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Ecology of Mite Phoresy on Mountain Pine Beetles

by

Guadalupe Haydeé Peralta Vázquez

A THESIS

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Abstract

Phoresy, a commensal interaction where smaller organisms utilize dispersive hosts for transmission to new habitats, is expected to produce positive effects for symbionts and no effects for hosts, yet negative and positive effects have been documented. This poses the question of whether phoresy is indeed a commensal interaction and demands clarification. In bark beetles (Scolytinae), both effects are documented during reproduction and effects on hosts during the actual dispersal are largely unknown. In the present research, I investigated the ecological mechanisms that determine the net effects of the phoresy observed in mites and mountain pine beetles (MPB), *Dendroctonus ponderosae* Hopkins.

Using flight mills, I found that MPB flight increased with beetle size and body condition but was not modified by mite abundance. Mites were initially more abundant on larger beetles in better condition, but their dispersal success was similar among all hosts. Host dispersal was costly for both host and mites. Beetles lost mass whereas mites exhibited mortality.

In the second study, I determined the number and survivorship of all juvenile stages of MPB development and found neutral effects of mites. Although I found a negative effect of mite abundance despite a positive effect of mite presence during host larval stage, there were no further effects during subsequent stages of beetle development. Moreover, I did not find effects of mites on the number or quality of adult beetle offspring.

When observing the distribution of phoretic mites and beetle hosts in the field, I found that the range-expanding behavior of MPB might determine symbiont loss at the leading front of beetle expansion. Three mites were common: *Tarsonemus ips*, *Proctolaelaps subcorticalis*, and *Trichouropoda australis*. Of these species, only *T. ips* was prevalent among all sites. However, both total mite abundance, considering all three species together, and *T. ips* abundance alone

were comparatively lower in the new area of MPB expansion. In addition, beetle body condition was similar in both historical and new areas of MPB distribution.

Given the results, MPB and its phoretic mites sustain a commensalism and the lower distribution of mites may be a consequence of MPB outbreak dynamics.

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CHAPTER 1: GENERAL INTRODUCTION

Symbioses

Permanent and close-living interactions between organisms of different species are known as symbioses (Paracer and Ahmadjian 2000). In general, the net outcome that a symbiosis yields determines its nature, which has facilitated classifying each into three common types. These include mutualism (+), parasitism (-), and commensalism (0) (Table 1.1). It is possible, however, that under certain conditions predation could be considered a symbiosis when predators are functionally equivalent to parasites (Raffel et al. 2008). In the case of parasitoidism, although the parasitoid spends a significant portion of its life feeding on its host, the host is inevitably ingested thus resembling predation. Yet some authors consider parasitoidism a type of parasitism, a parasite strategy, or a trophic strategy of natural enemies (Lafferty and Kuris 2002, Poulin 2011, Schmid-Hempel 2011).

The distribution of fitness effects in each symbiosis differs between the interacting species (Table 1.2). In mutualistic symbioses, fitness benefits (i.e. increases in reproduction and/or survival) are for all participants involved. These benefits are based in the trade of resources or services, mutual exploitation of resources, and/or access to resources that host and symbionts do not obtain efficiently (Bronstein 1994). In parasitic and commensal symbioses, however, the fitness benefits are only for the symbiont. The difference between parasitism and commensalism is the effect that a symbiont produces on its host by obtaining such benefits. While a parasite consumes, exploits, or utilizes its hosts causing fitness losses to the host (i.e. reduced reproduction and/or survival), a commensal symbiont obtains resources, or access to resources

and/or services from a host without increasing or reducing host fitness (Schmid-Hempel 2011). Therefore no fitness effects on hosts are expected in a commensal symbiosis. This however, has hardly been demonstrated in empirical examples as studies documenting effects of commensals seem to range from parasitic to mutualistic and some authors have equated commensals with low-virulence parasites or highly altruistic mutualists (Leung and Poulin 2008, Skelton et al. 2016).

Ecological Determinants of Symbioses

It has been certainly demonstrated that the outcome of a symbiosis is not fixed as it has been traditionally assumed and instead the strength and outcome of a symbiosis can shift as a result of dynamic abiotic and biotic ecological factors (Bronstein 1994, Thrall et al. 2007, Leung and Poulin 2008, Leimar and Hammerstein 2010). This context-dependency has been demonstrated in many studies; for example, some have shown that third party species can influence the fitness and population dynamics in pairwise interactions (Hofstetter et al. 2006, Leung and Poulin 2008, Okabe and Makino 2008, Sanders and van Veen 2012). Some other authors, however, have argued that the shifting nature of a symbioses relates instead to exclusive changes in the magnitude of the cost-benefit balance such that a shift in the net outcome of an interaction can produce a different conditional outcome (Bronstein 1994). Not surprisingly, the shifting nature of symbioses has prompted to development of several theoretical models to explain the evolutionary and ecological transitions among the symbioses with no clear unifying theory (Ewald 1987, Athias-Binche and Morand 1993, Yamamura 1993, Holland and DeAngelis 2009).

Some of the ecological factors include aspects related to level of dependency (whether an interacting species is needed for the reproduction of survival of the other), mode of transmission (the type of dispersal mode a symbiont has between hosts), host and symbiont population size, host and symbiont age structure, geographical patterns of species distribution and environmental quality (Thrall et al. 2007, Vicente et al. 2007, Gundel et al. 2011, Skelton et al. 2016). These factors can be summarized in a general set that includes:

a) Environmental quality
b) Species population structure
c) Species life history.

Level of Dependency

Different levels of dependency exist within a symbiosis yet the symbiont is usually the organism whose reproduction and survival is mostly dependent on the host. In general, two types of dependency are found among symbionts: facultative symbionts, those symbionts that are not required for host survival; and obligate symbionts, those who are required for host survival (on a mutualistic, and 'host-centric' context) and/or symbionts that require a host for their own survival (i.e. parasites and commensals) (Brownlie and Johnson 2009). In mutualistic symbioses dependency comes from both sides of the interaction: both symbiont and host require each other to gain resources (or access to resources) in order to attain and/or increase reproduction and survival. Yet symbionts can be facultative or obligate. On one hand, facultative mutualists can be either absent or replaced for other facultative mutualists present in the environment with no deadly consequences for hosts (Simon et al. 2007). Facultative mutualists are commonly involved in the exchange of services, which includes cleaning symbioses, farming symbioses and

protection symbioses (Biani et al. 2009, Stier et al. 2012, Hulcr and Stelinski 2017). A classic example of a facultative mutualism includes the symbiosis between aphids and bacteria (Oliver et al. 2010). In the pea aphid, *Acyrthosiphon pisum*, facultative mutualists such as bacteria provide protection (increased resistance) against parasitoids and heat stress (Montllor et al. 2002, Oliver et al. 2003). On the other hand, obligate mutualists include those organisms whose reproduction or survival is at risk if separated from their host with similar consequences for hosts if they are separated from their symbionts. Obligate symbioses are usually based in mutual exploitation of resources, and/or access to resources (i.e. nutrient capture) that otherwise organisms would not be able to obtain individually. Most obligate mutualisms are nutritional symbioses although other resource-based symbioses are also common. Typical examples include rhizobial and mycorrhizal symbioses (Denison and Kiers 2011).

In parasitic and commensal symbioses the level of dependency is predominantly unidirectional (only the symbiont depends entirely on the host) and both facultative and obligate symbionts are common to both symbioses (Poulin 2011, Schmid-Hempel 2011). Obligate parasites have produced sophisticated levels of detrimental dependency to hosts primarily related to their phenological requirements. In some cases, for instance, parasites utilize more than one host to complete development and reproduction (Poulin 2011, Schmid-Hempel 2011). On the other hand, although commensal symbionts also present a unidirectional interaction with their hosts similar to parasites, their dependency is not detrimental to the host or at least not as detrimental as in the case of parasites and in some cases commensals are consider a type of mild parasites (Schmid-Hempel 2011).

One of the critical assumptions about commensalism is that commensal fitness depends on hosts. In most cases commensals depend entirely on their host for development, reproduction, and survival (Houck and OConnor 1991, Hooper and Gordon 2001). However, their dependency can also be nutritional as in the case of gut bacteria of many mammals (including humans) and insects, where both facultative and obligate commensals are common. (Hooper and Gordon 2001, Brownlie and Johnson 2009, White 2011). In other cases, commensals also depend on their host for access to resources or basic services such as, dispersal, overwintering shelter, and habitats for development completion and mating (Houck and OConnor 1991, Leung and Poulin 2008, Liu et al. 2016). Some examples of these commensals are found in phoretic symbioses where mites (Arachnida: Acari) have mastered phoresy with outstanding behavioral, morphological, and phenological adaptations such as dormant stages (hypopus), simple gnathosoma, lack of digestive tract, caudoventral attachment organs, and various other attachment structures for dispersal on hosts (Binns 1982, Houck and OConnor 1991, Athias-Binche 1993, Athias-Binche and Morand 1993, Krantz 2009, Norton 2009, Bajerlein and Przewoźny 2012).

Mode of Transmission

Symbioses are partially maintained because symbionts have evolved mechanisms to propagate and persist to the next generation (Ewald 1987, Bright and Bulgheresi 2010). One ecological implication of this relates to the way symbionts are transmitted from host to host in time and space. Symbiont transmission in an ecological sense refers to the effective dispersal or transfer of symbionts, which includes its establishment and reproduction (Bright and Bulgheresi 2010, Antonovics et al. 2017). In general, two major modes of symbiont transmission occur: vertical

and horizontal, although various particularities within each transmission mode exist (Bright and Bulgheresi 2010, Antonovics et al. 2017). Vertical transmission, which is the direct transfer of symbionts from parents to offspring (either internally or externally), and horizontal transmission, which occurs among unrelated hosts (mostly through the environment or social structures of hosts) (Ewald 1987, Stewart et al. 2005). Both transmission modes are commonly utilized by symbionts regardless of type of symbioses but it was not recognized until recently that some symbionts use a combination of both transmission modes (Mangin et al. 1995, Inoue and Ushida 2003, Kikuchi et al. 2007, Ebert 2013).

Symbiont transmission plays out a critical role in host and symbiont ecology because it can determine how the life history and population dynamics of both participants will unfold (Bright and Bulgheresi 2010). For instance, in parasitic symbioses, it matters when parasites are encountered during a host's life because this determines how host energy will be allocated between particular activities and thus trade-offs are expected to occur (Sheldon and Verhulst 1996, Agnew et al. 2000). In mammals, birds, and invertebrates, for instance, when hosts are parasitized at maturity (or before reproduction), trade-offs between current and future reproduction happen affecting both host fitness and the probability of symbiont transmission (Sheldon and Verhulst 1996). In mutualistic symbioses, on the other hand, symbiont prevalence can affect the probability of symbiont transmission particularly when this mechanism incurs positive effects on hosts (Skelton et al. 2013). In commensals, there is little evidence that suggests symbiont transmission dynamics can play an important role modulating the population density of other interacting species (Hofstetter et al. 2006).

Symbiont Abundance

Another important aspect relates to the numeric distribution of symbionts within a host population, which can modify the net outcome in a symbiosis. Symbiont distribution is usually highly aggregated such that only few hosts carry the majority of symbionts. This is an essential attribute structuring a host population (Poulin 2007, 2013). For instance, it can explain sex biases in infection rates in rodents or symbiont size dependency in commensals (Bajerlein and Przewoźny 2012, Patterson et al. 2015). Moreover, symbiont abundance that is highly aggregated can also change the outcome in a symbiosis. For instance, high initial density of symbionts in crayfish hosts can change the outcome from a mutualism to a parasitism, or from a commensalism to a mutualism (Lee et al. 2009, Brown et al. 2012, Skelton et al. 2013, Skelton et al. 2016).

Phoresy

Phoresy is usually viewed as a short-term commensal interaction expected to benefit the symbionts without either benefiting or harming their hosts where smaller organisms with limited dispersal capabilities (the phoretic organisms) utilize active dispersal hosts to locate new colonization habitats (Mitchell 1970, Binns 1982, Houck and OConnor 1991, Benton and Bowler 2012). Phoresy could therefore be considered part of the life-history of phoretic symbionts (Houck and OConnor 1991). In some particular cases phoresy is not only limited to the dispersal portion of the interaction, it extends to a symbiosis in which phoretic organisms coexist in the same habitat with their host to complete development, find mates, reproduce and embark in the next dispersal event (Walter and Proctor 2013). It is therefore important to distinguish that in particular cases phoresy could be only a short-term interaction, one that solely involves the

dispersal of symbionts (Athias-Binche and Morand 1993) with phoretic and post-phoretic stages, and in other cases this interaction is a symbiosis in which phoretic organisms are in permanent and close-living association with their hosts (Walter and Proctor 2013).

The relevance of phoresy can be recognized through all the complex mechanisms phoretic mites seem to have evolved to locate their host, which include various behavioural, physiological and morphological adaptations (Binns 1982, Houck and OConnor 1991, Kaliszewski et al. 1995, Mercado et al. 2014). Examples include attachment-detachment structures (i.e. anal suckers, anal pedicels and enlarged legs for attachment) (Houck and OConnor 1991), mite physiological synchronization to host life cycle (Hunter and Rosario 1988), and discriminatory behaviors based on chemical cues or host traits (Niogret et al. 2006, Grossman and Smith 2008, Nehring and Muller 2009, Niogret and Lumaret 2009). All these phoresy-related adaptations have been suggested to increase mite fitness (Binns 1982).

Phoresy in Insects

Phoretic organisms (or phoronts) are common symbionts of several alate insect taxa particularly of those insect groups that inhabit ephemeral habitats (Hunter and Rosario 1988, Athias-Binche and Morand 1993, Perotti and Braig 2009). Among terrestrial phoretic organisms, mites (Acari) have been documented as the most successful radiating species where different taxa from both superorders, Parasitiformes and Acariformes, have independently evolved adaptations for host attachment and detachment during dispersal (Hunter and Rosario 1988, Houck and OConnor 1991, Walter and Proctor 2013). Different species of Mesostigmata and Astigmata mites sustain phoretic interactions with various insect species belonging to Coleoptera, Hymenoptera, Diptera,

and Lepidoptera (Binns 1982, Hunter and Rosario 1988, Houck and OConnor 1991, Walter and Proctor 2013). Classic examples of phoresy between mites and insects are found in bark beetles (Coleoptera: Curculionidae, Scolytinae), burying beetles (Coleoptera), and several groups of social insects (Hymenoptera) (Eickwort 1990, O'Connor 1993, Klimov et al. 2007, Mori et al. 2011).

Although phoretic mites have sophisticated mechanisms to locate and discriminate insect hosts (i.e. discrimination between sexes and good condition hosts), they are limited in their dispersal capabilities (i.e. phoretic mites are wingless) and thus rely entirely on their hosts to disperse long distances (Binns 1982, Niogret et al. 2006, Grossman and Smith 2008, Nehring and Muller 2009, Niogret and Lumaret 2009, Niogret et al. 2009). From a phoretic symbiont viewpoint dispersal is a crucial life-history characteristic, and it has several implications for individuals and for the population as a whole (Binns 1982, Benton and Bowler 2012). For instance, dispersal reduces the likelihood of interacting with relatives thus avoiding inbreeding and competition (Hamilton and May 1977). Furthermore, dispersal increases under unfavorable conditions such as habitat variability and local extinction thus providing higher reproductive potential in new colonization sites (Comins et al. 1980, McPeek and Holt 1992). Moreover, dispersal can be very costly, as active dispersers can succumb to costs related to energetic investment prior or during the transfer (i.e. physiological or metabolic), and mortality due to increased predation and establishment in unsuitable habitats (Bonte et al. 2012). In insects there is ample evidence of the physiological and reproductive costs associated to their dispersal (i.e. reduced reproductive ouput) (Roff 1977, Rankin and Burchsted 1992, Nespolo et al. 2008, Niitepold and Boggs 2015). Therefore, phoresy can be seen as an effective alternative for mites to maximized their fitness without paying the

costs (or at least to reduce the costs) associated to dispersal by utilizing insects (Binns 1982, Bonte et al. 2014).

Bark Beetles and Phoretic Symbionts

One common insect taxon that epitomizes the use of ephemeral habitats are bark beetles (Coleoptera: Curculionidae, Scolytinae). These beetles are common insects where most species are specialized to feed on the subcortical region of debilitated trees (Lindgren and Raffa 2013). Recently, this group of organisms has been of special interest since some species have reached an epidemic status feeding also on healthy pine hosts in forests of North America (i.e. *Dendroctonus* spp.). In particular, in western Canada, mountain pine beetle (MPB, *Dendroctonus ponderosae*) has recently extended its epidemic distribution from the south central British Colombia to the north east of Alberta, which has the potential to threaten different species of pines (*Pinus* spp.) (Safranyik et al. 2012). Bark beetles have a particular biology (colonization of trees, brood development, and emergence) that is always accompanied by various symbionts including different species of phoretic mites (Table 1.3), several species of mycangial fungi, various phoretic and parasitic nematodes, yeast and bacteria (Safranyik and Carroll 2006, Hofstetter 2011, Six 2013, Susoy and Herrmann 2014).

Mountain Pine Beetle Biology

Mountain pine beetle (MPB) colonization of pine hosts begins in mid-late July when immigrant MPB females disperse and locate a suitable pine and breeding aggregations are formed when beetles release specific pheromones that attract conspecifics. These breeding aggregations consist of females that have recently established a nuptial chamber in the phloem of the pine

subsequently followed by males that arrive approximately 24-72 hours after to mate with them. After mating, MPB females usually oviposit an average of 80 eggs along the maternal gallery. While these activities occur, beetle parents also inoculate two blue stain fungi species *Grosmannia clavigera* and *Ophiostoma montium*, which contribute to overcoming pine host defenses and eventually feed beetle larvae and pupae (Bleiker and Six 2007). These beneficial fungi are transported in the beetle mycangia, a specialized structure associated with the transportation of beneficial fungus spores (Bleiker and Six 2007). Once beetle eggs have hatched, the larvae feed on the phloem laterally from the maternal gallery. During this time it is common to find phoretic mites of juvenile cohorts wandering through the gallery system tunneled by the beetle mother and the larvae (personal observation). The larval stage is critical for the success of beetle population since this is the life stage that overwinters (Lachowsky and Reid 2014). Under benign conditions larvae normally survive the winter by metabolizing glycerol. However, larvae numbers can be reduced during extreme winter temperatures (Bentz and Mullins 1999). The successful larvae that have reached the pupa stage would mature from approximately June to July and would eventually emerge as adults by mid July to early September (Safranyik and Carroll 2006). During this period of time MPB dispersal occurs. Beetles emerge and disperse from the natal tree once their exoskeleton is completely hardened and melanized, and food resources are depleted. MPB are active dispersers that power their own flight and can also be wind-aided (Safranyik and Carroll 2006). Adults are capable of short and long distance dispersal and may fly just a few hundred meters or up to 19 km in laboratory conditions (Evenden et al. 2014). Body size positively affects MPB dispersal but sex does not (Evenden et al. 2014). In other *Dendroctonus* species, however, there seems to exist a sexual dimorphism in dispersal, where females can fly longer distances than males (Chen et al. 2011).

Dispersal in *Dendroctonus* species can be costly and in MPB initial body mass, particularly lipid content, appears to influence dispersal capacity (Williams and Robertson 2008, Chen et al. 2011, Evenden et al. 2014).

Biology and Ecology of Phoretic Mites of Mountain Pine Beetle

Once MPB concludes dispersal, phoretic mites disembark in the beetle nuptial chamber. It is common to find different species of phoretic mites coexisting with adults and juvenile stages of MPB, once beetles have mated and larvae started the larval gallery system. *Proctolaelaps subcorticalis*, *Trichouropoda australis, Histiogaster arborsignis*, and *Histiostoma* spp. are often found wandering in the gallery system in the maternal and larval galleries, whereas *Tarsonemus ips* mites are more commonly found staying in the vicinity of MPB pupal chambers (personal observation). Beetle and mite progeny coexist together until a new emigration event to new trees occurs.

Although phoretic mites are common symbionts of mountain pine beetle, there are few exhaustive studies documenting their taxonomy, diversity and abundance for Canada (Lindquist 1969, Mori et al. 2011). One of the most recent papers documenting diversity and abundance of phoretic mites by Mori et al. (2011), reported five mite species (*Proctolaelaps subcorticalis*, *Histiogaster arborsignis*, *Tarsonemus ips*, *Proctolaelaps* spp. and *Macrocheles schaeferi*) for northwestern Alberta, Canada (new area of expansion of MPB); however, only three species were consistently present in laboratory and field (*Proctolaelaps subcorticalis*, *Histiogaster arborsignis* and *Tarsonemus ips*). Some of these phoretic mites have been previously documented in other bark beetle species too (see Table 1.3 for detailed information). For

example, *H. arborsignis* is a common symbiont of the spruce beetle (*Dendroctonus rufipennis*) (Cardoza et al. 2008); whereas *T. ips* is commonly found in two other *Dendroctonus* species including *D. rufipennis* and *D. frontalis* (Lombardero et al. 2000, Hofstetter et al. 2006). Although a few other studies have also documented the presence of phoretic mites in MPB, these should be considered with caution since they have only reported the presence of mites on few beetle specimens and this do not reflect the actual diversity and abundance of phoretic mites associated with MPB (Lindquist 1969, 1971, Magowski 2010).

Among the three most common species that have been documented for MPB, *Tarsonemus ips* is a particularly abundant and interesting phoretic mite as its biology is highly linked to beetle biology (Moser 1985, Magowski 2010, Mori et al. 2011). This is a fungivorous mite whose life cycle seems to depend on the life cycle of its host. When mountain pine beetles disperse from the natal habitat, mites disperse hitchhiking on the ventral side of beetle's integument and under beetle elytra (personal observation). There is no evidence of a parasitic life-style in this mite species during or after dispersal of hosts. For instance, it does not reproduce or feed on adult beetles while in transit and it does not feed on beetle progeny while present in the breeding resource (Magowski 2010). In addition, during dispersal this particular mite species does not have piercing chelicera like other parasitic mites of the same group do (Kaliszewski et al. 1995); all their legs have strong tarsal claws associated to host attachment except for leg IV (Magowski 2010). Once beetles arrive to a breeding resource, mites detach, complete development, and reproduce in the gallery created by the beetle mother or the galleries carved by the beetle offspring.

T. ips females oviposit an average of 30 eggs (personal observation), and although they lay eggs along the beetle maternal gallery and larval gallery system, mite juvenile and adult stages remain close to the beetle pupal chambers where fungus seems to concentrate (personal observation). *T. ips* has a flap-like structure, the sporothecae, associated with the transportation of fungus spores (Moser 1985). In southern pine beetle, *Dendroctonus frontalis*, *T. ips* contributes to the transportation and farming (inoculation and cultivation) of its own food, *Ophiostoma minus* (a blue stain fungi), while doing its reproductive activities (Bridges and Moser 1983, Moser 1985, Hofstetter et al. 2006). There is evidence that this blue stain fungus has negative consequences in southern pine beetle juveniles; it outcompetes the beneficial mycangial fungus of the beetle (Hofstetter et al. 2006). In MPB however, there are no records of transportation of antagonist fungi. The two species of blue stain fungi associated with MPB, *Grosmannia clavigera* and *Ophiostoma montium*, do not compete but rather complement beetle larvae diet (Bleiker and Six 2009). In this scenario, it is possible that *T. ips* associated with MPB contributes to the transportation and farming of MPB beneficial fungi.

Once MPB development is completed, phoretic mite offspring hitchhike on beetle offspring and together disperse to a new colonization pine host. This type of symbiotic transmission would suggest a vertical transmission of phoretic mites in MPB (from beetle parents to offspring). However, there is no clear evidence of life cycle synchronization between phoretic mites and MPB. Phoretic mites can be facultative in other insects (Polak 1996). Moreover, there is evidence that *T. ips* also associates with other bark beetles such as pine engravers (*Ips pini*) (Lindquist 1969) that often co-occur with mountain pine beetles. Nonetheless, there are no

records of *T. ips* hitchhiking on other subcortical insects that also inhabit the same breeding resource (i.e. Cerambycids) suggesting a specific phoretic interaction between bark beetles and *T. ips*.

Thesis Outline

The aim of this thesis is to identify the ecological mechanisms that determine the net effects of phoresy observed in mountain pine beetles and mites. I confirmed that the outcome of phoresy is commensal. I provide evidence from different ecological angles that support my assertion. The thesis is organized in five chapters. Two chapters, Chapter 1 and Chapter 5, pertain to the general introduction, and the general discussion and concluding remarks of the thesis. The core findings of my research, Chapter 2, Chapter 3 and Chapter 4 are concerned with three aspects of the ecology of mite phoresy on mountain pine beetles. Specifically, Chapters 2 and 3 investigate ecological aspects at the individual level, such as dispersal and reproduction respectively, whereas Chapter 4 investigates a population level aspect of phoresy pertaining geographic patterns of distribution and abundance of symbionts.

In Chapter 2, I investigated the dispersal portion of this phoresy. I demonstrate that there are no negative effects of carrying phoretic mites for mountain pine beetles during dispersal. Instead, my results revealed that although there is symbiont mortality during host dispersal, dispersing in hosts that are in better condition mitigates that mortality cost. I found that beetle dispersal is a phenotype-dependent activity, and higher numbers of phoretic mites do not decrease host flight capacity regardless of host condition. This part of the research let me conclude that the dispersal portion of phoresy is indeed a commensal interaction for beetle hosts where mites have the same

probability beetles have to arrive to the breeding site.

In Chapter 3, I investigated the role of phoretic mites during host ontogeny. I was particularly interested in clarifying whether symbionts may incur positive, negative or both type of effects during beetle development and how these initial effects influence the net outcome of phoresy at the end of reproduction and beetle development in hosts. I demonstrate that despite the variation of phoretic mite effects at early stages of beetle development, these do not account for increases or decreases in beetle fitness (offspring production and/or offspring quality) at the end of beetle development; instead, this portion of the interaction is neutral for the host and positive for the phoretic mites.

Finally in Chapter 4, I investigated phoretic mite and beetle dynamics at the population level. This chapter is particularly concerned with the distribution and abundance of symbionts associated to the range expansion of mountain pine beetle. I collected field data from both parts of beetle distribution: the new area of expansion in northern British Columbia (Valemount) and northwestern Alberta (Grande Prairie and Peace River), and the historical range of distribution in central-south British Columbia (Yoho, Kootenay and Penticton). I show that the expansion of mountain pine beetle has reduced the distribution of its phoretic mites particularly of *T. ips*, the most common phoretic symbiont, in the new area of MPB expansion.

Table 1.1. Typical classification of symbiotic and non-symbiotic interspecific interactions between individuals of different species. This classification is organized by the net outcome. The left-hand sign indicates the fitness effect on the symbiont. The right-hand sign indicates the fitness effect on the host.

Type of Interaction	**Symbiotic**	**Non-symbiotic**
Positive (+,+)	Mutualism	Pollination
Antagonist (+,-)	Parasitism	Predation and parasitoidism
Neutral (+,0)	Commensalism	-

Table 1.2. Classification of interspecific interactions distinguishing by the effects on each pariticipant species.

	Species A		
	0	**-**	**+**
Species B			
0	Neutralism		
-	Amensalism	Competition	
+	Commensalism	Predation/Parasitism	Mutualism

Table 1.3. Brief review of common phoretic mites associated with bark beetles from North America.

