The Good, the Bad, and the Mites: Unraveling the relationship between burying beetles and phoretic mites

By

Anjalie St-Louis-Hodgins

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ABSTRACT

Interspecific interactions can shift along a spectrum from mutualism to antagonism depending on ecological context, but the mechanisms and consequences of these shifts remain poorly understood. The relationship between burying beetles (*Nicrophorus orbicollis*) and their phoretic mites (*Poecilochirus* spp.) exemplifies this complexity. Despite a co-evolutionary history spanning millions of years and decades of research, whether these mites are threats, harmless passengers, or beneficial partners remains unresolved, with evidence to date suggesting a highly context-dependent relationship. I investigated how environmental factors influence the beetle-mite relationship using competitive trials in which two same-sex but size-mismatched beetles competed for a mouse carcass – an essential reproductive resource. I first ran baseline trials without mites to confirm that, under standard conditions, the larger individual typically wins, and to determine the size difference where the smaller beetle has a non-zero probability of winning. I then ran trials in which one beetle carried a typical pre-breeding mite load, alongside controls where neither beetle carried mites, at two temperatures (15°C or 25°C). Mites affected the outcome of these competitive interactions by preferentially transferring to the larger beetle and reducing their competitive success, while temperature had no detectable effect. In a complementary study designed to assess whether the beetles' innate response to mites is tolerant or aversive, I conducted a behavioural assay, and found that beetles exposed to mites engaged more in self-grooming and escape behaviours relative to controls, suggesting an evolved aversive response. Together, these results suggest that *Poecilochirus* mites impose a net fitness cost on *N. orbicollis*, despite occasional context-dependent benefits. My research adds to the literature by demonstrating that even long-standing associations can shift along the mutualism-antagonism spectrum depending on context and illustrating the value of a simple behavioural assay to evaluate the net fitness effects of interspecific interactions over evolutionary timespans.

CO-AUTHORSHIP

Author contributions: Anjalie St-Louis-Hodgins and Dr. Frances Bonier conceived and designed the study. Anjalie St-Louis-Hodgins and Shannon Marginson collected the data. Dr. Frances Bonier and Anjalie St-Louis-Hodgins conducted the statistical analyses and prepared tables and figures. Anjalie St-Louis-Hodgins wrote the manuscript, with input from Dr. Frances Bonier and Shannon Marginson.

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AICc	Akaike's Information Criterion corrected for small sample size
GLM	Generalized Linear Model
LM	Linear Model
RH	Relative Humidity
LME	Linear Mixed-Effects Model
HSD	Honestly Significant Difference
PCA	Principal Components Analysis

CHAPTER 1: INTRODUCTION

Ecosystems encompass networks of species interactions that can influence an individual's survival and performance. From mutualistic partnerships to intense competition, the fitness of individuals is often tied to the outcome of their interactions with other species (Bronstein, 1994; Mathis & Bronstein, 2020). These interspecific interactions span a continuum: from *mutualism*, where both species benefit from the interaction, to *commensalism*, where one species benefits while the other's fitness is unaffected, to *antagonism*, where one benefits at the other's expense (Mathis & Bronstein, 2020). The fitness outcomes of these interactions can be dynamic, shifting in response to changes in environmental conditions, population dynamics, resource availability, and individual traits (Pekkonen et al., 2013; Mathis & Bronstein, 2020; de Bruin et al., 2024). Given this variability, the long-term net fitness outcomes of interspecific interactions might help us better understand their evolutionary impact. Categorizing interactions in this way could clarify the nature of these relationships and the selective pressures they impose, potentially capturing their dynamics more accurately than short-term fitness measures (Blüthgen et al. 2006; Carrara et al. 2015).

Intrinsic characteristics can shape the fitness outcomes of interspecific interactions, shifting them along the spectrum from mutualism to antagonism. Traits that influence an individual's quality or condition can be especially important, particularly in antagonistic interactions such as predation or parasitism, where lower-quality individuals might face greater risks and incur higher costs (Rózsa & Garay, 2023). Among intrinsic factors, body size stands out as one of the most broadly significant due to its influence on biological form and function, including biomechanics, physiology, and life history (Gillooly et al., 2001; Bonner, 2006; Chown & Gaston, 2010), all of which can shape interspecific interactions. For instance, in plant–herbivore interactions, the effect of plant-feeding insects can vary depending on host size and vigour (Price, 1991). In some cases, herbivores have minimal impact on large, robust plants but cause significant damage to smaller or less vigorous individuals (Price, 1991). This size-dependent variation in outcomes suggests that the same interaction can shift along the mutualism–antagonism spectrum depending on intrinsic host traits like body size. Additionally, life history stages can be important in determining the fitness outcome of interspecies interactions. While an interaction might be antagonistic at one life stage for either species, it can shift to being mutualistic or commensal at another, and vice-versa. This type of shift is illustrated in the association between brachyuran crabs (*Cancer gracilis*) and their pelagic jellyfish hosts (*Phacellophora camtschatica*): while younger crab instars are considered parasitic, actively feeding on host tissue, their diet shifts as they mature to primarily consuming the amphipod *H. medusarum,* a parasitoid that is harmful to the jellyfish (Towanda & Thuesen, 2006). By consuming this amphipod, *C. gracilis* shifts from a parasite to a mutualist, forming a valuable cleaning symbiosis with its host (Towanda & Thuesen, 2006). This interaction highlights how ontogenetic changes can influence the fitness outcome of interspecific relationships. Thus, understanding how intrinsic factors influence outcomes is essential to understanding the fitness consequences of interspecific interactions.

Extrinsic factors can also play a role in determining the outcomes of interspecific interactions by influencing organism behaviour and altering the strength of these interactions. Temperature, for instance, can affect resource uptake rates, competitive behaviours, and physiological traits that can alter the nature of species interactions (García et al., 2018; Lin et al., 2024). This concept is clearly illustrated in the interaction between the marine diatom *Skeletonema costatum* and bacteria from the genus *Tamlana*. At low temperature, *Tamlana* coexists harmlessly with the diatom, and may even promote its growth (Lin et al., 2024).

However, as temperatures rise, the relationship shifts dramatically: *Tamlana* becomes antagonistic, lysing algal cells and causing high mortality in *S. costatum* (Lin et al., 2024). A similar pattern is observed in the relationship between wild plants, including grasses and herbaceous forbs, and mycorrhizal fungi: as temperature increases from subambient to ambient levels, the interaction tends to shift from commensal or weakly mutualistic to strongly mutualistic, resulting in increases in plant biomass (Duarte & Maherali, 2022). As such, temperature can transform the fitness consequences of species interactions by altering partner behaviour and physiology. Additionally, the availability of essential resources, such as food or water, often mediates the intensity and fitness outcomes of interspecific interactions. Mutualisms can persist when resources are plentiful but might shift towards antagonism as resource availability declines and partners begin acting in self-interest. This pattern is observed in seed dispersal systems, where animals typically gain food in exchange for transporting seeds away from the parent plant, benefiting both parties (Zwolak et al., 2024). However, when alternative food resources are scarce, or granivores are abundant, these same animals might shift towards consuming a greater proportion of seeds, reducing plant reproductive success and shifting the relationship towards antagonism (Zwolak et al., 2024). This shift in fitness outcome can also occur through different mechanisms. Resource availability can mediate the balance of costs and benefits in species interactions, driving shifts along the mutualism-antagonism spectrum. This dynamic is evident in the relationship between mycorrhizal fungi and certain plant species (including wildflowers and grasses): when soil nutrients such as nitrogen and phosphorus are scarce, mycorrhizae enhance nutrient uptake and promote plant growth (Johnson et al., 1997; Friede et al., 2016). However, when nutrients are abundant, the net benefit to the plant diminishes, and mycorrhizae can act as net carbon sinks, extracting carbohydrates without providing compensatory benefits to the plants (Johnson et al., 1997; Friede et al., 2016). In some cases, such as under high phosphorus conditions, this shift to parasitism can even result in reduced plant growth (Friede et al., 2016). Finally, the number of interacting individuals can shape the outcomes of interspecific interactions, with species abundance and density influencing interaction intensity and the fitness outcome of these interactions. This pattern is especially evident in parasite-host systems, where low parasite loads may have little to no impact on host fitness, but high loads can be extremely detrimental, impacting fecundity and survival (Pohlink et al., 2020). However, this pattern is also found in other systems, such as in plant-pollinator interactions, wherein a disproportionate pollinator density can sometimes trigger a shift from mutualism to antagonism (Peralta et al. 2020). When pollinator densities are high, plants might experience more visits from pollinators than optimal, leading to damaged floral structures, depleted nectar, or pollen removal without effective transfer (Sáez et al., 2014; Sáez et al., 2017; Peralta et al. 2020). These effects impose costs on the plant, ultimately reducing their reproductive success and fitness, despite the interaction remaining beneficial to the pollinators (Sáez et al., 2014; Sáez et al., 2017; Peralta et al. 2020). Overall, these examples illustrate how the fitness outcomes of interspecific interactions can often be influenced by variable intrinsic and extrinsic factors, highlighting the importance of context in evaluating these interactions.

While the immediate fitness effects of interspecific interactions can be shaped by environmental context in the short-term, over evolutionary timescales their net fitness effects should drive selection for traits that enhance beneficial relationships or mitigate harmful ones. In mutualistic interactions, species often evolve traits that promote cooperation and maximize shared benefits. For instance, plants have evolved specialized floral structures and chemical signals to attract pollinators (Martos et al., 2015; Proffit et al., 2020), while host fish exhibit aggressive behaviours that prevent cleaner fish from cheating, enforcing cooperation and reinforcing the mutualistic partnership (Johnstone & Bshary, 2002). In contrast, interactions with neutral or minimal fitness effects (i.e., commensalism) might favour the evolution of traits that promote tolerance or energy conservation, avoiding unnecessary investment in interactions that neither harm nor benefit the organism. However, in antagonistic interactions with negative fitness consequences for one of the species, we see adaptations aimed at resistance or mitigation, such as immune responses to pathogens, anti-predator strategies like camouflage or escape behaviours, and physical defences like spines or toxins (Chaplin, 2010; Marples et al., 2018; Kikuchi et al., 2023). Consequently, despite sometimes dynamic and context-dependent fitness outcomes, we might be best able to categorize the position of interspecific interactions along the continuum from mutualism to antagonism by assessing the presence of traits that facilitate or reduce the frequency and intensity of these interactions. If one partner exhibits defences against an interaction, that suggests the relationship generally does not benefit that species. These traits can thus serve as signatures of selection, indicating whether a given interaction is typically harmful or beneficial over time.

