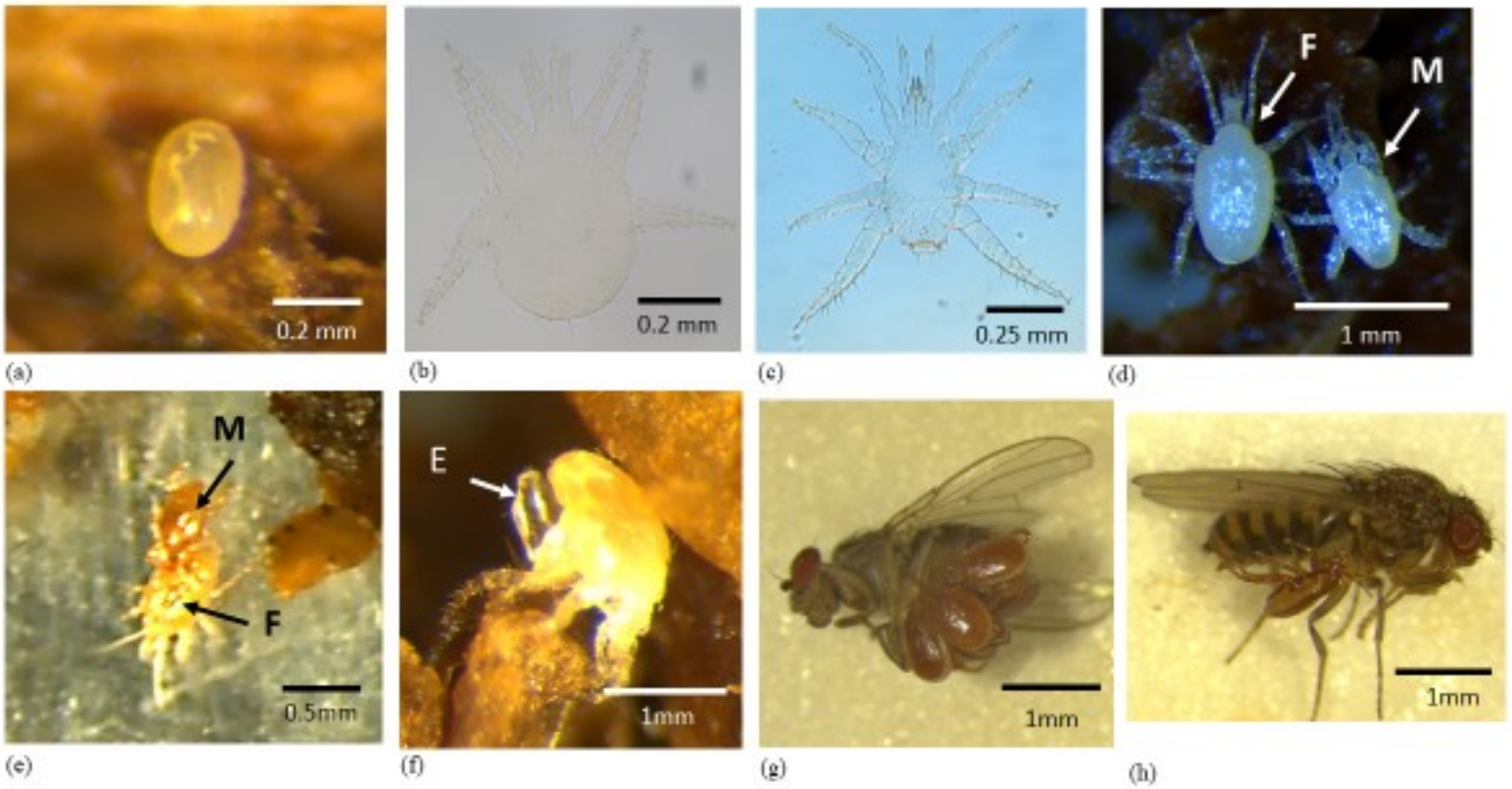
We include the mite culture's location of origin, the temperature (Temp.), relative humidity (RH) and light: dark cycle (L:D) the culture was maintained at in the laboratory and the diet the culture was sustained on: *Musca domestica* eggs (Eggs), *Musca domestica* larvae (Larvae) and nematodes (Nems). We report female *M. muscaedomesticae* total development time and stage durations in mean days ± SE (unless otherwise indicated). Total development refers to the time in days for development from egg to adult female. We also indicate the number of mites from which each mean observation was calculated (N mites).

Location	Temp.	RH	L:D	Diet	n mites	Total Development	Egg Duration	Larva Duration	Protonymph Duration	Deutonymph Duration	Author
Canada	25°C	70%	12:12	Nems	39	~3	--	--	--	--	this study
USA	26.7°C	55-60%	15:09	Eggs	25	2.35 ± 0.14 95%CI	0.29 ± 0.02 95%CI	0.34 ± 0.05 95%CI	0.76 ± 0.09 95%CI	0.96 ± 0.13 95%CI	Wade and Rodriguez 1961
Italy	28°C	75%	--	Eggs + Nems	34	2.50 ± 0.01	--	--	--	--	Filipponi *et al*. 1971 Expt 1
Italy	30°C	75%	--	Eggs + Nems	52	1.76 ± 0.02	--	--	--	--	"
Italy	32°C	75%	--	Eggs + Nems	40	2.06 ± 0.03	--	--	--	--	"
Italy	34°C	75%	--	Eggs + Nems	39	2.08 ± 0.04	--	--	--	--	"
Iran	28°C	65%	14:10	Eggs	27	2.84 ± 0.03	0.61 ± 0.02	0.18 ± 0.01	0.96 ± 0.02	1.09 ± 0.02	Farahi *et al.* 2018
Egypt	28°C	90%	--	Eggs	5	4	0.7	--	--	--	Abo-Taka *et al*. 2014
Egypt	28°C	90%	--	Larvae	5	7	0.8	--	--	--	"

Table 5.2 Reproductive data comparison of *Macrocheles muscaedomesticae* cultures from our studies to that of other published studies

We include the mite culture's location of origin, the temperature (Temp.), relative humidity (RH) and light: dark cycle (L:D) the culture was maintained at in the laboratory and the diet the culture was sustained on: *Musca domestica* eggs (Eggs), *Musca domestica* larvae (Larvae), nematodes (Nems) and *Drosophila hydei* (*hydei*). We report adult female *M. muscaedomesticae* longevity in days ± SE (unless otherwise indicated), daily fecundity as the mean ± SE (unless otherwise indicated) number of nymphs (larva-deutonymph; nn) or adult offspring (ad), and lifetime fecundity as the mean ± SE (unless otherwise indicated) number of eggs, nymphs (larva-deutonymph; nn) or adult offspring (ad) produced in a single female's lifetime.