Phoretic mite species	Bark beetle species	Source
Dendrolaelaps isodentatus	*Dendroctonus frontalis*	(Moser and Roton 1971)
Dendrolaelaps neocornutus	*D. frontalis, D. rhizophagus, D. valens, Ips bonanseai*	(Moser and Roton 1971, Chaires-Grijalva et al. 2015)
Dendrolaelaps neodisetus	*D. adjunctus, D.s frontalis, I. bonanseai, Pseudips mexicanus*	(Moser and Roton 1971, Moser 1975, Chaires-Grijalva et al. 2015)
Dendrolaelaps quadrisetosimilis	*D. frontalis, I. pini*	(Moser and Roton 1971)
Dendrolaelaps quadrisetus	*D. adjunctus, rufipennis, I. bonanseai, I.pini, I. typographus, Pityokteines curvidens, P. spinidens, P. vorontzowi,*	(Cardoza et al. 2008, Pernek et al. 2008, Takov et al. 2009, Pfammatter et al. 2013, Chaires-Grijalva et al. 2015)
*Histiogaster arborsignis**	*D. frontalis, D. rufipennis,* ***D. ponderosae****, D. terebrans I. avulsus, I. calligraphus, I. grandicollis, I.pini*	(Moser and Roton 1971, Moser 1975, Cardoza et al. 2008, Mori et al. 2011, Pfammatter et al. 2013)
*Histiostoma conjuncta**	*D. frontalis,* ***D. ponderosae***	(Hofstetter 2011)
Macrocheles boudreauxi	*D. frontalis, I. calligraphus, I. grandicollis*	(Moser and Roton 1971, Kinn and Witcosky 1977)
*Macrocheles schaeferi**	***D. ponderosae***	(Mori et al. 2011)
Proctolaelaps dendroctoni	*D. frontalis, I. acuminatus, I. avulsus, I. calligraphus, I. sexdentatus, Orthomicus longicollis,*	(Moser and Roton 1971, Moser 1975, Kinn 1983, Trach and Khaustov 2017)
Proctolaelaps fiseri	*D. frontalis, Hylastes opacus, I. acuminatus, I. typographus, Pityogenes chalcographus, Polygraphus proximus, Po. subopacus, Tomicus piniperda*	(Moser and Roton 1971, Takov et al. 2009, Trach and Khaustov 2017)
*Proctolaelaps hystricoides**	*D. frontalis,* ***D. poderosae****, D. rufipennis, Hylurgops glabratus, I. subelongatus, I. typographus, Pityokteines spp., Po. proximus, Pi. chalcographus,*	(Moser and Roton 1971, Cardoza et al. 2008, Trach and Khaustov 2017)
*Proctolaelaps subcorticalis**	***D. ponderosae****, Trypodendron lineatum*	(Lindquist 1971, Mori et al. 2011)
Schizosthetus lyriformis	*D. brevicomis, D. frontalis, D. simplex, D. valens, I. confusus, I. pini, Onthotomicus latidens,*	(Al-Atawi et al. 2002, Hofstetter et al. 2009)
*Tarsonemus ips**	*D. frontalis,* ***D. ponderosae****, D.rufipennis, I. acuminatus, I. confusus, I. lecontei, I. montanus, I. pini, I. plastographus*	(Lindquist 1969, Moser and Macías-Sámano 2000, Cardoza et al. 2008, Mori et al. 2011)
Tarsonemus krantzi	*D. frontalis*	(Moser 1976b, a)
Trichouropoda australis	*D. frontalis, I. pini*	(Moser 1976a, b, Pfammatter et al. 2013)

The symbol (*) indicates MPB phoretic mites. MPB scientific name is highlighted in bold.

CHAPTER 2: DO PHORETIC MITES IMPAIR MOUTAIN PINE BEETLE DISPERSAL?

Introduction

Phoresy, which is the dispersal of symbionts with limited movement capacity on dispersal hosts, is typically viewed as a short-term commensal interaction expected to benefit symbionts without either benefiting or harming their hosts (Binns 1982, Houck and OConnor 1991, Athias-Binche 1993, Athias-Binche and Morand 1993, Benton and Bowler 2012). This interaction is particularly prevalent in patchy environments, such as decaying materials (Hunter and Rosario 1988, Houck and OConnor 1991), where the large majority of phoretic symbionts lack specialized locomotory apparatus (phoretic mites are wingless) to abandon those environments (Binns 1982, Houck and OConnor 1991). In this sense, phoresy allows mobilization of organisms with limited dispersal capacity out of an environment that imposes a constant risk of local extinction (Wiens 1976, Benton and Bowler 2012, Fronhofer et al. 2013, O'Sullivan et al. 2014).

In invertebrates, many of the evolutionary and ecological advantages of phoresy from a symbiont standpoint are fairly well established. However, these benefits suggest that phoresy is a much longer interaction (i.e. a symbiosis) that do not relate to the actual dispersal or short-term aspects of phoresy (Binns 1982, Hunter and Rosario 1988, Houck and OConnor 1991, Athias-Binche 1993, Houck and Cohen 1995, Benton and Bowler 2012, Fronhofer et al. 2013, O'Sullivan et al. 2014, Skelton et al. 2015). For instance, there is evidence that phoresy is required to reach suitable habitats where symbionts can complete development (Hunter and Rosario 1988, Houck

and OConnor 1991), find reproductive sites and prospective mates (Hunter and Rosario 1988, Houck and OConnor 1991), allow inter-patch movement (O'Sullivan et al. 2014), enhance the probability of gene flow (Benton and Bowler 2012), and assure population persistence (Athias-Binche 1993, Fronhofer et al. 2013). Although most of these studies validate advantages of phoresy for symbionts after dispersal has occurred, they ignore the implications of the actual movement for both symbionts and hosts. For instance, phoresy may be costly for both symbionts and hosts, even when it is implied as a neutral association for hosts (whether phoretic mites are a substantial burden for hosts during dispersal). This is particularly critical for hosts because they are the organisms that exhibit the actual movement and therefore bear all the cost of dispersal (Bonte et al. 2012).

Curiously, from a host perspective, the literature provides little insight about the neutral outcome of phoresy for hosts. Most of the available evidence among invertebrates shows that this interaction can produce either negative or positive effects on hosts suggesting that phoresy might not be a commensal interaction, although much of this evidence relates to the settlement, a part of the interaction that follows dispersal (Wilson and Knollenberg 1987, Polak 1996, Lombardero et al. 2003, Hofstetter et al. 2006, Okabe and Makino 2008, Hofstetter et al. 2009, Rocha et al. 2009, Hodgkin et al. 2010, Mazza et al. 2011, De Gasperin et al. 2015, De Gasperin and Kilner 2015b). For instance, there is some evidence that suggests host reproductive success in insects (production of more and in better condition progeny) increases with phoretic symbiont prevalence. (Wilson and Knollenberg 1987, Okabe and Makino 2008, Hodgkin et al. 2010). Other studies have found detrimental effects on hosts not just during its reproduction (Polak 1996, Lombardero et al. 2003, Hofstetter et al. 2006, Okabe and Makino 2008, Hodgkin et al.

2010), or lifespan (Mazza et al. 2011), but also on host dispersal attributes (Rocha et al. 2009). For instance, phoretic mites can interfere with dispersal departure by reducing host flight preparation and takeoff in a grain borer beetle (Rocha et al. 2009). Phoretic organisms, however, are distinguished from ectoparasites in that they do not extract resources from their host for development or reproduction when they are attached to the host as ectoparasites naturally do (Bajerlein and Przewoźny 2012). The negative impact of phoretic organisms during host dispersal is related to the physical burden they impose on their host and not because they feed on it (Luong et al. 2015). In addition, other studies have simultaneously found both negative and positive effects on hosts, particularly during host reproduction (Okabe and Makino 2008, Hodgkin et al. 2010, De Gasperin et al. 2015, De Gasperin and Kilner 2015b). To the best of my knowledge, there is only a single recent study confirming the commensal nature of phoresy on a bark beetle but only during host reproduction (Pfammatter and Raffa 2015).

In this study, I focus on the short-term and dispersal aspects of phoresy to determine whether this interaction is commensal during the transit of both host and symbionts using a species of bark beetle (mountain pine beetle; *Dendroctonus ponderosae*) and its mites as my study system Phoresy is a very common interaction in bark beetles (Pernek et al. 2008, Hofstetter et al. 2009, Moser et al. 2010, Mori et al. 2011, Pfammatter et al. 2013, Mercado et al. 2014, Chaires-Grijalva et al. 2015). Most of the mites associated to beetle hosts during dispersal are presumably phoretic (Mercado et al. 2014), although some mites species have been reported as parasites but only after dispersal has occurred (Hofstetter et al. 2009, Hodgkin et al. 2010). Mountain pine beetle generally transports at least three different species of mites that usually attach to the ventral side, and occasionally to the dorsal side of beetles (Mori et al. 2011, Mercado et al.

2014), and only detach once beetles have arrived to a breeding resource (personal observation). However, only two of those mite species (*Proctolaelaps subcorticalis* and *Tarsonemus ips*) seem to be the most common mites associated to mountain pine beetle during dispersal (Mori et al. 2011). Mite abundance varies greatly among mountain pine beetles, with few beetles carrying a large proportion of mites and most beetles carrying no mites (Mori et al. 2011), suggesting that host traits might play an important role at explaining host variation in mite abundance.

Host dispersers usually vary in their dispersal ability (Kisdi et al. 2012), and long-distance dispersal (i.e. reduced intra and inter-specific competition for colonization sites) is frequently seen in those individuals with the greatest energy reserves, body size or body condition, a dispersal aspect of individuals known as condition-dependent dispersal or phenotype-dependent dispersal (Roff 1977, Bowler and Benton 2005, Clobert et al. 2009, Bonte et al. 2014). In mountain pine beetles, dispersal ability varies with body condition (Latty and Reid 2009, Evenden et al. 2014). For instance, beetles in better condition fly longer distance, which suggests that long-distance dispersal in mountain pine beetle is advantageous (Kisdi et al. 2012, Evenden et al. 2014). This is relevant from a phoretic symbiont perspective since it would be advantageous to disperse on those hosts in better condition, eventually increasing fitness prospects for symbionts. However, few studies have shown that phoretic symbionts select their dispersal host based on host body condition or size (Grossman and Smith 2008, Skelton et al. 2015). If it is considered that phoretic symbionts rely entirely on the dispersal ability of their host to complete essential components of their life history (Hunter and Rosario 1988, Houck and OConnor 1991, Benton and Bowler 2012), it can be assumed that the same aspects that predict host dispersal success should predict phoretic symbiont dispersal success too (Hopkins et al.

2015). Conversely, phoretic mites could be detrimental to beetles and therefore be considered as dispersal parasites. For instance, if phoretic symbionts represent a significant load to a beetle host, the host may experience increased cost of dispersal when carrying an extra burden as dispersal progresses. For instance, high mite abundance can reduce host survival or components of host dispersal behavior (Rocha et al. 2009).

In the present study, I was interested in clarifying the nature of the interspecific interaction between mountain pine beetle and its phoretic symbionts during host dispersal. The commensal hypothesis states that the same set of variables that explain long-distance dispersal in hosts also predict symbiont dispersal propensity and symbiont dispersal success, and that symbiont abundance will not interfere with components of host dispersal, assuming that long-distance dispersal in mountain pine beetle can be advantageous, I predict that the same set of variables that explain long-distance dispersal in beetles (i.e. beetle body condition and/or body size) should predict symbiont dispersal propensity (i.e. initial phoretic mite abundance) and symbiont dispersal success of arrival (number of mites post-flight relative to pre-flight number of mites), and that symbiont abundance will not interfere with components of host dispersal. I specifically expect that both initial mite abundance on a host individual that is prepared to disperse (symbiont dispersal propensity), and the proportion of mites post-flight (symbiont dispersal success of arrival) should correlate positively with beetle body condition and body size. Additionally, components of beetle dispersal, such as distance and velocity, will not correlate negatively with mite abundance. In contrast, the parasite-to-be hypothesis predicts that if symbiont abundance is costly to dispersal hosts, I expect that beetles with mites should disperse poorly and this cost should be particularly higher for poor condition beetles than for better condition beetles. More

specifically, beetles with higher mite abundance should fly shorter distances and fly slower as a consequence of carrying more mites and this should be more pronounced for beetles in poor condition.

Materials and Methods

Study species biology

Mountain pine beetle (*Dendroctonus ponderosae*) life-history includes a dispersal phase that occurs after emergence from the natal tree and prior to reproduction. It primarily uses lodgepole pine (*Pinus ponderosae*) as a resource for reproduction. This activity commences when female beetles colonize a pine, attract potential partners, and start a reproductive chamber. Males usually arrive within 24 and 72 hours after female colonization. Male and females mate in the reproductive chamber and both exhibit parental care. This consists of females building a main gallery where eggs are laid and males packing the phloem debris that those females leave behind. While doing these activities parents also inoculate blue stain fungi, which contributes to overcoming pine host defenses and eventually feeds beetle larvae and pupae (Bleiker and Six 2007). Male parental care is influenced by female resource characteristics rather than female body condition or size while female parental care is influenced by the pine host characteristics (Reid and Baruch 2010). Females lay their eggs on a vertical gallery and newly hatched larvae feed on the phloem and fungus perpendicular to their mother's main gallery. Larvae then hibernate as pupa and later hatch as teneral adults. When newly adult offspring do not have sufficient fungus in their pupal chamber they remain under the bark and feed on more phloem extensively before abandoning the pine host (Bleiker and Six 2007). It takes a year for beetle

offspring to complete development and disperse from the natal pine host (Safranyik and Carroll 2006).

Tarsonemus ips is a relatively abundant phoretic mite of mountain pine beetle that depends on beetle dispersal to spread to new colonization sites (Mori et al. 2011, Mercado et al. 2014). When mountain pine beetle departs from the natal habitat, mites disperse hitchhiking usually on the ventral side of beetle's integument. There is no evidence of a parasitic life-style in this mite species during and after dispersal of hosts. For instance, it does not reproduce or feed on adult beetles while in transit and it does not feed on beetle progeny while present in the breeding resource (Magowski 2010). In addition, during dispersal *Tarsonemus* mites do not have piercing chelicera like other parasitic mites do; all their legs have strong tarsal claws associated to host attachment except for leg IV (Magowski 2010). Once beetles arrive to a breeding resource, mites detach and complete or complement their life cycle in the gallery created by the beetle mother or the galleries carved by the beetle offspring. *T. ips* females usually lay eggs along the beetle maternal gallery and its juvenile and adult stages stay in the vicinity of beetle pupal chambers. There is evidence that *T. ips* contributes to the transportation and farming (inoculation and cultivation) of its own food, the blue stain fungus, while doing its reproductive activities (Bridges and Moser 1983, Magowski and Moser 2003, Hofstetter et al. 2006). In fact, *T. ips* has a sporothecae, a flap-like structure associated to the transportation of fungus spores (Moser 1985). Curiously, MPB has a specialized structure, the mycangium, also associated with two species of blue stain fungi, *Grosmannia clavigera* and *Ophiostoma montium*. When mountain pine beetle carries both blue stain fungi they do not outcompete but rather complement beetle larvae diet (Bleiker and Six 2009). However, there is evidence in another species of bark beetle,

Dendroctonus rufipennis, that *T. ips* can carry a blue stain fungus that outcompetes the beneficial fungus associated to that beetle species (Hofstetter et al. 2006). Thus, mites and beetles both contribute spreading and farming blue stain fungi to feed their own offspring. Once beetle offspring development is completed mites hitchhike on them and both species disperse together to a new colonization pine, where they commence the cycle again.

Beetle and mite culture preparation

All beetles and mites used for this study were the laboratory-born offspring from two parental beetle populations collected from the field during winter and summer 2014 respectively. The first field parental population came from logs cut from three naturally infested lodgepole pines with beetle brood from Exshaw, AB, cut on February 10, 2014. I immediately transported these infested logs to the laboratory at the University of Calgary and placed them in screened enclosures with collection jars for the duration of beetle development (approximately four weeks). I checked the collection jars daily for freshly emerging phototactic adults. These adults were separated by sex (males produce a distinct stridulation while females do not), and mite presence/absence upon emergence for subsequent treatment preparation. The second field parental population consisted of adult beetles collected in Yoho National Park, BC, on August 16, 2014. This time I used pheromone-specific baited traps to collect dispersing beetles. I transported these beetles to the laboratory and immediately separated them by sex and mite presence/absence for subsequent treatment preparation. For both field populations I arbitrarily paired couples of beetles that were naturally mite-infested to establish two parental treatments: mite and no-mite. This manipulation allowed me to create natural variation of mites. Parents

from the no-mite treatment were gently cleaned with a fine paintbrush to detach all external mites (mites under elytra were not removed as this manipulation has proved damaging to beetle wings; personal observation) prior to implantation into fresh logs for breeding, while parents from the mite treatment were gently touched with the paintbrush to ensure all were treated similarly. Each implantation consisted of one log of 30 cm length with a single couple of adult beetles that were allowed to reproduce. Females were added to microcentrifuge enclosures glued to one side of each log to initiate a nuptial chamber in the phloem 24 h before males were presented. I considered an implantation successful when there was no sign of male activity at the surface and frass had plugged the microcentrifuge tube. Each successful implantation was placed in a cardboard enclosure modified with a collection jar for the duration of beetle offspring development. All implantations were kept in the laboratory at room temperature (20 ^{o}C) for the duration of beetle development (approximately 7 weeks). Out of 8 mite and 13 no-mite implantations that I prepared, 6 mite and 10 no-mite implantations produced offspring. These laboratory-born offspring with natural variation of mites were used for flight mill tests. Because direct manipulation of mite numbers in lab was difficult, my approach was to manipulate parental number of mites so that I could produce beetle offspring naturally infested with mites and naturally mite-free. Although this approach proved imperfect, as all laboratory-born offspring had associated mites regardless of treatment, I was able to produce beetles with natural variation of mites.

The phoretic mites associated to beetles use in this study were cleared and mounted in slides and later identified using differential interface microscopy at 1000 times magnification (Lindquist 1969, Magowski and Moser 2003, Magowski 2010). Mite slides were deposited in the insect

collection of the Reid/Cartar laboratory. Although two mite species were identified in this study (one Mesostigmatid species –*Proctolaelaps* spp., and *Tarsonemus ips*), only beetles carrying *Tarsonemus ips* were included in the present study because less than five beetles carried *Proctolaelaps* spp.

Beetle measurements and mite counts

Once adult offspring started to emerge, I kept them individually in microcentrifuge vials fitted with a perch at 4C in the fridge until they were measured and prepared for flight mill tests. I used beetles that had emerged within 17 days. Prior to each flight mill test, I recorded body mass, body size and mite abundance of each beetle. Beetle body mass was measured to the nearest 0.01 mg. I consider body volume as our measurement of body size. In order to calculate body volume we first measured length and pronotum width of each beetle at the nearest 0.2mm using a dissecting microscope fitted with a micrometer eyepiece. Length and pronotum width then allowed us to calculate beetle volume such that volume = (4/3) [π x (length/2) x (width/2)2] assuming beetle body as an ellipsoid shape. With the previous measurements, I was able to determine a beetle body condition index (hereafter body condition) as the body mass residual with respect to the regression of body mass against body volume (Schulte-Hostedde et al. 2005). I finally recorded mite abundance (number of mites attached externally on beetles' integument) according to species. Once all measurements were taken, a 3.5 mm wire tether was glued to the beetle's pronotum using crazy glue. This last procedure made beetles ready for flight mill test (see below). After each flight mill test ended (8 hours approximately), I immediately detached beetles, removed any residue of glue and recorded again their mass and mite abundance. All beetles were sexed after this procedure to avoid beetle damaging prior to flight mill test. I

distinguished between males and females using acoustical cues (male beetles stridulate while females do not), and I additionally dissected those individuals whose sex was doubted to be males.

Flight performance test

Eight custom-made flight mills were utilized to perform dispersal capacity tests. These flight mills and their data collection software were designed in the Science Workshop of University of Calgary in 2013 to measure bark beetle flight behavior. Flight mills are a common device to study different aspects of insect flight behavior under laboratory conditions (Attisano et al. 2015). Each of our flight mills consisted of a rectangular frame of acrylic glass adapted with two central magnetic pivots and two optical sensors attach to the upper and lower sides of the flight mill, where a stainless steel rotational arm is suspended. This arm was fitted on one end with a micro tubing connector that allows the insertion of the tethered insect while the other end had a counterweight flag. The friction-less flight mill arm worked when the flying of tethered insects triggered the optical sensors and collected 2 counts as a result of one revolution. Flight mills counts and their timing were recorded using LabView software, which allowed me to calculate distance flown and average velocity my measurements of dispersal success for each individual. The flight mill system was designed to collect information from each device every 16 seconds. Unfortunately, this introduced some unwanted noise in the data and I decided to only consider for analysis those individuals that flew longer than 4 min (see results). Each flight mill test consisted of an eight h flight recording of a tethered individual that was previously measured (see previous section). Every beetle was flown only once. Each test consisted of eight beetles per trial, per day, and started at midday and finished at 8pm. This time frame was chosen to resemble

a typical daily flying behavior of a mountain pine beetle in the field. Beetles typically fly between 12pm and 8pm during hot summer days in the boreal forest (Safranyik and Carroll 2006). All flight tests were performed under laboratory conditions at a constant temperature of 24C.

Statistical Analysis

I analyzed the data using the statistical program JMP (v. 12.1.0; SAS Institute Inc., Cary, NC, 1989-2007). I used linear mixed models for hosts and symbionts. I performed three linear mixed models for hosts and each included log identity as a random variable. I always included beetle volume, beetle body condition, beetle age, and mite abundance preflight as explanatory variables for all three hosts' models. I examined three components of beetle flight that could be affected by mites. When analyzing two of those response variables, total distance flown and mean velocity, each of these models also included as explanatory variables both the interaction between mite abundance preflight and beetle body volume, and the interaction between mite abundance preflight and beetle body condition. The third host model, weight loss as response variable, included as additional explanatory variables total distance flown and mean velocity.

To test whether mites are distributed non-randomly with respect to host traits I performed three linear mixed models for symbionts and each included log number as a random variable. The response variables that I used for each of these models were mite abundance preflight, proportion of successful mites (number of mites post-flight relative to pre-flight number of mites) and mite mortality (number of mites lost during host dispersal). I always included beetle age, beetle body condition, and beetle volume as explanatory variables for all three symbiont models. The model

that included mite abundance preflight as response variable did not include any other additional explanatory variables. The two other symbiont models, proportion of successful mites and mite mortality, also included total distance flown, mean velocity and mite abundance preflight as explanatory variables. I decided to include both total distance flown and mean velocity in these two models as there was no evidence of collinearity between them. Additionally, these two models excluded all those beetles that did not have mites preceding the flight tests.

I tested the assumptions of all models by examining the residuals. I transformed four variables for the final models. Mite abundance preflight, total distance flown, and mean velocity were all log transformed. In addition I arcsine square-root transformed the proportion of weight loss. Furthermore, I did not include sex in any of the models because I did not detect sexual differences associated to any of the components of beetle dispersal.

Results

A total of 147 beetles flew on the flight mills, however, 38% (n= 56) that flew less than 4 min were excluded from the analyses. Of the remaining 62% (n=91) that were considered for the analyses, 12% (n=11) flew more than 9 km over an 8 h period. Overall, the distance flown ranged from 0.16 to 16 km (mean= 3.611, SD=3.78; Fig. 2.1). This maximum distance flown was achieved by a single female, nonetheless, I did not detect any differences in the total distance flown attributed to sex (*t*-test: t_{91}=-1.41, *P*=0.1595). The fastest beetle was also a female that flew at 2.6 km/h on a single flight event and the slowest beetle flew 0.16 km/h. The average speed was 0.74 km/h (SD=0.41 km/h) and there were no differences associated to sex either (*t*-test: t_{88}=-0.88, *P*=0.3799). Beetles lost 0.915 mg mass in average relative to initial mass preflight and this was the same for males and females (*t*-test: t_{91}=-1.81, *P*=0.0722).

Host dispersal

Host dispersal was a phenotype–dependent and costly activity. Beetles in better body condition flew farther and faster (Fig. 2.2a,b; Table 2.1). Similarly, larger beetles flew farther (Fig. 2.3) although not faster (Table 2.1). However, those beetles in better condition that flew farther lost more mass (Fig. 2.4a; Table 2.2). This was not the case for larger beetles as they lost less mass (Fig. 2.4c; Table 2.1). Interestingly, even when both distance flown and mean velocity explained mass loss, only mean velocity had a negative effect on mass loss (Fig 2.4b; Table 2.1). In other words, those beetles that flew slower lost more mass (Fig. 2.4). Mite abundance preflight and beetle age did not influence any aspect of host flight (Table 2.1).

Symbiont dispersal

Of the 91 beetles that I considered for the analysis, 25% (n=23) did not have mites attached naturally before flight mill test. The remaining 75% (n=68) had between 1 and 74 mites (mean=10, SD=15). There was no difference between males and females in the number of mites attached preflight (*t*-test: t_{67}=0.99, *P*=0.3239). Beetles lost an average of 4 mites (SD= 8) after 8 h flight mill test. There were no sexual differences associated to beetles in mite mortality after the flight mill test either (*t*-test: t_{67}=1.16, *P*=0.2483).

Symbiont dispersal was partially a host phenotype-dependent activity for symbionts. There were significantly more mites preflight on better condition and younger beetles (Fig. 2.2c and 2.5a; Table 2.2). When analyzing mite dispersal success of arrival (proportion of successful mites at the end of beetle flight test relative to mite abundance preflight, and excluding beetles that

naturally did not have mites preflight), none of the explanatory variables included in the model had a significant effect (Table 2.2). On the other hand, when looking at mite mortality (number of mites lost during host dispersal), only mite abundance before host flight explained the number of mites lost (Table 2.2). The more mites the beetles had to start with, the more mites they lost (Fig. 2.5c). No other variables, such as host mean velocity and distance flown, explained mite mortality at the end of the dispersal exercise (Table 2.2).

Discussion

Host dispersal is a phenotype-dependent activity in mountain pine beetle and this only predicts symbiont dispersal propensity but not symbiont dispersal success of arrival. The commensal hypothesis stated that the same set of variables that predict long-distance dispersal in hosts also predict symbiont dispersal propensity and symbiont dispersal success of arrival and that symbiont abundance will not interfere with host dispersal rate. On one hand, my findings confirm the first part of this hypothesis. Larger and in better condition beetles endured long-distance and faster dispersal (phenotype-dependent dispersal). Positive relationships between measurements of body size or body mass and dispersal capacity are commonly found in animals (Roff 1977, Dingle et al. 1980, Karlsson and Johansson 2008) but positive relationships between body condition and dispersal are of recent appearance (Bonte and De la Peña 2009, Debeffe et al. 2012, Kisdi et al. 2012, Baines et al. 2015).

My results showed that both body size (beetle volume) and body condition were good predictors of beetle dispersal success or long-distance dispersal. A previous study done in mountain pine beetle too found that dispersal distance increases with preflight weight (Evenden et al. 2014).

Both body size and body weight are good predictors of body condition and this is consistent with studies of phenotype-dependent dispersal where individuals in better condition are more likely to disperse (Kisdi et al. 2012). Although previous studies exploring bark beetle flight behavior have used preflight weight, body size and body volume as surrogates for body condition, none has included an index of body condition to predict long-distance dispersal as in the present study (Atkins 1961, Williams and Robertson 2008, Evenden et al. 2014).

Interestingly, beetle condition only predicted symbiont dispersal propensity (mite abundance preflight) but not symbiont dispersal success of arrival (proportion of successful mites). My results showed that younger and in better condition beetles had more mites at departure (right before going to the flight mills). However, this relationship was not present at the end of the flight mill test. For example, I did not detect any relationship (either negative or positive) between the proportion of successful mites and host characteristics and/or host dispersal capacity. These results suggest both that symbiont dispersal strategy is variable and that mites have the same probability beetles have to arrive to the breeding site. Thus host phenotype-dependent dispersal is only important for symbionts during host departure and other beetle traits or mechanisms might be responsible for symbiont dispersal success of arrival during transfer. An alternative mechanism that could explain this is that mites could achieve an ideal free distribution (Fretwell and Lucas 1970). That is, mites load up more in better beetles (long-distance dispersers), and in the end have the same fitness (i.e. the percentage of successful mites at the end of dispersal -those that make it to the breeding resource- is the same in all long-distance dispersers). This, however, requires of further exploration.

A previous study that focused in symbiont dispersal strategies revealed that not only host body size but intra and inter specific competition for high quality host microhabitats (attachment locations on a dispersal host) play a determinant role in symbiont dispersal success (Skelton et al. 2015). The study found that from a symbiont perspective, avoidance of intra and interspecific competition is the mechanism behind symbiont choosiness for high quality hosts during dispersal of horizontally transmitted symbionts (Skelton et al. 2015). Even though my study focuses on hosts and symbionts that are vertically transmitted (from parents to offspring) and in the absence of secondary bark beetles, my results provide sufficient evidence of a symbiont dispersal strategy dependent on the host phenotype at least during dispersal initiation. However, I cannot discard that other mechanisms, such as intraspecific competition for preferred attachment sites, may occur during the transfer stage of dispersal in vertically transmitted symbionts as well. This may explain why I did not find a connection between host phenotype-dependent dispersal and symbiont dispersal success. This could be explored further in my study system if flight mill tests were interrupted at different times and mite abundance recorded. In addition and most importantly, my results demonstrated that phoretic mites did not hamper beetle distance flown or mean velocity. Evidence that confirms such outcome during dispersal is scarce. Although there is one recent study that confirmed neutral effects of mites on their bark beetle host, this study only focused on the effects of mites after dispersal has occurred, during the reproduction of their host (Pfammatter and Raffa 2015).