One trait that reflects attempts to defend against an interspecific interaction is grooming, a fundamental behaviour that extends beyond basic hygiene and often functions as a defence against ectoparasites (Sachs, 1988; Madden & Clutton-Brock, 2009; Kenny et al., 2017). Although it can carry fitness costs, including energy expenditure, reduced vigilance, and thermoregulatory challenges (Mooring, 1996; Mooring, 2024), grooming can substantially mitigate harm by removing ticks, lice, and other ectoparasites that can transmit diseases or drain their host's resources (Akinyi et al., 2013; Wren et al., 2021; Lacroux et al., 2023). The importance of grooming is made clear in systems such as impalas (*Aepyceros melampus*), where individuals prevented from grooming harboured up to 20 times more ticks than those allowed to groom (Mooring, 1996). Notably, when grooming is insufficient or impossible, animals might adopt other strategies, such as spatial avoidance or escape behaviours, to limit parasite exposure (Hart, 1990; Hart, 1992; Amano et al., 2008). By contrast, behaviours aimed at reducing contact are rarely directed at mutualistic partners. Hosts often tolerate, or even facilitate, the presence of symbionts that provide net benefits, despite occasional costs. For example, in marine systems, cleaner fish, (e.g., *Labroides dimidiatus*), remove parasites from client fish including butterflyfish (*Chaetodon uriga*) and yellow tang (*Zebrasoma flavescens*), and these clients spread their fins to provide better access to areas with high parasite loads (Losey, 1979). Even when cleaner fish occasionally consume host tissue, the benefits of parasite removal outweigh the minor damage caused (Cheney & Côté, 2005; Caves, 2021). These examples demonstrate how grooming reflects behavioural plasticity shaped by fitness consequences of different interactions. When parasitism imposes substantial costs, grooming effort is expected to intensify, as seen in greater mouse-eared bats (*Myotis myotis*), which groom more when infested with mites, despite an increase metabolic burden (Giorgi et al., 2001). When relationships are mutualistic, grooming might instead be reduced to preserve the net benefits of the partnership, even if this results in minor harm. Although grooming is well-documented across taxa, its potential as an indicator of the nature of interspecific interactions remains underexplored. Studies often examine the frequency and effectiveness of grooming (e.g., Mooring et al., 1996; Akinyi et al., 2013) but, to my knowledge, none have used measures of grooming responses to better understand whether an interaction is parasitic, mutualistic, or commensal. Because hosts are expected to groom actively in response to parasites and reduce grooming when tolerance of a mutualist yields greater benefits, grooming could serve as a practical and observable metric for inferring the net fitness effects of interspecific relationships.

Among the many species that rely on grooming, insects are avid groomers, using specialized behaviours to maintain hygiene, remove debris, and eliminate parasites (Valentine, 1973; Zhukovskaya et al., 2013). These grooming adaptations are crucial for reducing risks posed by external contaminants and parasites, directly contributing to survival and reproductive success (Zhukovskaya et al., 2013; Nadein & Gorb, 2022). Beetles, in particular, exhibit a diverse array of grooming behaviours, such as cleaning their antennae, legs, and wings using mouthparts or by rubbing body parts together (Valentine, 1973). For example, Japanese beetles (*Popillia japonica*) engage in frequent self-grooming to remove parasites including vigorously brushing and rubbing their bodies (Gaugler et al., 1994; Alvandi et al., 2017). Given the widespread role of grooming in parasite defence, it might offer a useful behavioural window into how insects respond to their symbionts, particularly in systems where the costs and benefits of the association remain unclear.

Nicrophorus beetles provide a compelling case for using grooming as a behavioural lens through which to examine insect-symbiont interactions. These beetles exhibit a wide range of grooming behaviours, are broadly distributed across the globe, and almost universally host phoretic mites (*Poecilochirus* spp.). Fossil evidence points to a co-evolutionary relationship between *Nicrophorus* beetles and *Poecilochirus* mites spanning millions of years (Canitz et al., 2021). Although *Nicrophorus* beetles can reproduce independently of mites, the life cycle of *Poecilochirus* mites is tightly intertwined with that of their beetle hosts. The mites rely on the beetles for transport to carcasses – critical reproductive sites for both species – making beetle reproductive behaviour central to mite fitness and survival (Eggert & Müller, 1998; Schwarz et al., 1998; Scott, 1998). Upon arrival at a carcass, *Nicrophorus* beetles often engage in intense competition, with the dominant pair – typically the largest individuals – securing the carcass, burying it, and preparing it for their offspring (Springett, 1968; Wilson & Fudge, 1984;

Schrempf et al., 2021; Vangenne et al., 2023). Once the beetle secures the carcass, mites disembark and reproduce alongside them, synchronizing their own development with that of the beetle brood (Springett, 1968; Schwarz & Muller, 1992). This reliance on beetle-mediated access to carcasses has produced a tightly coordinated life cycle shaped by a shared reliance on ephemeral resources.

Despite this long-standing history, the fitness consequences of the *Nicrophorus*-*Poecilochirus* interaction remain ambiguous and heavily debated. Mites benefit from hitching a ride to and from carcasses, and their presence can influence beetle reproductive success in ways that vary across environmental, behavioural, and life history contexts. Some evidence from various *Nicrophorus* species suggests that mites can benefit their beetle hosts, indicating mutualistic dimensions of the relationship (Springett, 1968; Blackman & Evans, 1994; Sun & Kilner, 2020). For instance, mites can reduce competition from fly larvae, which would otherwise consume the carrion resource essential for beetle reproduction (Springett, 1968; Blackman & Evans, 1994; Nagel et al., 2025). Some experimental evidence also suggests that mites might enhance the competitive success of relatively small beetles by raising their body temperature during intraspecific contests for carrion (Sun et al., 2019). However, evidence also points to antagonism, as mites can feed on beetle eggs, directly reducing reproductive success, and heavy mite loads can compromise beetle mobility (Beninger, 1993; Blackman & Evans, 1994; De Gasperin & Kilner, 2015; Nagel et al., 2025). In some contexts, the relationship appears commensal, where mites benefit from transportation without significantly impacting the beetles' reproductive success (Nagel et al., 2025). Additionally, several studies have demonstrated the importance of considering broader ecological contexts. For instance, Sun and Kilner (2020) found that mites feeding on fly eggs can yield net fitness benefits for the beetles, but only under certain conditions such as elevated temperature or intense competition (Sun &

Kilner, 2020). Other work has shown that phoretic mites can impose contrasting fitness effects within a single beetle brood, sometimes harming one parent while sparing the other (De Gasperin & Kilner, 2015). Put together, these decades of research suggest that the fitness outcomes of this relationship are not static, but instead highly context dependent, influenced by intrinsic factors (e.g., beetle body size) and extrinsic factors (e.g., presence of third-party competitors or resource availability). No research has thus far revealed the net, long-term fitness effects of this relationship.

One way to address the current gaps in our understanding of the dynamic relationship between *Nicrophorus* and phoretic mites is to study both the *direct, short-term effects* of their interactions and the *evolved responses to long-term effects* that might reveal net fitness effects over evolutionary timescales. This dual approach offers a comprehensive framework for understanding how mites influence beetle performance and behaviour in both immediate and longer-term contexts.

To investigate the short-term effects of the beetle-mite interaction, I conducted a series of competitive dominance trials using *Nicrophorus orbicollis* beetles and their associated *Poecilochirus* spp. mites to test how beetle body size, ambient temperature, and the presence or absence of mites influence competitive success. Competition is a particularly relevant behavioural context in this system: for *Nicrophorus* beetles, reproduction depends on securing a carcass, often through intense inter- and intraspecific competitions (Wilson & Fudge, 1984; Schrempf et al., 2021; Vangenne et al., 2023). Because access to a carcass is a prerequisite for reproduction, competitive success is directly tied to individual fitness (Scott, 1998; Schrempf et al., 2021). The outcomes of these encounters are shaped by intrinsic traits like body size (Otronen, 1988) and could also be influenced by extrinsic factors like temperature (Abram et al., 2016; Wettlaufer et al., 2022). In semi-natural experiments, for example, the largest beetle typically dominates smaller rivals in contests for carrion (Otronen, 1988; Schrempf et al., 2021; Vangenne et al., 2023). Despite its central role in determining reproductive success, the effects of *Nicrophorus*-mite interactions on competition remains relatively underexplored. Yet, intraspecific competition for carrion represents a critical ecological context where fitness effects of the beetle-mite interaction might be amplified, masked, or even reversed. Given that mites often co-occur with beetles during these high-stakes competitive interactions, their presence could directly or indirectly shift competitive dynamics, altering which individuals gain access to breeding resources. Despite this, only one study (Sun et al., 2019), has examined the effects of mites during competition, with most others focusing solely on their effects during breeding, with competition excluded due to study design (i.e., pairs are provided a carcass, so do not need to compete). Notably, no research has explored how mites and temperature together influence competitive outcomes, a surprising gap, given that both factors are likely to interact in shaping reproductive success. Because securing a carcass is essential for reproduction, any mite-driven shifts, especially temperature-dependent ones, might influence competitive outcomes, with possible downstream effects on reproductive success.

To explore how intrinsic (body size) and extrinsic (temperature) factors influence competitive success, I conducted a series of competitive trials among beetles of the same sex, but different sizes, under varying temperature conditions. Body size is a well-established predictor of competitive outcomes (Otronen, 1988; Safryn & Scott, 2000; Schrempf et al., 2021), while temperature can affect key physiological and behavioural traits that affect competition (Sun et al., 2019; Wettlaufer et al., 2022). For example, higher temperatures might increase the activity of smaller individuals, potentially altering competitive dynamics between beetles (Sun et al., 2019). These trials provide insight into immediate performance outcomes in contests for carcasses and were designed to test whether beetle competitive ability is influenced by temperature, the presence of mites, or an interaction between the two. Specifically, I tested the null hypothesis that neither factor affects competitive ability (H_0), an additive hypothesis that each factor might have independent effects (H_1), and an interaction hypothesis that the effects of mites might be temperature dependent (H_2).

While competitive trials offer insight into immediate performance outcomes, they reveal only part of the story. To understand the longer-term evolutionary consequences of the beetle-mite relationship, we must also consider how selection has shaped behavioural traits like grooming, which could reflect more persistent patterns over time. To explore the beetles' evolved behavioural responses to mites, I conducted grooming assays, given that grooming serves as a primary mechanism for reducing ectoparasite loads. In the assays, each beetle's behaviour was observed both before and after mite exposure in the treatment groups, while control beetles underwent the same protocol, but were never exposed to mites. This within-subjects design allowed me to detect behavioural shifts that might reflect evolved responses to the presence of mites, while controlling for baseline variation. Because grooming functions as a targeted behavioural defence, it offers a window into how beetles might have adapted to persistent interactions with their symbionts, particularly when short-term fitness effects are subtle, context-dependent, or difficult to measure. I tested whether grooming and related anti-parasitic behaviours remain stable or decrease in the presence of the mites, as expected under commensal or mutualistic conditions (H_0), or whether they increase in response to mites, consistent with a defensive response to a costly partner (H_1)

Together, the competitive trials and behavioural assays respectively capture the context-dependence and long-term fitness effects of the beetle-mite relationship. By disentangling these dynamics, this research not only contributes to our understanding of how environmental factors influence the fitness effects of host-symbiont interactions, but also provides a more nuanced perspective on how these relationships shift along the interaction spectrum, from mutualism to commensalism or antagonism. Taken together, these approaches help clarify both the variability and the consequences of such interactions, adding to existing frameworks for understanding complex interspecific relationships in dynamic ecosystems.