Location	Temp.	RH	L:D	Diet	n mites	Adult Female Longevity	Daily Fecundity	Lifetime Fecundity	Author
Canada	25°C	70%	12:12	Nems	19	11.0 ± 1.2	4.13 ± 0.22 nn	35.05 ± 5.05 nn	this study
Canada	25°C	70%	12:12	Nems+*hydei*	20	12.8 ± 1.1	4.41 ± 0.25 nn	48.95 ± 4.64 nn	"
USA	26.7°C	55-60%	15:09	Eggs	20	23.9 ± 5.4 95%CI	--	61.4 ± 11.2 eggs	Wade and Rodriguez 1961
Italy	28°C	75%	--	Eggs + Nems	8	11.0 ± 1.0	19.65 ± 1.25 ad	157.8 ± 8.8 ad	Filipponi *et al*. 1971 Expt 2
Italy	28°C	75%	--	Eggs + Nems	8	11.6 ± 1.1	19.96 ± 0.81 ad	161.5 ± 3.0 ad	"
Italy	30°C	75%	--	Eggs + Nems	8	10.9 ± 1.2	16.73 ± 1.99 ad	123.6 ± 13.8 ad	"
Italy	30°C	75%	--	Eggs + Nems	8	12.6 ± 0.6	18.98 ± 0.33 ad	140.5 ± 4.7 ad	"
Italy	32°C	75%	--	Eggs + Nems	8	11.1 ± 0.3	16.86 ± 1.85 ad	123.8 ± 12.3 ad	"
Italy	32°C	75%	--	Eggs + Nems	8	12.8 ± 0.9	17.73 ± 0.80 ad	130.6 ± 5.2 ad	"
Italy	34°C	75%	--	Eggs + Nems	8	12.0 ± 0.8	9.06 ± 1.72 ad	73.8 ± 14.5 ad	"
Italy	34°C	75%	--	Eggs + Nems	8	11.0 ± 0.7	12.35 ± 0.77 ad	91.3 ± 5.2 ad	"
Iran	28°C	65%	14:10	Eggs	27	38.63 ± 0.68	4.04 nn	128.51 ± 1.4SE nn	Farahi *et al*. 2018



1592

Figure 5.1 Images of *Macrocheles muscaedomesticae* life history

1593 Images of *Macrocheles muscaedomesticae* life history stages: (a) egg; (b) larva; (c) protonymph; (d) one female (F) and male (M) deutonymph; (e) adult male
1594 (M) mate-guarding a female deutonymph (F); (f) an adult female emerging from her deutonymph exuvia (E); (g) 3 adult females attached to a *Drosophila*
1595 *hydei*;(h) single adult female attached to a *D. hydei*.

1596

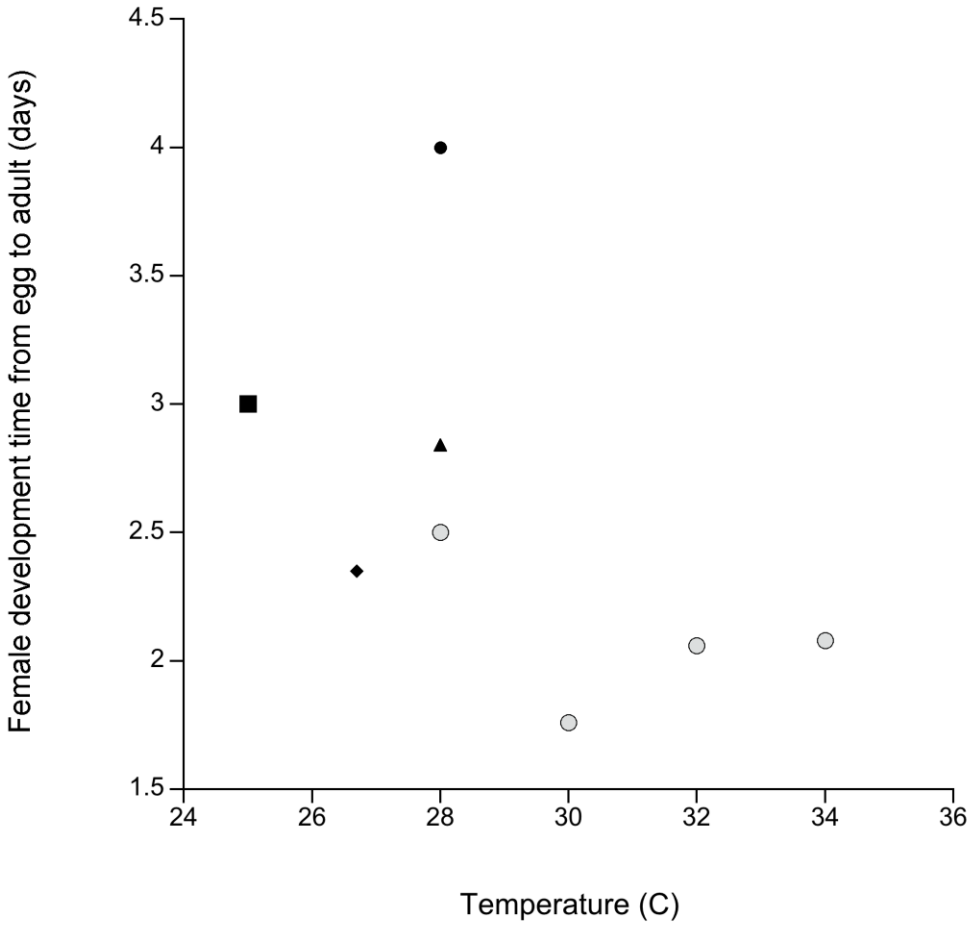
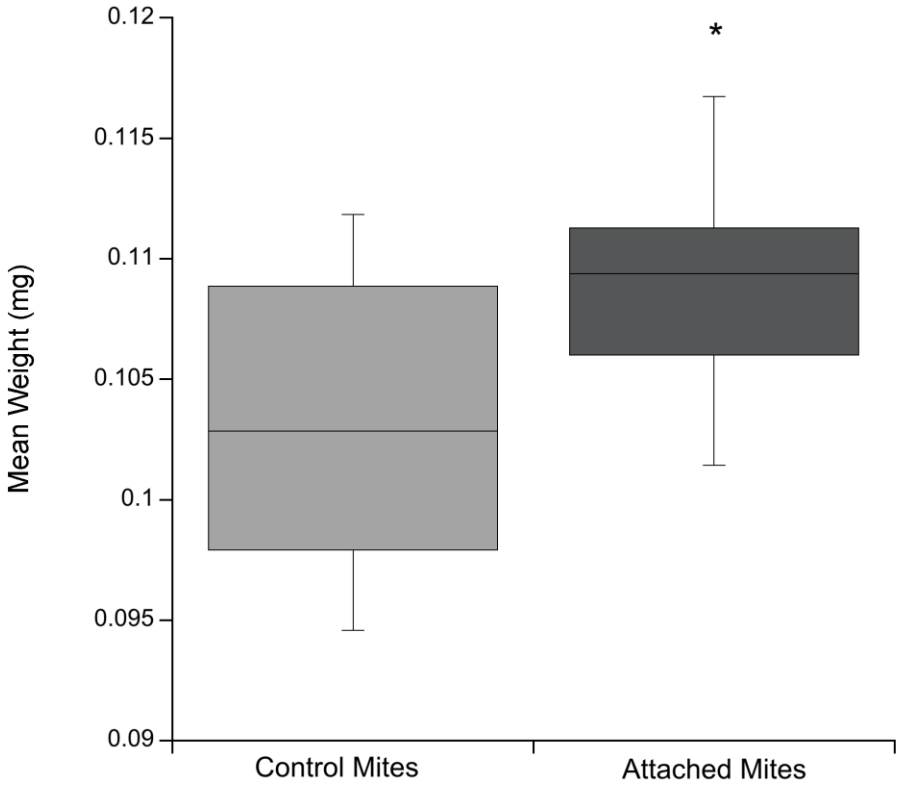