I also observed that dispersal for both hosts and symbionts was costly, but not in the way I speculated. My parasite-to-be hypothesis stated that if symbiont abundance were costly to dispersal hosts, I would expect beetles with mites to disperse poorly and this cost would have

been particularly higher for poor condition beetles than for better condition beetles. Contrary to my expectations, I only detected intrinsic costs of dispersal for both hosts and symbionts. On one hand, costs of dispersal for beetle hosts were merely physiological, in terms of mass loss (energy costs). The more they flew the more weight they lost and this was most likely due to energy depletion as has been suggested on a previous study (Evenden et al. 2014). On the other hand, I observed mortality costs for the symbionts. Both energy and mortality costs during transit are well-documented currency costs in both active and passive dispersers (Bonte et al. 2012). Active dispersers, particularly long-distance dispersers, such as my study hosts, are more likely to reduce their energetic reserves (lipids and carbohydrates) as a consequence of movements to fuel flight, and less prone to experience direct mortality costs due to, for example, predation (Baines et al. 2015). Interestingly, my results exposed that dispersal was costly for hosts as a response of their own behavioral strategy and not as a consequence of carrying mites, suggesting that the effect of mites on host dispersal is insignificant. In contrast, passive dispersers such as mites are more likely to experience direct mortality costs due to declining physiological traits, such as dehydration (Bowler and Benton 2009, O'Sullivan et al. 2014). Curiously, host dispersal didn't affect symbiont dispersal mortality either. I didn't find a negative or positive relationship between total distance flown or mean velocity and mite mortality. Only mite abundance preflight explained mite mortality. In other words, the variation in symbiont dispersal propensity explained the variation in symbiont mortality; the more mites the host carried the more mites it lost. Thus, host dispersal success does not increase or decrease the likelihood of symbiont mortality during dispersal. An interesting question is whether other mechanisms are responsible for the mortality costs I observed in mites during host transfer (i.e. intraspecific competition for preferred attachment sites). It is possible, for example, that certain host behaviors, such as host

grooming responses against mite loads during the flight mill tests could occur. (Skelton et al. 2016). In addition, subtle changes in the flight behavior of hosts may explain mite mortality too. For example, variable periods of acceleration and deceleration could explain mite losses that my flight mill system was unable to capture. Further observations of monitored grooming behavior during flight and/or supervised flight behavior at a finer scale could help to understand a possible behavioral mechanism responsible of mite mortality. Alternatively, symbiont dehydration or starvation could explain mite mortality during host dispersal. There is some evidence that suggests mite dehydration and starvation as factors responsible of symbiont mortality during dispersal (Bowler and Benton 2009, O'Sullivan et al. 2014). Additional monitoring of mite body condition could help to test this hypothesis.

In brief, my results showed that host dispersal is a phenotype-dependent activity that partially predicted symbiont dispersal propensity. Beetle body condition and volume predicted long-distance dispersal and also explained mite abundance preflight. In addition, mean velocity and total distance flown by beetles had a neutral outcome on proportion of successful mites, and similarly, symbiont abundance preflight did not influence host long-distance dispersal either, suggesting that phoretic mites and mountain pine beetle have a genuine commensal interaction during host dispersal.

Table 2.1. Linear models for beetle flight behavior.

Response/Explanatory	**Estimate**	**SE**	***dfDen***	***t***	***P***
Total distance flown (km) (N= 91)					
Volume (mm^3)	0.1632	0.0514	64.67	3.17	**0.0023***
Condition *units*	0.4668	0.1235	83.49	3.78	**0.0003***
Mite abundance preflight[a]	-0.0164	0.1172	31.65	-0.14	0.8900
Beetle age *units*	-0.0297	0.0208	22.85	-1.43	0.1671
Mite abundance preflight[a]*Volume	-0.0269	0.0380	76.59	-0.71	0.4802
Mite abundance preflight[a]*Condition	-0.0779	0.0807	84.00	-0.97	0.3369
Mean velocity (km/h) (N= 88)					
Volume (mm^3)	0.0074	0.0098	78.28	0.76	0.4522
Condition	0.0543	0.0232	76.33	2.33	**0.0223***
Mite abundance preflight[a]	-0.0337	0.0242	80.81	-1.39	0.1671
Beetle age	-0.0071	0.0050	67.89	-1.42	0.1601
Mite abundance preflight[a]*Volume	-0.0132	0.0072	77.90	-1.82	0.0727
Mite abundance preflight[a]*Condition	-0.0094	0.0147	74.11	-0.64	0.5236
Weight loss[b] (N= 89)					
Volume (mm^3)	-0.0044	0.0019	74.78	-2.36	***0.0210****
Condition	-0.0032	0.0046	80.31	-0.70	0.4890
Beetle age	-0.0015	0.0009	50.07	-1.66	0.1022
Mite abundance preflight[a]	0.0040	0.0046	76.99	0.89	0.3771
Total distance flown (km)[a]	0.0294	0.0040	74.56	7.30	**<0.0001***
Mean velocity (km/hr)[a]	-0.0832	0.0186	81.84	-4.48	***<0.0001****

The symbol ([a]) indicates a log transformed variable. The symbol ([b]) indicates a proportion that was arcsine square-root transformed.

Table 2.2 Linear models for symbionts.

Response/Explanatory	**Estimate**	**SE**	***dfDen***	***t***	***P***
Mite abundance preflight[a] (N= 91)					
Beetle age	-0.1000	0.0199	67.13	-5.03	***<0.0001****
Condition	0.2685	0.0956	81.12	2.80	**0.0064***
Volume (mm^3)	-0.0125	0.0419	80.93	0.30	0.7666
Proportion of successful mites[b] (N= 67)					
Mean velocity (k/hr)[a]	0.3618	0.2117	55.90	1.71	0.0930
Mite abundance preflight[a]	-0.1029	0.0644	55.19	-1.60	0.1157
Volume (mm^3)	0.0323	0.0248	58.66	1.30	0.1981
Condition	-0.0244	0.0541	57.54	-0.45	0.6531
Total distance flown (km)[a]	0.0040	0.0496	59.41	0.08	0.9359
Beetle age	-0.0007	0.0122	42.75	-0.06	0.9491
Mite mortality (N= 67)					
Mite abundance preflight[a]	0.8247	0.1092	56.58	7.55	**<0.0001***
Mean velocity (k/hr)[a]	-0.6705	0.3603	56.57	-1.86	0.0680
Condition	0.0694	0.0919	58.30	0.76	0.4532
Volume (mm^3)	0.0074	0.0421	59.20	0.18	0.8598
Beetle age	-0.0021	0.0208	45.23	-0.10	0.9183
Total distance flown (km)[a]	-0.0015	0.0836	58.90	-0.02	0.9856

The symbol ([a]) indicates a log-transformed variable. The symbol ([b]) indicates a proportion that was arcsine square-root transformed.

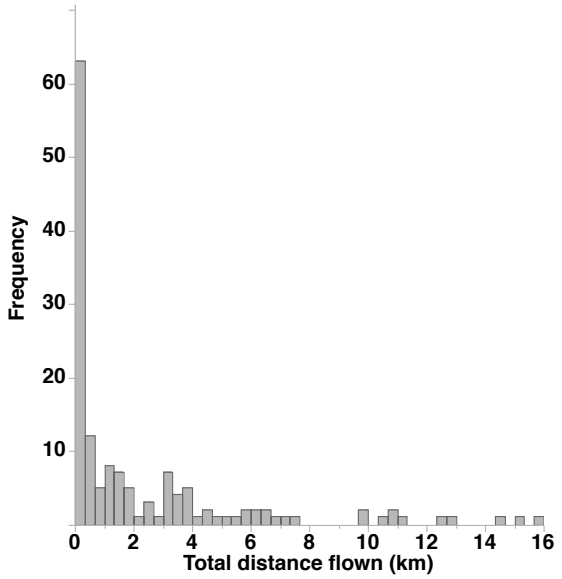

Fig. 2.1. The frequency distribution of 147 measures of distance flown (km) of adult mountain pine beetles.

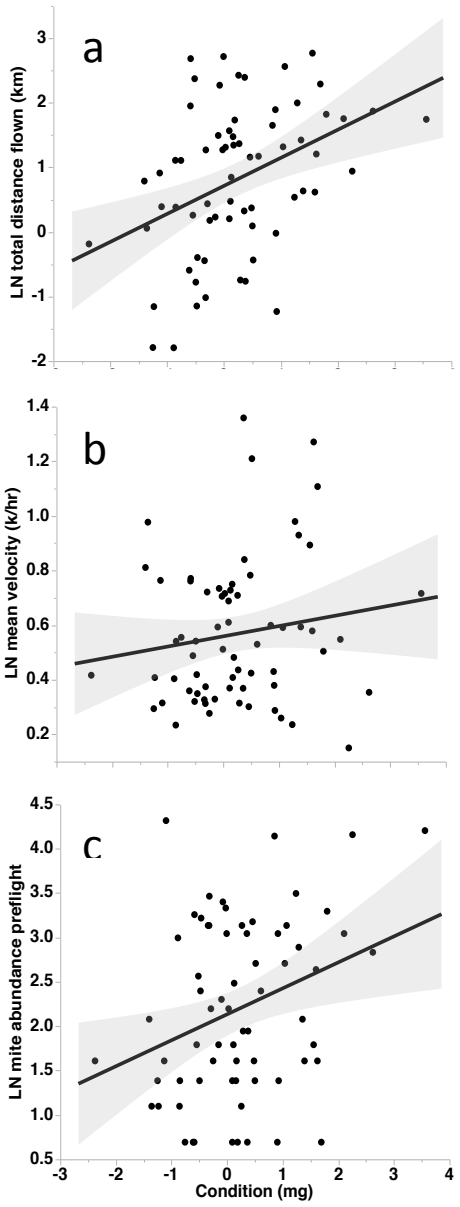

Fig. 2.2. The relationship between beetle body condition and components of beetle flight. Beetle body condition was calculated as the body mass residual with respect to the regression of body mass against body volume. Panel a) shows the relationship between body condition and total distance flown. Panel b) shows the relationship between body condition and mean velocity. Panel c) shows the relationship between body condition and mite abundance pre-flight. Total distance flown, mean velocity, and mite abundance pre-flight were log transformed. Each data point represents a different individual beetle host. The least square regression line is shown with 95% confidence interval for each figure.

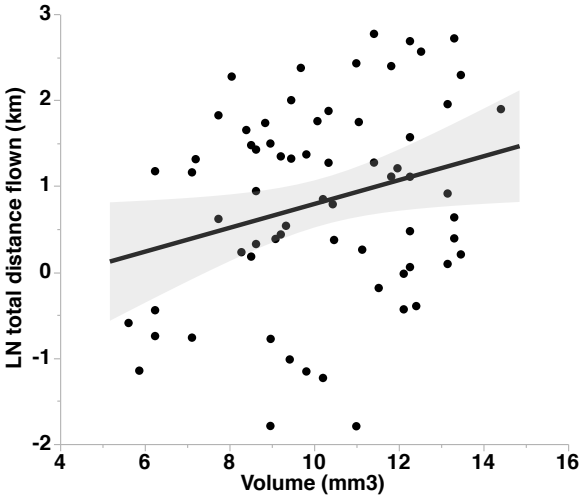

Fig. 2.3. The relationship between host volume and total distance flown by hosts. Total distance flown data was log transformed. Each datapoint represents a different individual host. The least square regression line is shown with 95% confidence interval.

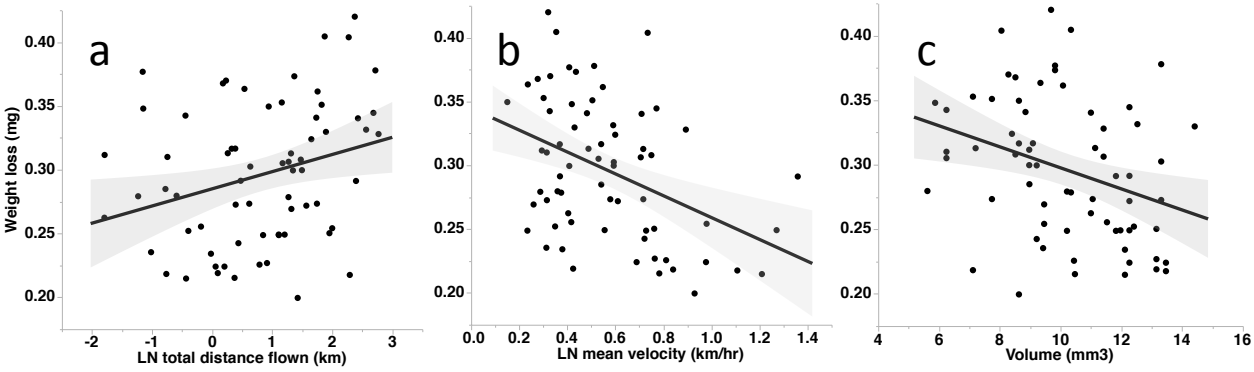

Fig. 2.4. The relationship between components of beetle flight, beetle volume, and weight loss. Panel a) shows the relationship between total distance flown and weight loss. Panel b) shows the relationship between mean velocity and weight loss. Panel c) shows the relationship between beetle volume and weight loss. Total distance and mean velocity were log transformed. Each datapoint represents a different individual host. The least square regression line is shown with 95% confidence interval.

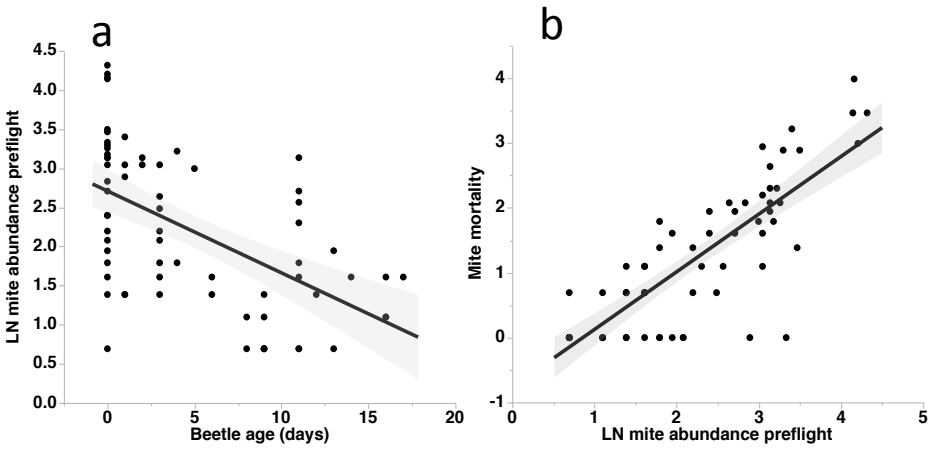

Fig. 2.5. The relationship between host age and mite abundance preflight, and between mite abundance preflight and mite mortality (number of mites lost after host dispersal). Mite abundance preflight and mite mortality were log transformed. Each data point depicts information on a different individual host. The least square regression line is shown with 95% confidence interval.

CHAPTER 3: EFFECTS OF MITES ON THE REPRODUCTION OF MOUNTAIN PINE BEETLE

Introduction

Phoretic symbionts are increasingly being recognized for the influential role they play at modifying particular aspects of their host life history. Accordingly, it is critical to assess the extent to which they modify host fitness and the mechanisms that lead to potential negative, positive or neutral net outcomes on phoretic symbioses. Of the many invertebrate taxa commonly associated to phoretic symbionts (Walter and Proctor 2013), insects have received increasing attention. Insect population dynamics and fitness may be responsive to the presence of phoretic symbionts because symbiont abundance might mediate the outcome between intra and interspecific interactions (Hofstetter et al. 2006, De Gasperin et al. 2015, De Gasperin and Kilner 2015a). Moreover, phoretic symbionts can also directly alter the reproductive success and survivorship of their insect hosts in some specific contexts (Wilson and Knollenberg 1987, Hodgkin et al. 2010).

As studies have increasingly focused on examination of final consequences of phoretic symbionts on adult insect hosts (i.e. number of offspring produce), the body of literature has shifted to the investigation of primary mechanisms that fuel the fluctuating nature of phoretic symbioses (Hodgkin et al. 2010, Pfammatter and Raffa 2015). For instance, a recent study suggested that both phoretic mites and their beetle host benefit each other as a by-product of coexisting in a mutually suitable environment instead of each associated species modifying each other's reproductive success (Pfammatter and Raffa 2015). Although this analysis focused on the

reproductive success (number of offspring produced) of host beetles associated with phoretic mites, the assessment of how this positive effect may occur was not clarified. While, it is reasonable to assess reproductive success after emergence of host adult offspring (most studies interested in the nature of symbioses do that), previous evidence has already suggested that a phoretic symbiosis between mites and insect hosts may differ over the course of the host's lifespan, implying that mites could interact differently with different host developmental stages (Hodgkin et al. 2010, De Gasperin and Kilner 2015a). For instance, mites could be parasites on adult hosts, and mutualistic when hosts are larvae, ultimately altering cost and benefits for both participant species during different moments of host ontogeny (Hodgkin et al. 2010, De Gasperin and Kilner 2015a). Interestingly, only one study has recently explored mite effects over the course of host life in the burying beetle *Nicrophorus vespilloides* and its phoretic mite *Poecilochirus carabi*. In this particular case the authors showed how inconsistent the effects of phoretic mites can be during beetle reproduction and parental care. (De Gasperin and Kilner 2015a).

Although different responses might arise when the influence of phoretic symbionts is examined through complete host ontogeny, the mechanisms that govern them may be the same. It has been shown, for instance, that phoretic symbionts can not only have negative and positive effects at the host adult stage (Wilson and Knollenberg 1987, Athias-Binche and Morand 1993, Houck and Cohen 1995, Hofstetter et al. 2006, Grossman and Smith 2008, Rocha et al. 2009, Hodgkin et al. 2010, Mazza et al. 2011, De Gasperin et al. 2015, De Gasperin and Kilner 2015a, b, Luong et al. 2015, Pfammatter and Raffa 2015), but also inflict negative effects (i.e. through predation) and/or have positive effects (i.e. increased survivorship through symbiont cleaning or nursing

services) on their hosts at certain developmental stages suggesting that phoretic symbionts influence the whole duration of host ontogeny if examined thoroughly (Kinn 1983, Okabe and Makino 2008, Biani et al. 2009, Hofstetter et al. 2009, Okabe 2013, Hofstetter and Moser 2014, De Gasperin and Kilner 2015a). Particularly for those species that cohabit in ephemeral habitats (i.e. decaying materials), negative and positive effects at both adult and early developmental stages could be particularly regulated by density-dependent mechanisms including, predation, symbiont intraspecific competition, host abundance among others (Wilson and Knollenberg 1987, Witte et al. 2008, Hodgkin et al. 2010, Fronhofer et al. 2013, Skelton et al. 2016). It has been shown, for instance, that an increase in the density of phoretic symbionts decreases host juvenile survival and adult production in southern pine beetle (Hofstetter et al. 2006, Witte et al. 2008, Hofstetter et al. 2009).

Particular biology and ecological requirements of phoretic symbiosis could explain differences in outcomes (Krantz 2009). In insects, juvenile stages of phoretic symbionts, such as mites, usually disperse on sexually immature hosts and remain with them for the whole duration of host lifespan including reproduction, parental care, overwintering, and subsequent dispersal events (Houck and OConnor 1991, Fronhofer et al. 2013, De Gasperin et al. 2015, Liu et al. 2016). During this time phoretic mites also complete development, and reproduce. Because mite generations are shorter than those exhibited in hosts, different age categories of both hosts and symbionts coexist and interact at the same time in the same habitat (Walter and Proctor 2013). It is thus likely that under such circumstances several interactions occur between host and symbionts, for instance predation or parasitism of host broods during the egg stage (Hofstetter et al. 2009) and mutualism during the larval stage (Biani et al. 2009) Moreover, it would not be

surprising that either an initial negative or positive effect may not persist during subsequent developmental stages if the feeding requirements of both symbionts and hosts change through development. Furthermore, phoretic symbioses are particularly abundant in ephemeral habitats increasing the likelihood of competitive interactions to occur, such as intraspecific competition (Binns 1982, Athias-Binche 1993). It is, thus, possible that under those circumstances this long lasting interaction may change over the course of host and symbiont ontogeny explaining the inconsistent nature of this type of symbiosis. Moreover, although theory agrees that oscillations among symbioses occur at both ecological and evolutionary scales, effects may actually be observable at a more immediate scale, for instance during the progression of a single host generation (Bronstein 1994). This could provide a measurable scale at which such shifts occur (Holland and DeAngelis 2009).

Here, I investigated how the experimental manipulation of phoretic mite presence (treatment) and abundance (number of initial and final mites) influences mountain pine beetle (*Dendroctonus ponderosae*) ontogeny. I was particularly interested in evaluating whether there is variation of effects during beetle ontogeny and whether this explains the net outcome at the end of the interaction. I predict that outcome variation during beetle development cancels out a mutualism or a parasitism and this should be reflected at beetle emergence. If phoretic mites do incur long-lasting positive or negative effects this should be reflected in the quality and production of brood adults, for instance a mutualistic symbiosis would yield a larger beetle production and/or beetles in better quality; if a parasitism were the result, I should find a reduced beetle production and/or beetles in poor quality. The ecology of the phoretic interaction between mountain pine beetle and one of its phoretic mites, *Tarsonemus ips*, has not been explored in

detail yet but there is evidence that suggests *T. ips* is a commensal of southern pine beetle, *Dendroctonus frontalis*, which could also be the case in mountain pine beetle but the mechanisms that lead to that outcome are unknown (Hofstetter et al. 2006).

Materials and Methods

To establish the parental beetle population, I collected beetle broods from five different infested lodgepole pines (*Pinus contorta*) from near Wapiti campground in Jasper National Park (52°50'15.7"N 118°03'50.8"W), AB, 5.4 km south of Jasper town on May 11 2015 . I transported four bolts from each infested pine to the laboratory at the University of Calgary in Calgary, AB and enclosed them in screened emergence cages that had illuminated glass jars for collecting positively phototactic adult beetles. I collected beetles daily for approximately four weeks. Upon collection, I immediately sexed beetles acoustically as males produce a distinctive stridulation (Safranyik and Carroll 2006). I kept beetles individually in 1.5 ml microcentrifuge tubes fitted with a perch at 5^{o}C degrees to immobilize them prior to their measurement and mite enumeration. I weighed beetles to the nearest 0.01 mg and measured beetle body size (length and pronotum width) at the nearest 0.2 mm using a dissecting microscope with a micrometer eyepiece. I recorded beetle mite abundance (number of mites attached externally on beetles' integument) and mite species identity. I calculated beetle volume as my measure of body size assuming an ellipsoid shape such that volume = 4/3 x pi x length/2 x $(\text{width}/2)^2$. I then determined a beetle body condition index as the body mass residual with respect to the regression of body mass against body volume (Schulte-Hostedde et al. 2005).

Mite treatments

Once parental beetles emerged, I chose those that carried mites. I subsequently divided beetles on those that naturally had fewer than five mites and those with equal or more than 5 mites. I established three treatments: low mite load (those beetles with less than five mites); high mite load (those beetles that had more than or equal to five mites); and high mite load with mites removed (those beetles that had more than or equal to five mites whose mites I removed). Parent beetles with high mite loads were randomly assigned to either high or removal treatments. Beetles from the removal treatment were cleaned with a fine paintbrush to detach all visible mites prior to parental breeding while beetles from the high and low treatments were simply poked with the paintbrush to make sure all beetles received the same handling. Note that I did not remove any mites that may have been under the elytra as this manipulation could be harmful to beetles.

Beetle rearing

I cut 27 bolts, each 30 cm long from one freshly-felled and healthy lodgepole pine from Bragg Creek (50°57'15.2"N 114°40'16.8"W), Alberta on May 12, 2015 to implant parental beetles. Implantations consisted of two beetle couples (one female, one male) that were allowed to breed separately on opposite sides of each log. I allowed females to establish a breeding site 24 hours before I introduced males. I considered a beetle couple successful when 24 hours after male introduction there was no sign of male activity outside the gallery entrance. After verifying that couples established successfully, I placed single implantation logs in individual cardboard enclosures fitted with a glass jar to collect phototactic adult offspring. Note that I was not able to associate the offspring to their own parents. However, I was able to trace back each

developmental stage to their parental source (see next procedure). The rearing temperature was maintained at 23C during the entire beetle development.

Offspring measurements

To quantify number of offspring produced, I collected all emerged offspring daily for seven weeks (from August 10 to September 27, 2015). Upon their emergence, I measured adult beetle length, pronotum width, mass, and recorded the mite abundance per individual beetle to quantify offspring quality following the same methods and protocol I previously described for the assessment of parental beetles. I similarly calculated beetle volume and beetle body condition index. Once I collected all offspring quality measurements and mite abundance, I sexed all beetles acoustically and by dissection (Safranyik and Carroll 2006). After all adult offspring had emerged I peeled the logs to measure mother gallery length and quantify offspring number and survival during development by recording number of larval trails and number of pupal chambers per couple, per log.

Statistical Analysis

I performed all statistical analysis using R (v. 3.4.0; R Development Core Team, 2012) and the package lme4 (Bates et al. 2015). I ran four different types of models for each fitness component of beetle ontogeny in order to distinguish mite influence from parental influence. Therefore, each model differentiated the predictor variables associated to either initial or final number of mites for each parent. Initial number of mites could indicate beetle quality whereas final number of mites would disclose whether there is influence from mites. The type of models were as follows: father volume and initial number of mites; mother volume and initial number of mites; father

volume and final number of mites; and mother volume and final number of mites. I did not include parent body condition because after data inspection this predictor variable did not have any influence in any of the models.

On a first model, I analyzed maternal gallery length using linear mixed-effects models. Predictor variables included parent body size (either mother or father), treatment, and number of mites (either initial or final). All these models included bolt number as a random effect.

When looking at number of larvae produced (number of larval trails) and larval survivorship (number of pupae chambers relative to number of larval trails), I used generalized linear mixed-effects models with a binomial distribution in the case of larval survivorship (number of pupal chambers/number of larval trails), and log-transformed number of larval trails when analyzing number of larvae produced. Predictor variables included parent body size (mother or father), bolt diameter, treatment, initial and final number of mites. I included bolt number as random variable in the aforementioned models.

I also analyzed number of pupae produced (number of pupal chambers) and pupae survivorship number of emerged offspring relative to number of pupal chambers) using generalized linear-mixed-effects models with binomial distribution in the case of pupae survivorship (emerged offspring/number of pupal chambers), and log transformed number of pupal chambers in the case of number of pupae produced. When I examined number of pupae produced, I used as predictors parent body size (mother and father) bolt diameter, treatment, initial and final number of mites. I looked for the effect of both initial and final mites separately maintaining all other predictors

constant. When looking at pupae survivorship (emerged offspring/number of pupae) I only used as predictor variables bolt diameter and treatment. I did not use parent characteristics for this specific model, as I was only able to relate emerged offspring per bolt. I included bolt number as random variable for all these models too.

Because I was interested in detecting whether mite presence (treatment) affected beetle offspring quality, I separately analyzed four aspects of newly emerged offspring (length, mass, volume, and condition) as response variables, using linear mixed-effects models maintaining all predictor variables constant for each model. Predictor variables included, bolt diameter and treatment, and bolt number was included as random effect.

I ran two more models with the number of mite offspring produced (the mites that were found hitchhiking on beetle offspring including mites under elytra) as response variable separately in order to detect what influenced mite production per bolt (mite production model), and how mites associated to beetles at emergence, for instance whether mites were more commonly associated to certain type of beetles (mite preference model). For the mite production model I used a standard least square model with the number of mite offspring produced as a response variable (log transformed) and number of beetle offspring and number of final parental mites as predictor variables. For the mite preference model, I used a generalized linear mixed model with the number of mites produced as response variable, but in this case the predictors included quality variables of beetle offspring, such as volume and condition. I additionally included bolt diameter and treatment in both models. Only the mite preference included bolt identity as random variable. I tested the assumptions of all models by examining the residuals.

Results

Patterns of mite occurrence in parents

Parent volume was significantly different between females and males ($t = -8.24$, df = 88, $p = <0.0001$; means of volume: females 14.55 ± 0.38 mm^3, males 9.81 ± 0.43 mm^3). Similarly the number of initial mites differed between sexes ($t = -2.97$, df = 88, $p = <0.0038$; means of log transformed mites: females 1.75 ± 0.17, males 1.10 ± 0.14). The number of initial mites (before implantation of parents) varied for each treatment ($F_{2,90} = 73.23$, $p = <0.0001$; least squared means of treatment: low 0.26 ± 0.12 log transformed mites, high 2.24 ± 0.11 log transformed mites, removal 1.55 ± 0.11 log transformed mites), and it was positively correlated with parent volume ($F_{1,90} = 30.17$, $p = <0.0001$) and parent condition ($F_{1,90} = 5.02$, $p = 0.0277$) (Table 3.1). These patterns remained after removal of mites: final mites varied with treatment ($F_{2,90} = 161.60$, $p = <0.0001$; least squared means of treatment: low 0.25 ± 0.10 log transformed mites, high 2.24 ± 0.10 log transformed mites, removal 0.001 ± 0.09 log transformed mites), and positively correlated with parent volume ($F_{1,90} = 11.92$, $p = 0.0009$) (Fig. 3.1, Table 3.1a), and parent condition ($F_{1,90} = 5.14$, $p = 0.0259$) (Fig. 3.1, Table 3.1b).