CHAPTER 2: METHODS

Study System:

Nicrophorus orbicollis (roundneck sexton beetles; Family: Silphidae carrion beetles) burying beetles are native to North America, predominantly found in the eastern United States and southern Canada, with some populations extending westward (Potticary et al., 2023). These beetles rely on small vertebrate carcasses, such as dead birds or mice, for reproduction. Upon locating a carcass, an individual beetle releases a pheromone to attract a mate, which can also draw in rival burying beetles (Scott 1998). The odours emitted from the carcass itself also attract a diverse array of scavengers, including flies, ants, other Silphid species, and vertebrates (Scott, 1998). As a result, carcasses can be detected by numerous organisms all vying to use it as a resource, and so *N. orbicollis* must compete fiercely, in both intra- and interspecific contests, for a chance to secure a carcass for breeding (Scott 1998; de Gasperin et al., 2015). If successful, the breeding pair of *N. orbicollis* prepares the carcass by burying it underground, removing its fur or feathers, and shaping it into a compact 'brood ball' (Fetherston et al., 1990; Scott, 1998). Within ~48 hours of securing the carcass, the female lays her eggs in the surrounding soil (Fetherston et al., 1990; Scott, 1998). Three to four days later, the larvae hatch and crawl to the prepared carrion. There, the larvae beg for food and are fed regurgitate by both parents (Fetherston et al., 1990; Scott, 1998; Panaitof et al., 2016). While both sexes contribute to care, females often spend more time feeding larvae (Scott, 1998; Fetherston et al., 1990). Males, although capable of performing the same caregiving behaviours as females, more frequently assume a guardian role early in larval development and tend to be absent from the brood chamber for longer periods (Fetherston et al., 1990). Actively defending the carcass from competitors – such as vertebrate and invertebrate scavengers, other beetles, and flies – is critical for burying beetles, as these intruders can steal the carcass, destroy eggs or larvae, or quickly consume the resource, all of which reduces the beetles' reproductive success (Scott, 1998). Typically, male *N. orbicollis* remain with the brood until a day or two after larvae reach the third instar stage (9-11 days post burial), whereas females stay until the larvae disperse, around 15-18 days after the carcass is buried (Milne & Milne, 1976; Wilson & Fudge, 1984; Scott, 1988).

Poecilochirus carabi mites are the most widespread mites found on *Nicrophorus* beetles, occurring on nearly every species of *Nicrophorus* beetle worldwide (Wilson & Knollenberg, 1987; Schwarz & Muller, 1992; Canitz et al., 2021). *P. carabi* forms a species complex with some variants exhibiting specific carrier species preferences, sometimes specializing on one or more *Nicrophorus* hosts (Wilson & Fudge, 1984; Schwarz & Muller, 1992; Nehring et al., 2017). These mites are phoretic (i.e., they rely on their beetle hosts for transportation), requiring their beetle hosts to locate and transport them to and from vertebrate carcasses, which serve as a feeding and reproductive site for both species. These mites travel on the beetle as deuteronymphs, an active, non-reproductive stage that allows them to remain mobile and feed alongside their hosts, thereby reducing the detachment risks faced by other species that enter a resting stage during transit (Hunter & Rosario, 1988; Schwarz & Muller, 1992). They remain on the beetle until it secures a carcass and buries it in a brood chamber, at which point the mites disembark, moult into adults, and begin reproducing. Female mites lay their eggs in the surrounding soil, burying them for protection (Hunter & Rosario, 1988; Schwarz & Muller, 1992). Mite development parallels that of the beetle larvae, with offspring feeding on the carcass, nematodes, and other small organisms within the *Nicrophorus* brood chamber (Schwarz et al., 1998). After hatching, the new mites remain in the chamber until reaching the deuteronymph stage, at which point they attach to the beetles for transport (Schwarz & Muller, 1992). The mites typically attach to the male beetle first; however, if they mature after the male departs, they will congregate on the female. Those that miss both adults will disperse on the larvae and remain with them until they eclose from their pupa as teneral adults, perpetuating the close interaction between beetles and mites across generations (Schwarz & Muller, 1992; Canitz et al., 2021).

Animal Maintenance and Husbandry:

I bred *Nicrophorus orbicollis* from wild-caught individuals collected near the Queen's University Biological Station and maintained under standardized laboratory conditions (22°C day / 19°C night; 16L:8D light cycle; 68% RH). I excluded sibling and cousin pairings to minimize inbreeding (F2-F5). To initiate breeding, I haphazardly paired an unrelated male and female in a 6L plastic container (34.1 × 20.8 × 11.9cm) containing ~2 cm of organic garden soil (with mycorrhizae) and a thawed mouse carcass (20-30g, ripened at room temperature for 48 hours). The shallow soil depth prevented carcass burial while still allowing reproductive behaviour. I stored breeding containers in complete darkness in ambient laboratory conditions (temperature: ~22°C) until larval dispersal to simulate natural subterranean environments. When larvae dispersed from the carcass, I transferred them to new 6L containers filled with ~10cm of soil to allow pupation and placed these containers in a Percival DR36VL environmental chamber (Percival Scientific, Perry, IA, USA) set to a reversed photoperiod (16L: 8D) to align nocturnal beetle activity with daytime observations. Beginning on day 45 after beetle pairing (i.e., day 0), I checked the containers daily and removed newly eclosed beetles to prevent sibling aggression. I housed each beetle individually in a 350mL plastic container (14 × 10 × 3.5cm) with a moistened paper towel as a water source and thawed ground beef (~0.5g). From eclosion onward, I provided beetles food and water twice weekly. On day 9 post-eclosion, I fed all beetles regardless of the schedule, and on day 10, I recorded morphological measurements and determined each beetle's sex. I measured their body mass using a digital jewelry scale (±0.001g, Smart Weigh GEM20), calibrated using a 10g standard and corrected as follows: measured beetle weight × (measured standard weight / 10g). I measured pronotum width (i.e., the hardened dorsal shield over the thorax; Figure 1A), which served as an estimate of body size (Figure A1) with digital calipers (±0.01cm, Marathon CO 030150 Electronic Digital Caliper) and determined sex by counting abdominal segments: males have five segments and females have four (Figure 1B). I collected three repeated measurements from 35 individuals prior to the experiment to assess measurement repeatability and error, given the central role of body size in subsequent analyses. I collected these measures in random order, with no identifying information available for each beetle, to increase independence of each measurement. These measurements revealed high precision, with a mean measurement error of <0.01cm and a repeatability of 0.97 [95% CI: 0.95-0.98] (Table A1).

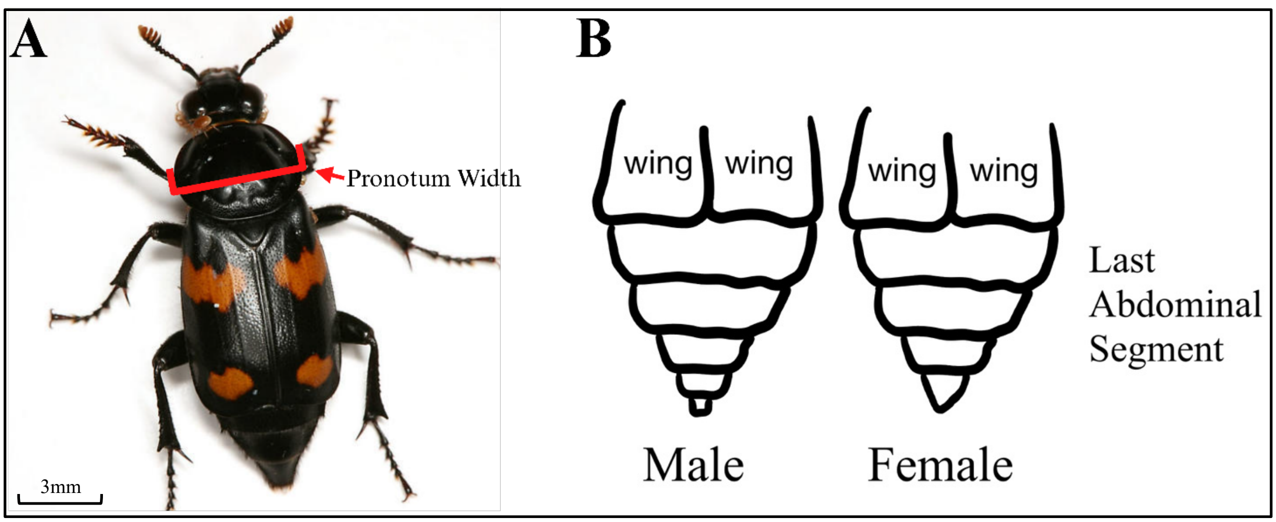

Figure 1. Measurement and sex determination in *Nicrophorus orbicollis*. (A) Diagram showing how pronotum width was measured (i.e., at the widest point of the pronotum) (adapted from Quinn, 2015). This measurement was used as a proxy for body size in all analyses. (B) Diagram depicting abdominal segmentation: males have five visible segments, while females have four.

I maintained *Poecilochirus* mites independently of the beetles in five 6L plastic containers with perforated lids and ~2cm of organic garden soil. I fed the mite colonies 1-2 thawed mouse carcasses per week, which served as both a food source and reproductive site. To maintain colony hygiene and prevent microbial buildup, I transferred the mites to new containers approximately every six months, or as needed based on observations of proliferating fungal growth. The original mite colonies were established from individuals collected off 4-5 *N. orbicollis* beetles captured in forested areas near the Queen's University Biological Station. I subsequently maintained the colonies under ambient laboratory conditions (temperature: 22°C). To minimize inbreeding, I routinely exchanged lids and mouse carcasses between containers to promote gene flow across colonies. When mite populations declined, I supplemented colonies by either introducing wild-caught individuals or breeding new mites. To do so, I paired a male and female *N. orbicollis* beetle in a separate breeding container under standard conditions allowing mites to complete their reproductive cycle alongside the beetles.

Experimental Methods

Competitive Dominance Baseline

I conducted preliminary competitive dominance trials (n = 43) to determine the range of body size differences (as estimated by pronotum width) between competitors for use in subsequent experiments. These baseline trials followed the same protocol as the main experiment (described below) but excluded mites and varied temperature conditions. The outcomes were used to identify the range of size differences where competition outcomes were variable (i.e., where the smaller beetle had a non-zero chance of winning), and to select a range of pairings for the experiment (centered around a mean pronotum width difference of 0.05cm, range: 0.01–0.15cm).

Competitive Dominance Trials:

<u>*Pre-Trial Preparation*</u>

I thawed mouse carcasses (20-30g; Canadian Mouseman, Medicine Hat, AB) at room temperature for 48 hours prior to use in competitive trials. Two days before each trial, I marked each beetle in a pair with a small dot of nail polish (either black or white) on the elytra to allow for visual individual identification. I alternated which beetle (smaller or larger) received which colour every 6 trials (3 warm, 3 cold) to control for potential colour effects. After the polish dried, I fed each beetle and provided them a moistened paper towel to ensure they began competitive trials with similar previous access to food and water, which could influence their motivation and ability to compete for a carcass.