Figure 5.2 The relationship between female *Macrocheles muscaedomesticae* egg to adult development time and temperature, including data from other published studies

The relationship between female *Macrocheles muscaedomesticae* egg to adult development time (in days) and temperature from our study (black square) and other published studies. Wade (1961) = black diamond , Farahi et al. (2018) = black triangle, Abo-Taka et al. (2014) = black circle and Filipponi (1971) = gray circles. See Table 5.1 for culture temperatures used in each study.



1603

Figure 5.3 Mean weights of *Macrocheles muscaedomesticae* mites that had and had not previously attached to a *Drosophila hydei* host.

1604 Mean weight of *Macrocheles muscaedomesticae* mites that had previously attached to a *Drosophila hydei* fly host
1605 for 4 hours ("attached"/dark gray) compared to mites that did not have access to a fly host ("control"/light gray).
1606 Mites that had previously attached to a host for four hours weighed significantly more than mites that did not.

1607

Chapter 6. Conclusions

6.1 Thesis conclusions

Although parasitism is a ubiquitous lifestyle, little direct experimental evidence exists for how and why parasitism evolves in free-living organisms. Analysis of phylogenetic relationships among taxa that exhibit a range of lifestyles (free-living to obligately parasitic), has aided in the development of a commonly proposed hypothesis for the evolution of parasitism: that transient host-associations served as evolutionary stepping-stones towards more obligate parasitism (Anderson 1984; Athias-Binche and Morand 1993; Krantz 1998; Dowling 2015).

Using experimental evolution (i.e. artificial selection) of the facultatively parasitic mite, *Macrocheles muscaedomesticae*, I was able to provide some of the first experimental evidence that supports an assumption for this hypothesis. The mites responded positively to selection for increased infectious behaviour (i.e. infectivity). We estimated that 16.6% of infection variation was due to additive genetic variation (Table 2.1). Furthermore, the mites selected for increased infectious behaviour continued to exhibit significantly higher levels of infection prevalence 20 generations post selection (Fig. 2.5). Although our results are strong, further studies in other systems are needed to provide additional evidence for additive genetic variation in infection behaviour. Furthermore, infectious behaviour is merely one trait that is essential to parasite evolution. Traits associated with host-reliance and parasite specialization (e.g. increased host-feeding, morphological modification for host attachment and feeding, etc.) are likely critical to parasite evolution. Investigation of the evolution of these traits in facultative parasites would generate a more-comprehensive understanding of parasite evolution from free-living organisms.

In order for parasitism to evolve by natural selection, there must be an associated fitness benefit (Darwin and Wallace 1858; Futuyma 2009). Indeed, I found that *M. muscaedomesticae*

produced significantly more offspring in their lifetime after they attached to a fly (Fig. 3.1a). These results were obtained from only one of the replicate lines and the fly that each mite was previously attached to was crushed and placed into the media with the mite; it's possible that mites obtained some nutrients from the fly carcass. Indeed, there is evidence that *M. muscaedomesticae* mites produce more offspring when fly eggs and larvae are included in their diet (Rodriguez et al. 1962). Increasing replication and restricting fly access after detachment would produce more substantial evidence for a fitness benefit associated with infection in *M. muscaedomesticae*.

Given the evidence for additive genetic variation in infection behaviour and the potential associated fitness benefit, I was curious as to how infection variation was maintained in *M. muscaedomesticae*. I hypothesized that evolutionary trade-offs (i.e. costs) associated with increased infectious behaviour might prevent the fixation of a single infection strategy, thus maintaining infection variation. Although I did not find evidence for trade-offs associated with increased infectious behaviour, I cannot reject my hypothesis because I did not test all possible trade-off situations.

Trade-offs can be context-dependent and manifest only in particular environments (Stearns 1989). Measuring potential trade-offs in a variety of environments, particularly stressful environments, would improve my ability to detect them. As mentioned previously, infectious behaviour is likely one of many traits involved in parasitism, and there could be costs associated with these other parasitic-traits, that maintain infection variation in *M. muscaedomesticae*.

There may be other means by which infection variation is maintained in nature. Plasticity could be responsible for the infection variation observed in *M. muscaedomesticae*. By adaptively responding to environmental fluctuations, mite populations will exhibit variation in infection

strategy. The results of my experiment suggest that infection plasticity is inherent in *M. muscaedomesticae*. More experiments in infection plasticity are necessary to understand its potential trade-offs and genetics. The ephemeral nature of *M. muscaedomesticae*'s habitat could also select for maintenance of variation in infection strategy. With constantly fluctuating environments, it is unlikely that a single strategy consistently achieves the highest fitness. In other words, the optimal infection strategy may depend on the environment. It is also important to note that infection plasticity and environmental stochasticity are not mutually exclusive hypotheses; both could be working in concert to maintain variation in infection strategy in nature.

My last objective was to provide a comprehensive overview of the biology and life history of the *M. muscaedomesticae*. As a part of this work, I also wanted to collect more evidence on the nature of *M. muscaedomesticae*'s relationship with its fly hosts. The mites used in my experiments displayed life-histories similar to those of other published studies (Table 5.1 and 5.2). I also found evidence that supports the hypothesis that *M. muscaedomesticae* do not merely attach to their host for dispersal (i.e. phoresy); mites that attached to a fly for 4 hours weighed significantly more than the mites that did not (Fig. 5.2). However, there is the possibility that only larger mites were physically able to attach to flies (i.e. the infectious mites were naturally larger mites). An experiment comparing the weights of mites that attached to flies without feeding to that of free mites could rule out this alternative hypothesis. Furthermore, experiments in which the host's hemolymph is tracked could provide definitive evidence for whether *M. muscaedomesticae* is truly parasitic.