Maternal gallery length

Of the original 54 couples that were prepared for implantations, only nine did not mate either because the female or the male did not enter the bolt. Of the remaining 45 couples, a further 3 couples had some incomplete information associated with either the mother or certain developmental stages of the offspring, this explains why subsequent models had different sample sizes. Over all, 13 couples of the low treatment established and produced offspring, whereas 16

couples of the high treatment, and another 16 of the removal treatment established and produced offspring too. For those maternal galleries that were included in the analysis none of the mite metrics (treatment, number of initial and final mites) had any effect on maternal gallery length (Table 3.2). However, mother volume had a negative effect on maternal gallery length (Fig. 3.2, Table 3.2) while there was no effect of father volume.

Beetle larvae production and survivorship

Fathers with more initial mites had more larval trails produced (Fig. 3.3a). Father volume, bolt diameter, treatment, and final number of mites did not have any effect on the number of larvae produced (Table 3.3). On the other hand, when looking at mother models, I found that bolt diameter had a marginally negative effect on the number of larvae produced. Mother volume, treatment, and initial and final mites did not have any effect on the number of larvae produced (Table 3.3).

However, when looking at larval survivorship, I found that final mite numbers of fathers and treatment had an effect on larval survivorship (Fig. 3.4a,b; Table 3.4). Specifically, survivorship of larvae was higher for fathers on the high mite treatment (Estimate = 2.35; SE = 0.93; z value = 2.52; P = 0.01), whereas, number of final mites of fathers had a negative effect on survivorship ($\chi^2_1 = 6.33$; P = 0.01), i.e., fathers with more mites had lower larval survivorship. I also detected that the volume of both parents had a consistent positive effect on larval survivorship (Fig. 3.5a,b; Table 3.4); however, when considering mother models I found that none of the mite predictor variables (mother number of initial and final mites, and treatment) had an effect on larval survivorship (Table 3.4).

Beetle pupae production and survivorship

Log diameter had a negative effect in models including mother traits, but not in models with father traits (Table 3.5). Body volume of mothers or fathers did not affect pupal production. Mite treatment and the final number of mites on either mothers or fathers did not affect the number of pupae produced, but the initial number of mites on fathers had a positive effect on the number of pupae produced (Fig.3.3b, Table 3.5). Pupal survivorship did not differ among mite treatments ($\chi^2_2 = 1.74$; P = 0.418), and was not affected by bolt diameter either ($\chi^2_1 = 1.45$; P = 0.228)

Beetle offspring quality and production

In general, when analyzing different offspring quality variables (length, mass, volume and condition), I did not find any effect of bolt diameter or parental mite treatment (Table 3.6).

Mite offspring production and mite preference

When I analyzed separately the number of mite offspring to distinguish between what influences mite production and how mites were associated with beetles at emergence (mite preference model), I found that when looking at what influences mite production, the number of mite offspring increased with the number of emerged beetle offspring (Fig. 3.6); however, the number of mite offspring did not vary with number of parental mites or mite treatment, or with the size of the bolt when mite production is considered per bolt (Table 3.7; mite production model). On the other hand when looking at mite production to detect how mites were associate with beetles at emergence, I found that larger beetle offspring seemed to carry more mites (Table 3.7; mite preference model) particularly for those beetles that came from the high treatment (Estimate =

2.20; SE = 0.95; z value = 2.31; P = 0.02). In this case offspring condition and bolt diameter did not have any effect on the number of mite offspring produced (Table 3.7).

Discussion

I determined the number and survivorship of juvenile stages as well as the number and quality of emerging adults of mountain pine beetle, *D. poderosae*, and did not find conclusive evidence that mites had a net positive or net negative effect on host fitness. Instead, the inconsistency of phoretic mite effects during host development and the absence of effects in emerging adults suggest that the interaction between pine beetle and its phoretic mites should be deemed as commensal (neutral for the host and positive for the symbiont). Although I detected a positive effect of father treatment in larvae survivorship when accounting for initial number of mites (mites before manipulation), final mite abundance on fathers (number of mites after removal) seemed to decrease it. These findings differ with the complete absence of mite effects I detected in later beetle developmental stages particularly when observing pupae survivorship and adult offspring production and quality thus supporting the hypothesis that early effects of this symbiosis do not remain during host ontogeny and this outcome fluctuation could explain the net outcome at the end of the interaction. These results contrast with previous knowledge, which showed either net positive or net negative effects of phoretic symbionts on host adult broods in other invertebrates (Wilson and Knollenberg 1987, Hofstetter et al. 2006, Hodgkin et al. 2010, De Gasperin and Kilner 2015a, Skelton et al. 2016). However, in the current study a negative effect of mite abundance was detected despite a positive effect of mite presence at least during host larval stage, and on males.

My results showed a negative effect of final mite load on fathers (number of mites after manipulation) on larvae survivorship despite the positive effect of mite treatment and parents volume when only accounting for initial number of mites. Although, this result seems contradictory (positive and negative effect at the same time), another recent study has suggested a similar conflicting mechanism in another bark beetle species. Pfammatter et al. (2015) showed that number of emerging adults decreased with mite density in *Ips grandicollis*. However, they did not find evidence of a density-dependent mechanism after manipulation of mite numbers on parental treatments, for instance, they did not find a negative effect of mite density in the number of emerging adult beetles (Pfammatter and Raffa 2015). It is possible that the authors could not identify a negative relationship in emerging offspring because this effect is only detectable in juveniles. The presumably negative effect in my study was detected exclusively in the larval stage and on males, which contrast with the lack of effect in pupae survivorship and the number of emerging offspring. In contrast, another previous study done with *I. grandicollis* found an opposite effect of maternal mites on emerging offspring: within females that had mites, those with higher mite densities were more fecund and produced larger offspring suggesting a positive effect of maternal mites in emerging beetles (Hodgkin et al. 2010). This study similarly to the previous one focused only on emerging adult broods. In my study, however, neither mother initial or final mites nor treatment had an effect on adult offspring production. Moreover, when only considering mother mites, the lack of effect of mites is consistent within all juvenile stages of beetle development.

The positive effect of both parents' volume was only obvious at the larval stage suggesting that parents in better condition might play a role at increasing chances of larvae surviving to pupae stage. This further confirms previous research that showed body condition and/or body size are important predictors of traits related to fitness maximization in insects (Bonte and De la Peña 2009, Latty and Reid 2009, Samejima and Tsubaki 2009, Kisdi et al. 2012, Saastamoinen and Rantala 2013, Baines et al. 2015, Seppälä et al. 2015, Skelton et al. 2015). Although maternal effects, such as the effect of mother volume on maternal gallery length and larval survivorship are not rare (Mousseau and Fox 1998), the positive effect of father volume on larval survivorship is surprising. A possible explanation could be that father parental care increases with father volume and therefore larvae survivorship increases too. Although there is no evidence of such behavioral mechanism in mountain pine beetle, previous research has shown that long parental care, presumably gallery cleaning, increases female reproductive rate in other bark beetle species (Reid and Roitberg 1994). Although gallery cleaning has not been confirmed in males of mountain pine beetle during brood development, it is possible that other modes of parental care can still explain the positive effects on larval survivorship in the present study (De Gasperin et al. 2015, De Gasperin and Kilner 2015a). Alternatively, paternal genetic quality could influence larval survival to pupation, but this has not been explored in mountain pine beetle yet. I suggest that parental care and its relationship with mite abundance and presence in mountain pine beetle should be investigated to clarify whether this explains increases in larval survivorship. Moreover paternal genetic quality of mountain pine beetle could also be another important venue of research (Reid and Roitberg 1994).

Although I was unable to perfectly manipulate the presence of parental mites (i.e. adult offspring from removal treatment had mites at emergence) in this study, the results imply that parental mite abundance, if important after further verification, may play a role at modifying survivorship of juveniles of mountain pine beetle during development. Interestingly, there is previous evidence of high mortality in juveniles of mountain pine beetle in nature (Safranyik et al. 2012). Moreover, a recent study looking at secondary sex ratios suggests that the female-biased ratio (2:1) in mountain pine beetle is due to male mortality occurring during beetle development (Lachowsky unpublished results). In her study the author identified that the primary sex ratios in mountain pine beetle is 1:1 and that the 2:1 ratio observed in adult beetles is achieved after high male mortality associated to early developmental stages in this species (Lachowsky unpublished results). Curiously, another previous study with mountain pine beetle suggested that the overwintering mortality of males is not the only mechanism responsible for the female-biased ratio in this species and other size-independent mechanisms may be responsible (Lachowsky and Reid 2014). Although my results do not provide conclusive evidence that high mite abundance may be the additional mechanism responsible of male larvae mortality specifically, I suggest that this could be a venue for further research.

Alternatively, negative effects of parental mites can be related to mite biology. On one hand, there is evidence of several predatory mites cohabitating with other bark beetle species (i.e. *Proctolaelaps* spp.). Some of these species predate on early developmental stages of beetle hosts, including eggs and pupae, although they may also feed on phoretic and parasitic nematodes with a possible beneficial effect for beetles (Walter and Proctor 2013). In the present study, it was very unlikely that predation of larvae or pupae occur. Parent beetles only had *Tarsonemus ips*

and this mite species is know as a fungivorous mite that feeds on, transports and spreads *Ophiostoma minus*, a type of blue stain fungus in southern pine beetle (*D. frontalis*). In this beetle, however, *O. minus* is antagonist of a beneficial mycangial fungus that beetle larvae feeds on (Hofstetter 2011). In this particular case, *T. ips* seems to indirectly affect southern pine beetle by increasing the abundance of *O. minus* which has negative consequences in beetle development (Hofstetter et al. 2006). Although, there is evidence that mountain pine beetle also carries *O. minus* externally on the exoskeleton (Six 2003), it is unknown whether *T. ips* also contributes to the spread of this fungus in the gallery system of mountain pine beetle. Moreover, it is unknown whether *T. ips* associated with mountain pine beetle increases the abundance of *O. minus* causing negative effects on beetle development too. It is known, however, that in mountain pine beetle several other mycangial fungi benefit beetle larvae, so the chances that *T. ips* of mountain pine beetle carries a beneficial fungus exist. To date there is no evidence of a mite-fungus symbiosis in bark beetles although some authors have presumed mites and fungi may sustain a symbiotic association. If this is the case, it is likely that this might be mutualistic; mites can benefit from feeding on the fungus, while the fungus could obtain the benefit of dispersal. However, all these ideas remain to be tested.

While the positive relationship between larval survivorship and parents' volume, and between maternal gallery length and mother volume, are obvious responses, the positive relationship between number of larvae and pupae produced and number of initial mites on fathers (mites before manipulation), is not. It is likely that this positive effect is due to most phoretic mites preferentially associating with hosts in better condition. Several studies have confirmed that phoretic mites do not associate with their hosts randomly (Niogret et al. 2006, Grossman and

Smith 2008, Bajerlein and Przewoźny 2012, Fronhofer et al. 2013). In fact, there is specific evidence that the number of phoretic mites increases with host body size (Bajerlein and Przewoźny 2012). In my study, it is possible that the number of initial mites could be an indication of father quality. Fathers with more initial mites, presumably of better quality, may contribute more to their brood through parental care (De Gasperin et al. 2015, De Gasperin and Kilner 2015a). Nonetheless, parental care in mountain pine beetle remains to be investigated. Alternatively, differential allocation in reproductive output from females relative to the condition of their male partner might also explain why larvae and pupae numbers seemed to increase with initial mites of fathers (Reid and Roitberg 1994, Reid and Baruch 2010), an hypothesis that remains to be investigated.

Interestingly, pupae survivorship, adult brood quality and adult brood production did not respond to parental mite abundance (either initial or final mites) or treatment. Particularly, the lack of mite effects on adult brood production confirms what a previous study showed in adult offspring of *I. gradicollis*, where phoretic mite abundance does not influence adult offspring production but a negative effect could possibly arise when mites are present in high numbers (Pfammatter and Raffa 2015). In their study the authors did not find significant correlations between number of beetle brood and brood mite abundance after manipulation of parental mite numbers. It is possible that the lack of phoretic symbiont effects in host adult broods might be a widespread phenomenon among other phoretic symbioses. This could explain why recent investigations had focused on symbiont life-history strategies (i.e. dispersal) rather than on investigating net effects on hosts (De Gasperin and Kilner 2015a, Skelton et al. 2015). In the present study, the lack of consistent positive or negative effects of phoretic symbionts through mountain pine beetle

development suggests that the cost, benefits, and the lack thereof in a phoretic symbiosis is also likely to change with host ontogeny (Yule et al. 2013). This is important because the fluctuating nature of a phoretic symbiosis has just started to be examined in manipulative studies (Skelton et al. 2014).

I also detected that mite offspring abundance increased with both the number of beetle adult offspring and beetle volume. In other words, the more beetles were produced the more mites were produced too, and the larger the beetles the more mites beetles carried. This finding is consistent with symbiont transmission dynamics observed in host-parasite symbioses. Theory predicts that parasite transmission depends on host population density, particularly, parasite abundance increases with host abundance and this relationship is also expected when parasites have a minor effect or do not have any negative effects at all (i.e. commensal and mutualistic symbionts) (Arneberg et al. 1998, Krasnov et al. 2002). This is confirmed in this study. My results suggests that even if mites had a negligible negative effect or if it is considered that they did not have a negative effect on their beetle hosts at all, mite abundance increased with host abundance similar to what has been predicted for host-parasite symbioses. Phoretic mites may have a minor or no effect in the development of mountain pine beetle, moreover, mites did not reduce survival of emerging adult beetles either, yet I found that symbiont density increased with host density at emergence. This mechanism relates to symbiont transmission or dispersal. This is a critical population-level process for persistence to the next generation of any phoretic symbiont population in the context of phoresy. The biological requirements of phoretic mites of mountain pine beetle require beetle dispersal to complete phoretic mite development. Once the pine resource has been depleted both species must abandon and subsequently colonize other suitable

pines. In this new resource phoretic mites complete their development and reproduce. It is therefore expected that if mite transmission depends on host dispersal, high host density would increase the probability that symbionts will be transmitted.

My results also showed a positive relationship between mite offspring production and beetle offspring volume, which is consistent with previous findings in other phoretic symbioses in insects and marine invertebrates (Bajerlein and Przewoźny 2012, Skelton et al. 2015). Moreover, there is evidence of phoretic symbiont choosiness based on host exudates and sex, suggesting there might be advantages of associating with particular individual hosts (Niogret et al. 2006, Grossman and Smith 2008, Niogret and Lumaret 2009, Bajerlein and Przewoźny 2012, Fronhofer et al. 2013, Skelton et al. 2015). That phoretic symbionts do not associate with their hosts randomly seems to be a general and advantageous mechanism for symbionts to achieve dispersal (Hopkins et al. 2015, Skelton et al. 2015). This is commonly observed in parasitic and mutualistic symbionts and phoretic symbionts might not be the exception. Advantages of non-random association of phoretic mites for dispersal (Fronhofer et al. 2013), could help explain why the number of symbionts seems to increase with host volume. This finding and my findings (including the positive relationship between larva survivorship and parents volume, and the positive effect between the number of larvae and pupae and initial mites) should be considered as correlational evidence of condition-dependent transmission of phoretic symbionts (Skelton et al. 2015). It remains to be scrutinized whether this mechanism is advantageous to phoretic mites of mountain pine beetle.

Table 3.1. Details of mite occurrence per sex and treatment for parents before and after their implantation.

	Initial mites						Final mites					
	Females			Males			Females			Males		
Treatment	NM	Mean	SE	NM	Mean	SE	NM	Mean	SE	NM	Mean	SE
Low	6	0.46	0.27	4	0.31	0.17	6	0.46	0.27	4	0.31	0.17
High	322	20.13	5.17	105	6.56	1.28	322	20.13	5.17	105	6.56	1.28
Removal	107	6.69	0.61	47	2.94	0.64	0	0	0	0	0	0

NM represents the total number of mites of each treatment. Low treatment had 13 couples; high treatment had 16 couples; and removal treatment had 16 couples.

Table 3.2. Linear mixed models predicting maternal gallery length with initial and final mites.

		Response variable: maternal gallery length							
		Initial mites model				Final mites model			
		Mother		Father		Mother		Father	
Predictor variable	d.f.	LRT: X^2	*P* value	LRT: X^2	*P* value	LRT: X^2	*P* value	LRT: X^2	*P* value
Num of mites[a]	1	0.8272	0.3631	3.7901	0.0516	1.8749	0.1709	1.5812	0.2086
Volume	1	4.4700	**0.0345***	0.1779	0.6732	5.0537	**0.0246***	1.1908	0.2752
Treatment	2	0.1297	0.9372	0.9217	0.6307	2.0752	0.3543	1.1471	0.5635
n		42		42		42		42	

[a] indicates that the variable was log transformed. Bolt identity was included as random variable.

Table 3.3. Linear mixed models for number of beetle larvae produced with initial and final mites. Models for fathers and mothers with both initial and final mites were done separately.

		Response variable: number of larvae produced							
		Initial mites model				Final mites model			
		Mother		Father		Mother		Father	
Predictor variable	d.f.	LRT: X^2	*P* value	LRT: X^2	*P* value	LRT: X^2	*P* value	LRT: X^2	*P* value
Num of mites[a]	1	0.3153	0.5744	6.7278	**0.0095***	0.7939	0.3730	0.8172	0.3660
Volume	1	1.9233	0.1655	0.2260	0.6345	2.1733	0.1404	1.4787	0.2240
Bolt diameter	1	3.7579	0.0526	1.8984	0.1682	4.0021	**0.0454***	1.9818	0.1592
Treatment	2	0.1596	0.9233	1.6429	0.4398	1.6518	0.4378	1.1338	0.5673
n		44		44		44		44	

[a] indicates that the variable was log transformed. Bolt identity was included as random variable.

Table 3.4. Generalized linear mixed models for beetle larvae survivorship with initial and final mites. Models for mothers and fathers both initial and final mites were done separately.

		Response variable: larvae survivorship							
		Initial mites model				Final mites model			
		Mother		Father		Mother		Father	
Predictor variable	d.f.	LRT: X^2	*P* value	LRT: X^2	*P* value	LRT: X^2	*P* value	LRT: X^2	*P* value
Num of mites[a]	1	0.272	0.6021	2.835	0.0923	3.726	0.0536	6.334	**0.0118***
Volume	1	4.049	**0.0442***	21.397	**<0.0001***	4.408	**0.0358***	20.218	**<0.0001***
Treatment	2	1.591	0.4514	4.767	0.0922	5.050	0.0800	8.254	**0.0161***
n		43		43		43		43	

[a] indicates that the variable was log transformed. Bolt identity was included as random variable.

Table 3.5. Linear mixed models for number of beetle pupae produced with initial and final mites. Models for fathers and mothers with both initial and final mites were done separately.

		Response variable: number of pupae produced							
		Initial mites model				Final mites model			
		Mother		Father		Mother		Father	
Predictor variable	d.f.	LRT: X^2	*P* value	LRT: X^2	*P* value	LRT: X^2	*P* value	LRT: X^2	*P* value
Num of mites[a]	1	0.5417	0.4617	6.3791	**0.0116***	1.1478	0.2840	1.0456	0.3065
Volume	1	2.1961	0.1384	0.9479	0.3303	2.4826	0.1151	2.5739	0.1086
Bolt diameter	1	4.4052	**0.0358***	2.3335	0.1266	4.7417	**0.0294***	2.4252	0.1194
Treatment	2	0.0250	0.9876	0.8944	0.6394	1.1689	0.5574	0.7501	0.6873
n		43		43		43		43	

[a] indicates that the variable was log transformed. Bolt identity was included as random variable.

Table 3.6. Linear mixed model for different beetle offspring quality variables and generalized linear mixed model for number of beetle offspring produced.

		Response variable							
		Offspring length		Offspring mass		Offspring volume		Offspring condition	
Predictor variable	d.f.	LRT: X^2	*P* value	LRT: X^2	*P* value	LRT: X^2	*P* value	LRT: X^2	*P* value
Log diameter	1	0.5828	0.4452	0.1870	0.6654	0.2988	0.5847	0.0016	0.9673
Treatment	2	4.8015	0.0906	4.7629	0.0924	4.2202	0.1212	1.4631	0.4812
n		653		653		653		653	

Bolt identity was included as random variable

Table 3.7. Least squares model for total number of mite offspring produced per bolt (mite production model), and generalized linear mixed model for number of mite offspring produced associated to the mite preference model.

		Response variable: number of mite offspring produced			
		Mite production		Mite preference	
Predictor variable	d.f.	F	P value	χ^2	P value
Number of beetle offspring[a]	1	11.7498	**<0.0032***	-	-
Number of parental mites[a]	1	0.3151	0.5819	-	-
Beetle offspring volume	1	-	-	4.2240	**0.0399***
Beetle offspring condition	1	-	-	2.0420	0.1530
Bolt diameter	1	0.1568	0.6970	0.6996	0.4072
Treatment	2	0.2517	0.7803	6.2241	**0.0445***
r^2	**0.5699***			-	
n	23			653	

Predictor variables indexed with an [a] were log transformed. Bolt number was included as a random variable for the mite preference model.

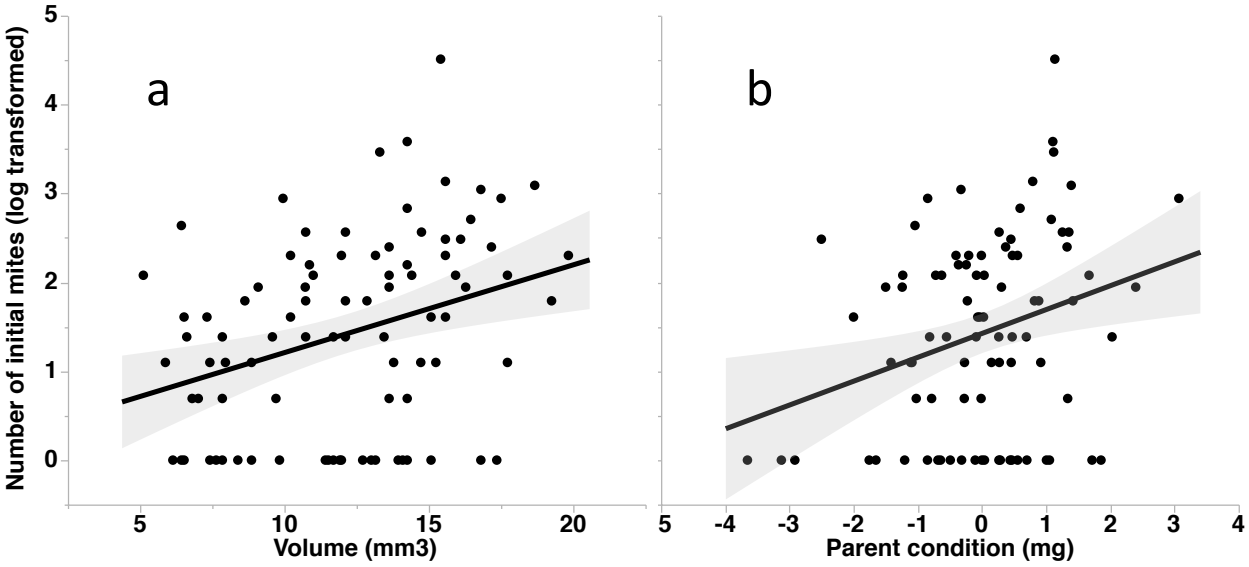

Fig. 3.1. The relationship between traits of parent quality and number of initial mites. Panel a shows the relationship between volume (mm^3) and number of initial mites. Panel b shows the relationship between condition (mg) and number of initial mites. Number of initial mites was log-transformed. The range of mite numbers was 0-90. The graph shows the linear regression for univariate analyses.

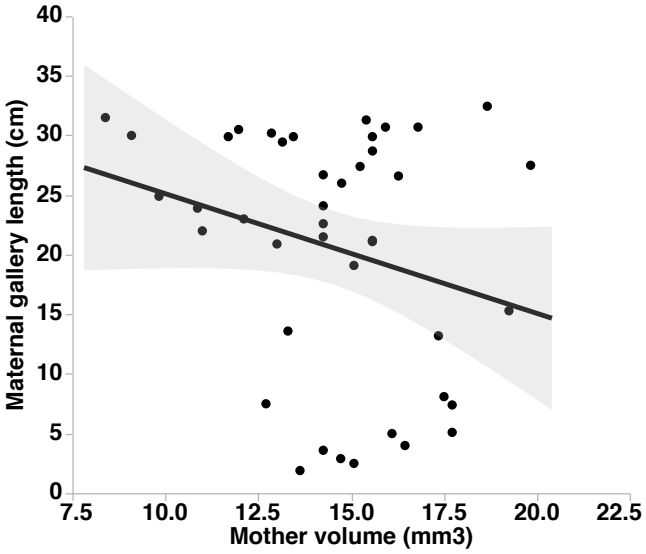

Fig. 3.2. The relationship between mother volume (mm^3) and maternal gallery length (cm). The graph shows the linear regression for the univariate analysis.

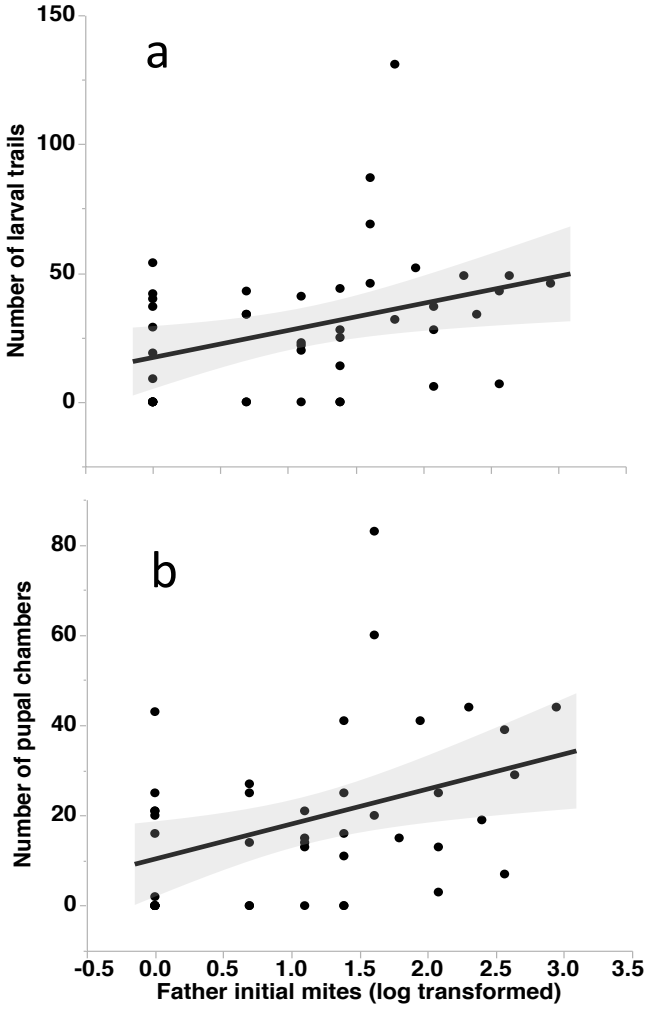

Fig. 3.3. The relationship between father initial mites and the number of two beetle juvenile stages. Panel a shows the relationship between initial mites and number of larval trails. Panel b shows the relationship between initial mites and number of pupal chambers. The graph shows the linear regression for univariate analyses for both response variables and the log-transformed values for number of initial mites.