I collected live mites from the colonies using a stainless-steel double ended spatula and transferred them into 14mL Falcon Tubes. I then placed the mites onto a CO_2 plate and counted out 35 live individuals for each mite-designated beetle and transferred them to a plastic container (120mL). This load was intentionally set above typical pre-breeding levels observed in field-caught beetles (10-30 mites; Springett, 1968; Schwarz & Muller, 1992; Grossman & Smith, 2008; Nagel et al., 2025) to ensure that at least 25-30 mites remained attached by the start of the trial (after the exposure phase detailed below), and to maximize the likelihood of detecting any potential effects. I then introduced each beetle into the container with 35 mites (mite treatment) or into an identical container without mites (control). All containers included a slightly moistened paper towel but no food. I held the beetles in these containers in an environmental chamber (22°C/19°C day-night cycle, 16L:8D) for approximately 40 hours prior to the start of competitive trials, to allow mites time to attach to beetles.

Experimental Design

Each trial involved a pair of same-sex but size-mismatched beetles (larger vs. smaller) competing for a mouse carcass under one of six treatment conditions. I haphazardly paired beetles for trials based on the difference in the width of their pronotums (mean = 0.05cm difference, range 0.01–0.15cm) ensuring that neither siblings nor cousins were paired. Each day, I selected six pairs and randomly assigned two to each mite treatment group (i.e., larger beetle, smaller beetle, or none) at a single temperature (15°C or 25°C) using a random number generator, alternating temperature treatments daily (Table 1). I assigned mite treatments without prior knowledge of size differences, resulting in a similar distribution of pronotum size differences across treatment groups. Sample size varied among treatment groups due to differences in beetle mortality during the 40-hour pre-experiment exposure period (described above) in an environmental chamber prior to the start of trials. I excluded trials if a winner could not be determined (e.g. both beetles were equidistant from the carcass at the end of the experiment; n = 6), resulting in a total of 126 successful trials (Table 1).

Table 1. Treatments and sample sizes (number of pairs) for competitive dominance trials.

Group	Temp. (°C)	Mites	Final N	Mean Size Difference (cm)
1	15 (Cool)	Larger	27	0.04
2	15 (Cool)	Smaller	21	0.05
3	15 (Cool)	None	20	0.06
4	25 (Warm)	Larger	18	0.05
5	25 (Warm)	Smaller	21	0.05
6	25 (Warm)	None	19	0.05
Total			**126**	**0.05**

Arena Setup & Trial Conditions

Each trial was conducted in a 6L plastic container filled with ~1cm of soil. I set the environmental chamber to the designated trial temperature (15°C or 25°C, both at 68% RH) and allowed it to stabilize before introducing the beetles. To allow observation without disrupting beetle behaviour, I affixed 2 red LED bicycle lights (Supercycle 10-Lumen Rear Light) to the sides of each container beneath the lid. These lights produced low-intensity red illumination (~10 lumens), to minimize disturbance to the nocturnal beetles while enabling trials to be video recorded. During the beetles' inactive period (daylight), I assessed each individual's condition (e.g., limb loss, wing damage, activity level). I then transferred each beetle to a new 120mL container and introduced the pair into the arena at the same time in different corners, along the side of the container opposite the carcass, so that each beetle was equidistant to the carcass.

I inspected the paper towels and original containers for any remaining mites and deducted those numbers to calculate starting mite load. To begin each trial, I simultaneously removed the cups from both beetles and then covered the arena with a ventilated lid. Trials ran for 8 hours under red light only, corresponding to the beetles' active nocturnal period.

Post-Trial Procedures

After 8 hours, still under red light conditions, I removed one container at a time from the environmental chamber. For each pair, I recorded the competition outcome, using the closest beetle to the carcass as a proxy for behavioural dominance (Schrempf et al., 2021). I also recorded activity level, positioning, survival, and carcass condition. For trials that included mites, I anesthetized both beetles using CO_2, brushed off any remaining mites, and recorded each beetle's final mite load (number of mites found on a beetle at the end of the trial).

Grooming Behaviour Assays:

Procedure & Pre-Trial Preparation

To assess the beetle's behavioural responses to phoretic mites, I measured grooming and escape behaviours of *N. orbicollis* under controlled conditions. I categorized behaviours in real time by activity type (grooming, escape, or other), body region, and locomotion status (locomoting or not) using standardized datasheets (Figure A2). The classification system I used was adapted from Valentine (1973), who described grooming movements in Coleoptera as cleaning, rubbing, and positioning, and from Vincent and Bertram (2010), who grouped behaviours by anterior and posterior regions. Grooming included any rubbing or wiping of body parts with legs or mouthparts. Behaviours that resembled grooming but occurred along with rapid locomotion, biting, or wall-rubbing were categorized as escape behaviours, while behaviours that fit into neither category, (e.g., antennae movement or head repositioning), were classified as "other".

The day before each trial, I fed the beetles and provided them a moistened paper towel. I assigned individuals to either a mite or control treatment, balancing for sex and body size (n = 40; 20 males and 20 females). To account for size variation, I categorized beetles into four pronotum width classes: <0.55cm, 0.56 – 0.60cm, 0.61 – 0.65cm, >0.65cm. I distributed size classes evenly across treatments. All beetles included in the assay were lab bred (F5-F6) and naïve to mites. Before each trial, I collected mites from lab-maintained colonies using a stainless-steel double-ended spatula and transferred them into a 14mL plastic Falcon tube. I anesthetized mites on a CO_2 plate, counted 30 live individuals, and transferred them to a clean, clear, 120mL plastic container.

<u>*Trial Setup & Observation Procedure*</u>

Each beetle was tested in two 120mL clear plastic containers: one empty pre-exposure container, followed by either a mite-treated or empty control container during the exposure phase. Each container included a ~5cm cotton swab (cotton tip removed) to aid beetle mobility.

Each assay consisted of two sequential 20-minute observation periods during which I recorded behaviour using instantaneous sampling at 10-second intervals (5 seconds observing the beetle's behaviour, 5 seconds recording the behaviour). I then transferred the beetle into the second container (with or without mites) and repeated the same sampling procedure during the 20-minute exposure phase. Behaviours were scored using a standardized sampling sheet (Figure A2) to minimize subjectivity; however, observers were not blind to treatment, as mite presence was obvious and could not be concealed. To reduce potential observer bias, final statistical models were conducted by a separate researcher blinded to treatment identity.

Following the trial, I anesthetized all beetles in the mite-exposed treatment group using CO_2 and counted the number of mites attached to each individual.

Statistical Analysis:

I performed all statistical analyses in R (version 4.3.3). I assessed fit and agreement with model assumptions for all global and top-ranked models using the *DHARMa* package to assess homoscedasticity, dispersion, zero inflation, and distribution of model residuals. I simplified all models using model selection based on Akaike's Information Criterion corrected for small sample size (AICc), ranking all recombinant versions of the global models, including a null, using the *dredge* function. I report the top-ranked model in the results and provide details on all models with ΔAICc < 2 in Tables A2-A13. I used the *emmeans* package to compare pairwise differences among levels of categorical variables involved in significant interactions using Tukey HSD.

Competitive Dominance Baseline

To assess whether larger beetles were generally more likely to win, I performed a one-way binomial test assessing the number of competitions won by the larger beetle out of the total number of trials. I also assessed whether size difference, sex, or their interaction predicted the competition outcome using a GLM with a binomial error distribution (logit link) with the outcome of competitive trials as the response variable and the difference in size of the competing beetles (as estimated by pronotum width difference), beetle sex, and their interaction as fixed effects in the global model.

Competitive Dominance Trials

I used GLMs with a binomial error distribution (logit link) to test whether mite treatment, temperature, sex, and pronotum width differences affected the outcome of competitive trials. I treated outcome (0 = small won, 1 = large won) as the binary response variable. I included treatment group (larger beetle with mites, smaller beetle with mites, or no-mite control), beetle sex, pronotum width difference, temperature (15°C or 25°C), and all two-way interactions as fixed effects in the global model.

After finding no differences between treatment groups where the larger or smaller beetle was exposed to mites, I assessed the overall effect of the presence of mites on competition outcome by pooling these two mite treatments into a single “mite-present” category and repeated the model selection procedure with treatment (mite-present vs. control), sex, temperature, and size difference, and all two-way interactions as fixed effects in the global model. A main effect of temperature or mite treatment would suggest that factor independently influences outcome (H_1), whereas an interaction between mite treatment and temperature would indicate context-dependence (H_2).

I conducted a number of follow-up analyses based on the results of the above analysis. To determine if mites preferentially attached to the winner or loser of trials, I used a paired t-test to compare the number of mites on winning vs. losing beetles, using only trials from the mite-present treatment groups. I also examined whether mites were more likely to transfer from small to large beetles by assessing the proportion of mites transferred between the beetle they started on and their opponent. I used a binomial GLM weighted by the initial number of mites present and included treatment, sex, size difference, outcome (larger or smaller won), and all two-way interactions as fixed effects. I predicted that if mites preferentially transferred based on size, a greater proportion of mites would finish on the larger beetle. Alternatively, if mites responded to the outcome of the contest, I expected a greater number on the winner. If both factors influenced transfer, I expected an interaction between outcome and beetle size.

Grooming Behaviour Assays

Prior to analysis, I assessed normality of the distributions of pronotum width and body mass using Shapiro-Wilk tests and histograms, and natural log (ln)-transformed both to improve distributional properties. Due to the strong correlation between pronotum width and mass ($r = 0.86$, $p < 0.001$), only pronotum width was retained as a covariate in analyses (Figure A1).

I examined grooming, escape, and other behaviour data distributions for normality and applied appropriate transformations identified using the *bestNormalize* package: a square-root transformation for grooming and escape behaviours, and a double reverse log transformation (base 10) for other behaviours. To assess correlations among the three behavioural metrics, I calculated Pearson correlation coefficients on their transformed values. Other behaviours were strongly negatively correlated with grooming ($r = –0.92$, $p < 0.001$) and escape ($r = –0.55$, $p < 0.001$), while grooming and escape were weakly positively correlated ($r = 0.21$, $p = 0.059$). Given this multicollinearity, I performed a principal component analysis (PCA) using the *prcomp* function. The first principal component (PC1) explained >72% of variation in the three behaviours and was retained as a general estimate of behaviour (original loadings: grooming = -

0.60, escape = -0.42, other = 0.67). For analysis, PC1 scores were multiplied by -1 so that higher scores reflected higher rates of grooming and escape behaviours, and lower rates of other behaviours Figure A3). All subsequent analyses used this PC rather than individual behavioural measures (separate analyses of grooming and escape behaviours are provided in Figures A4 and A5).