Historically, parasitism was thought to be a static relationship between parasite and host. My thesis work, along with the work of other researchers, highlights the dynamic nature of

parasitism. Evidence for parasites successfully adapting to new environments is illustrated by their shifts in geographical range (Parmesan and Yohe 2003; Khatchikian et al. 2015), host range (i.e. zoonoses; Polley and Thompson 2009), and virulence (i.e. emerging disease; Fisher et al. 2012). A more comprehensive understanding of the evolution and plasticity of parasitism will have an immense impact on the way we understand, treat and control parasites.

6.2 Future directions

To tackle further questions in this system, I think a more comprehensive understanding of *M. muscaedomesticae* is necessary. One potential avenue of research could investigate the repeatability of attachment in mites. If some mites maintain infectious behaviour through time and across contexts, some researchers would argue that these mites exhibit "infectious" personalities (Wolf and Weissing 2012). Perhaps "infectious personalities" also play an important role in parasite evolution from free-living organisms.

I also think it is important to find clear evidence for whether *M. muscaedomesticae* feed on fly hemolymph while attached. There is data that supports the hypothesis that they feed (see Jalil and Rodriguez 1970; Farish and Axtell 1971; Chapter 5), but alternative hypotheses cannot be ruled out. If there were evidence of fluid transfer from fly to the mite, feeding could serve as another trait to explore in the context of parasite evolution; and potentially provide empirical evidence for the evolution of parasitism in closely related ticks.

There are many opinions on what defines an organism as a parasite, yet there doesn't exist a single unifying characteristic. Zelmer (1998) suggested that parasites are unified by their ability to evade their host's immune response and that this characteristic was key in all evolutionary transitions to parasitism. Others have argued that parasitic ex-aptations were necessary in order to form relationships with potential hosts which were later shaped by natural

selection (Rothschild and Clay 1952; Poulin 2007; Dowling 2015). Although host evasion, and characteristics that assist in forming symbiotic relationships are critical, one might wonder how a free-living organism might develop such critical parasitic characteristics given their lifestyle. According to the modern synthesis of evolution, necessary parasitic traits could manifest in free-living population due to genetic mutation or be introduced through gene flow (Darwin and Wallace 1858; Futuyma 2009). However, it's possible that phenotypic plasticity is also an important factor in parasite evolution.

Phenotypic plasticity is expected to evolve when environmental heterogeneity is persistent, reliable cues about the environment exist, phenotypic plasticity confers a fitness benefit, and the population exhibits additive genetic variation in phenotypic plasticity (Via et al. 1995; Garland and Kelly 2006). Host heterogeneity is ubiquitous (Hudson et al. 2002), and similar to variable environments, it is unlikely that a single, fixed strategy works best for all potential hosts (Via et al. 1995). There is also evidence that parasites are capable of detecting information about their host(Reece et al. 2008; Birget et al. 2017), or even the absence of a potential host(Lagrue and Poulin 2007). Furthermore, there is evidence for the conferred fitness benefits in plastic parasites (Lagrue and Poulin 2007; Reece et al. 2008; Birget et al. 2017). Is it possible that one of the unifying traits of parasites is their phenotypic plasticity? Could phenotypic plasticity be one of the proposed ex-aptations required for the transition to parasitism? This question could only be answered by looking for phenotypic plasticity in a wide-range of parasites.

Comparative studies of the phenotypic plasticity among closely related species that exhibit differing levels of parasitic activity could provide some insight on its importance in parasite evolution. If phenotypic plasticity were an important 'preadaptation' to parasitism, I

would predict species that exhibit more transient host associations relative to obligate parasite species to display higher levels of phenotypic plasticity. However, my research suggests facultative parasites might experience the Baldwin effect and I would then expect greater or maintained levels of phenotypic plasticity in more obligately parasitic species. Again, I think it is important to note that my data were collected from facultative parasites. Might some obligate parasites exhibit fixed infection strategies? If yes, some obligate parasites may represent examples of genetic assimilation in infection strategy from their more variable relatives. Just as Suzuki and Nijhout (2006) experimented with *Manduca sexta*, might it be possible to stress parasites that exhibit fixed infection strategies to generate ancestral phenotypic plasticity?

Beyond providing evidence for one of the assumpations to facultative parasites serving as evolutionary stepping-stones in the evolution of parasitism from free-living ancestors, this thesis work emphasizes the importance of infection variation in facultative parasites. Thus, because of its ability to generate variation, phenotypic plasticity might be an important component to parasite evolution. Furthermore, phenotypic plasticity might be a defining characteristic in some of the most successful and persistent parasites. Future research that focuses on the phenotypic plasticity of parasites may prove critical to our understanding infectious diseases and the development of successful control programs.