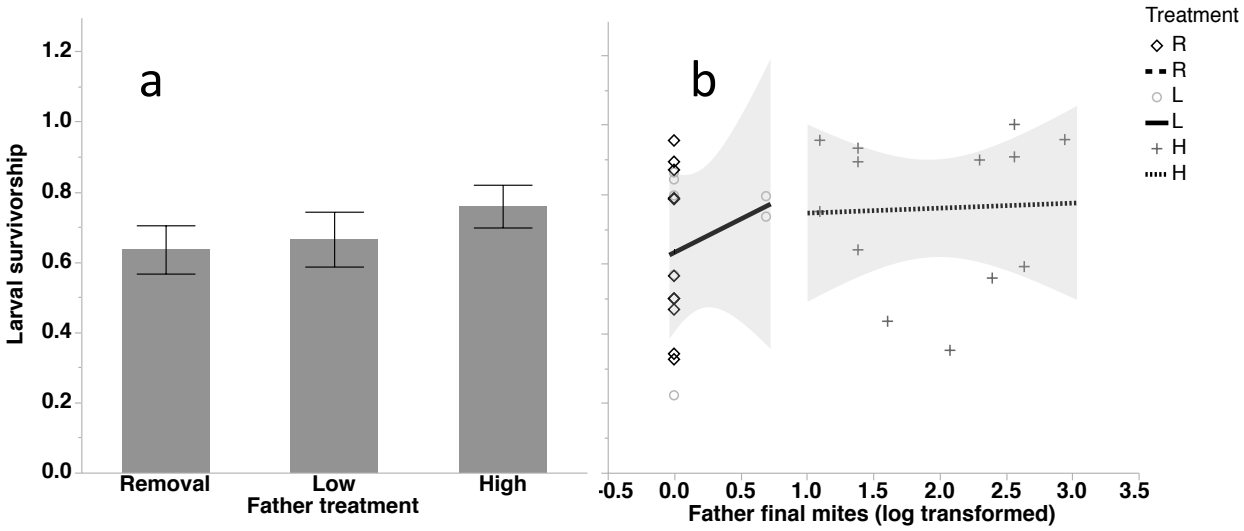

Fig. 3.4. The relationship between father traits and larval survivorship. Panel a shows the mean larval survivorship per treatment and their associated SE. Panel b shows the linear regression between the log-transformed values for father final mites and the proportional values of larval survivorship. Panel b shows the linear regression for the univariate analysis

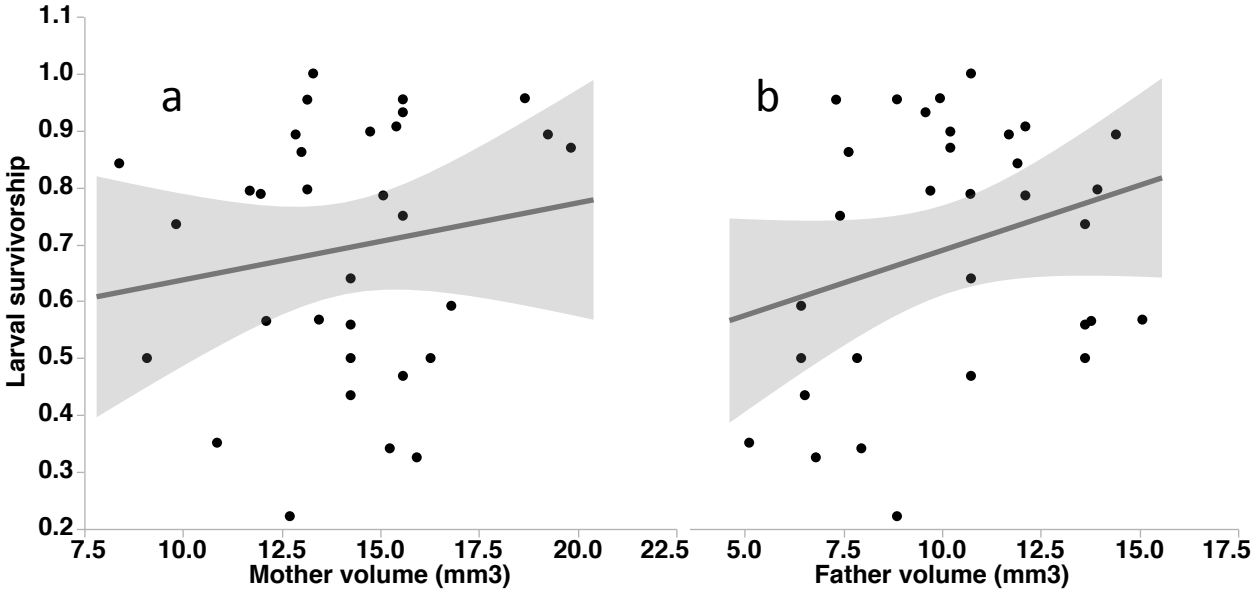

Fig. 3.5. The relationship between parent volume (mm^3) and larval survivorship. Panel a shows the relationship between mother volume (mm^3) and larval survivorship. Panel b shows the relationship between father volume (mm^3) and larval survivorship. The graph shows the linear regression for univariate analyses between the proportional values of survivorship and the raw values of mother and father volume (mm^3).

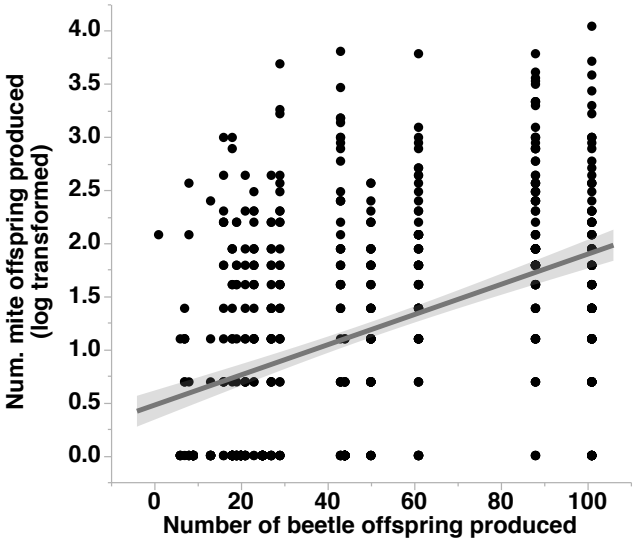

Fig. 3.6. The relationship between the number of beetle offspring produced and mite offspring produced. The graph shows the linear regression for the univariate analysis.

CHAPTER 4: EFFECTS OF HOST RANGE EXPANSION ON PHORETIC SYMBIONT DISTRIBUTIONS

Introduction

Spatial shifts in the range of species not only modify the population and community ecology of range-shifting species themselves but it also disrupts ecological attributes and processes with other associated species (Parmesan 2006, Safranyik et al. 2012, Stewart et al. 2015). Range-expanding species usually migrate with their symbionts. During this process models suggest abundance patterns can also be altered for symbionts (Reuter et al. 2005, Phillips et al. 2010a, Phillips et al. 2010b, Van der Putten et al. 2010, Coates et al. 2017). For instance, in host-parasite systems, a range-expanding host can either acquire and increase prevalence of new parasites at the leading edge of expansion (Coates et al. 2017), or decrease the abundance and even experience entire loss of commonly associated parasites (Reuter et al. 2005, Phillips et al. 2010a, Phillips et al. 2010b). This process of loss, retention and gain of symbionts has several implications for hosts. These can include the altered ability of a host to expand its range further (Reuter et al. 2005, Stanton-Geddes and Anderson 2011), and changes in host life-history traits and/or phenology (Parmesan 2006, Van der Putten et al. 2010). However, consequences for symbionts themselves are possibly more dramatic, since their persistence depends entirely on the host ability to disperse and find new colonization habitats during periods of rapid expansion. In some severe cases, reduced symbiont abundance can hamper their persistence and ultimately drive symbionts to extinction at the leading edge of host advancement (Reuter et al. 2005, Cahill et al. 2013). This altered spatial distribution of symbionts as a consequence of host range

expansion is similar for both parasites and mutualists (Berg et al. 2010), suggesting that it should be analogous for other type of symbionts as well.

The loss or retention of symbionts during periods of range expansion may depend on the mode and efficiency of their transmission, and the effect of symbionts on their host (Douglas 1998, Herre et al. 1999, Gundel et al. 2008). In mutualistic symbioses, for instance, a host is likely to retain its symbionts if reproductive success of host and symbionts is fully linked. This partner fidelity is primarily exhibited between host and symbionts that sustain an obligatory symbiosis or interactions that are repeated or long-term whose symbionts are usually transmitted vertically (Husseneder et al. 2010, Leimar and Hammerstein 2010). In such case, retention of symbionts at leading fronts of expansion should be more common. It is, however, possible symbionts that meet such criteria but that exhibit imperfect vertical transmission (i.e. mixed modes of transmission) are at risk of being lost from host populations (Jaenike 2012, Yule et al. 2013). In mutualistic symbioses that involved facultative symbionts, these could be lost if they are not indispensable for host growth and reproduction, or if they are mostly horizontally transmitted. In such cases, hosts could establish novel interactions with local symbionts that are functionally equivalent (Wooding et al. 2013). In parasitic symbioses, however, it seems that vertically transmitted symbionts (i.e. obligate parasites) are more likely to be lost during periods of host range expansion than those that are horizontally transmitted because differences in virulence due to an enemy release process driven by founder events and followed by higher host investment in traits that enhance reproduction and dispersal (Phillips et al. 2010a, Phillips et al. 2010b).

Although in commensal symbioses the aforementioned mechanisms are not well understood yet, it is reasonable to assume that same processes regulate whether symbionts may be lost or retained at leading fronts of host expansion. Commensal symbionts can exhibit all sorts of effects in their hosts either positive, negative, both or none (Wilson and Knollenberg 1987, Hodgkin et al. 2010, Pfammatter and Raffa 2015). However, they are not necessarily required for reproduction of hosts. Most of the times they sustain a unidirectional interaction where only symbionts depend on the host for services such as dispersal (Benton and Bowler 2012). Under such circumstances, it is possible that lost or retention of commensal symbionts may occur depending on the level of dependency more than the associated effects they incur on hosts (effects on host can be negligible, see chapter two and three). Similar processes that explain symbiont loss in obligate parasites may occur in commensals, for instance, a symbiont release process also driven by founder events and higher investment in traits that enhance reproduction and dispersal of hosts (Phillips et al. 2010a, Phillips et al. 2010b). The transmission mode of commensal symbionts, however, seems less consistent. They can exhibit both transmission modes with no clear predictability (Binns 1982). It is thus also possible that commensal symbionts may instead exhibit a host switching mechanism (i.e. switching between phylogenetically related hosts) (Susoy and Herrmann 2014), which is observed among symbionts that have either imperfect vertical transmission or mixed modes of transmission in other parasitic or mutualistic symbioses (Ebert 2013).

Phoretic symbionts, commensals that use dispersive hosts to power their own dispersal to new colonization sites, are also commonly associated with range-shifting hosts but they have received less attention (Hofstetter et al. 2006, Hulcr and Dunn 2011, Six and Wingfield 2011). These

symbionts are ubiquitous and are generally found cohabiting with their dispersive hosts (Wiens 1976, Krantz 2009). This type of symbiosis, also known as phoresy, usually starts when phoretic symbionts hitchhike on hosts for the duration of the transit phase of dispersal that in some cases prolongs to subsequent stages of host life including reproduction and development (Binns 1982, Hunter and Rosario 1988, Houck and OConnor 1991). When hosts and phoretic symbionts breed together their phenology is usually synchronized to host phenology (Belozerov 2009, Okabe 2013). Phoretic symbionts may also play a key role in indirectly regulating population dynamics of range-expanding species during periods of host advancement (Hofstetter et al. 2006). For instance, in the southern pine beetle (*Dendroctonus frontalis*), phoretic mites can reduced the survival of developing beetle brood by increasing the abundance of an antagonist fungus (*Ophiostoma minus*) that also disperse with the beetle (Hofstetter et al. 2006). However, dispersive hosts might have a more serious impact on the dynamics of phoretic symbionts if range-expanding hosts also have the potential to modify distribution and abundance of associated phoretic symbionts just as it happens with other type of symbionts. Because phoretic symbionts must continually recolonize new habitats in order to persist, any declines in their distribution and abundance are expected to increase the possibility of local extinction particularly on the leading edge of host range expansion.

Mountain pine beetle, which exhibits outbreak population dynamics in western North America, is frequently associated with phoretic mites (Acari) (Safranyik and Carroll 2006, Mori et al. 2011). Beetle range has rapidly extended from central-south British Columbia towards north and eastern Alberta since the early 2000. It is predicted that the outbreak will eventually extend through the boreal forest from Alberta to eastern Canada (Safranyik et al. 2012). Although it is a

native bark beetle of western North America, its outbreak progression is likely to modify the distribution and abundance of other interacting species (Mori et al. 2011, Six and Wingfield 2011). For instance, there is evidence that suggests mountain pine beetle has the potential to establish novel interactions, for instance *Ophiostoma montium*, a type of beneficial blue stain fungus (note not to be confused with *O. minus* which is an antagonist), is of recent acquisition in mountain pine beetle (Six and Paine 1999). Furthermore, it has been suggested that the association between certain phoretic mites and mountain pine beetle in Northern Alberta, Canada could have been established recently as well (Mori et al. 2011). Phoretic mites of bark beetles do not seem to be randomly distributed among bark beetles and in mountain pine beetle seems to be primarily determined by environmental seasonality (Mori et al. 2011, Pfammatter and Raffa 2015). For instance, phoretic mites of mountain pine beetle from the leading edge of expansion (northern Alberta) seemed to be more abundant at the beginning of the flying season although this depended on the mite species (Mori et al. 2011). However, this might not be the only mechanism if other ecological requirements of phoretic mites are considered (i.e. unidirectional level of dependency and transmission mode). In this sense, phoretic mites might be subject to other processes at the leading front of expansion of mountain pine beetle. For instance, the diversity of phoretic mite species associated with mountain pine beetle at the leading edge of beetle expansion in Western Canada seems to be less than that normally associated with other bark beetle species in core areas of beetle distribution, suggesting that the recent range expansion of mountain pine beetle might decrease phoretic mite diversity and therefore abundance (Hofstetter 2011, Mori et al. 2011, Pfammatter et al. 2013).

In the present study I was interested in detecting whether there is symbiont loss in the new range of mountain pine beetle expansion in western Canada. I particularly predict a decrease in the abundance, prevalence, and intensity of phoretic mites associated with mountain pine beetle from the new area of expansion in northern British Columbia (Valemount) and northwestern Alberta (Grande Prairie and Peace River), when compared with the historical range of beetle distribution in central-south British Columbia (Yoho, Kootenay and Penticton). Furthermore, if a symbiont release process were in place, I would expect hosts from the leading range of expansion to be of better quality and carry fewer mites.

Materials and methods

Beetle collections

This study was performed during the mountain pine beetle flight season of two consecutive years, 2011 and 2012. I established seven sites in the core historical range and 10 in the new expansion area (Table 4.1). Although I tried to keep the sampling sites consistent for both years of collections, I was only able to resample five sites from the historical part of the range in both years. I set up three 12-unit Lindgren funnel traps (Con-tech Enterprises Inc., Delta, British Columbia) lured with *D. ponderosae* specific pheromones (Con-tech Enterprises Inc.) at each site. I placed each trap in between two lodgepole pines in a linear transect at 20 m intervals. Traps were set up and uninstalled on a same day of collection (from 10am to 7pm approximately) to avoid mite loss. Both years of collections were completed between mid July and mid September each year. Beetles and their phoretic mites were recovered from the traps at the end of collection day, and placed in micro-centrifuge tubes individually for further inspection

in the laboratory. I recorded external mite species and number per individual beetle. I pooled all beetles from the three traps at one site on one collection day.

Beetle measurements

I measured each beetle's length and pronotum width to the nearest 0.2 mm using a dissecting microscope with a micrometer eyepiece in the laboratory. I used these beetle measurements to calculate body volume, which was the measurement I used for body size. For this purpose body volume was calculated assuming an ellipsoid shape such that volume = 4/3 x pi x length/2 x $(\text{width}/2)^2$. I also dissected each beetle to identify beetle sex, and recorded mites located under the elytra after external inspection and measurement of beetles. I also checked each micro-centrifuge to make sure all mites that an individual beetle carried were counted. Finally, I dehydrated all beetles for 24 hours at 50 ^{o}C to quantify dry body mass at the nearest 0.1 mg. I finally calculated an index of body condition, which is determined as the body mass residual with respect to the regression of body mass against body volume (Schulte-Hostedde et al. 2005). The mites used for taxonomic identification were removed from beetles and mounted on slides. The primary identification of phoretic mites was done with support of Dr. David Walter from the University of Alberta in early summer 2011 and later during the same summer with help from Dr. Hans Klompen from the Ohio State University and Dr. Ronald Ochoa from the Agricultural Research Service (ARS) of the United States Department of Agriculture (USDA) (refer to Apprendix A). Further verification of mite species was done with support from Dr. Diana Six who provided a personal collection of specimens identified by Dr. John Moser.

Statistical analysis

I performed all the analyses in the statistical program JMP® (Version 13. SAS Institute Inc., Cary, NC, 1989-2007). In this study, I calculated phoretic mite prevalence as the proportion of beetle hosts with mites (those with at least one mite). Mean abundance was calculated as the average number of phoretic mites present within a host sample, while mean intensity was calculated as the average number of phoretic mites present only in infested beetles in the sample (Bush et al. 1997). For descriptive purposes, I compared mite prevalence between sexes, and between parts of beetle spatial distribution using a contingency analysis for presence/absence of mites.

In order to investigate patterns of phoretic mite occurrence between the historical area of beetle outbreak and the new area of expansion, I examined each of the three response variables described above (prevalence, abundance, and intensity) in three individual models. These are three commonly used measurements to describe host population structure. The first model to look for differences in total mite prevalence (all three mite species included), I performed a generalized linear model with a binomial distribution, where the presence/absence of phoretic mites was the response variable and explanatory variables included range status, region, site, year, and beetle condition. To examine differences in total mite abundance (all species of phoretic mites included), I conducted a least square model using log transformed number of mites as the response variable with the explanatory variables of range status, region, site, year, and beetle condition. For the third model to look for differences in total mite intensity, I used the number of phoretic mites present among only infested beetles for each of the samples as response variable and explanatory variables included range status, region, site, year, and beetle

condition. I also performed three additional models to look for patterns of prevalence per individual species. In this case I performed a generalized linear model with a binomial distribution, where the presence/absence of mites was the response variable and explanatory variables included range status, region, site, year, and beetle condition. I included site and region as nested effects within outbreak status in all models I performed.

Results

Overall patterns of mite abundance

I collected 1186 adult beetles from sites within the new and the historical area of expansion of mountain pine beetle during a two-year period of collections (Fig. 4.1,Table B.1). Of these, 536 beetles carried phoretic mites (45% prevalence) (Table B.2). Phoretic mite prevalence was similar between males and females (χ^2= 2.180, P=0.1398) (Table B.2). I therefore did not include sex in subsequent models. In general, mite prevalence contrasted significantly between both parts of the range (χ^2= 6.214, P=0.0127) (Table B.3). The most prevalent mite species was *Tarsonemus ips* with 36% of hosts infected, followed by *Proctolaelaps subcorticalis* with 10%, and *Trichouropoda australis* with 7% (Fig. 4.1; Table B.4). Only three sites had no phoretic mites detected, and they were all in the new area of expansion of mountain pine beetle (Table B.1). Similarly, *T. ips* was commonly distributed in most sites of both parts of mountain pine beetle range except for three sites in the new area of expansion (Table B.5). Both *P. subcorticalis* and *T. australis* had lower mite prevalence, abundance and intensity among sites compare to *T. ips* and additionally they were mostly absent from sites of the new area of expansion (Table B.6 and B.7 respectively).

Phoretic mite prevalence, abundance and intensity in the new and historic range of mountain pine beetles

Phoretic mite prevalence and abundance significantly varied between the historical area of beetle outbreak and the new area of expansion (Table 4.1), being higher in the historical part than in the new part of mountain pine beetle expansion (Fig. 4.2 and 4.3). However, mite intensity was the same between historic and new parts of their range (Table 4.1). Within each of the two range types, prevalence and abundance, but not intensity, also differed among regions, sites and years of sampling (Table 4.1; Fig. 4.4 and 4.5). This is mostly because Penticton and different sites in Yoho had more beetles infested with mites (Table B.1 and 4.1). This was not the case for mite intensity since it was only significantly different for sites regardless of region or year (Table 4.1). Beetle condition did not influence the occurrence of mites (Table 4.1).

When looking at individual prevalence of each species *T. ips* prevalence was significantly different between the two parts of beetle expansion (Table 4.2), being higher in the historic area than in the new area (Fig. B.6). Additionally, *T. ips* prevalence was significantly different among regions, sites, and between years (2011 had higher prevalence than 2012). *P. subcorticalis* prevalence on the other hand, didn't differ between parts of beetle distribution, among sites, or between years (Fig. 4.7, Table 4.2). Instead, *P. subcorticalis* prevalence was significantly higher among regions and on those beetles in better condition. In the case of *T. australis* prevalence, I found significant differences among regions and between years only (Fig 4.1). For this particular species 2011 had less prevalence than 2012.

Discussion

Phoretic mites are common symbionts of mountain pine beetle although each species had different abundance and prevalence. *Tarsonemus ips*, *Proctolaelaps subcorticalis*, and *Trichouropoda australis* were present in most of the sites I sampled except for three sites located within the new area of expansion. These species are known to be regular associates of bark beetle hosts including different species of *Dendroctonus* (Mori et al. 2011). In this study, *T. ips* was the most common phoretic mite present in most of the sites I collected within both parts of mountain pine distribution and in relatively higher prevalence and intensity whereas *P. subcorticalis* and *Tr. australis* were rarely found. This is similar to what previous studies have found with respect to *T. ips* (Hofstetter et al. 2006, Hofstetter 2011, Mori et al. 2011, Hofstetter and Moser 2014). *T. ips* is a particularly important commensal symbiont that regulates the interaction between antagonist fungi associated with southern pine beetle (Hofstetter et al. 2006).

The main findings of this study suggest that the abundance and prevalence of phoretic mites were significantly lower in the new area of mountain pine beetle distribution. The new range of expansion, which includes northeast of British Columbia (Valemount) and northwestern Alberta (Grande Prairie and Peace River), is characterized for the outbreak advancement of mountain pine beetle in the last 30 years approximately (Safranyik et al. 2012). This rapid expansion of mountain pine beetle could have reduced the distribution of its phoretic symbionts particularly of *T. ips*. Nonetheless, the intensity of infestation was similar for both sites of beetle distribution. Only one previous study has also observed lower levels of abundance and prevalence of phoretic symbionts in mountain pine beetle in the new area of expansion, although not between the historical and new are of beetle range (Mori et al. 2011).

The loss of symbionts in range-shifting hosts in new areas of expansion could be a common phenomenon among different type of symbioses. Symbiont loss has been observed primarily in host-parasite symbioses (Reuter et al. 2005, Phillips et al. 2010b, Yang et al. 2010), plant-herbivores (Phillips et al. 2010a), and plant-fungal symbionts (Gundel et al. 2008, Gundel et al. 2011). My results suggest that the loss of symbionts, if common, could also extend to phoretic symbionts of insects. Although this symtiont loss has not been observed in other insect-phoretic mite symbiosis, it has been documented in certain commensal symbioses including insect-gut bacteria and insect-nematode symbioses (Yang et al. 2010, Wooding et al. 2013, Susoy and Herrmann 2014)

Some mechanisms have been proposed to explain how symbiont loss occurs. The enemy release hypothesis partially explains the absence of symbionts at leading fronts of expansion in host-parasite systems (Phillips et al. 2010a). Two components characterized this process, founder events followed by higher investment in traits associated to reproduction and dispersal. In the present study is possible that the lower abundance and prevalence of phoretic mites may be associated to founder events occurring during the rage expansion of mountain pine beetle. In fact there is evidence of founder effects in *Leptographium longiclavatum*, a fungal beneficial symbiont of mountain pine beetle which exhibits reduced genetic diversity in populations located near Valemount, BC (part of the new range of beetle distribution) (Tsui et al. 2014). This is particularly interesting in terms of the biology and potential for association between *T. ips* (the most common mite in my study) and fungal symbionts of mountain pine beetle. It is known that *T. ips* is a fungivore mite that helps spreading, *Ophiostoma montium*, in the southern pine beetle

(Lombardero et al. 2003). Moreover, previous evidence suggests that the abundance of *O. montium* increases with *T. ips* abundance in natural populations (Lombardero et al. 2003, Hofstetter et al. 2006). Although there is no record of this symbiosis to be obligate, it opens the possibility of whether this phoretic mite species may also spread other fungal symbionts of mountain pine beetle. To the best of my knowledge this is unknown and I suggest this being subject of further research.

The enemy release hypothesis also establishes that the lower density of symbionts at leading fronts ultimately release hosts from natural enemies (parasites and pathogens), allowing host to allocate higher investment in dispersal and reproductive traits (Phillips et al. 2010a, Phillips et al. 2010b). Higher body condition at sites of range expansion could be used as a surrogate for higher investment in host dispersal and reproductive traits (Kisdi et al. 2012). In fact, a previous study and my results on flight behaviour of mountain pine beetle (Chapter 2) confirmed that dispersal of this insect is a condition or phenotype-dependent activity, in other words individuals in better condition fly longer distances (Evenden et al. 2014). I would have some support for a similar "symbiont release mechanism", if body condition had been significantly higher at sites from the new area of expansion in addition to lower prevalence and abundance of phoretic mites. My results do not suggest such mechanism (Table 4.9). Instead, my results showed that body condition was similar in both parts of mountain pine beetle distribution, suggesting that beetle populations are not distributed by dispersal ability at the leading range of expansion precisely the way a "symbiont release mechanism" would predict (Phillips et al. 2010a).

One other alternative, relates to variability of symbiont transmission in mountain pine beetle habitat. Usually other species of bark beetles, such as pine engraver (*Ips pini*) and their symbionts colonize pine trees that have been previously attacked by mountain pine beetles. Although, pine engraver have a shorter life cycle than mountain pine beetle (i.e. reproduction an development of *I. pini* offspring is archive in the same summer), individuals of both species cohabit and reproduce at the same time providing the right conditions for interspecific transmission of phoretic mites to occur. It is likely, for instance, that *P. subcorticalis* and *T. australis* disperse with pine engravers instead of mountain pine beetle. These species could exhibit horizontal transmission (transmission among unrelated hosts) thus reducing or diluting the contact rate between those mite species and mountain pine beetles. This could explain why these two species are similarly found in low abundance and prevalence in both parts of mountain pine beetle distribution. There is actually evidence that indicates these two species are also present in different bark beetles species (Blackwell et al. 1988, Pfammatter et al. 2013, Pfammatter and Raffa 2015), suggesting that they may be horizontally transmitted.

In the particular case of *T. ips,* this species exhibits characteristics of an obligate and vertically transmitted symbiont, this particular mite biology could exhibit higher levels of mortality in the new area of beetle expansion. *T. ips* seems to be almost exclusively associated to *Dendroctonus* species including southern pine beetle (*Dendroctonus frontalis*), spruce beetle (*Dendroctonus rufipennis*) and mountain pine beetle (*Dendroctonus ponderosae*) suggesting a high level of dependency on *Dendroctonus* beetles. In southern pine beetle *T. ips* exhibits higher densities. In my study, although this particular species was the most abundant and prevalent when compared to the other two species it is present in lower numbers in the new area of expansion (Mori et al.

2011) and this could be to related to the nature of *T. ips* transmission mode. This mite species depends entirely on mountain pine beetle for development, reproduction, dispersal (see Introduction Chapter and Chapters two and three), and thus colonization of new habitats (Magowski 2010). Curiously, when mites are attached to pine beetle they do not abandon their host when a choice to switch hosts is offered, unless mites sense the chemical cues from pine phloem (personal observations), suggesting that these mites do not transfer horizontally. Furthermore *T. ips* individuals exhibit high mortality during beetle flight under laboratory conditions (see Chapter 3). Additionally mountain pine beetles showed lower abundances in the new area of expansion suggesting that founder effects may be in place. It is possible that, under such circumstances and considering *T. ips* biology together, individuals of this species experience higher mortality in the new area of expansion. Nonetheless it would be informative to explore whether different bark beetle species at the new area of expansion are also dispersing *T. ips* individuals. Lower levels of abundance and prevalence at the new area of expansion could also be related to high mite mortality in drier places, for instance, dehydration is prevalent and a common cause of mortality in the field in other mite species (Houck and OConnor 1991, Houck and Cohen 1995, Yoder et al. 1999). These ideas could help explain lower symbiont abundance and prevalence during periods of host advancement or at new areas of host expansion but they have not been tested yet.

Table 4.1. Generalized linear models for mite prevalence and standard least square models for mite abundance and intensity.