To assess the effect of mite treatment and exposure period (pre vs. post exposure) on beetle behaviour, I fit an LME using the *lme* function from the *lme4* package. I modeled behaviour (PC1) as a function of treatment (mite or no-mite), time period (pre- or post-exposure), pronotum width (ln transformed), and sex, along with all two- and three-way interactions as fixed effects in the global model. I included beetle ID as a random effect, to account for repeated measures of behaviour within individuals across time periods. I predicted that grooming and escape behaviours would increase if the net fitness effect of the mites on the beetle is negative (i.e., antagonistic) (H_1). Conversely, if the relationship is commensal or mutualistic, I expected no change or a decrease in these behaviours (H_0).

CHAPTER 3: RESULTS

Baseline Competitive Dominance Trials

The larger beetle won baseline competitive trials more often than the smaller beetle (*binomial test*, n = 35, larger beetle win rate = 77%, [95% CI: 0.63, 1.00], p = 0.001; Figure 2). Beetle sex and size difference (as estimated by difference in pronotum width) were both retained in the top-ranked model explaining variation in the outcome of baseline competitive trials (Table A2). Larger males tended to be less likely to win their contests than larger females (*GLM*, n = 35, β = -1.78 [95% CI: -3.93, -0.03], z = -1.85, p = 0.064). Size difference, although retained in the top-ranked model, was not a strong predictor of outcome, but the effect estimate was positive, suggesting the beetles with a greater size advantage won more often (*GLM,* n = 35, β = 21.19 [95% CI: -1.24, 56.26], z = 1.50, p = 0.134). When the size difference exceeded 0.1cm, the larger beetle always won, but at size differences between 0.01cm and 0.08cm, outcomes were more variable, indicating a range where dominance was not entirely determined by size (Figure 2). Three other models were similarly likely as the top-ranked model ($\Delta AICc \leq 2$, Table A3), with the second ranked model retaining only sex, and the third ranked model retaining only size difference. The null model ranked 4th ($\Delta AICc = 1.70$).

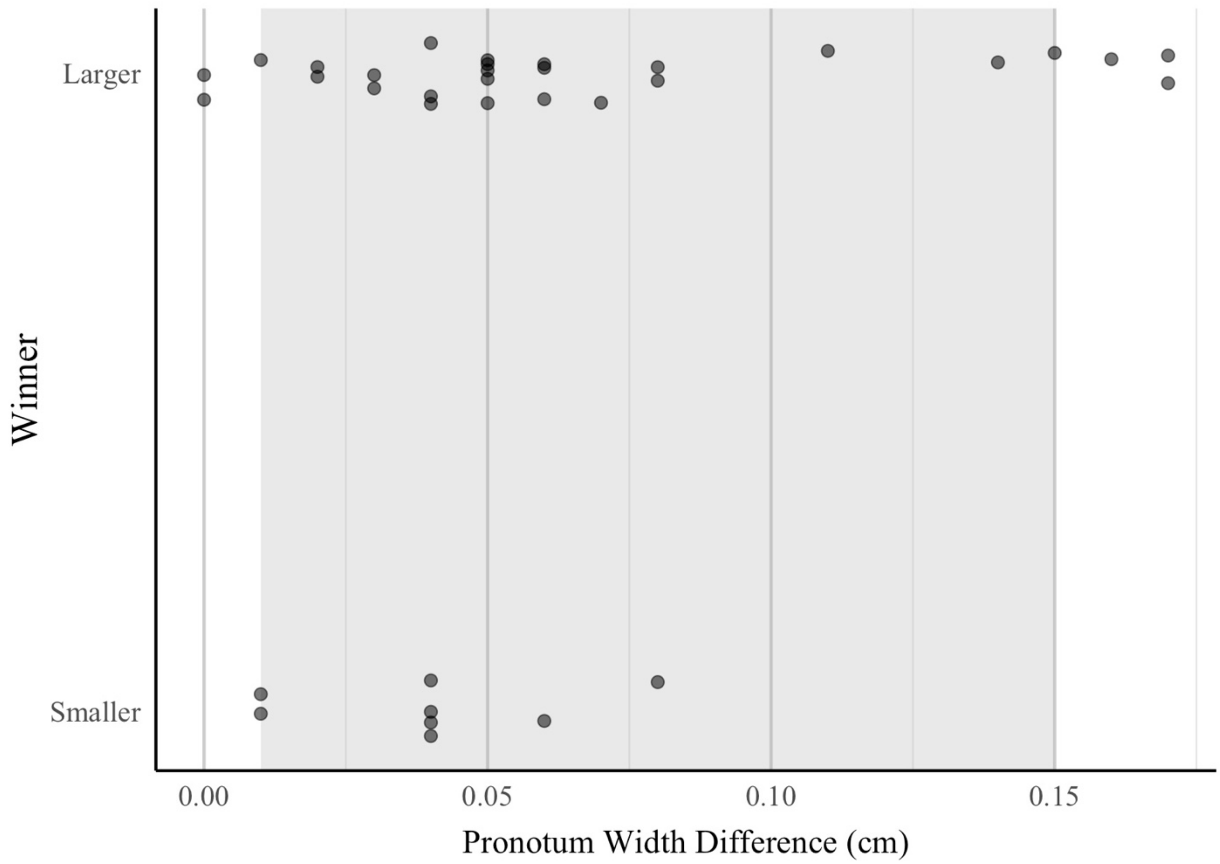

Figure 2. The outcomes of baseline competitive trials were not strongly predicted by the difference in body size (as estimated by difference in pronotum width) of *Nicrophorus orbicollis* competitors, although larger beetles won 27 of 35 trials. Each point represents a single trial, with the y-axis indicating which beetle won (larger or smaller) and the x-axis showing the difference in pronotum width (cm). Points are jittered along the y-axis to improve visualization, with darker points indicating overlap. The shaded area depicts the range of size differences used in subsequent experiments. See main text for statistical support.

Influence of Mites & Temperature on Competition

Experimental exposure to mites and beetle sex predicted the outcome of competitive trials between two beetles, but temperature and size difference did not (Table A4, Table A5). The larger beetle was more likely to win in control trials without mites than in trials where the smaller beetle started with mites (*GLM,* n = 126, $\beta = -1.20$ [95% CI: -2.30, -0.19], $z = -2.26$, $p = 0.024$) and tended to be more likely to win when the larger beetle started with mites (*GLM,* n = 126, $\beta = -0.81$ [95% CI: -1.90, 0.20], $z = -1.54$, $p = 0.124$). In contrast to the result of the baseline trials, larger males tended to win their trials more often than larger females (*GLM*, n = 126, β = 0.68 [95% CI: -0.10, 1.49], z = 1.69, p = 0.091). Four other models were similarly likely as the top-ranked model (ΔAICc≤2, Table A5). Sex and mite treatment were retained in 3 of the 5 top models, while temperature was not retained until the 4^{th}-ranked model (ΔAICc = 1.79). No interactions between factors were retained in any of the top 5 models. Competitive outcomes did not differ based on whether the larger or smaller beetle started with mites (*emmeans contrast, smaller vs. larger:* β = 0.39 [95% CI: -0.68, 1.46], z = 0.85, p = 0.670; Figure A6), so these treatment groups were combined into a single "mites present" group for subsequent analyses.

In this follow-up analysis, the top-ranked model (Table A6) retained the fixed effects of sex and mite treatment (mites present or absent), but not temperature or size differences. The larger beetle won 82% of contests in the absence of mites, but only 63% of contests when mites were present (*GLM*, n = 126, β = -1.00 [95% CI: -2.00, -0.11], z = -2.10, p = 0.036; Figure 3). Larger males tended to win more often than larger females (*GLM*, n = 126, β = 0.64 [95% CI: -0.13, 1.44], z = 1.62, p = 0.106). Five other models were similarly likely as the top-ranked model (ΔAICc≤2, Table A7). Mite treatment was retained in all 6 of these models, while sex was retained in 5 of them.

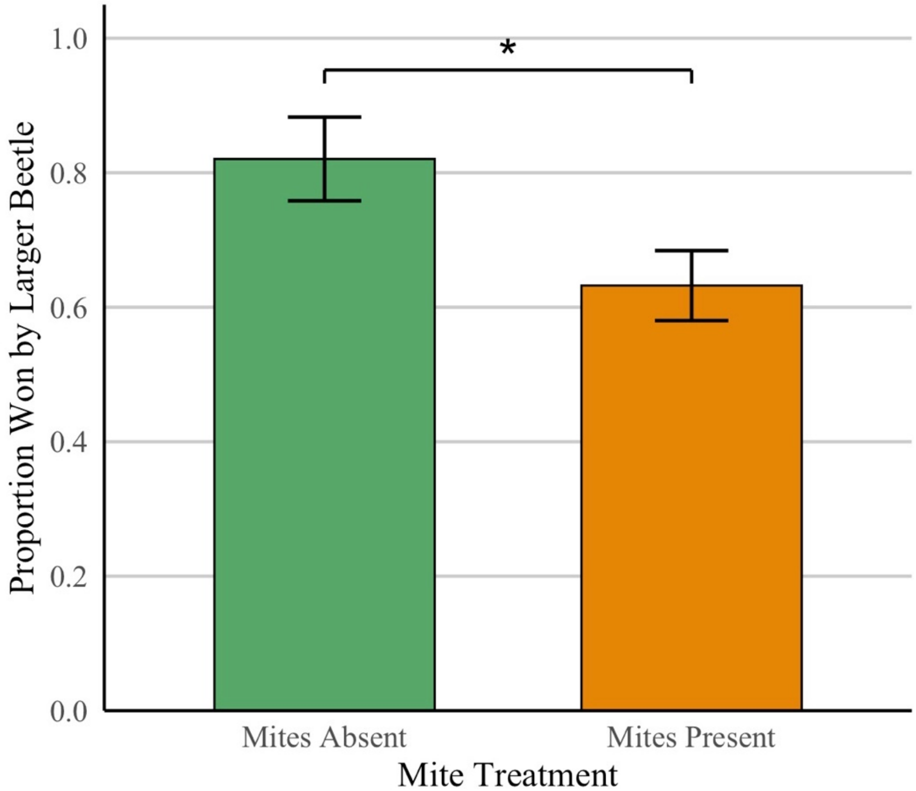

Figure 3. The larger *Nicrophorus orbicollis* competitor won less often in trials when *Poecilochirus carabi* mites were present (orange bar), relative to trials without mites (green bar). Bars show the proportion of contests won by the larger individual (y-axis: 1 = all contests won by the larger beetle, 0 = all contests won by the smaller beetle). Error bars show ± 1 SE. Asterisk indicates a statistically significant difference between groups (p = 0.036). See main text and Table A5 and A6 for statistical support.

When considering only trials that included mites, losers carried fewer mites than winners by the end of the trial (*t*-test, n = 87, mean difference between winners and losers = -1.85 mites [95% CI: -3.26, -0.44], t = -2.61, df = 86, p = 0.010; Figure 4). Mite transfer during competition was predicted not only by the trial's outcome, but also by beetle size. The top-ranked model (Table A8) explaining variation in the proportion of mites that transferred from the beetle they started on to their competitor retained mite treatment (larger or smaller started with mites), sex, size difference, the outcome of competition (larger or smaller won), and the interaction between mite treatment and outcome. Mite transfer was lowest when mites started on the larger beetle and it won and highest when mites started on the smaller beetle and it lost (Table A9; Figure 5).