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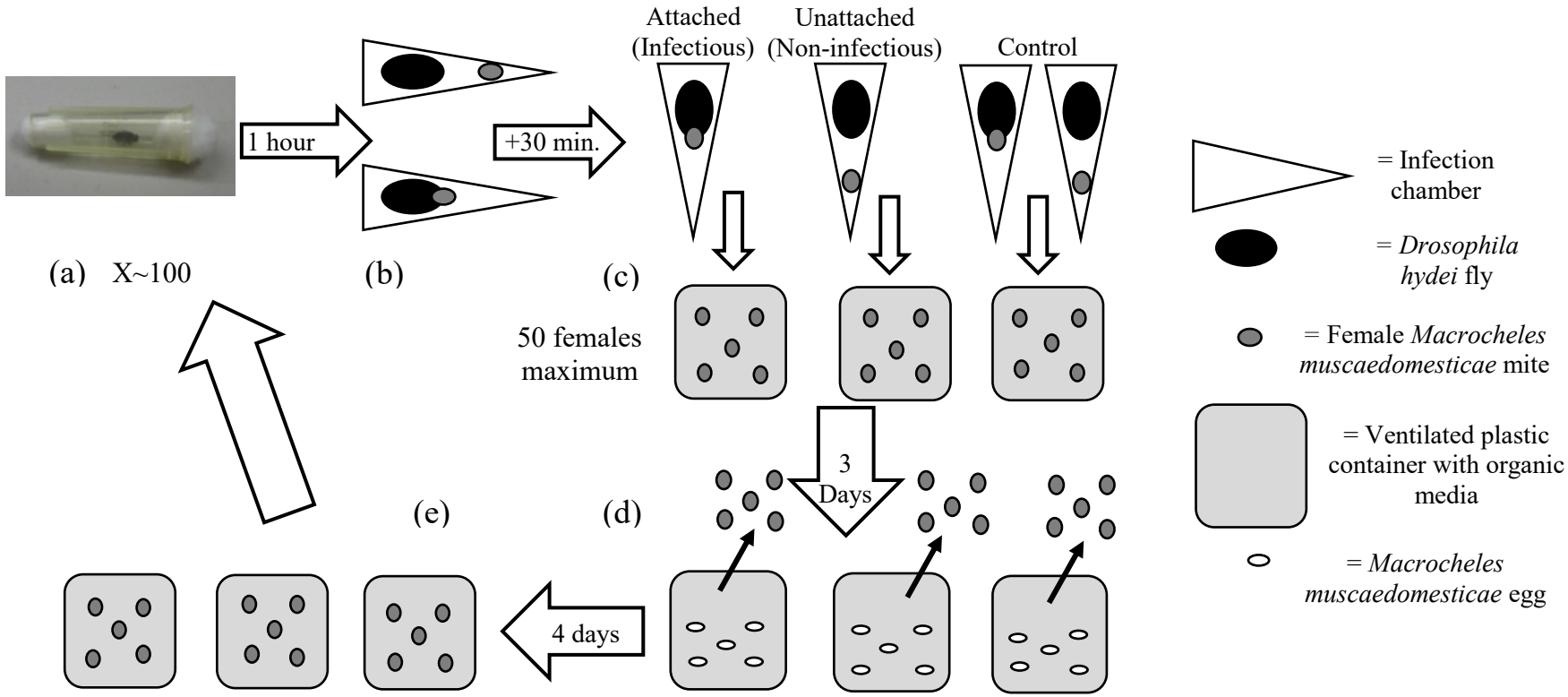
Appendix 1

This supplemental figure accompanies Chapter 2. Experimental evolution of infectious behaviour in a facultative ectoparasite.



Supplemental Figure S2.1 Artificial selection protocol

Selection began by individually placing 100 adult female *Macrocheles muscaedomesticae* mites in an infection chamber with a single female *Drosophila hydei* (a). After 60 minutes, mites were scored as either "attached" or "unattached" to the fly (b). Mites remained in the chambers for an additional 30 minutes. Mites that remained attached the entire 90 minutes were used to seed the "infectious" selected lines, mites that remained unattached the entire 90 minutes were used to seed the "uninfectious" selected lines. Mites used to seed the control lines were randomly selected regardless of their attachment. A maximum of 50 female mites were placed in organic media to lay eggs for 3 days (c). The adult females were then discarded, and the eggs were allowed to develop in the media for 4 days (d). 100 of the newly developed female offspring were then used for the subsequent round of selection.

These supplemental tables and figures accompany Chapter 3: Evolutionary trade-offs do not constrain the evolution of infectivity in a facultative ectoparasite.

Supplemental table S3.1 Life table calculations for replicate line 1's unselected control *Macrocheles muscaedomesticae* mites that did not have access to a *Drosophila hydei* fly host

Line 1

Control: Non-Parasitic

X (days) (age class)	S_X (survivors)	D_X (dying)	q_X (death rate)	p_X (survival rate)	l_X (survival to age x)	m_X (birth rate)	$l_X m_X$
0	10	0	0	1	1	0	0
2	10	0	0	1	1	4.50	4.50
4	10	0	0	1	1	8.20	8.20
6	10	1	0.10	0.90	1	7.80	7.80
8	9	1	0.11	0.89	0.90	9.88	8.89
10	8	3	0.38	0.63	0.80	5.37	4.30
12	5	1	0.20	0.80	0.50	5.80	2.90
14	4	2	0.50	0.50	0.40	2.00	0.80
16	2	0	0	1.00	0.20	3.00	0.60
18	2	1	0.50	0.50	0.20	4.50	0.90
20	1	1	1	0	0.10	0	0
22	0				0		
24							
26						$\sum l_X m_x = 38.88$	
28							

Supplemental table S3.2 Life table calculations for replicates line 1's unselected control *Macrocheles muscaedomesticae* mites that did have access to a *Drosophila hydei* fly host

Line 1

Control: Parasitic

X (days) (age class)	S_X (survivors)	D_X (dying)	q_X (death rate)	p_X (survival rate)	l_X (survival to age x)	m_X (birth rate)	$l_X m_X$
0	10	0	0.00	1.00	1.00	0.00	0.00
2	10	0	0.00	1.00	1.00	8.50	8.50
4	10	1	0.10	0.90	1.00	10.70	10.70
6	9	0	0.00	1.00	0.90	6.88	6.19
8	9	1	0.11	0.89	0.90	6.55	5.90
10	8	2	0.25	0.75	0.80	4.75	3.80
12	6	2	0.33	0.67	0.60	4.83	2.90
14	4	2	0.50	0.50	0.40	4.50	1.80
16	2	0	0.00	1.00	0.20	4.00	0.80
18	2	2	1.00	0	0.20	0.00	0.00
20	0				0.00		
22							
24							
26							
28							
						$\sum l_X m_x = 40.59$	

Supplemental table S3.3 Life table calculations for replicate line 1's *Macrocheles muscaedomesticae* mites selected for increased infectivity that did not have access to a *Drosophila hydei* fly host

Line 1

Selected: Non-parasitic

X (days) (age class)	S_X (survivors)	D_X (dying)	q_X (death rate)	p_X (survival rate)	l_X (survival to age x)	m_X (birth rate)	$l_X m_X$
0	10	0	0	1.00	1.00	0.00	0.00
2	10	0	0	1.00	1.00	4.60	4.65
4	10	0	0	1.00	1.00	10.00	10.00
6	10	0	0	1.00	1.00	7.30	7.30
8	10	0	0	1.00	1.00	7.22	7.22
10	10	3	0.30	0.70	1.00	5.70	5.70
12	7	1	0.14	0.86	0.70	6.57	4.60
14	6	3	0.50	0.50	0.60	3.00	1.80
16	3	1	0.67	0.67	0.30	0	0
18	2	0	1.00	1.00	0.20	0	0
20	2	0	1.00	1.00	0.20	0	0
22	2	1	0.50	0.50	0.20	0	0
24	1	1	0	0.00	0.10	0	0
26	0				0	$\sum l_X m_x = 41.22$	
28							

Supplemental table S3.4 Life table calculations for replicate line 1's *Macrocheles muscaedomesticae* mites selected for increased infectivity that did have access to a *Drosophila hydei* fly host