		Response variable						
		Mite prevalence		**Mite abundance**		**Mite intensity**		
Explanatory variables	**d.f.**	X^2	***P* value**	**F**	***P* value**	**d.f**	**F**	***P* value**
Host range	1	28.10	**<0.0001***	13.58	**0.0002***	1	1.14	0.2865
Region [Range]	4	87.40	**<0.0001***	12.36	**<0.0001***	4	0.61	0.6581
Site [Range, Region]	11	87.90	**<0.0001***	6.28	**<0.0001***	8	2.69	**0.0065***
Year	1	7.40	**0.0066***	5.15	**0.0234***	1	0.08	0.7710
Condition	1	1.56	0.2103	1.09	0.2955	1	0.014	0.9034
r^2			**<0.0001***	0.1275	**<0.0001***		0.0614	**0.0041***
n		1186		1186			536	

Table 4.2. Generalized linear models for mite prevalence for each species.

		Prevalence					
		T. ips		***P. subcorticalis***		***T. australis***	
Explanatory variables	**d.f.**	X^2	***P* value**	X^2	***P* value**	X^2	***P* value**
Host range	1	28.68	**<0.0001***	1.30	0.2548	0	1.00
Region	4	51.36	**<0.0001***	103.40	**<0.0001***	33.79	**<0.0001***
Site	11	91.44	**<0.0001***	2.39	0.9966	17.73	0.0880
Year	1	16.93	**<0.0001***	1.96	0.1612	7.42	**0.0064**
Condition	1	0.005	0.9424	6.42	**0.0113**	3.72	0.0536
r^2		170.16	**<0.0001***	152.77	**<0.0001***	108.29	**<0.0001***
n		1186		1186		1186	

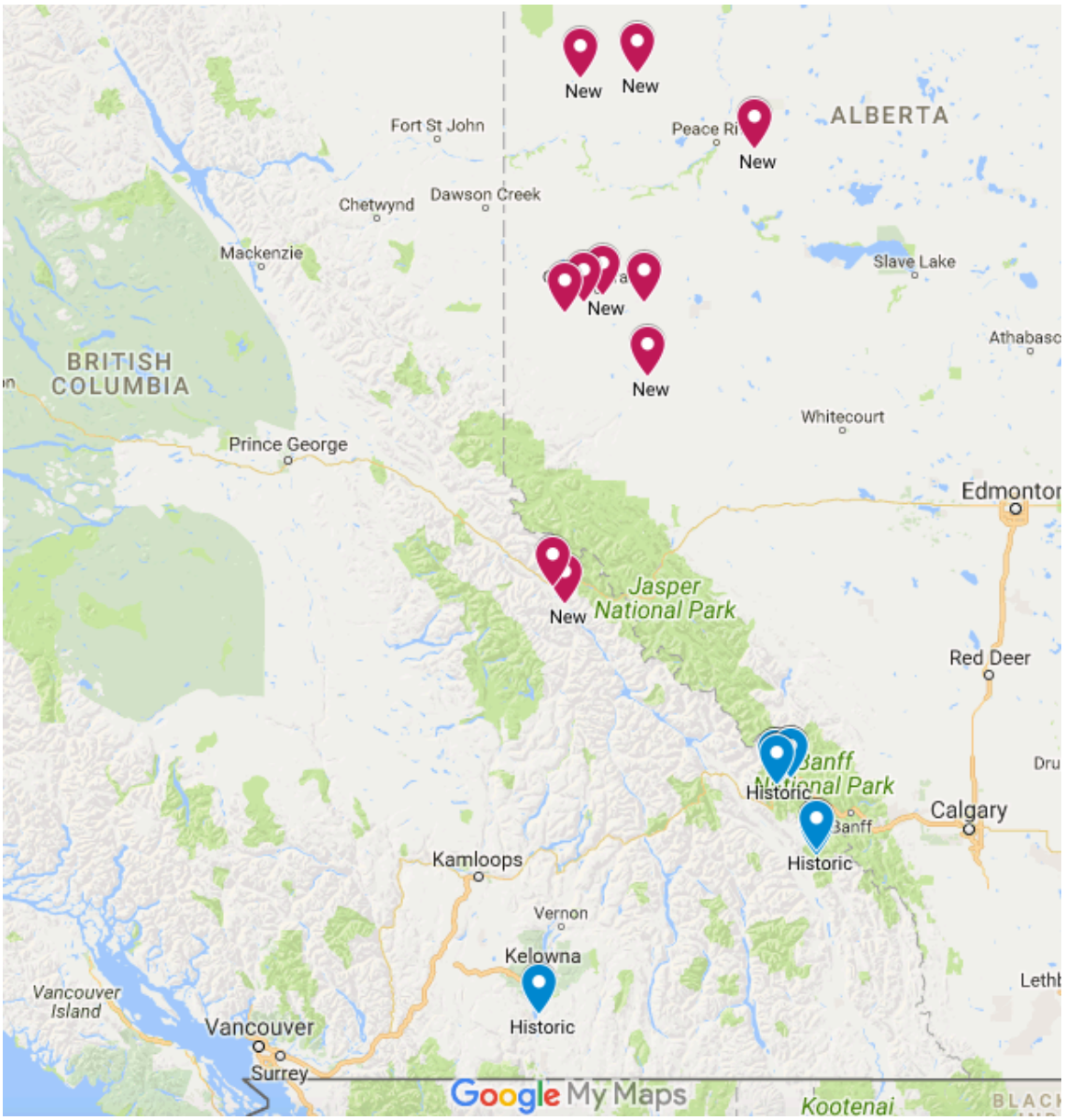

Fig. 4.1. Location map of sites where the study was conducted. Mountain pine collections were done over a period of two years, 2011 and 2012. Figure indicates both the new (red landmarks) and the historical range (blue landmarks) of mountain pine beetle expansion in western Canada.

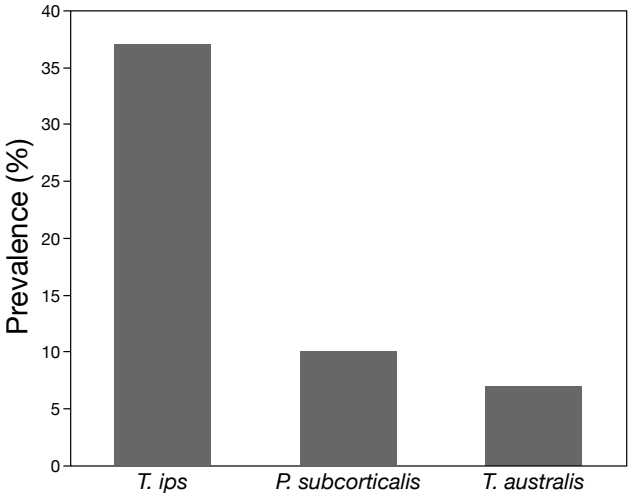

Fig. 4.2. Total mite prevalence per species. Prevalence is indicated in percentages.

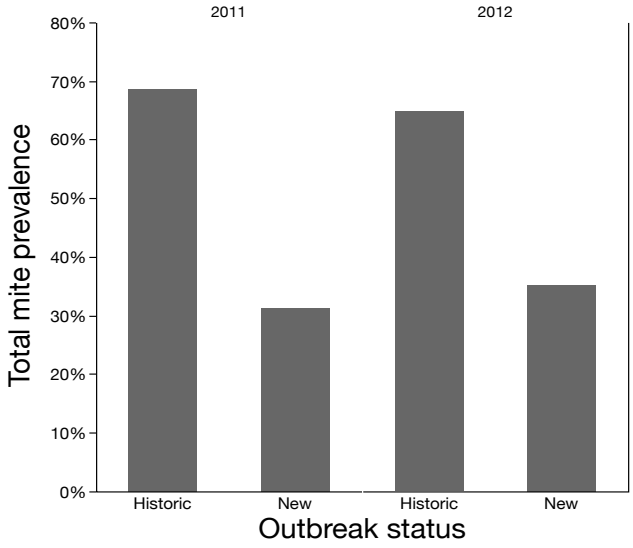

Fig. 4.3. Total mite prevalence distinguishing between range status and year. Prevalence is indicated in percentages.

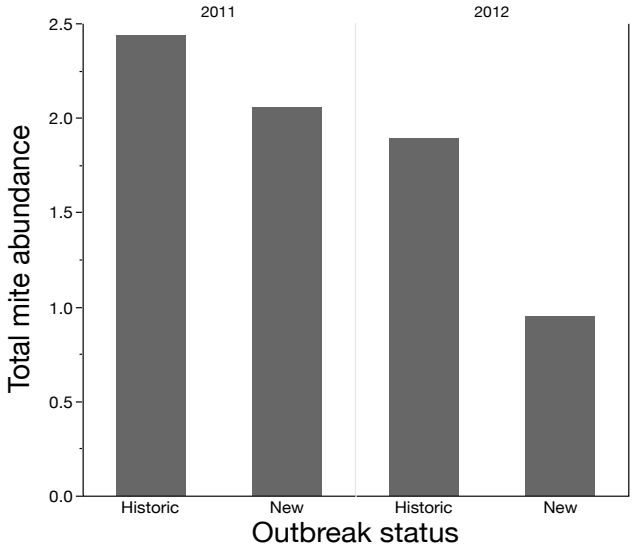

Fig. 4.4. Total mite abundance per range status for both years of study.

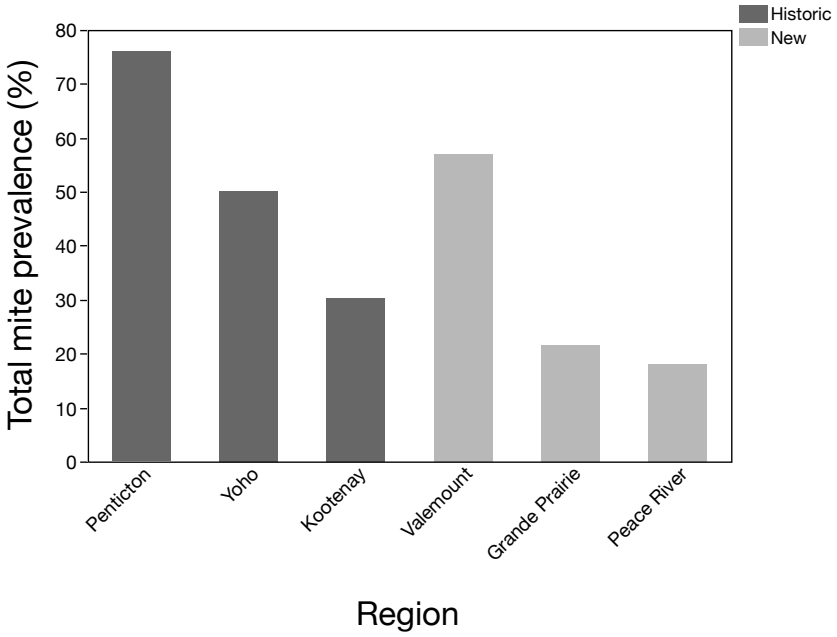

Fig. 4.5. Total mite prevalence per region and range status for both years of study. Prevalence is indicated in percentages.

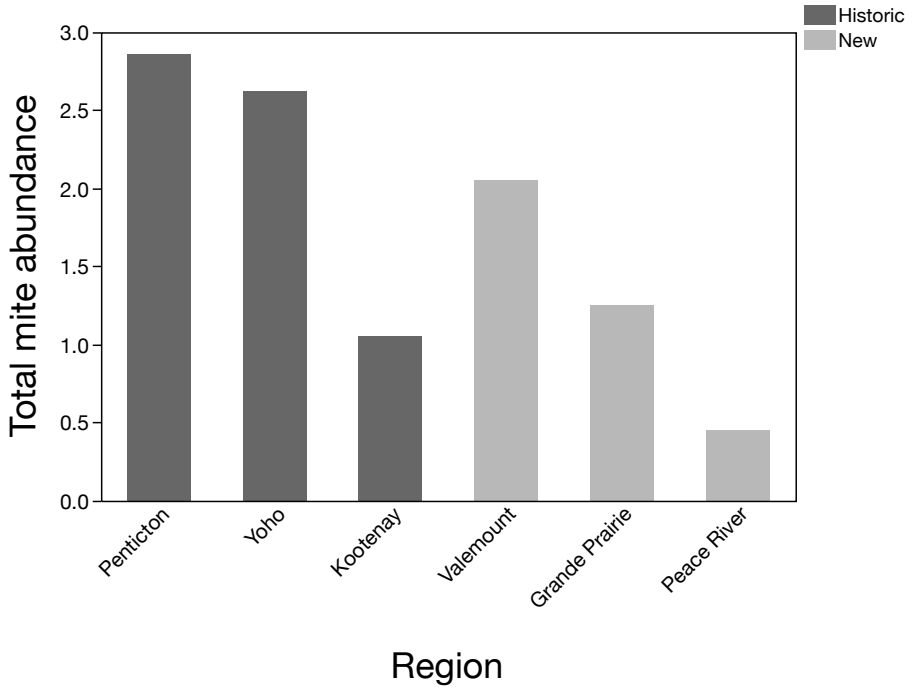

Fig. 4.6. Total mite abundance per region and range status for both years of study.

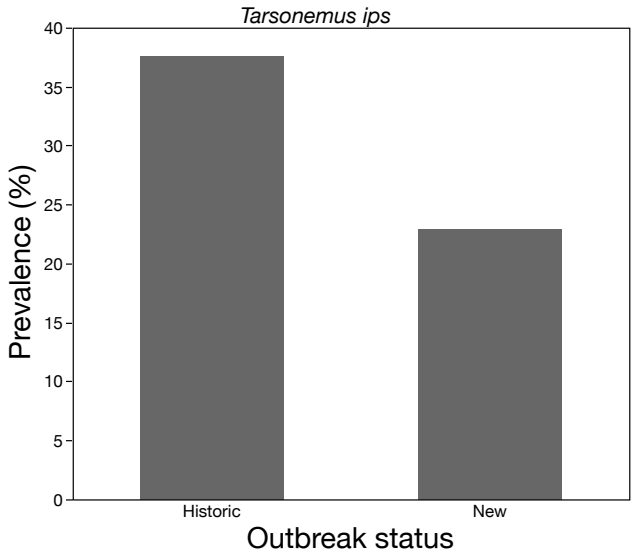

Fig. 4.7. *Tarsonemus ips* prevalence per range status. Prevalence in indicated in percentages.

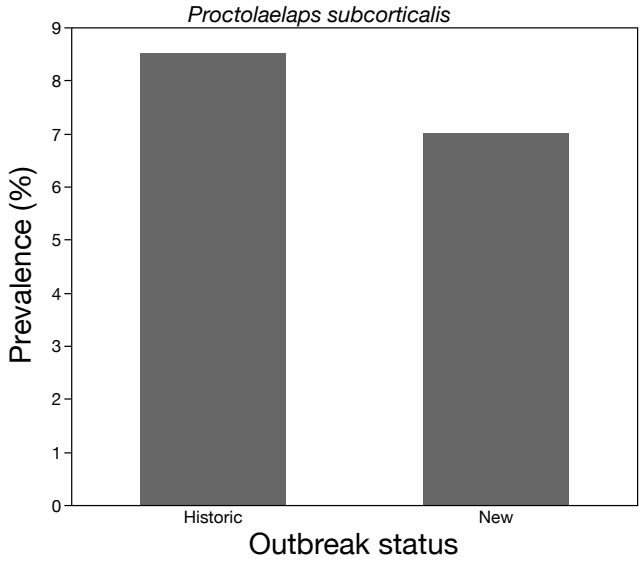

Fig. 4.8. *Proctolaelaps subcorticalis* prevalence per range status. Prevalence is indicated in percentages.

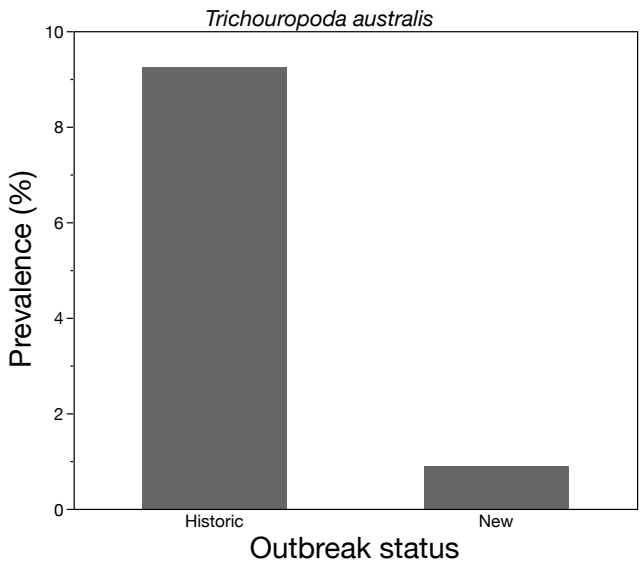

Fig. 4.9. *Trichouropoda australis* prevalence per range status. Prevalence is indicated in percentages

CHAPTER 5: CONCLUSION

Phoresy, typically considered as a short-term commensal interaction where phoretic symbionts utilize dispersive hosts for transmission to new colonization habitats, is expected to produce positive effects for symbionts and no effects for hosts, yet negative and positive effects have been documented. This poses the question of whether phoresy is indeed a commensal interaction and demands clarification. In bark beetles this is of particular relevance because both negative and positive effects have been documented primarily during beetle reproduction. Curiously, the effects on hosts during the actual dispersal event are largely unknown. In the present research, I investigated the ecological mechanisms that determine the net effects of the phoresy observed in mites and MPB. Given the results presented in this study, I am confident to say that mite phoresy in MPB is genuinely commensal and the implications of this symbiosis permeates in the existing distribution of phoretic mites exhibited in both parts (historic and new range of expansion) of MPB distribution. I conducted three empirical studies that support those views. I tested three main hypotheses that helped to clarify whether phoresy is a commensal symbiosis in MPB: a) long-distance dispersal of beetles predicts mite dispersal propensity and dispersal success of arrival; mite abundance does not impair host dispersal; b) variation of mite effects during beetle ontogeny explains the net outcome at the end of beetle development; and c) whether mite loss at the new range of mountain pine beetle expansion is explained by a 'symbiont release mechanism'.

Summary of Findings

In the first part of this research I determined that MPB dispersal was a phenotype-dependent activity and this only predicted dispersal propensity (mite abundance preflight) but not dispersal success of arrival in phoretic mites. Long-distance dispersal of MPB increased with beetle body size and body condition. In addition, younger and in better condition beetles had more mites at departure. However, dispersal success of arrival in phoretic mites did not depend on beetle traits or flight characteristics. For instance proportion of successful mites after flight did not vary with total distance flown or mean velocity. Furthermore, host dispersal was costly for both hosts and symbionts. Beetles exhibited a physiological cost in terms of mass loss as a response of its own behavioral activity and not as a consequence of carrying mites. In contrast, beetle dispersal was costly for mites. I detected mite mortality but this was not related to beetle characteristics or beetle dispersal components. Instead mite mortality was associated to mite abundance preflight. In summary, phoretic mites did not impeded beetle dispersal. These results certainly suggest that phoretic mites are genuinely commensal during MPB dispersal.

In the second portion of this research, I investigated the effects of phoretic mites during MPB reproduction. I determined the number and survivorship of all juvenile stages of MPB development and found neutral effects of phoretic mites on beetle fitness. On one hand, beetle fathers from the mite treatment had a positive effect on beetle larval survivorship but those fathers with more mites exhibited a comparatively lower larval survivorship. These results contrast with the lack of mite effects in subsequent beetle developmental stages. In addition, mites did not have any effect on the adult quality or numbers of emerging beetles. Nonetheless, mite offspring abundance increased with both the number of beetle adult offspring and beetle

volume. The lack of continuous positive or negative effect of phoretic mites on beetle fitness and the positive effects of beetles on mite fitness, suggests that the interaction between MPB and its phoretic mites should be deemed as commensal during the reproduction of MPB

In the third part of this research, I examined the dynamics of phoretic mites and beetle hosts at the population level in the field and I found that the range-expanding behavior of mountain pine beetle might explain symbiont loss at the leading front of beetle expansion. Three phoretic mites were commonly found: *Tarsonemus ips*, *Proctolaelaps subcorticalis*, and *Trichouropoda australis*. Of these species, *T. ips* was the most prevalent mite. However, both the total mite abundance, when considering all three species together, and *T. ips* abundance alone were comparatively lower in the new area of MPB expansion. In addition, beetle body condition was similar in both parts of beetle distribution, suggesting that beetle populations are not distributed by dispersal ability at the leading range of expansion.

Ecological Implications of Phoresy at the Individual Level

Dispersal

Phoretic organisms can be distinguished from ectoparasites in that they do not extract resources from their host for development or reproduction when they are attached to the host as ectoparasites naturally do during host dispersal. If phoretic mites were parasites, I would have detected that mite density was physiologically costly and/or a physical burden for beetles. In particular, poor quality beetles would have exhibited lower dispersal ability than beetles in better phenotypic condition. However, it is not easy to infer how a mutualistic scenario would have looked like during dispersal of MPB. A mutualistic effect would be more accurately detectable

during beetle reproduction but this was not the case in this research (Chapter 3; see next subsection).

Dispersal is a very costly life-history trait for any organism and its costs are present at any stage of the dispersal process (Bonte et al. 2012). In my study beetles and mites seemed to have paid the costs of dispersal in different fitness currencies. Beetles experienced the physiological costs of movement whereas phoretic mites exhibited the mortality costs associated to the transit. In my study there were no other biotic influences that could explain mortality costs in mites, such as increased predation or settlement in unsuitable habitats (Bonte et al. 2012). It is likely that mite mortality could be associated to physiological changes, such as dehydration (Houck and OConnor 1991). Other explanations pertain to mortality associated to density-dependent effects at the infrapopulation level where competition between mites for better attachment sites in a host can occur (Skelton et al. 2016). These ideas are not exhaustive and require of careful exploration.

Beyond the mortality cost mites exhibited, the individual response of mites during this particular fragment of the interaction with MPB is likely to have implications in terms of mite transmission. Each portion of this symbiosis can impose very specific ecological pressures for mites. The inability of long-distance dispersal in phoretic mites and the deterioration of the habitat are two potential pressures that may select for a symbiont dispersal strategy. These pressures could select for higher transmissibility of these commensals. In contrast, I did not detect higher transmissibility of phoretic mites. Although mites seemed to respond to initial beetle traits that predict long-distance dispersal (younger, larger and in better condition beetles carried more mites preflight), the proportion of successful mites at the end of the flight mill

exercise was not explain by either beetle traits or flight characteristics. Phoretic mites of MPB had the same success of arrival as beetles had. It is possible that the mite mortality I detected can be responsible of no negative effects for beetles during dispersal.

Reproduction

The majority of studies that have looked at the effects of phoretic mites in insects have focused on the settlement part of this phoretic symbiosis (Wilson and Knollenberg 1987, Hofstetter et al. 2006, Grossman and Smith 2008, Hodgkin et al. 2010, Fronhofer et al. 2013, De Gasperin and Kilner 2015a, b, Pfammatter and Raffa 2015). Settlement refers to what occurs after host dispersal, which includes reproduction. This is a critical portion of a phoretic symbiosis because this determines the fitness of both host and symbionts before the next dispersal event. From a host perspective, the literature provides contradictory evidence; phoretic mites can produce either negative or positive effects on hosts suggesting that phoresy might not be a commensal interaction. In the mutualistic scenario, production of more and in better condition progeny in hosts increases with phoretic symbiont prevalence. (Wilson and Knollenberg 1987, Okabe and Makino 2008, Hodgkin et al. 2010). In the parasitic scenario, phoretic symbionts have detrimental effects on host development, reduced offspring production and lower offspring quality (Polak 1996, Lombardero et al. 2003, Hofstetter et al. 2006, Okabe and Makino 2008, Hodgkin et al. 2010). In contrast, I detected a neutral effect of phoretic mites in the fitness of MPB.

There are several implications to these findings. First, the lack of consistency in the effects of phoretic mites through host ontogeny (positive-negative during larval stage and then neutral in

later stages of brood development) suggests that outcome fluctuations during development could also be present in phoretic symbioses (De Gasperin and Kilner 2015a, Skelton et al. 2016). Not only parasitic or mutualistic symbioses produce outcome fluctuations, commensal symbioses may too and this could be more common than previously thought. Although theory from both ends of the literature spectrum of symbioses (parasitism and mutualism) has argued the ubiquity of outcome fluctuations within single symbioses across space and time (Bronstein 1994, Leung and Poulin 2008), it is not clear how this occurs at a more immediate ecological scale, for instance during host ontogeny as my study showed.

Secondly, although parents' quality influence the number and survival of developing offspring, my study showed that phoretic mites may still influence beetle host development at least partially. It should be noted, however, that the influence of mites (presence and/or abundance) and beetle quality might not be mutually exclusive. The non-random association between phoretic mites and MPB (larger beetles had more mites) for both parents and offspring could be ecologically and perhaps evolutionary advantageous. Interestingly, this apparent condition-dependent transmission of phoretic symbionts did not translate into higher chances of transmission. Phoretic mites have the same success of arrival hosts have (Chapter 2) (Schwarz and Müller 1992, Benton and Bowler 2012, Kisdi et al. 2012, Fronhofer et al. 2013, Yule et al. 2013, Skelton et al. 2015).

Third, host parents may contribute differently to offspring development and this could be influence or be signaled by mites. In MPB, females share a big portion of the reproductive costs that perhaps overlooks the potential influence fathers have upon reproduction, parental care and

transmission of symbionts (De Gasperin et al. 2015, De Gasperin and Kilner 2015a). The challenge detecting this mechanism seem to be related to the inability of detecting sexual differences in phoretic mite abundance. For instance, I did not detect sexual differences in the number of mites in the field (Chapter 4) or in the number of mites preflight (Chapter 2).

Fourth, even if phoretic mites had negligible effects during MPB development, the fact that mite abundance increased with host abundance similar to what has been predicted for host-parasite symbioses implies that this mechanism of apparent condition-dependent transmissibility is a critical population-level process for the dispersal and thus persistence of phoretic symbiont populations as well (Benton and Bowler 2012, Fronhofer et al. 2013).

Ecological Implications of Phoresy at the Population Level

The rapid expansion of mountain pine beetle seemed to have reduced the distribution of its phoretic symbionts particularly of *T. ips.* In parasitic and mutualistic symbioses, symbionts can regulate host populations, if they cause a decrease or increase in host fecundity and/or densities respectively, and in the case of parasitic symbionts, if they can incur as well higher mortality (increase in virulence) in host populations. However, in the case of commensal symbiosis this seems to occur the opposite way: hosts regulate symbiont populations.

In the field, total mite abundance and prevalence (when considering all three species), and *T. ips*. abundance and prevalence alone were lower in the new range of beetle expansion. This can be suggestive of a 'symbiont release' mechanism similar to what has been observed in parasites and pathogens: founder events followed by higher investment in traits associated to reproduction

and dispersal (Phillips et al. 2010a). In my particular study, if mite transmission is density dependent (Chapter 3), it is possible that the low prevalence and/or abundance of phoretic mites of MPB at the new range of beetle expansion may be the response of a founder effect. This mechanism should be complemented by higher host investment in traits associated to reproduction and dispersal in the leading range of expansion. Curiously, MPB exhibited phenotype-dependent dispersal in the laboratory: larger and in better condition beetles flew longer distances (Chapter 2). However, when I compared the size and condition of beetles (surrogates of higher investment) between the two parts of beetle distribution (Chapter 4), I did not detect such relationship. Evidence of founder events in one of MPB fungal symbionts exists, *Leptographium longiclavatum*, from near Valemount, BC part of the new range of beetle distribution(Tsui et al. 2014). Founder events, however, remain to be confirmed through genetic work for phoretic mites of MPB.

General Implication of this Study

The unprecedented impact MPB has caused in the ecological integrity of western Canadian forests has prompted remarkable efforts to understand and predict its outbreak dynamics and factors influencing its life-history, ecology and behavior (Safranyik and Carroll 2006, Six and Wingfield 2011, Safranyik et al. 2012, Six 2013, Cooke and Carroll 2017). A consequence of this outbreak dynamic is the rapid expansion from the northeast front of beetle historical range to the boreal forest posing a serious threat to not only other pine host species but also to the dynamics of other coexisting species. As a result, several aspects of beetle ecology and life-history have been the focus of current study, including habitat selection (Latty and Reid 2009), dispersal capacity (Evenden et al. 2014), mating behavior (Reid and Baruch 2010), sex ratios

(Lachowsky and Reid 2014), interactions with fungal symbionts (Bleiker et al. 2012), and interactions with other organisms (Adams and Six 2008)

The early works of M.D. Atkins on the effects of phoretic symbionts in another bark beetle (*Dendroctonus pseudotsugae*) established the foundation for the modern study of phoretic symbionts in bark beetles (Atkins 1959, 1960, 1961). His works partly aimed to understand whether phoretic nematodes and mites could affect aspects of beetle dispersal. He pioneered the utilization of a basic flight mill to determine components of beetle flight (Atkins 1961). Although his research did not find conclusive evidence of negative effects of nematodes and mites on the dispersal of *D. pseudotsugae*, his works opened the possibility to implement an improved version of the flight mill in the research of biocontrol insects and other forests pests including MPB (Blackmer et al. 2004, Williams and Robertson 2008, Elliott and Evenden 2009, Chen et al. 2011, Elliott and Evenden 2012, Evenden et al. 2014, Khuhro et al. 2014, Lopez et al. 2014, Attisano et al. 2015, Gaudon et al. 2016, Lopez et al. 2017).