Additionally, mite transfer was higher in male-male contests, relative to trials between females (*GLM*, n = 87, $\beta = 0.50$ [95% CI: 0.28, 0.72], z = 4.42, $p < 0.001$). Mites also tended to transfer more often in trials with a greater size difference between the two beetles (*GLM*, n = 87, $\beta = 6.41$ [95% CI: -0.17, 12.96], z = 1.91, p = 0.056). Two other models were similarly likely as the top-ranked model (ΔAICc≤2, Table A10). All three top models retained mite treatment, outcome, sex, and the interaction between mite treatment and the outcome of competition.

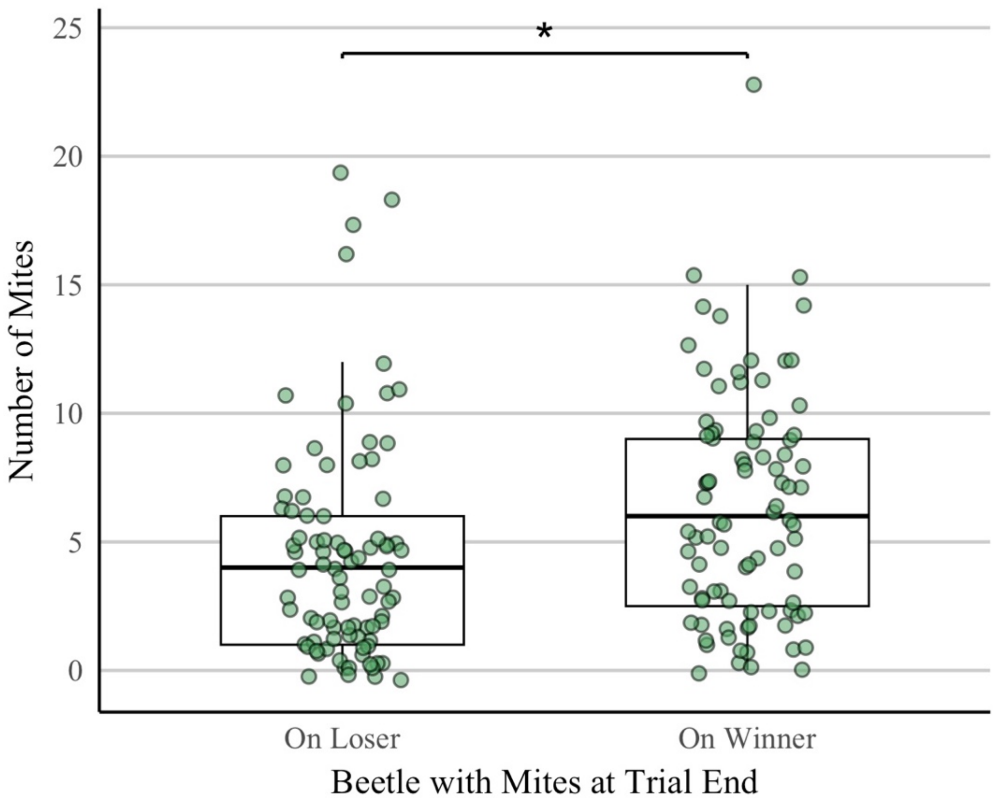

Figure 4. *Nicrophorus orbicollis* beetles that lost competitive trials carried fewer *Poecilochirus carabi* mites at the trial's end than winning beetles. Points represent individual beetles and are jittered along the x-axis for better visualization. Boxes represent the interquartile range (25th – 75th percentile), with the median shown as a bold line. Whiskers extend to the most extreme values within 1.5x the interquartile range. Asterisk denotes a statistically significant difference (p = 0.011). See the main text for full statistical support.

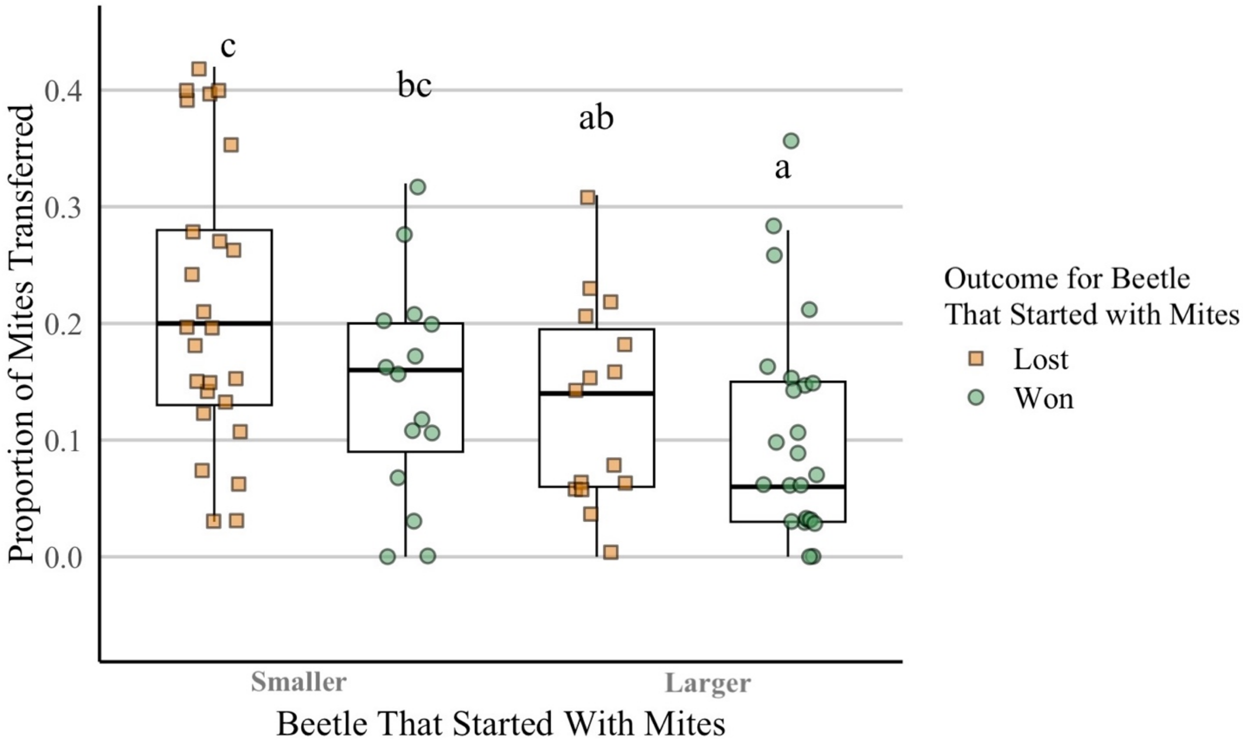

Figure 5. The proportion of *Poecilochirus carabi* mites that transferred during competitive trials depended on both the size of the *Nicrophorus orbicollis* beetle they started the trial on (x-axis, larger or smaller) and which beetle won the trial. Mite transfer was highest when mites started on the smaller beetle and the larger beetle won, and lowest when mites started on the larger beetle and it won. Points represent individual trials and are jittered along the x-axis for better visualization. Green circles represent trials where the beetle that started with mites won; orange squares represent trials where the beetle that started with mites lost. Boxes represent the interquartile range (25^{th} – 75^{th} percentile), with the median shown as a bold line. Whiskers extend to the most extreme values within 1.5x the interquartile range. Different letters above boxes indicate statistically significant differences among groups (*emmeans*, $p < 0.05$). See the main text and Table A9 and Table A10 for statistical support.

<u>Grooming Behaviour Assay:</u>

Beetles showed evidence of increased aversive behaviours when exposed to mites (see Figure A4 and A5 for separate analyses of grooming and escape behaviours). The top-ranked model (Table A11) retained treatment, time period (pre- or post-exposure), and their interaction, while size difference and sex were not retained. One other model was similarly likely as the top-ranked model (ΔAICc≤2, Table A12). This model retained the same factors as the top model, along with size difference. The effect of treatment depended on time period (*LME,* Treatment ×

Time Period interaction: n = 80, β = -1.87 [95% CI: -2.81, -0.94], t = –3.92, df = 38, $p < 0.001$; Figure 6; Table A13). During the exposure phase, beetles in the mite treatment group exhibited increased grooming and escape behaviours relative to their behaviour before exposure and also relative to control beetles during both phases (Figure 6; Table A13).

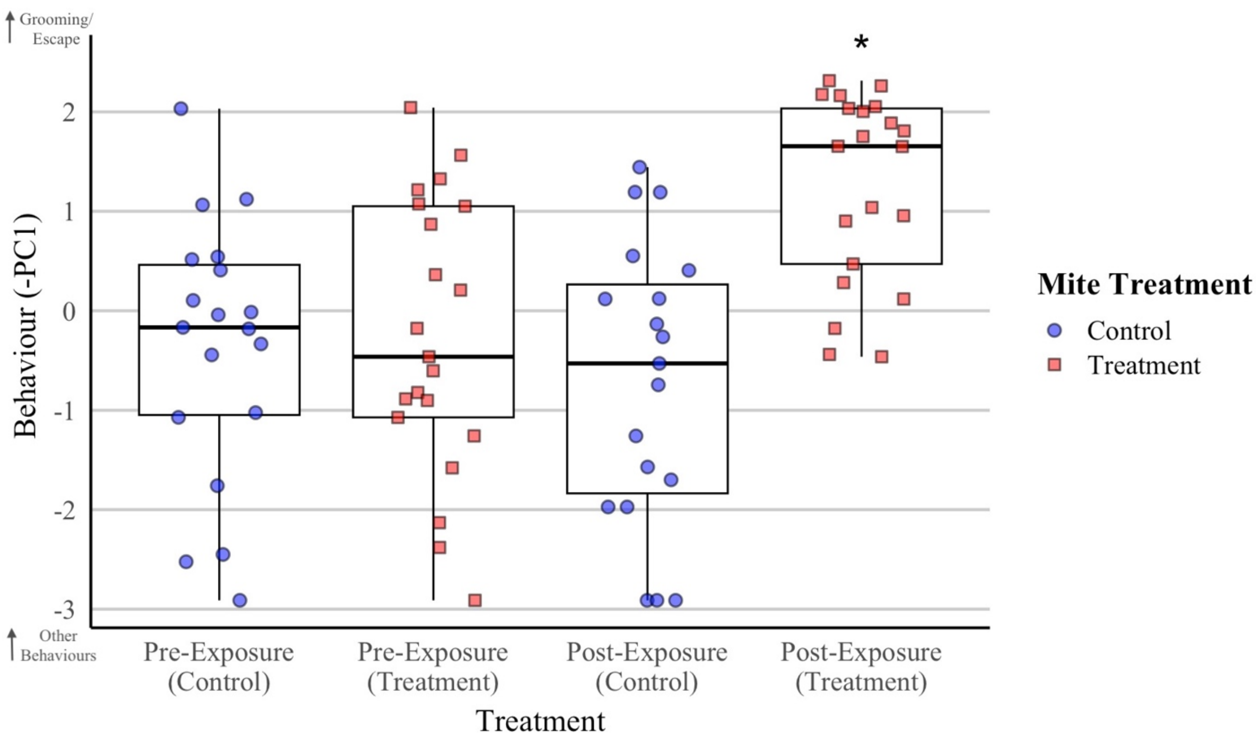

Figure 6. *Nicrophorus orbicollis* beetles increased aversive (escape and grooming) behaviours in response to exposure to *Poecilochirus carabi* mites. Behaviour is summarized using PC1 scores (y-axis), where higher values reflect more grooming and escape behaviours and lower values reflect increased rates of other behaviours. The x-axis shows the treatment each beetle received and time period of observations (pre- or post-exposure). Points represent individual beetles and are jittered along the x-axis for better visualization. Blue circles represent control beetles; red squares represent mite-exposed beetles. Boxes represent the interquartile range (25^{th} – 75^{th} percentile), with the median shown as a bold line. Whiskers extend to the most extreme values within 1.5x the interquartile range. The asterisk denotes a statistically significant difference from all other groups (*emmeans* $p < 0.05$). See the main text and Table A12 and Table A13 for statistical support; separate plots for grooming and escape behaviours are presented in Figures A4 and A5.