Line 1

Selected: Parasitic

X (days) (age class)	S_X (survivors)	D_X (dying)	q_X (death rate)	p_X (survival rate)	l_X (survival to age x)	m_X (birth rate)	$l_X m_X$
0	10	0	0	1.00	1.00	0.00	0.00
2	10	0	0	1.00	1.00	6.60	6.60
4	10	0	0	1.00	1.00	12.00	12.00
6	10	1	0.10	0.90	1.00	8.70	8.70
8	9	2	0.22	0.78	0.90	8.62	7.76
10	7	1	0.14	0.86	0.70	4.57	3.20
12	6	0	0	1.00	0.60	4.83	2.90
14	6	1	0.17	0.83	0.60	4.00	2.40
16	5	1	0.20	0.80	0.50	0.80	0.40
18	4	2	0.50	0.50	0.40	0.25	0.10
20	2	0	0	1.00	0.20	0	0
22	2	1	0.50	0.50	0.20	0	0
24	1	0	0	1.00	0.10	0	0
26	1	1	1.00	0.00	0.10	0	0
28	0				0	$\sum l_X m_x = 44.06$	

Supplemental table S3.5 Life table calculations for replicate line 2's unselected control *Macrocheles muscaedomesticae* mites that did not have access to a *Drosophila hydei* fly host

line 2

control: non-parasitic

x (days) (age class)	s_x (survivors)	d_x (dying)	q_x (death rate)	p_x (survival rate)	l_x (survival to age x)	m_x (birth rate)	$l_x m_x$
0	10	0	0	1.00	1.00	0.00	0.00
2	10	2	0.20	0.80	1.00	8.60	8.60
4	8	0	0	1.00	0.80	11.62	9.30
6	8	0	0	1.00	0.80	9.62	7.70
8	8	3	0.38	0.63	0.80	5.00	4.00
10	5	2	0.40	0.60	0.50	5.40	2.70
12	3	1	0.33	0.67	0.30	4.33	1.30
14	2	1	0.50	0.50	0.20	8.00	1.60
16	1	0	0	1.00	0.10	6.00	0.60
18	1	0	0	1.00	0.10	6.00	0.60
20	1	0	0	1.00	0.10	2.00	0.20
22	1	1	1.00	0	0.10	0	0.00
24	0				0		
26						$\sum l_X m_x = 36.59$	
28							

Supplemental table S3.6 Life table calculations for replicate line 2's unselected control *Macrocheles muscaedomesticae* mites that did have access to a *Drosophila hydei* fly host

Line 2

Control: Parasitic

X (days) (age class)	S_X (survivors)	D_X (dying)	q_X (death rate)	p_X (survival rate)	l_X (survival to age x)	m_X (birth rate)	$l_X m_X$
0	10	0	0	1.00	1.00	0	0
2	10	0	0	1.00	1.00	5.80	5.80
4	10	1	0.10	0.90	1.00	16.00	16.00
6	9	0	0	1.00	0.90	14.55	13.10
8	9	2	0.22	0.78	0.90	11.55	10.40
10	7	0	0	1.00	0.70	7.71	5.40
12	7	1	0.14	0.86	0.70	7.00	4.90
14	6	1	0.17	0.83	0.60	3.16	1.90
16	5	2	0.40	0.60	0.50	1.60	0.80
18	3	1	0.33	0.67	0.30	0	0
20	2	2	1.00	0	0.20	0	0
22	0				0		
24							
26						$\sum l_X m_x = 58.28$	
28							

Supplemental table S3.7 Life table calculations for replicate line 2's *Macrocheles muscaedomesticae* mites selected for increased infectivity that did not have access to a *Drosophila hydei* fly host

Line 2

Selected: Non-parasitic

X (days) (age class)	S_X (survivors)	D_X (dying)	q_X (death rate)	p_X (survival rate)	l_X (survival to age x)	m_X (birth rate)	$l_X m_X$
0	10	0	0	1.00	1.00	0	0
2	10	0	0	1.00	1.00	12.80	12.80
4	10	0	0	1.00	1.00	12.30	12.30
6	10	3	0.30	0.70	1.00	8.80	8.80
8	7	1	0.14	0.86	0.70	5.85	4.10
10	6	3	0.50	0.50	0.60	3.66	2.20
12	3	0	0	1.00	0.30	2.33	0.70
14	3	1	0.33	0.67	0.30	2.00	0.60
16	2	0	0	1.00	0.20	0.50	0.10
18	2	1	0.50	0.50	0.20	0.50	0.10
20	1	0	0	1.00	0.10	0	0
22	1	1	1.00	0	0.10	0	0
24	0				0		
26						$\sum l_X m_x = 41.69$	
28							

Supplemental table S3.8 Life table calculations for replicate line 2's *Macrocheles muscaedomesticae* mites selected for increased infectivity that did have access to a *Drosophila hydei* fly host

Line 2

Selected: Parasitic

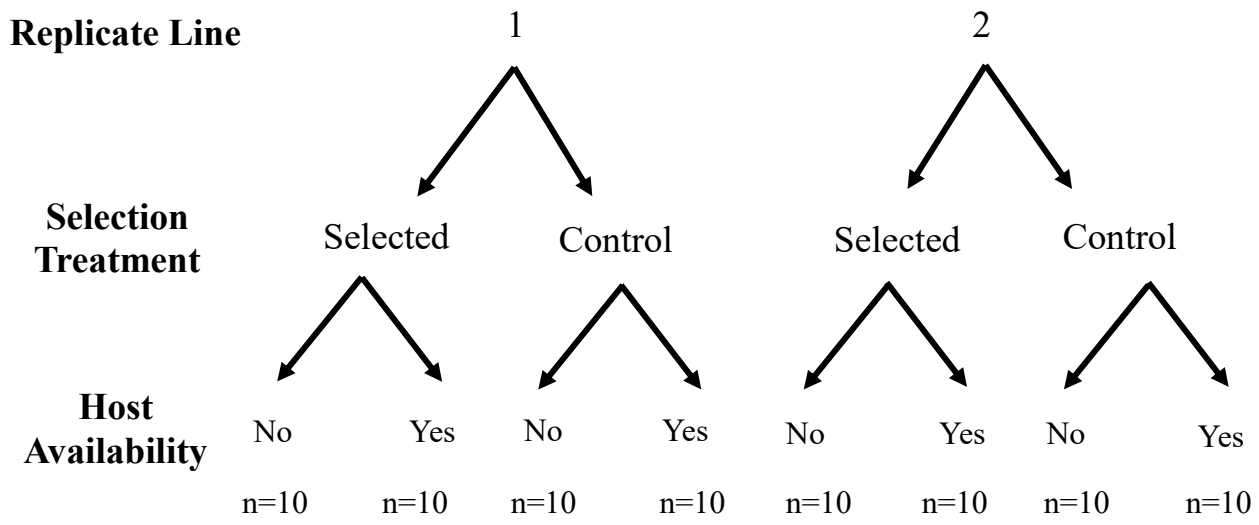
X (days) (age class)	S_X (survivors)	D_X (dying)	q_X (death rate)	p_X (survival rate)	l_X (survival to age x)	m_X (birth rate)	$l_X m_X$
0	9	0	0	1.00	1.00	0	0
2	9	1	0.11	0.89	1.00	5.88	5.88
4	8	0	0	1.00	0.89	19.12	17.00
6	8	0	0	1.00	0.89	21.37	19.00
8	8	2	0.25	0.75	0.89	12.62	11.22
10	6	1	0.17	0.83	0.67	12.66	8.44
12	5	1	0.20	0.80	0.56	14.40	8.00
14	4	2	0.50	0.50	0.44	4.25	1.89
16	2	1	0.50	0.50	0.22	2.50	0.56
18	1	1	1.00	0	0.11	0	0
20	0				0		
22							
24							
26							
28						$\sum l_X m_x = 71.97$	