The interest in using phoretic mites as a biological control of forest pests increased as a consequence of exceptional outbreaks of southern pine beetle (*Dendroctonus frontalis*) in the States during the 1960's. Drs. Kinn, Lindquist and Moser, to mention some examples, produced exceptional work in mite taxonomy and aspects of mite biology and ecology (Lindquist 1969, 1971, Moser and Roton 1971, Moser 1975, 1976a, Kinn and Witcosky 1977, Kinn 1983). Although a prototype for biological control in the southern pine beetle using mites was not conclusive, this effort continues and has extended to investigating phoretic mites of other bark beetles including, pine engraver (*Ips pini*) and MPB.

Biocontrol denotes a method of controlling pest species with the introduction of an antagonist species. An antagonist species could be a parasite, a predator, or a parasitoid. This practice has been useful in the control of pests of agricultural importance using mites. In bark beetles this practice has not been successful but knowledge in mite biology and ecology is still lacking. This has implications particularly for the current MPB outbreak in western Canada. Phoretic mites of MPB, particularly *Tarsonemus ips*, might not be a good candidate for biocontrol. Its effects on the dispersal (Chapter 2) and reproduction of MPB (Chapter 3) are negligible. In southern pine beetle, *Tarsonemus ips*, contributes with beetle mortality through spreading of an antagonist fungus (Hofstetter et al. 2006). In this case *Tarsonemus ips* does represent a potential candidate for biocontrol. In contrast, in MPB is still unknown whether adults also carry an antagonist fungus that mites could spread. The information available in this regard indicates that MPB carries only mutualistic fungus (Bleiker and Six 2007), suggesting that phoretic mites could contribute to the spreading of beneficial fungus of MPB. Moreover, phoretic mites of MPB did not have a detectable negative or positive effect on its reproductive success (Chapter 3), which has implications in the light of the current outbreak. The neutral effects phoretic mites of MPB had in this study could also account for the current success of its outbreak. With no natural enemies that can control MPB advancement selection pressures can become weak. MPB could allocate resources to other life-history traits (i.e. immune system) that can influence their rapid advancement.

Unlike the null biocontrol potential of phoretic mites of MPB, they are likely to be major predictors of MPB population dynamics. In general, MPB carries lower diversity and abundance

of phoretic mites in comparison to what has been documented for other major forest pests in North America (Moser and Roton 1971, Cardoza et al. 2008, Hofstetter 2011, Mori et al. 2011, Pfammatter et al. 2013, Chaires-Grijalva et al. 2015). This dissimilarity is likely an indication of the rapid progression MPB has experienced over the past 20 years in comparison to the relatively gradual expansion of southern pine beetle over the past 50 years. Yet the existing phoretic mite distribution is likely the response of local processes that remain largely unexplored. Effort should be directed to consider aspects that affect the endemic ecology of MPB and its phoretic mites.

LITERATURE CITED

Adams, A. S., and D. L. Six. 2008. Detection of host habitat by parasitoids using cues associated with mycangial fungi of the mountain pine beetle, *Dendroctonus ponderosae*. Can Entomol **140**:124-127.

Agnew, P., J. C. Koella, and Y. Michalakis. 2000. Host life history responses to parasitism. Microbes and Infection **2**:891-896.

Al-Atawi, F., H. Klompen, and J. C. Moser. 2002. Redescription of *Schizosthetus lyriformis* (McGraw and Farrier, 1969) (Parasitiformes: Parasitidae), with revision of the genus. International Journal of Acarology **28**:341-360.

Antonovics, J., A. J. Wilson, M. R. Forbes, H. C. Hauffe, E. R. Kallio, H. C. Leggett, B. Longdon, B. Okamura, S. M. Sait, and J. P. Webster. 2017. The evolution of transmission mode. Philos Trans R Soc Lond B Biol Sci **372**.

Arneberg, P., A. Skorping, B. Grenfell, and A. F. Read. 1998. Host densities as determinants of abundance in parasite communities. Proc Biol Sci **265**:1283-1289.

Athias-Binche, F. 1993. Dispersal in varying environments: The case of phoretic uropodid mites. Can. J. Zool. **71**:1793-1798.

Athias-Binche, F., and S. Morand. 1993. From phoresy to parasitism: The example of mites and nematodes. Research and Reviews in Parasitology **53**:73-79.

Atkins, M. D. 1959. A study of the flight of the douglas-fir beetle *Dendroctonus pseudotsugae* Hopk. (Coleoptera: Scolytidae) I. Flight preparation and response. The Canadian Entomologist **91**:283-291.

Atkins, M. D. 1960. A study of the flight of the douglas-fir beetle *Dendroctonus pseudotsugae* Hopk. (Coleoptera: Scolytidae) II. Flight movements. The Canadian Entomologist **92**:941-954.

Atkins, M. D. 1961. A study of the flight of the douglas-fir beetle *Dendroctonus pseudotsugae* Hopk. (Coleoptera: Scolytidae) iii. Flight capacity. The Canadian Entomologist **93**:467-474.

Attisano, A., J. T. Murphy, A. Vickers, and P. J. Moore. 2015. A simple flight mill for the study of tethered flight in insects. J Vis Exp:e53377.

Baines, C. B., S. J. McCauley, and L. Rowe. 2015. Dispersal depends on body condition and predation risk in the semi-aquatic insect, *Notonecta undulata*. Ecol Evol **5**:2307-2316.

Bajerlein, D., and M. Przewoźny. 2012. When a beetle is too small to carry phoretic mites? A case of hydrophilid beetles (Coleoptera: Hydrophilidae) and *Uropoda orbicularis* (Acari: Mesostigmata). Canadian Journal of Zoology **90**:368-375.

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software **67**.

Belozerov, V. N. 2009. Diapause and quiescence as two main kinds of dormancy and their significance in life cycles of mites and ticks (Chelicerata: Arachnida: Acari). Part 1. Acariformes. Acarina **16**:79-130.

Benton, T. G., and D. E. Bowler. 2012. Dispersal in invertebrates: Influences on individual decisions. Pages 41-49 *in* J. Clobert, M. Baguette, T. G. Benton, and J. M. Bullock, editors. Dispersal Ecology and Evolution. Oxford University Press.

Bentz, B. J., and D. E. Mullins. 1999. Ecology of mountain pine beetle (Coleoptera: Scolytidae) cold hardening in the intermountain west. Environ Entomol **28**:577–587.
Berg, M. P., E. T. Kiers, G. Driessen, M. van der Heijden, B. W. Kooi, F. Kuenen, M. Liefting, H. A. Verhoef, and J. Ellers. 2010. Adapt or disperse: Understanding species persistence in a changing world. Global Change Biology **16**:587-598.
Biani, N. B., U. G. Mueller, and W. T. Wcislo. 2009. Cleaner mites: Sanitary mutualism in the miniature ecosystem of neotropical bee nests. Am Nat **173**:841-847.
Binns, E. S. 1982. Phoresy as migration - some functional aspects of phoresy in mites. Biol Rev **57**:571-620.
Blackmer, J. L., S. E. Naranjo, and L. H. Williams. 2004. Tethered and untethered flight by *Lygus hesperus* and *Lygus lineolaris* (Heteroptera: Miridae). Environ Entomol **33**:1389-1400.
Blackwell, M., J. C. Moser, and J. Wisniewski. 1988. Ascospores of *Pyxidiophora* on mites associated with beetles in trees and wood. Mycol Res **92**:397-403.
Bleiker, K. P., S. E. Potter, C. R. Lauzon, and D. L. Six. 2012. Transport of fungal symbionts by mountain pine beetles. The Canadian Entomologist **141**:503-514.
Bleiker, K. P., and D. L. Six. 2007. Dietary benefits of fungal associates to an eruptive herbivore: Potential implications of multiple associates on host population dynamics. Environ Entomol **36**:1384-1396.
Bleiker, K. P., and D. L. Six. 2009. Competition and coexistence in a multi-partner mutualism: Interactions between two fungal symbionts of the mountain pine beetle in beetle-attacked trees. Microb Ecol **57**:191-202.
Bonte, D., and E. De la Peña. 2009. Evolution of body condition-dependent dispersal in metapopulations. J Evol Biol **22**:1242-1251.
Bonte, D., A. D. Roissart, N. Wybouw, and T. V. Leeuwen. 2014. Fitness maximization by dispersal: Evidence from an invasion experiment. Ecology **95**:3104-3111.
Bonte, D., H. Van Dyck, J. M. Bullock, A. Coulon, M. Delgado, M. Gibbs, V. Lehouck, E. Matthysen, K. Mustin, M. Saastamoinen, N. Schtickzelle, V. M. Stevens, S. Vandewoestijne, M. Baguette, K. Barton, T. G. Benton, A. Chaput-Bardy, J. Clobert, C. Dytham, T. Hovestadt, C. M. Meier, S. C. Palmer, C. Turlure, and J. M. Travis. 2012. Costs of dispersal. Biol Rev Camb Philos Soc **87**:290-312.
Bowler, D. E., and T. G. Benton. 2005. Causes and consequences of animal dispersal strategies: Relating individual behaviour to spatial dynamics. Biological Reviews **80**:205-225.
Bowler, D. E., and T. G. Benton. 2009. Variation in dispersal mortality and dispersal propensity among individuals: The effects of age, sex and resource availability. J Anim Ecol **78**:1234-1241.
Bridges, R. J., and J. C. Moser. 1983. Role of two phoretic mites in transmission of bluestain fungus, *Ceratocystis minor*. Ecological Entomology **8**:9-12.
Bright, M., and S. Bulgheresi. 2010. A complex journey: Transmission of microbial symbionts. Nat Rev Microbiol **8**:218-230.
Bronstein, J. L. 1994. Conditional outcomes in mutualistic interations. Trends Ecol Evol **9**:214-217.
Brown, B. L., R. P. Creed, J. Skelton, M. A. Rollins, and K. J. Farrell. 2012. The fine line between mutualism and parasitism: complex effects in a cleaning symbiosis demonstrated by multiple field experiments. Oecologia **170**:199-207.

Brownlie, J. C., and K. N. Johnson. 2009. Symbiont-mediated protection in insect hosts. Trends Microbiol **17**:348-354.
Bush, A. O., K. D. Lafferty, J. M. Lotz, and A. W. Shostak. 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. The Journal of Parasitology **83**:575-583.
Cahill, A. E., M. E. Aiello-Lammens, M. C. Fisher-Reid, X. Hua, C. J. Karanewsky, H. Y. Ryu, G. C. Sbeglia, F. Spagnolo, J. B. Waldron, O. Warsi, and J. J. Wiens. 2013. How does climate change cause extinction? Proc Biol Sci **280**:20121890.
Cardoza, Y. J., J. C. Moser, K. D. Klepzig, and K. Raffa. 2008. Multipartite symbioses among fungi, mites, nematodes, and the spruce beetle, *Dendroctonus rufipennis*. Community and Ecosystem Ecology **37**:956-963.
Chaires-Grijalva, M. P., E. Estrada-Venegas, A. Equihua-Martínez, J. C. Moser, and S. R. Blomquist. 2015. Ácaros digamáselidos (Acari: Mesostigmata: Digamasellidae) asociados a descortezadores en México. Entomología Mexicana **2**:100-105.
Chen, H., Z. Li, S. H. Bu, and Z. Q. Tian. 2011. Flight of the Chinese white pine beetle (Coleoptera: Scolytidae) in relation to sex, body weight and energy reserve. Bull Entomol Res **101**:53-62.
Clobert, J., J. F. Le Galliard, J. Cote, S. Meylan, and M. Massot. 2009. Informed dispersal, heterogeneity in animal dispersal syndromes and the dynamics of spatially structured populations. Ecol Lett **12**:197-209.
Coates, A., L. K. Barnett, C. Hoskin, and B. L. Phillips. 2017. Living on the edge: Parasite prevalence changes dramatically across a range edge in an invasive gecko. Am Nat **189**:178-183.
Comins, H. N., W. D. Hamilton, and R. M. May. 1980. Evolutionarily stable dispersal strategies. J Theor Biol **82**:205-230.
Cooke, B. J., and A. L. Carroll. 2017. Predicting the risk of mountain pine beetle spread to eastern pine forests: Considering uncertainty in uncertain times. Forest Ecology and Management **396**:11-25.
De Gasperin, O., A. Duarte, and R. M. Kilner. 2015. Interspecific interactions explain variation in the duration of paternal care in the burying beetle. Animal Behaviour **109**:199-207.
De Gasperin, O., and R. M. Kilner. 2015a. Friend or foe: Inter-specific interactions and conflicts of interest within the family. Ecological Entomology **40**:787-795.
De Gasperin, O., and R. M. Kilner. 2015b. Interspecific interactions change the outcome of sexual conflict over prehatching parental investment in the burying beetle *Nicrophorus vespilloides*. Ecol Evol **5**:1-9.
Debeffe, L., N. Morellet, B. Cargnelutti, B. Lourtet, R. Bon, J. M. Gaillard, and A. J. Mark Hewison. 2012. Condition-dependent natal dispersal in a large herbivore: Heavier animals show a greater propensity to disperse and travel further. J Anim Ecol **81**:1327.
Denison, R. F., and E. T. Kiers. 2011. Life histories of symbiotic rhizobia and mycorrhizal fungi. Curr Biol **21**:R775-785.
Dingle, H., N. R. Blakley, and E. R. Miller. 1980. Variation in body size and flight performance in milkweed bugs (*Oncopeltus*). Evolution **34**:371-385.
Douglas, A. E. 1998. Host benefit and the evolution of specialization in symbiosis. Heredity (Edinb) **81**:599–603.
Ebert, D. 2013. The epidemiology and evolution of symbionts with mixed-mode transmission. Annual Review of Ecology, Evolution, and Systematics **44**:623-643.

Eickwort, G. C. 1990. Associations of mites with social insects. Annu Rev Entomol **35**:469-188.
Elliott, C. G., and M. L. Evenden. 2009. Factors influencing flight potential of *Choristoneura conflictana*. Physiological Entomology **34**:71-78.
Elliott, C. G., and M. L. Evenden. 2012. The effect of flight on reproduction in an outbreaking forest lepidopteran. Physiological Entomology **37**:219-226.
Evenden, M. L., C. M. Whitehouse, and J. Sykes. 2014. Factors influencing flight capacity of the mountain pine beetle (Coleoptera: Curculionidae: Scolytinae). Environ Entomol **43**:187-196.
Ewald, P. W. 1987. Transmission modes and evolution of the parasitism-mutualism continuum. Ann N Y Acad Sci **503**:295-306.
Fretwell, S. D., and H. L. J. Lucas. 1970. On territotial behviour and other factors influencing habitat distribution in birds. Acta Biotheoretica **19**:16-36.
Fronhofer, E. A., E. B. Sperr, A. Kreis, M. Ayasse, H. J. Poethke, and M. Tschapka. 2013. Picky hitch-hikers: Vector choice leads to directed dispersal and fat-tailed kernels in a passively dispersing mite. Oikos **122**:1254-1264.
Gaudon, J. M., L. J. Haavik, C. J. K. MacQuarrie, S. M. Smith, and J. D. Allison. 2016. Influence of nematode parasitism, body size, temperature, and diel period on the flight capacity of *Sirex noctilio* F. (Hymenoptera: Siricidae). Journal of Insect Behavior **29**:301-314.
Grossman, J. D., and R. J. Smith. 2008. Phoretic mite discrimination among male burying beetle (*Nicrophorus investigator*) hosts. Annals of the Entomological Society of America **101**:266-271.
Gundel, P. E., W. B. Batista, M. Texeira, M. A. Martinez-Ghersa, M. Omacini, and C. M. Ghersa. 2008. *Neotyphodium* endophyte infection frequency in annual grass populations: Relative importance of mutualism and transmission efficiency. Proc Biol Sci **275**:897-905.
Gundel, P. E., J. A. Rudgers, and C. M. Ghersa. 2011. Incorporating the process of vertical transmission into understanding of host-symbiont dynamics. Oikos **120**:1121-1128.
Hamilton, W. D., and R. M. May. 1977. Dispersal in stable habitats. Nature **269**:578-581.
Herre, E., N. Knowlton, U. Mueller, and S. Rehner. 1999. The evolution of mutualisms: Exploring the paths between conflict and cooperation. Trends Ecol Evol **14**:49-53.
Hodgkin, L. K., M. A. Elgar, and M. R. E. Symonds. 2010. Positive and negative effects of phoretic mites on the reproductive output of an invasive bark beetle. Australian Journal of Zoology **58**:198-204.
Hofstetter, R. W. 2011. Mutualists and phoronts of the southern pine beetle. Pages 161-181 *in* R. N. Coulson and K. D. Klepzig, editors. Southern Pine Beetle II.
Hofstetter, R. W., J. T. Cronin, K. D. Klepzig, J. C. Moser, and M. P. Ayres. 2006. Antagonisms, mutualisms and commensalisms affect outbreak dynamics of the southern pine beetle. Oecologia **147**:679-691.
Hofstetter, R. W., and J. C. Moser. 2014. The role of mites in insect-fungus associations. Annu Rev Entomol **59**:537-557.
Hofstetter, R. W., J. C. Moser, and R. McGuire. 2009. Observations on the mite *Schizosthetus lyriformis* (Acari: Parasitidae) preying on bark beetle eggs and larvae. Entomological News **120**:397-400.

Holland, J. N., and D. L. DeAngelis. 2009. Consumer-resource theory predicts dynamic transitions between outcomes of interspecific interactions. Ecol Lett **12**:1357-1366.
Hooper, L. V., and J. I. Gordon. 2001. Commensal host-bacterial relationships in the gut. Science **292**:1115-1118.
Hopkins, S. R., L. J. Boyle, L. K. Belden, and J. M. Wojdak. 2015. Dispersal of a defensive symbiont depends on contact between hosts, host health, and host size. Oecologia **179**:307-318.
Houck, M. A., and A. C. Cohen. 1995. The potential role of phoresy in the evolution of parasitism: radiolabelling (tritium) evidence from an astigmatid mites. Exp Appl Acarol **19**:677-694.
Houck, M. A., and B. M. OConnor. 1991. Ecological and evolutionary significance of phoresy in the Astigmata. Annu Rev Entomol **36**:611-636.
Hulcr, J., and R. R. Dunn. 2011. The sudden emergence of pathogenicity in insect-fungus symbioses threatens naive forest ecosystems. Proc Biol Sci **278**:2866-2873.
Hulcr, J., and L. L. Stelinski. 2017. The ambrosia symbiosis: From evolutionary ecology to practical management. Annu Rev Entomol **62**:285-303.
Hunter, P. E., and R. M. T. Rosario. 1988. Associations of Mesostigmata with other arthropods. Annu Rev Entomol **33**:393-417.
Husseneder, C., H.-Y. Ho, and M. Blackwell. 2010. Comparison of the bacterial symbiont composition of the formosan subterranean termite from its native and introduced range. The Open Microbiology Journal **4**:53-66.
Inoue, R., and K. Ushida. 2003. Vertical and horizontal transmission of intestinal commensal bacteria in the rat model. FEMS Microbiology Ecology **46**:213-219.
Jaenike, J. 2012. Population genetics of beneficial heritable symbionts. Trends Ecol Evol **27**:226-232.
Kaliszewski, M., F. Athias-Binche, and E. E. Lindquist. 1995. Parasitism and parasitoidism in Tarsonemina (Acari: Heterostigmata) and evolutionary considerations. **35**:335-367.
Karlsson, B., and A. Johansson. 2008. Seasonal polyphenism and developmental trade-offs between flight ability and egg laying in a pierid butterfly. Proc Biol Sci **275**:2131-2136.
Khuhro, N. H., A. Biondi, N. Desneux, L. Zhang, Y. Zhang, and H. Chen. 2014. Trade-off between flight activity and life-history components in *Chrysoperla sinica*. BioControl **59**:219-227.
Kikuchi, Y., T. Hosokawa, and T. Fukatsu. 2007. Insect-microbe mutualism without vertical transmission: A stinkbug acquires a beneficial gut symbiont from the environment every generation. Appl Environ Microbiol **73**:4308-4316.
Kinn, D. N. 1983. The life cycle of *Proctolaelaps dendroctoni* Lindquist and Hunter (Acari: Ascidae): A mite associated with pine bark beetles. International Journal of Acarology **9**:205-210.
Kinn, D. N., and J. J. Witcosky. 1977. The life cycle and behaviour of *Macrocheles boudreauxi* Krantz. Zeitschrift für Angewandte Entomologie **84**:136-144.
Kisdi, E., M. Utz, and M. Gyllenberg. 2012. Evolution of condition-dependent dispersal. Page 462 *in* J. Clobert, M. Baguette, T. G. Benton, and J. M. Bullock, editors. Dispersal Ecology and Evolution. Oxford Unversity Press, Oxford, UK.
Klimov, P. B., B. Vinson, and B. M. OConnor. 2007. Acarinaria in associations of apid bees (Hymenoptera) and chaetodactylid mites (Acari). Invertebrate Systematics **21**:109-136.

Krantz, G. W. 2009. Habits and habitats. Pages 64-82 *in* G. W. Krantz and D. E. Walter, editors. A manual of acarology. Texas Tech University Press, Lubbock, Tex.
Krasnov, B. R., I. S. Khokhlova, and G. I. Shenbrot. 2002. The effect of host density on ectoparasite distribution: An example of a rodent parasitized by fleas. Ecology **83**.
Lachowsky, L. E., and M. L. Reid. 2014. Developmental mortality increases sex-ratio bias of a size-dimorphic bark beetle. Ecol Entomol **39**:300-308.
Lafferty, K. D., and A. M. Kuris. 2002. Trophic strategies animal diversity and body size. Trends Ecol Evol **17**:507-513.
Latty, T. M., and M. L. Reid. 2009. Who goes first? Condition and danger dependent pioneering in a group-living bark beetle (*Dendroctonus ponderosae*). Behavioral Ecology and Sociobiology **64**:639-646.
Lee, J. H., T. W. Kim, and J. C. Choe. 2009. Commensalism or mutualism: Conditional outcomes in a branchiobdellid-crayfish symbiosis. Oecologia **159**:217-224.
Leimar, O., and P. Hammerstein. 2010. Cooperation for direct fitness benefits. Philos Trans R Soc Lond B Biol Sci **365**:2619-2626.
Leung, T. L. F., and R. Poulin. 2008. Parasitism, commensalism, and mutualism: Exploring the many shades of symbioses. VIE ET MILIEU - LIFE AND ENVIRONMENT **58**:107-115.
Lindgren, B. S., and K. F. Raffa. 2013. Evolution of tree killing in bark beetles (Coleoptera: Curculionidae): Trade-offs between the maddening crowds and a sticky situation. The Canadian Entomologist **145**:471-495.
Lindquist, E. E. 1969. New species of *Tarsonemus* (Acarina: Tarsonemidae) associated with bark beetles. The Canadian Entomologist **101**:1291-1314.
Lindquist, E. E. 1971. New species of Ascidae (Acarina: Mesostigmata) associated with forest insect pests. The Canadian Entomologist **103**:919-942.
Liu, S., J. Li, K. Guo, H. Qiao, R. Xu, J. Chen, C. Xu, and J. Chen. 2016. Seasonal phoresy as an overwintering strategy of a phytophagous mite. Sci Rep **6**:25483.
Lombardero, M. J., M. P. Ayres, R. W. Hofstetter, J. C. Moser, and K. D. Klepzig. 2003. Strong indirect interactions of Tarsonemus mites (Acarina: Tarsonemidae) and *Dendroctonus frontalis* (Coleoptera: Scolytidae). Oikos **102**:243-252.
Lombardero, M. J., K. D. Klepzig, J. C. Moser, and M. P. Ayres. 2000. Biology, demography and community interactions of Tarsonemus (Acarina: Tarsonemidae) mites phoretic on *Dendroctonus frontalis* (Coleoptera: Scolytidae). Agricultural and Forest Entomology **2**:193-202.
Lopez, V. M., M. S. Hoddle, J. A. Francese, D. R. Lance, and A. M. Ray. 2017. Assessing flight potential of the invasive asian longhorned beetle (Coleoptera: Cerambycidae) with computerized flight mills. J Econ Entomol **110**:1070-1077.
Lopez, V. M., M. N. McClanahan, L. Graham, and M. S. Hoddle. 2014. Assessing the flight capabilities of the goldspotted oak borer (coleoptera: Buprestidae) with computerized flight mills. Journal of Economic Entomology **107**:1127-1135.
Luong, L. T., L. R. Penoni, C. J. Horn, and M. Polak. 2015. Physical and physiological costs of ectoparasitic mites on host flight endurance. Ecological Entomology **40**:518-524.
Magowski, W. L. 2010. *Schaarschmidtia*, a new subgenus of the genus *Tarsonemus* Canestrini & Fanzago, 1876 (Acari: Heterostigmatina: Tarsonemidae) - A systematic review of the

mite taxon associated with subcortical coleopteran insects, with notes on its host affiliation, ecology and biogeography. Pol. J. Entomol. **79**:1-176.
Magowski, W. L., and J. C. Moser. 2003. Redescription of *Tarsonemus minimax* and definition of its species-group in the genus *Tarsonemus* (Acari: Tarsonemidae) with descriptions of two new species. Annals of the Entomological Society of America **96**:345-368.
Mangin, K. L., M. Lipsitch, and D. Ebert. 1995. Virulence and transmission modes of two microsporidia in *Daphnia magna*. Parasitology **111**:133-142.
Mazza, G., A. Cini, R. Cervo, and S. Longo. 2011. Just phoresy? Reduced lifespan in red palm weevils *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae) infested by the mite *Centrouropoda almerodai* (Uroactiniinae: Uropodina). Italian Journal of Zoology **78**:101-105.
McPeek, M. A., and R. D. Holt. 1992. The evolution of dispersal in spatially and temporally varying environments. Am Nat **140**:1010-1027.
Mercado, J. E., R. W. Hofstetter, D. M. Reboletti, and J. F. Negrón. 2014. Phoretic symbionts of the mountain pine beetle (*Dendroctonus ponderosae* Hopkins). Forest Science **60**:512-526.
Mitchell, R. 1970. An analysis of dispersal in mites. Am Nat **104**:425-431.
Montllor, C. B., A. Maxmen, and A. H. Purcell. 2002. Facultative bacterial endosymbionts benefit pea aphids *Acyrthosiphon pisum* under heat stress. Ecol Entomol **27**:189-195.
Mori, B. A., H. C. Proctor, D. E. Walter, and M. L. Evenden. 2011. Phoretic mite associates of mountain pine beetle at the leading edge of an infestation in Northwestern Alberta, Canada. The Canadian Entomologist **143**:44-55.
Moser, J. C. 1975. Mite predators of the southern pine beetle. Annals of the Entomological Society of America **68**:1113-1116.
Moser, J. C. 1976a. Phoretic carrying capacity of flying southern pine beetles (Coleoptera: Scolytidae). The Canadian Entomologist **108**:807-808.
Moser, J. C. 1976b. Surveying mites (Acarina) phoretic on the Southern pine beetle (Coleoptera: Scolytidae) with sticky traps. The Canadian Entomologist **108**:809-813.
Moser, J. C. 1985. Use of sporothecae by phoretic *Tarsonemus* mites to transport ascospores of coniferous bluestain fungi. Transactions of the British Mycological Society **84**:750-753.
Moser, J. C., H. Konrad, S. R. Blomquist, and T. Kirisits. 2010. Do mites phoretic on elm bark beetles contribute to the transmission of Dutch elm disease? Naturwissenschaften **97**:219-227.
Moser, J. C., and J. E. Macías-Sámano. 2000. Tarsonemid mite associates of *Dendroctonus frontalis* (coleoptera: Scolytidae): Implications for the historical biogeography of *D. frontalis*. The Canadian Entomologist **132**.
Moser, J. C., and L. M. Roton. 1971. Mites associated with Southern pine bark beetles in Allen Parish, Louisiana. The Canadian Entomologist **103**:1775-1798.
Mousseau, T. A., and C. W. Fox. 1998. The adaptive significance of maternal effects. Trends Ecol Evol **13**:403-407.
Nehring, V., and J. K. Muller. 2009. Social environment affects the life history tactic of a phoretic mite. J Evol Biol **22**:1616-1623.
Nespolo, D. A., D. Roff, and D. J. Fairbairn. 2008. Energetic trade-off between maintenance costs and flight capacity in the sand cricket (*Gryllus firmus*). Functional Ecology **22**:624-631.