CHAPTER 4: DISCUSSION

I investigated how phoretic mites (*Poecilochirus carabi*) influence behaviour and performance in *Nicrophorus orbicollis*, using both competitive trials and assays of grooming and escape behaviours. Mites reduced the likelihood of winning for larger beetles by preferentially transferring to them mid-contest, allowing smaller individuals to gain a relative advantage due to their opponent's increased mite burden. In parallel, beetles exposed to mites exhibited elevated rates of grooming and escape behaviours, consistent with an aversive response. While neither outcome directly estimates fitness, both suggest that mites impose net costs on their hosts that might affect fitness over evolutionary timescales, despite occasionally conferring context-dependent benefits.

In the absence of mites, larger *N. orbicollis* competitors consistently outcompeted smaller rivals, consistent with earlier work on size-based competitive hierarchies in both intra- and interspecific contests among burying beetles (Otronen, 1988; Schrempf et al., 2021; Vangenne et al., 2023). However, this size-based advantage was disrupted in the presence of *P. carabi* mites: mites transferred preferentially to the larger beetle mid-contest, resulting in a higher final mite load relative to their smaller opponent. This asymmetric burden reduced the larger beetle's likelihood of winning, allowing smaller individuals to succeed in contests they would typically lose. These results suggest that mites can alter competitive outcomes by targeting the likely winner, rather than directly improving the performance of smaller beetles.

Mite transfer tended to occur most frequently in male-male contests and in trials with a greater size difference between opponents. This pattern suggests that mites respond dynamically to cues associated with both beetle sex and size. One possibility is that mites cue on traits linked to male life history, such as earlier dispersal from the brood chamber, which might increase the mites' likelihood of reaching a future breeding site (Schwarz & Muller, 1992; Canitz et al., 2021). Importantly, however, these contests for carcasses occur prior to breeding, and mites will ultimately disembark to reproduce regardless. Another possibility is that behavioural differences between males and females influence transfer rates, with males perhaps engaging in more aggressive interactions or spending more time in contact with the carcass, both behaviours that could facilitate mite movement. Testing this would require detailed behavioural observations; although video recordings are available for some trials, their quality might limit the ability to reliably score fine-scale behaviours. Notably, mite transfer tended to increase in contests with greater size asymmetry, suggesting that mites might actively cue on beetle size, and, by extension, competitive dominance, when switching hosts. Temperature, by contrast, did not affect contest outcomes or modulate the effect of mites.

Although my findings parallel those of Sun et al. (2019), who also reported increased competitive success of smaller *Nicrophorus vespilloides* beetles in the presence of *Poecilochirus* mites, the underlying mechanisms appear to differ substantially. Sun et al. attributed this effect to thermal enhancement, proposing that mites raise their host's body temperature – particularly in smaller individuals – thereby improving their competitive ability (Sun et al., 2019). In contrast, my results support a behavioural mechanism: mites dynamically transfer to the larger individual (i.e., the likely winner) mid-contest, imposing an asymmetrical burden that reduces their likelihood of winning. This shift likely arises not from improved performance in smaller beetles, but from a reduction in their opponent's competitive performance – potentially arising from biomechanical handicapping, increased grooming effort, distraction, and/or carcass abandonment due to increased mite load. Several factors might help explain this divergence. First, species differences are important: *N. orbicollis*, which is larger-bodied, North American, and breeds later in the season, might be less sensitive to small thermal changes than its European counterpart, *N. vespilloides*. *N. orbicollis* also buries carcasses more deeply, reducing exposure to blow fly predation and competition (Wilson & Knollenberg, 1987). By contrast, *N. vespilloides* faces stronger pressure from blow flies, which might intensify the mutualistic dimension of its association with mites (Wilson & Knollenberg, 1987). Second, experimental design differed: my trials ran for longer periods (8hrs) under stable soil temperatures and allowed time for mites to attach and transfer both before and after competition, whereas Sun et al. (2019) focused on shorter contests (30mins intervals, ending once the same beetle was observed on the carcass in two consecutive checks). These ecological and experimental contrasts might help explain why Sun et al. (2019) reported that 97.5% of mites remained attached to their host beetle throughout contests. Nevertheless, previous work on *N. vespilloides* and other *Nicrophorus* species has demonstrated that mites frequently transfer between beetles when they encounter one another at carcasses, even though they do not fully disembark until burial and brood-chamber entry (Wilson & Knollenberg, 1984; Otronen, 1988; Muller & Schwarz, 1992). In particular, Muller & Schwarz (1992) showed that mites on *N. vespilloides* can redistribute between competitors, effectively evening out densities across beetles at a carcass. Consistent with this behavioural flexibility, Sun et al.'s (2019) field data also indicated that mites were more abundant on large *N. vespilloides* and appeared to prefer them, in line with the behavioural mechanism I observed. Thus, while both studies describe similar ecological outcomes, the differing interpretations of mechanism illustrate how variation in experimental context or observational detail can shape our understanding of species interactions.

Together, these findings suggest that benefits to smaller beetles arise not through enhanced performance, but through the selective disruption of their opponent's dominance.

Rather than directly interfering with contests, mites impose asymmetrical costs by preferentially transferring to larger individuals mid-contest, effectively altering the competitive landscape. This form of behavioural interference, where symbionts alter interaction outcomes by impairing host condition, is conceptually similar to the logic of the Handicap Hypothesis, which proposes that costly traits honestly signal condition by handicapping the bearer (Penn & Számadó, 2019). Here, however, the 'handicap' is not an evolved signal but a parasite-imposed burden that diminishes fitness or performance – in this case, competitive ability. For example, microsporidian infections in amphipods reduced shelter defence and displaced infected individuals from contested shelters, shifting competitive hierarchies and allowing subordinate species to coexist with dominant ones that would otherwise displace them (Kobak et al, 2021). More broadly, a recent meta-analysis revealed that parasites can reduce, amplify, or have no effect on competition (Hasik et al., 2022), suggesting that such handicapping effects might be widespread but context-dependent. However, this synthesis also reveals how fragmented the existing evidence remains across systems and approaches. Going forward, identifying the specific pathways by which mite burden impairs performance, whether through mechanical constraint, increased grooming, or decision-making costs, will be essential for understanding the broader ecological implications of these interactions.

In addition to altering competitive outcomes, *P. carabi* mites elicited a consistent behavioural shift in beetle hosts. Beetles exposed to mites exhibited increased grooming and escape behaviours, which were positively correlated and negatively correlated with other observed behaviours. This pattern suggests a reallocation of effort toward parasite avoidance and removal. Given the energetic and opportunity costs of grooming (e.g., reduced foraging or mating time; Mooring, 1996; Mooring, 2024), such responses are unlikely to persist without corresponding selective benefits. Thus, the presence of these aversive behaviours across individuals supports the interpretation that *P. carabi* imposes sufficient costs to select for behavioural resistance, in contrast to the tolerance expected, and often observed, in commensals or mutualists (Mooring, 1996; Kohari et al., 2020; Plotz & Linklater, 2020).

Across taxa, grooming is a well-documented behavioural defence against parasitic ectosymbionts and pathogens (Mooring, 1996; Lacroux et al., 2023). For instance, primates, rodents, and bats all show elevated grooming in response to mite and tick infestation, even when doing so increases metabolic demands (Giorgi et al., 2001; Akinyi et al., 2013). In contrast, hosts engaged in mutualistic relationships often reduce grooming effort or actively tolerate certain symbionts, despite minor costs. Black rhinoceroses (*Diceros bicornis*), for example, actively groom away parasitic stable flies (*Stomoxys calcitrans*) while tolerating oxpeckers (*Buphagus erythrorhynchus*), which offer net benefits such as parasite removal and predator detection, despite occasional costs (Kohari et al., 2020; Plotz & Linklater, 2020). This distinction, between grooming as a resistance trait and tolerance of beneficial interactions provides a useful framework for interpreting host behaviour. In this context, the consistent increase in antiparasitic behaviours among mite-exposed beetles provides strong evidence that *N. orbicollis*'s interaction with *P. carabi* is generally antagonistic. To my knowledge, this is the first study to use grooming behaviour as a behavioural indicator of the evolutionary costs associated with an interspecific interaction. Because grooming is widespread, easily measured, and responsive to selective pressures, it represents a promising behavioural metric for quantifying the fitness impact of interspecific relationships in both lab and field contexts. Future work could assess the beetles' ability to adjust aversive responses to allow colonization by mites in contexts where they might be beneficial (e.g., when competitors like carrion flies are present in high numbers, Nagel et al., 2025).

Together, the competitive trials and grooming assays paint a coherent picture of the *Nicrophorus-Poecilochirus* relationship: *P. carabi* mites reduce the competitive success of larger beetles through preferential transfer based on size and sex, and impose sufficient fitness costs to elicit consistent antiparasitic responses across beetle hosts. While smaller individuals might occasionally benefit from mite presence, these benefits are context-dependent and asymmetrically distributed. The widespread expression of grooming and escape behaviours instead signals long-term selection for resistance, a clearer indicator of the evolutionary pressures shaping this interaction than short-term shifts in contest outcomes.