Supplemental table S3.9 Deviances and p-values for all variables and interaction terms in the analysis of the weighted fecundity schedule data.

Variable	**Deviance**	***p***
Replicate Line (Line)	-0.0153	0.698
Selection TRT	0.0721	0.394
Fly TRT	0.746	0.00663
Mite Age (Age)	-58.211	2.2e-16
Age^2	74.128	2.2e-16
Fly TRT:Line	-0.378	0.0506
Age:Line	-0.167	0.195
Selection TRT:Fly TRT	-0.130	0.250
Selection TRT:Line	-0.0308	0.577
Age:Fly TRT	-0.0156	0.693
Age:Selection TRT	-0.000750	0.931
Age:Selection TRT:Fly TRT	0.125	0.265
Age:Selection TRT:Line	0.111	.295
Age:Fly TRT:Line	-0.0995	0.320
Selection TRT:Fly TRT:Line	-0.0257	0.616
AgexSelection TRT:Fly TRT:Line	-0.00813	0.779

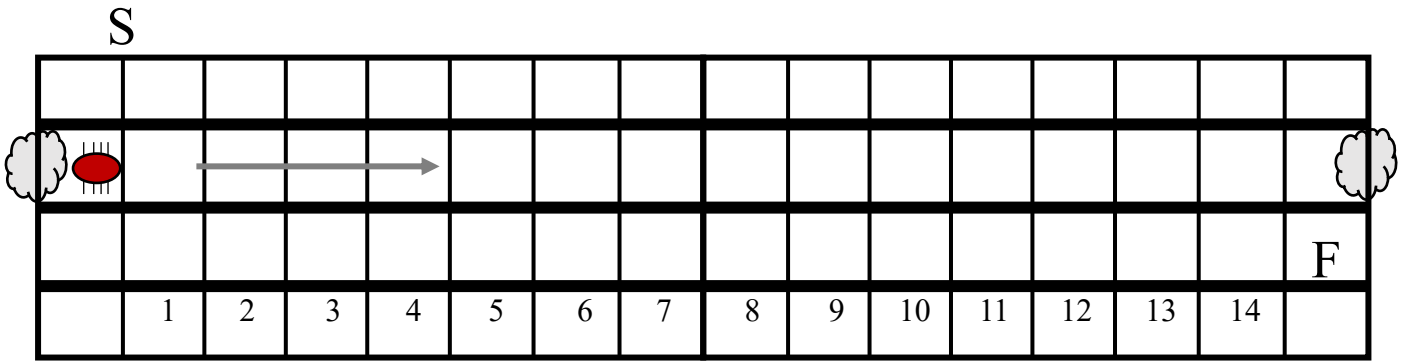
Supplemental table S3.10 Deviances and p-values for all variables and interaction terms in the analysis of the mite longevity data.

Variable	**Deviance**	***p***
Replicate Line (Line)	-0.519	0.0920
Selection TRT	-0.156	0.358
Fly TRT	-0.126	0.407
Fly TRT:Line	-0.290	0.210
Selection TRT:Fly TRT	-0.0716	0.537
Selection TRT:Line	-0.192	0.309
Selection TRT:Fly TRT:Line	-0.142	0.387



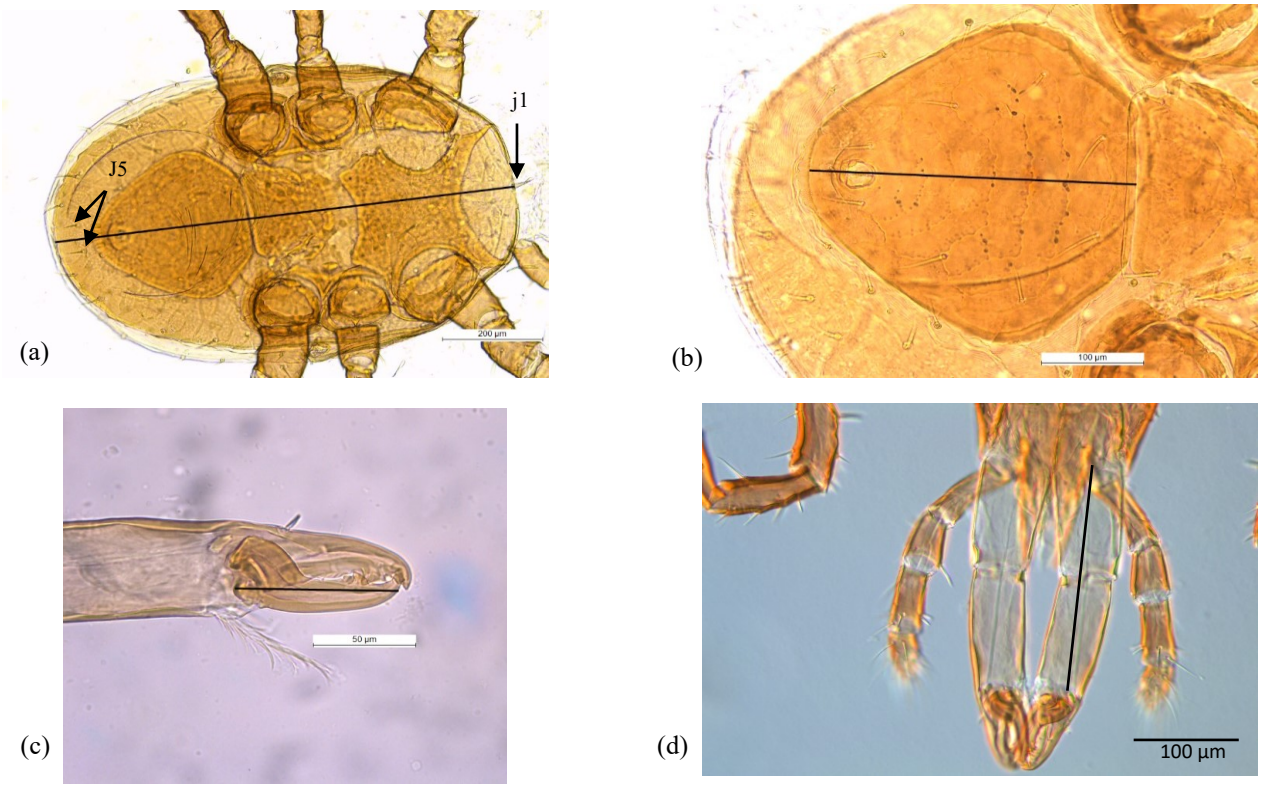
Supplemental Figure S3.1 Experimental design for "host access" treatments