Niitepold, K., and C. L. Boggs. 2015. Effects of increased flight on the energetics and life history of the butterfly *Speyeria mormonia*. PLoS One **10**:e0140104.
Niogret, J., J.-P. Lumaret, and M. Bertrand. 2006. Semiochemicals mediating host-finding behaviour in the phoretic association between *Macrocheles saceri* (Acari: Mesostigmata) and *Scarabaeus* species (Coleoptera: Scarabaeidae). Chemoecology **16**:129-134.
Niogret, J., and J. P. Lumaret. 2009. Identification of the cues used in the host finding behavior during the phoretic association *Ceroptera rufitarsis* (Diptera: Sphaeroceridae) and dung beetles (Coleoptera: Scarabaeidae). Journal of Insect Behavior **22**:464-472.
Niogret, J., J. P. Lumaret, and M. Bertrand. 2009. Generalist and specialist strategies in macrochelid mites (Acari: Mesostigmata) phoretically associated with dung beetles (Coleoptera: Scarabaeidae). Trends in Acarology:343-347.
Norton, R. A. 2009. Observations on phoresy by oribatid mites (Acari: Oribatei). International Journal of Acarology **6**:121-130.
O'Connor, B. M. 1993. The mite community associated with *Xylocopa latipes* (Hymenoptera: Anthophoridae: Xylocopinae) with description of a new type of acarinarium. International Journal of Acarology **19**:159-166.
O'Sullivan, D., T. G. Benton, and T. C. Cameron. 2014. Inter-patch movement in an experimental system: The effects of life history and the environment. Oikos **123**:623-629.
Okabe, K. 2013. Ecological characteristics of insects that affect symbiotic relationships with mites. Entomological Science **16**:363-378.
Okabe, K., and S. Makino. 2008. Parasitic mites as part-time bodyguards of a host wasp. Proc Biol Sci **275**:2293-2297.
Oliver, K. M., P. H. Degnan, G. R. Burke, and N. A. Moran. 2010. Facultative symbionts in aphids and the horizontal transfer of ecologically important traits. Annu Rev Entomol **55**:247-266.
Oliver, K. M., J. A. Russel, N. A. Moran, and M. S. Hunter. 2003. Facultative bacterial symbionts in aphids confer resistance to parasitic wasps. Proc Natl Acad Sci U S A **100**.
Paracer, S., and V. Ahmadjian. 2000. Introduction. Symbiosis: An Introduction to Biological Associations. Oxford University Press, New York.
Parmesan, C. 2006. Ecological and evolutionary responses to recent climate change. Annual Review of Ecology, Evolution, and Systematics **37**:637-669.
Patterson, J. E., P. Neuhaus, S. J. Kutz, and K. E. Ruckstuhl. 2015. Patterns of ectoparasitism in North American red squirrels (Tamiasciurus hudsonicus): Sex-biases, seasonality, age, and effects on male body condition. Int J Parasitol Parasites Wildl **4**:301-306.
Pernek, M., B. Hrasovec, D. Matosevic, I. Pilas, T. Kirisits, and J. C. Moser. 2008. Phoretic mites of three bark beetles (*Pityokteines* spp.) on Silver fir. Journal of Pest Science **81**:35-42.
Perotti, M. A., and H. R. Braig. 2009. Phoretic mites associated with animal and human decomposition. Exp Appl Acarol **49**:85-124.
Pfammatter, J. A., J. C. Moser, and K. F. Raffa. 2013. Mites phoretic on *Ips pini* (Coleoptera: Curculionidae: Scolytinae) in Wisconsin red pine stands. Annals of the Entomological Society of America **106**:204-213.
Pfammatter, J. A., and K. F. Raffa. 2015. Do phoretic mites influence the reproductive success of *Ips grandicollis* (Coleoptera: Curculionidae)? Environ Entomol **44**:1498-1511.

Phillips, B. L., G. P. Brown, and R. Shine. 2010a. Life-history evolution in range-shifting populations. Ecology **91**:1617-1627.
Phillips, B. L., C. Kelehear, L. Pizzatto, G. P. Brown, D. Barton, and R. Shine. 2010b. Parasites and pathogens lag behind their host during periods of host range advance. Ecology **91**:872-881.
Polak, M. 1996. Ectoparasitic effects on host survival and reproduction: The *Drosophila-Macrocheles* association. Ecology **77**:1379-1389.
Poulin, R. 2007. Are there general laws in parasite ecology? Parasitology **134**:763-776.
Poulin, R. 2011. The many roads to parasitism: A tale of convergence. Adv Parasitol **74**:1-40.
Poulin, R. 2013. Explaining variability in parasite aggregation levels among host samples. Parasitology **140**:541-546.
Raffel, T. R., L. B. Martin, and J. R. Rohr. 2008. Parasites as predators: Unifying natural enemy ecology. Trends Ecol Evol **23**:610-618.
Rankin, M. A., and J. C. A. Burchsted. 1992. The cost of migration in insects. Annu Rev Entomol **37**:533-559.
Reid, M. L., and O. Baruch. 2010. Mutual mate choice by mountain pine beetles: Size-dependence but not size-assortative mating. Ecological Entomology **35**:69-76.
Reid, M. L., and B. D. Roitberg. 1994. Benefits of prolonged male residence with mates and brood in pine engravers (Coleoptera: Scolytidae). Oikos **70**:140-148.
Reuter, M., J. S. Pedersen, and L. Keller. 2005. Loss of *Wolbachia* infection during colonisation in the invasive Argentine ant *Linepithema humile*. Heredity (Edinb) **94**:364-369.
Rocha, S. L., E. Pozo-Velázquez, L. R. D. A. Faroni, and R. N. C. Guedes. 2009. Phoretic load of the parasitic mite *Acarophenax lacunatus* (Cross & Krantz) (Prostigmata: Acarophenacidae) affecting mobility and flight take-off of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae). Journal of Stored Products Research **45**.
Roff, D. 1977. Dispersal in dipterans: Its costs and consequences. Journal of Animal Ecology **46**:446-456.
Saastamoinen, M., and M. J. Rantala. 2013. Influence of developmental conditions on immune function and dispersal-related traits in the glanville fritillary (*Melitaea cinxia*) butterfly. PLoS One **8**:e81289.
Safranyik, L., and A. L. Carroll. 2006. The biology and epidemiology of the mountain pine beetle in lodgepole pine forests. Pages 3-66 *in* L. Safranyik and B. Wilson, editors. The Mountain Pine Beetle A Synthesis of Biology, Management, and Impacts on Lodgepole Pine.
Safranyik, L., A. L. Carroll, J. Régnière, D. W. Langor, W. G. Riel, T. L. Shore, B. Peter, B. J. Cooke, V. G. Nealis, and S. W. Taylor. 2012. Potential for range expansion of mountain pine beetle into the boreal forest of North America. The Canadian Entomologist **142**:415-442.
Samejima, Y., and Y. Tsubaki. 2009. Body temperature and body size affect flight performance in a damselfly. Behavioral Ecology and Sociobiology **64**:685-692.
Sanders, D., and F. J. van Veen. 2012. Indirect commensalism promotes persistence of secondary consumer species. Biol Lett **8**:960-963.
Schmid-Hempel, P. 2011. The diversity and natural history of parasites. Pages 18-51 Evolutionary parasitology. The integrated study of infections, immunology, ecology, and genetics. Oxford Univerisity Press, United States.

Schulte-Hostedde, A. I., B. Zinner, J. S. Millar, and G. J. Hickling. 2005. Restitution of mass-size residuals: Validating body condition indices. Ecology **86**:155-163.
Schwarz, H. H., and J. K. Müller. 1992. The dispersal behaviour of the phoretic mite *Poecilochirus carabi* (Mesostigmata, Parasitidae): Adaptation to the breeding biology of its carrier *Necrophorus vespilloides* (Coleptera, Silphidae). Oecologia **89**:487-493.
Seppälä, O., K. Leicht, and J. Blount. 2015. Quality attracts parasites: Host condition-dependent chemo-orientation of trematode larvae. Functional Ecology **29**:791-795.
Sheldon, B. C., and S. Verhulst. 1996. Ecological immunology: Costly defences and trade-offs in evolutionary ecology. Trends Ecol Evol **11**:317-321.
Simon, J. C., M. Sakurai, J. Bonhomme, T. Tsuchida, R. Koga, and T. Fukatsu. 2007. Elimination of a specialised facultative symbiont does not affect the reproductive mode of its aphid host. Ecological Entomology **32**:296-301.
Six, D. L. 2003. A comparison of mycangial and phoretic fungi of individual mountain pine beetles. Canadian Journal of Forest Research **33**:1331-1334.
Six, D. L. 2013. The bark beetle holobiont: Why microbes matter. J Chem Ecol **39**:989-1002.
Six, D. L., and T. D. Paine. 1999. Phylogenetic comparison of ascomycete mycangial fungi and *Dendroctonus* bark beetles (Coleoptera: Scolytidae). Annals of the Entomological Society of America **92**:159–166.
Six, D. L., and M. J. Wingfield. 2011. The role of phytopathogenicity in bark beetle-fungus symbioses: A challenge to the classic paradigm. Annu Rev Entomol **56**:255-272.
Skelton, J., R. P. Creed, and B. L. Brown. 2014. Ontogenetic shift in host tolerance controls initiation of a cleaning symbiosis. Oikos **123**:677-686.
Skelton, J., R. P. Creed, and B. L. Brown. 2015. A symbiont's dispersal strategy: Condition-dependent dispersal underlies predictable variation in direct transmission among hosts. Proc Biol Sci **282**.
Skelton, J., S. Doak, M. Leonard, R. P. Creed, and B. L. Brown. 2016. The rules for symbiont community assembly change along a mutualism-parasitism continuum. J Anim Ecol **85**:843-853.
Skelton, J., K. J. Farrell, R. P. Creed, B. W. Williams, C. Ames, B. S. Helms, J. Stoekel, and B. L. Brown. 2013. Servants, scoundrels, and hitchhikers: Current understanding of the complex interactions between crayfish and their ectosymbiotic worms (Branchiobdellida). Freshwater Science **32**:1345-1357.
Stanton-Geddes, J., and C. G. Anderson. 2011. Does a facultative mutualism limit species range expansion? Oecologia **167**:149-155.
Stewart, A. D., J. J. M. Logsdon, and S. E. Kelley. 2005. An empirical study of the evolution of virulence under both horizontal and vertical transmission. Evolution **59**:730-739.
Stewart, A. J. A., T. M. Bantock, B. C. Beckmann, D. H. Botham, and D. B. Roy. 2015. The role of ecological interactions in determining species ranges and range changes. Biological Journal of the Linnean Socitey **115**:647-663.
Stier, A. C., M. A. Gil, C. S. McKeon, S. Lemer, M. Leray, S. C. Mills, and C. W. Osenberg. 2012. Housekeeping mutualisms: Do more symbionts facilitate host performance? PLoS One **7**:e32079.
Susoy, V., and M. Herrmann. 2014. Preferential host switching and codivergence shaped radiation of bark beetle symbionts, nematodes of *Micoletzkya* (Nematoda: Diplogastridae). J Evol Biol **27**:889-898.

Takov, D. I., D. Pilarska, and J. C. Moser. 2009. Phoretic mites associated with spruce bark beetle *Ips typographus* L. (Curculionidae: Scolytinae) from Bulgaria. Acta Zool. Bulg. **61**:293-296.

Thrall, P. H., M. E. Hochberg, J. J. Burdon, and J. D. Bever. 2007. Coevolution of symbiotic mutualists and parasites in a community context. Trends Ecol Evol **22**:120-126.

Trach, V. A., and A. A. Khaustov. 2017. Mites of the genus *Proctolaelaps* Berlese, 1923 (Acari: Mesostigmata: Melicharidae) associated with bark beetles in Asian Russia. Acarina **25**:151-163.

Tsui, C. K., L. Farfan, A. D. Roe, A. V. Rice, J. E. Cooke, Y. A. El-Kassaby, and R. C. Hamelin. 2014. Population structure of mountain pine beetle symbiont *Leptographium longiclavatum* and the implication on the multipartite beetle-fungi relationships. PLoS One **9**:e105455.

Van der Putten, W. H., M. Macel, and M. E. Visser. 2010. Predicting species distribution and abundance responses to climate change: Why it is essential to include biotic interactions across trophic levels. Philos Trans R Soc Lond B Biol Sci **365**:2025-2034.

Vicente, J., U. Hofle, I. G. Fernandez-De-Mera, and C. Gortazar. 2007. The importance of parasite life history and host density in predicting the impact of infections in red deer. Oecologia **152**:655-664.

Walter, D. E., and H. C. Proctor. 2013. Mites: Ecology, evolution & behaviour; life at a microscale. 2nd edition. Springer Science+Business Media.

White, J. A. 2011. Caught in the act: Rapid, symbiont-driven evolution. Bioessays **33**:823-829.

Wiens, J. A. 1976. Population responses to patchy environments. Ann Rev Ecol Syst **7**:81-120.

Williams, W. I., and I. C. Robertson. 2008. Using automated flight mills to manipulate fat reserves in Douglas-fir beetles (Coleoptera: Curculionidae). Environ Entomol **37**:850-856.

Wilson, D. S., and W. G. Knollenberg. 1987. Adaptive indirect effects: The fitness of burying beetles with and without their phoretic mites. Evolutionary Ecology **1**:139-159.

Witte, V., A. Leingärtner, L. Sabaß, R. Hashim, and S. Foitzik. 2008. Symbiont microcosm in an ant society and the diversity of interspecific interactions. Animal Behaviour **76**:1477-1486.

Wooding, A. L., M. J. Wingfield, B. P. Hurley, J. R. Garnas, P. de Groot, and B. Slippers. 2013. Lack of fidelity revealed in an insect-fungal mutualism after invasion. Biol Lett **9**:20130342.

Yamamura, N. 1993. Vertical transmission and evolution of mutualism from parasitism. Theor Popul Biol **44**:95-109.

Yang, C.-C., Y.-C. Yu, S. M. Valles, D. H. Oi, Y.-C. Chen, D. Shoemaker, W.-J. Wu, and C.-J. Shih. 2010. Loss of microbial (pathogen) infections associated with recent invasions of the red imported fire ant *Solenopsis invicta*. Biol Invasions **12**:3307-3318.

Yoder, J. A., D. Sammataro, J. A. Peterson, G. R. Needham, and W. A. Bruce. 1999. Water requirements of adult females of the honey bee parasitic mite, *Varroa jacobsoni* (Acari: Varroidae) and implications for control. International Journal of Acarology **25**:329-335.

Yule, K. M., T. E. X. Miller, and J. A. Rudgers. 2013. Costs, benefits, and loss of vertically transmitted symbionts affect host population dynamics. Oikos **122**:1512-1520.

APPENDIX A: LIST OF PHORETIC MITE SPECIES

Table A.1. List of phoretic mites species identified in this study.

Mite species	Host range	Occurrence	Feeding behavior	Fungi vector	Reference
Tarsonemus ips Lindquist (Acari: Prostigmata: Tarsonemidae)	New, Historic	Common	Fungivorous	Yes	(Lindquist 1969)
Proctolaelaps subcorticalis Lindquist (Acari: Mesostigmata: Melicharidae)	New, Historic	Common	Nematofagous	Yes	(Lindquist 1971)
Trichouropoda australis Hirschmann (Acari: Mesostigmata: Trematuridae)	Historic	Uncommon	Nematofagous	Unknown	(Moser 1975)
Histiogaster arborsignis Woodring (Acari: Astigmatina: Acaridae)	Historic	Rare	Fungivorous	Yes	(Moser and Roton 1971)
Histiostoma spp. Kramer (Astigmatina: Histiostomatidae)	Historic	Rare	Fungivorous, Microbial feeders	Yes	(Hofstetter and Moser 2014)
Macrocheles spp. (Mesostigmata: Macrochelidae)	New	Rare	Nematofagous, Predacious	Unknown	(Moser 1975)

The mites used for taxonomic identification were removed from beetles, clear in lactic acid and mounted on slides. These holotypes are deposited in the arthropods collection of the Ried/Cartar Laboratory of Univeristy of Calgary. The primary identification of phoretic mites was done with support of Dr. David Walter from the University of Alberta from samples I provided in early summer 2011. Later during the same summer Dr. Hans Klompen from the Ohio State University, and Dr. Ronald Ochoa from the Agricultural Research Service (ARS) of the United States Department of Agriculture (USDA) provided further support with taxonomic keys and guidance during the Acarology Summer Program 2011 attended by myself in Ohio State University. Further verification of mite species was done with support from Dr. Diana Six during summer 2012 that provided a personal collection of specimens identified by Dr. John Moser.

APPENDIX B: DESCRIPTIVE STATISTICS (CHAPTER 4)

Table B.1. Details of phoretic mite occurrence in mountain pine beetle sampled at 22 sites located in six geographical regions of both parts of beetle range.

Year	HR	Region	Site	Longitude	Latitude	TH	IH	NM	Prev. (%)	Abnd.	Ints.
2011	Historic	Kootenay	dollyvarden	-116.0147	50.8262	114	31	79	27	0.69	2.55
2011	Historic	Kootenay	kootenaycrossing	-116.0227	50.8555	101	56	280	55	2.77	5.00
2011	Historic	Yoho	emeraldlake	-116.5377	51.4334	101	63	390	62	3.86	6.19
2011	Historic	Yoho	field	-116.4867	51.3937	107	55	271	51	2.53	4.93
2011	Historic	Yoho	greatdividelodge	-116.3531	51.4426	100	55	233	55	2.33	4.24
2011	New	Valemount	canoeroad	-119.2166	52.8014	100	58	231	58	2.31	3.98
2011	New	Valemount	jackmanflats	-119.3871	52.9412	50	28	90	56	1.80	3.21
2012	Historic	Kootenay	dollyvarden	-116.0147	50.8262	61	9	23	15	0.38	2.56
2012	Historic	Kootenay	kootenaycrossing	-116.0227	50.8555	34	8	13	24	0.38	1.63
2012	Historic	Penticton	penticton	-119.5466	49.5297	50	38	143	76	2.86	3.76
2012	Historic	Yoho	emeraldlake	-116.5377	51.4334	5	2	16	40	3.20	8.00
2012	Historic	Yoho	field	-116.4867	51.3937	48	13	22	27	0.46	1.69
2012	Historic	Yoho	greatdividelodge	-116.3531	51.4426	62	42	293	68	4.73	6.98
2012	Historic	Yoho	naturalbridge	-116.5303	51.3841	50	24	62	48	1.24	2.58
2012	New	GrandePrairie	evergreenpark	-118.7347	55.1157	11	3	25	27	2.27	8.33
2012	New	GrandePrairie	forestrytrunk	-118.2130	55.0670	24	1	1	4	0.04	1.00
2012	New	GrandePrairie	groovedale	-118.9784	55.0656	50	6	7	12	0.14	1.17
2012	New	GrandePrairie	iriquois	-119.2252	54.9955	15	0	0	0	0	-
2012	New	GrandePrairie	wapitiskicentre	-118.1708	54.5166	26	17	99	65	3.81	5.82
2012	New	PeaceRiver	harmonvalley	-116.8079	56.1681	3	0	0	0	0	-
2012	New	PeaceRiver	runninglake	-119.0309	56.6680	50	27	68	54	1.36	2.52
2012	New	PeaceRiver	sulfurlake	-118.3066	56.7078	24	0	0	0	0	-
			Total			**1186**	**536**	**2346**			

HR represents the host range of distribution of mountain pine beetle. Information about the location of each site is also provided (longitude and latitude). TH represents the number of total hosts sampled. IH represents the number of infested hosts in the sample. NM represents the total number of phoretic mites. Information about percentage of prevalence (Prev. %), abundance (Abnd.), and intensity (Ints.) is provided.

Table B.2. Mite prevalence and percentage of uninfected individuals per sex.

Sex	Num. infested	Num. non infested	Total	Prev. (%)	Non infested (%)
Females	303	395	698	43	57
Males	233	255	488	48	52
Females + males	536	650	1186	45	55

Table B.3. Mite prevalence and percentage of uninfected individuals per outbreak status.

Host range	Num. infested	Non infested	Total	Prev. (%)	Non infested (%)
Historic	396	437	833	47.5	52.5
New	140	213	353	40	60
Total	536	650	1186	45	55

Table B.4. Mite prevalence and percentage of uninfected individuals per species of phoretic mite.

Species	Num. infested	Num. non infested	Total	Prev. (%)	Non infested (%)
Tarsonemus ips	434	752	1186	37	63
Proctolaelaps subcorticalis	122	1064	1186	10	90
Trichouropoda australis	87	1099	1186	7	93
All three species	536	650	1186	45	55

Table B.5. *Tarsonemus ips* patterns of infestation per site. Rows in italic indicate absence of mites for those sites. Rows in bold indicate the highest prevalence observed.

Tarsonemus ips								
Year	**HR**	**Region**	**Site**	**TH**	**IH**	**Prev. (%)**	**Abnd.**	**Ints.**
2011	Historic	Kootenay	dollyvarden	114	29	25	0.65	2.55
2011	Historic	Kootenay	kootenaycrossing	101	54	53	2.54	4.76
2011	Historic	Yoho	emeraldlake	101	55	54	3.28	6.02
2011	Historic	Yoho	field	107	45	42	2.05	4.87
2011	Historic	Yoho	greatdividelodge	100	43	43	1.97	4.58
2011	New	Valemount	canoeroad	100	37	37	1.12	3.03
2011	New	Valemount	jackmanflats	50	16	32	0.68	2.13
2012	Historic	Kootenay	dollyvarden	61	4	7	0.08	1.25
2012	Historic	Kootenay	kootenaycrossing	34	6	18	0.29	1.67
2012	Historic	Penticton	penticton	50	35	70	1.92	2.74
2012	Historic	Yoho	emeraldlake	5	2	40	3.20	8.00
2012	Historic	Yoho	field	48	8	17	0.23	1.38
2012	Historic	Yoho	greatdividelodge	62	31	50	2.60	5.19
2012	Historic	Yoho	naturalbridge	50	16	32	0.44	1.38
2012	New	GrandePrairie	evergreenpark	11	3	27	2.27	8.33
2012	New	GrandePrairie	forestrytrunk	24	1	4	0.04	1.00
2012	New	GrandePrairie	groovedale	50	6	12	0.14	1.17
2012	*New*	*GrandePrairie*	*iriquois*	*15*	*0*	*0*	*0*	*0*
2012	New	GrandePrairie	wapitiskicentre	26	17	65	3.81	5.82
2012	*New*	*PeaceRiver*	*harmonvalley*	*3*	*0*	*0*	*0*	*0*
2012	New	PeaceRiver	runninglake	50	26	52	1.34	2.58
2012	*New*	*PeaceRiver*	*sulfurlake*	*24*	*0*	*0*	*0*	*0*

HR represents the host range of distribution of mountain pine beetle. TH represents the number of total hosts sampled. IH represents the number of infested hosts in the sample. Information about percentage of prevalence (Prev. %), abundance (Abnd.), and intensity (Ints.) is provided.

Table B.6. *Proctolaelaps subcorticalis* patterns of infestation per site. Rows in italic indicate absence of mites for those sites. Rows in bold indicate the highest prevalence observed.

Proctolaelaps subcorticalis								
Year	**HR**	**Region**	**Site**	**TH**	**IH**	**Prev. (%)**	**Abnd.**	**Ints.**
2011	Historic	Kootenay	dollyvarden	114	1	1	0.02	2.00
2011	Historic	Kootenay	kootenaycrossing	101	3	3	0.13	4.33
2011	Historic	Yoho	emeraldlake	101	6	6	0.09	1.50
2011	Historic	Yoho	Field	107	16	15	0.41	2.75
2011	Historic	Yoho	greatdividelodge	100	7	7	0.14	2.00
2011	New	Valemount	canoeroad	100	36	36	1.18	3.28
2011	New	Valemount	jackmanflats	50	16	32	1.04	3.25
2012	Historic	Kootenay	dollyvarden	61	3	5	0.10	2.00
2012	Historic	Kootenay	kootenaycrossing	34	2	6	0.09	1.50
2012	Historic	Penticton	penticton	50	16	32	0.94	2.94
2012	*Historic*	*Yoho*	*emeraldlake*	*5*	*0*	*0*	*0*	*0*
2012	Historic	Yoho	Field	48	4	8	0.08	1.00
2012	Historic	Yoho	greatdividelodge	62	9	15	0.24	1.67
2012	Historic	Yoho	naturalbridge	50	2	4	0.04	1.00
2012	*New*	*GrandePrairie*	*evergreenpark*	*11*	*0*	*0*	*0*	*0*
2012	*New*	*GrandePrairie*	*forestrytrunk*	*24*	*0*	*0*	*0*	*0*
2012	*New*	*GrandePrairie*	*groovedale*	*50*	*0*	*0*	*0*	*0*
2012	*New*	*GrandePrairie*	*iriquois*	*15*	*0*	*0*	*0*	*0*
2012	*New*	*GrandePrairie*	*wapitiskicentre*	*26*	*0*	*0*	*0*	*0*
2012	*New*	*PeaceRiver*	*harmonvalley*	*3*	*0*	*0*	*0*	*0*
2012	New	PeaceRiver	runninglake	50	1	2	0.02	1.00
2012	*New*	*PeaceRiver*	*sulfurlake*	*24*	*0*	*0*	*0*	*0*

HR represents the host range of distribution of mountain pine beetle. TH represents the number of total hosts sampled. IH represents the number of infested hosts in the sample. Information about percentage of prevalence (Prev. %), abundance (Abnd.), and intensity (Ints.) is provided.

Table B.7. *Trichouropoda asutralis* patterns of infestation per site. Rows in italic indicate absence of mites for those sites. Rows in bold indicate the highest prevalence observed.

Trichouropoda australis								
Year	**HR**	**Region**	**Site**	**TH**	**IH**	**Prev. (%)**	**Abnd.**	**Ints.**
2011	Historic	Kootenay	dollyvarden	114	3	3	0.03	1.00
2011	Historic	Kootenay	kootenaycrossing	101	6	6	0.10	1.67
2011	Historic	Yoho	emeraldlake	101	15	15	0.50	3.33
2011	Historic	Yoho	field	107	5	5	0.07	1.60
2011	Historic	Yoho	greatdividelodge	100	14	14	0.22	1.57
2011	New	Valemount	canoeroad	100	1	1	0.01	1.00
2011	New	Valemount	jackmanflats	50	4	8	0.08	1.00
2012	Historic	Kootenay	dollyvarden	61	3	5	0.20	4.00
2012	*Historic*	*Kootenay*	*kootenaycrossing*	*34*	*0*	*0*	*0*	*0*
2012	*Historic*	*Penticton*	*penticton*	*50*	*0*	*0*	*0*	*0*
2012	*Historic*	*Yoho*	*emeraldlake*	*5*	*0*	*0*	*0*	*0*
2012	Historic	Yoho	field	48	3	6	0.15	2.33
2012	Historic	Yoho	greatdividelodge	62	23	37	1.89	5.09
2012	Historic	Yoho	naturalbridge	50	10	20	0.76	3.80
2012	*New*	*GrandePrairie*	*evergreenpark*	*11*	*0*	*0*	*0*	*0*
2012	*New*	*GrandePrairie*	*forestrytrunk*	*24*	*0*	*0*	*0*	*0*
2012	*New*	*GrandePrairie*	*groovedale*	*50*	*0*	*0*	*0*	*0*
2012	*New*	*GrandePrairie*	*iriquois*	*15*	*0*	*0*	*0*	*0*
2012	*New*	*GrandePrairie*	*wapitiskicentre*	*26*	*0*	*0*	*0*	*0*
2012	*New*	*PeaceRiver*	*harmonvalley*	*3*	*0*	*0*	*0*	*0*
2012	*New*	*PeaceRiver*	*runninglake*	*50*	*0*	*0*	*0*	*0*
2012	*New*	*PeaceRiver*	*sulfurlake*	*24*	*0*	*0*	*0*	*0*

HR represents the host range of distribution of mountain pine beetle. TH represents the number of total hosts sampled. IH represents the number of infested hosts in the sample. Information about percentage of prevalence (Prev. %), abundance (Abnd.), and intensity (Ints.) is provided.