Experimental set-up for comparing the fecundity and longevity of *Macrocheles muscaedomesticae* mites selected for increased infectious behaviour and unselected control mites with and without access to a host. The mites used in this experiment came from selection experiment A which continued for 15 and 17 generations in replicate lines 1 and 2 respectively (Durkin and Luong 2018). Selected and control mites were further divided into two groups based on whether they had access to a fly host (*Drosophila hydei*).



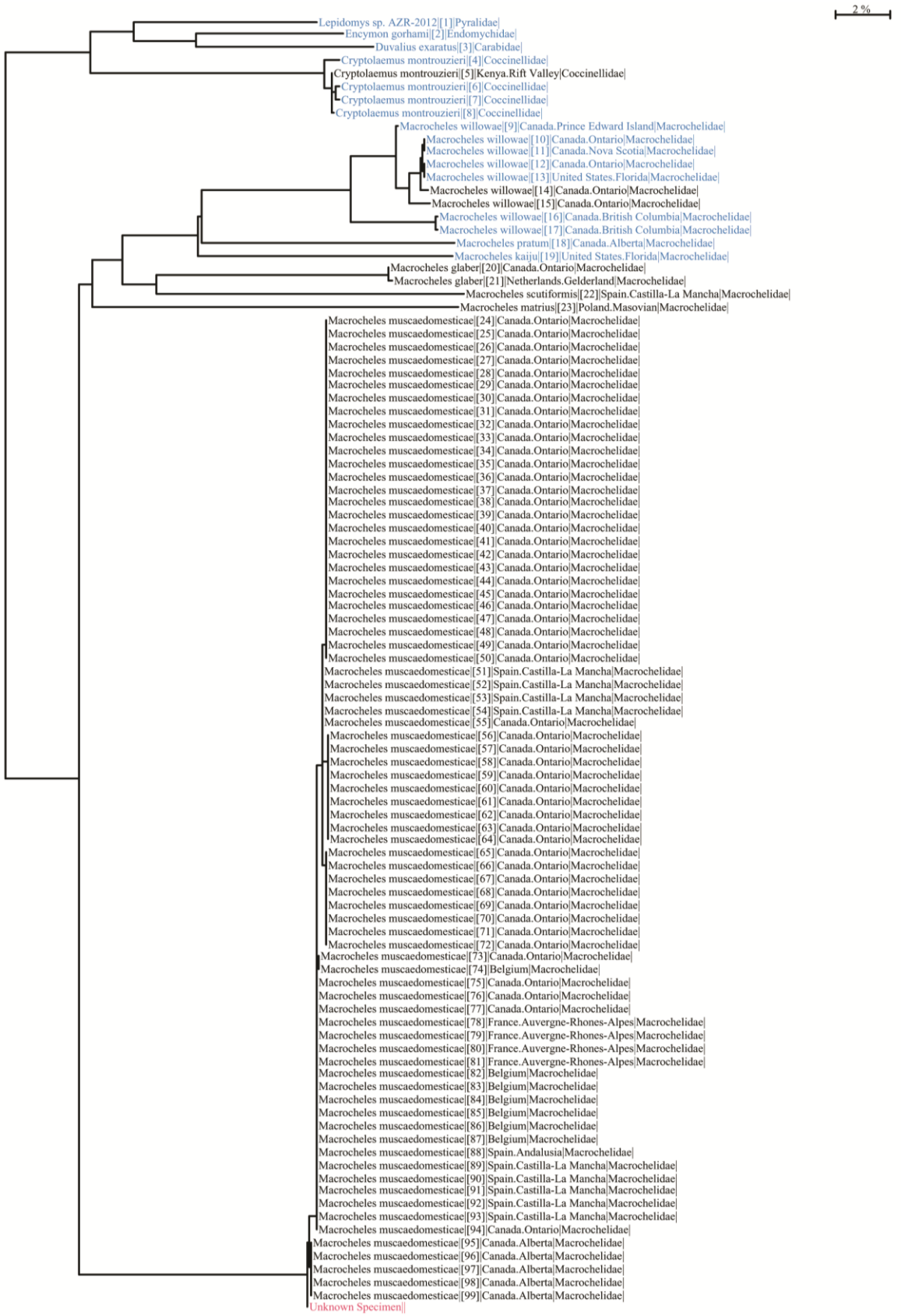
Supplemental Figure S3.2 Racetrack used to compare the motility of *Macrocheles muscaedomesticae* mites selected for increased infectious behaviour and unselected control mites

Mites used in this experiment came from selection experiment B (Durkin and Luong 2018)The track was constructed by sandwhiching 3 wooden applicator sticks (to create lanes) between four (2.6 x 7.6 cm; 1mm thick) microscope slides. Mites were singly raced in the same lane which was rinsed out with water before each race. Mites were placed in the starting end of the lane and the lane was then closed with cotton. Timing began when the mite completely crossed the start line (S). Timing ceased when the mite completely crossed the 8cm line while traveling to the finish end of the ractrack (F).



Supplemental Figure S3.3 Morphometrics used to compare the body sizes between *Macrocheles muscaedomesticae* female mites selected for increased infectivity and unselected control mites

The mites used in these comparisons came from selection experiment B (Durkin and Luong 2018). The dorsal shield was measured from the j1 setae on the anterior end to the posterior end through the J5 setae (a). The ventrianal shield was measured from the anterior end the anterior-most pair of setae through to the posterior end (between the posterior-most pair of setae) (b). This measurement ended at the beginning of the cribiform plate (circled). The moveable digit of the chelicerae was measured from its proximal attachment to the distal tip (c). Lastly, the length of the first and second digits of the chelicerae were measured (d).



Supplemental Figure S.5.1 *Macrocheles muscaedomesticae* neighbor-joining tree

Our *M. muscaedomesticae* COI sequences were compared to other available *M. muscaedomesticae* COI sequences using the Barcode of Life Database (BOLD) (http://www.boldsystems.org/). This neighbor-joining tree was generated by BOLD.