My findings help clarify a long-standing debate within the burying beetle literature. The nature of the *Nicrophorus-Poecilochirus* relationship has been variously described as mutualistic, commensal, or parasitic depending on ecological conditions (Springett, 1968; Blackman & Evans, 1994; Sun & Kilner, 2020). For instance, mites can deter heterospecific competitors, offering short-term benefits under certain circumstances (Springett, 1968; Blackman & Evans, 1994; Nagel et al., 2025). However, such context-dependent effects have made it difficult to assess the net direction of selection shaping the interaction. By linking behavioural resistance with asymmetric ecological costs, my study suggests that, over evolutionary timescales, the relationship is best characterized as antagonistic. Reviews of dung beetle-mite (Scarabaeinae-Acari) associations similarly emphasize that interactions once assumed to be simple phoresy in fact span a continuum from mutualism to parasitism, shaped by ecological context (Noriega et al., 2022). Situating my findings within this broader pattern underscores that ambiguity in beetle-mite interactions is not unique, but part of a wider challenge in classifying symbioses that shift along the mutualism-antagonism gradient. However, behavioural evidence – such as grooming and escape behaviours – can help resolve these uncertainties by indicating the net direction of selection over time. Future experiments manipulating mite number could help disentangle these effects, particularly given that previous studies have shown fitness consequences often scale with mite burden, with reproductive and developmental costs typically emerging only above a key threshold (Wilson & Knollenberg, 1987; Sun et al., 2019). Such manipulations would also help determine the ecological relevance of the observed patterns, as my study used a relatively high mite load to maximize detection of potential effects. Likewise, testing grooming responses across related *Nicrophorus* species, or under varying mite densities, would clarify how broadly grooming can serve as a proxy for host investment in defence, and under what conditions interactions shift along the mutualism antagonism spectrum.

Beyond this specific system, my study underscores how context-dependent interactions can still give rise to predictable evolutionary outcomes. While ecological conditions might shift the apparent costs and benefits of a symbiont, consistent host responses (such as behavioural avoidance or grooming) can offer insight into the long-term selective pressures at play. This highlights the value of integrating behavioural ecology studies of symbioses: rather than relying solely on reproductive output or survival metrics, we can examine how animals respond in real time to their partners. As context-dependence continues to challenge how we classify mutualisms, commensalism, and parasitism, incorporating behavioural indicators might provide a more tractable and evolutionarily meaningful framework for comparing interspecific relationships across systems.

My study demonstrates how behavioural ecology can sharpen our understanding of symbiotic relationships, revealing selective pressures that might be overlooked when focusing on ecological outcomes alone. By pairing behavioural assays with competition experiments, my work shows how context-dependent interactions can still produce consistent evolutionary signals, offering a clear lens on how hosts experience their partners. The finding that mites effectively ‘handicap’ their hosts points to a broader pattern: parasite-imposed handicaps likely shape competition and other ecological interactions, potentially to an extent that we do not fully appreciate. Greater focus on the behavioural and physiological pathways driving these effects will be critical for understanding how symbionts shape individual contests and other aspects of host performance, and how those dynamics might ultimately ripple out to influence ecological communities. This work underscores the value of looking at what animals *do* as well as what they gain or lose, providing a framework that could help resolve long-debated interactions across a wide range of taxa.

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APPENDIX A: SUPPLEMENTARY FIGURES

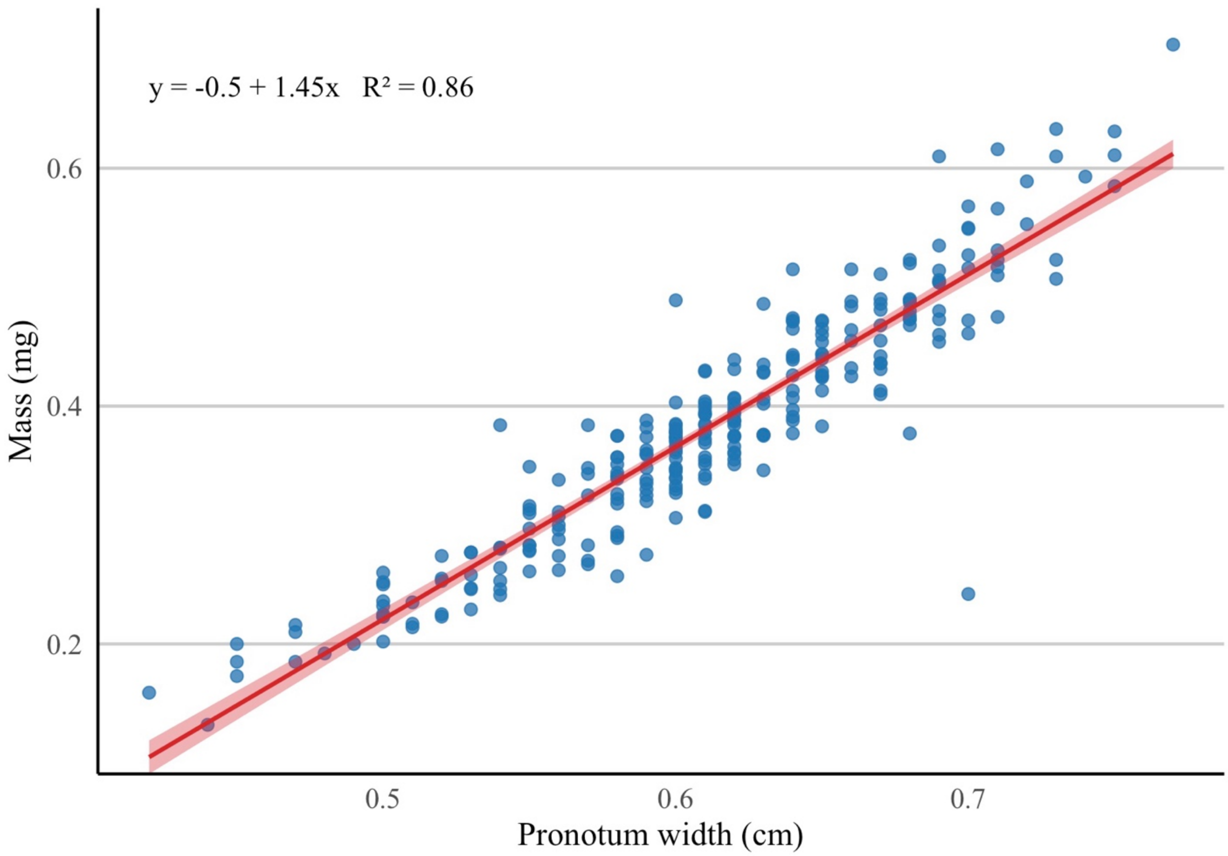

Figure A1. Relationship between pronotum width (in cm, x-axis) and body mass (in mg, y-axis) in *Nicrophorus orbicollis*. Mass increased linearly with pronotum width ($R^2 = 0.86$). Each point represents a single beetle (n = 266). The shaded area represents the 95% confidence interval around the fitted regression line.

Date:			Sex:		Mites remaining post tx:
Beetle Number:			Protonum Width:		
Treatment:			Weight:		

Pre-Treatment

Time	Antenna			Head				Pronotum				Abdomen				Legs			Wings					Mobile		Immobile		Other
	Mouth	Front Leg	Move	Front leg	Bite	Rub	Move	Front leg	Mid leg	Rub	Move	Mid leg	Hind leg	Rub	Move	Move	Tap	Rub	Mid leg	Hind leg	Partial	Full	Flare	Base	Top	Base	Top	
0:00																												
0:10																												
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Figure A2. Example of instantaneous sampling data sheet used in grooming behaviour assays for *Nicrophorus orbicollis*. Observations were recorded every 10s, with 5s spent observing the beetle's behaviour and 5s used to complete the sheet (for a total of 20 minutes for each phase of the trials). All sheets followed the same format across treatments and timepoints (control vs. treatment; pre- and post-treatment). Behaviours were categorized by body region (antennae, head, pronotum, abdomen, legs, and wings). Locomotion status and location within the arena (mobile [green] or immobile [red]; base or top) were recorded for all timepoints. Additional notes were written in the "Other" column.

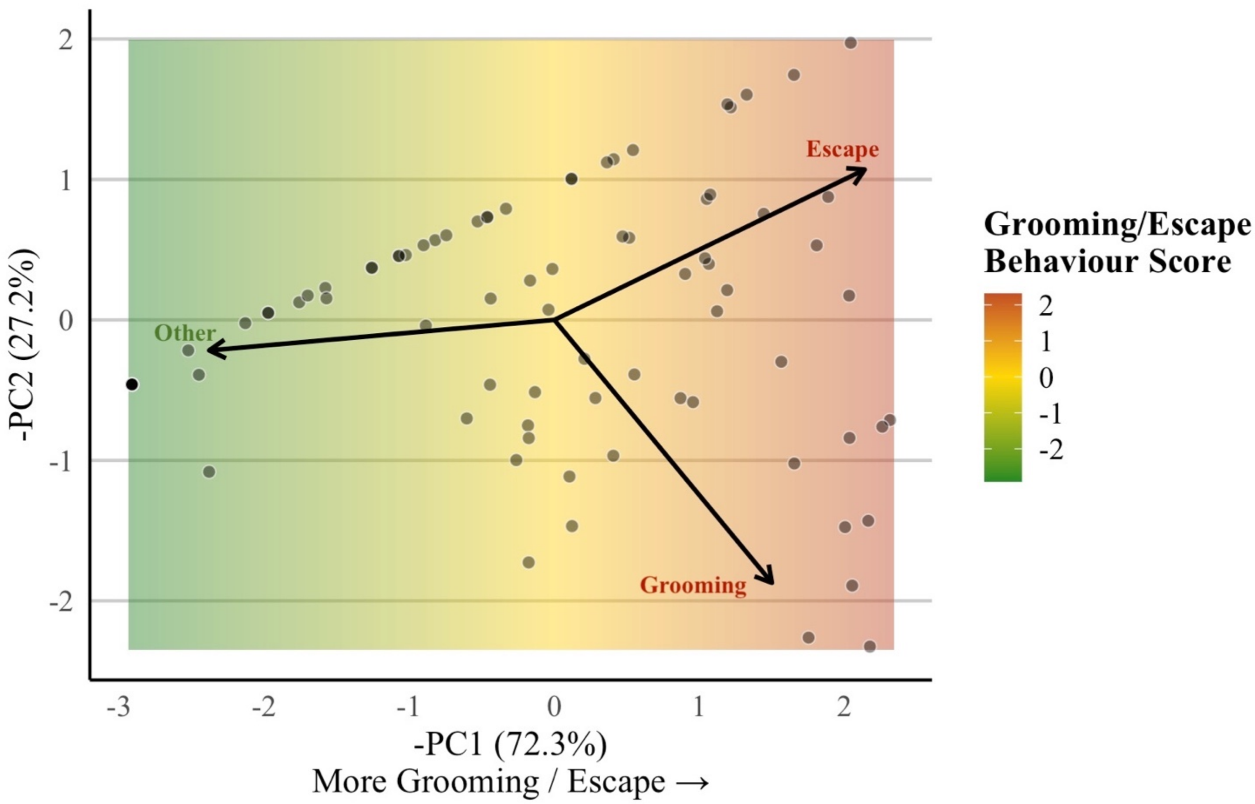

Figure A3. Biplot of principal component analysis (PCA) of behavioural data in *Nicrophorus orbicollis*. The three measured behaviours (grooming, escape, and other) are summarized along axes for two principal components. PC1 (x-axis) explains 72.3% of the variance and illustrates a trade-off, with negative loadings for “other” behaviours, and positive loadings for grooming and escape behaviours. PC2 (y-axis) accounts for an additional 27.2% of the variance but was not analyzed. Each point represents an individual beetle in a single phase of the trial. Background shading represents the behavioural gradient along PC1.

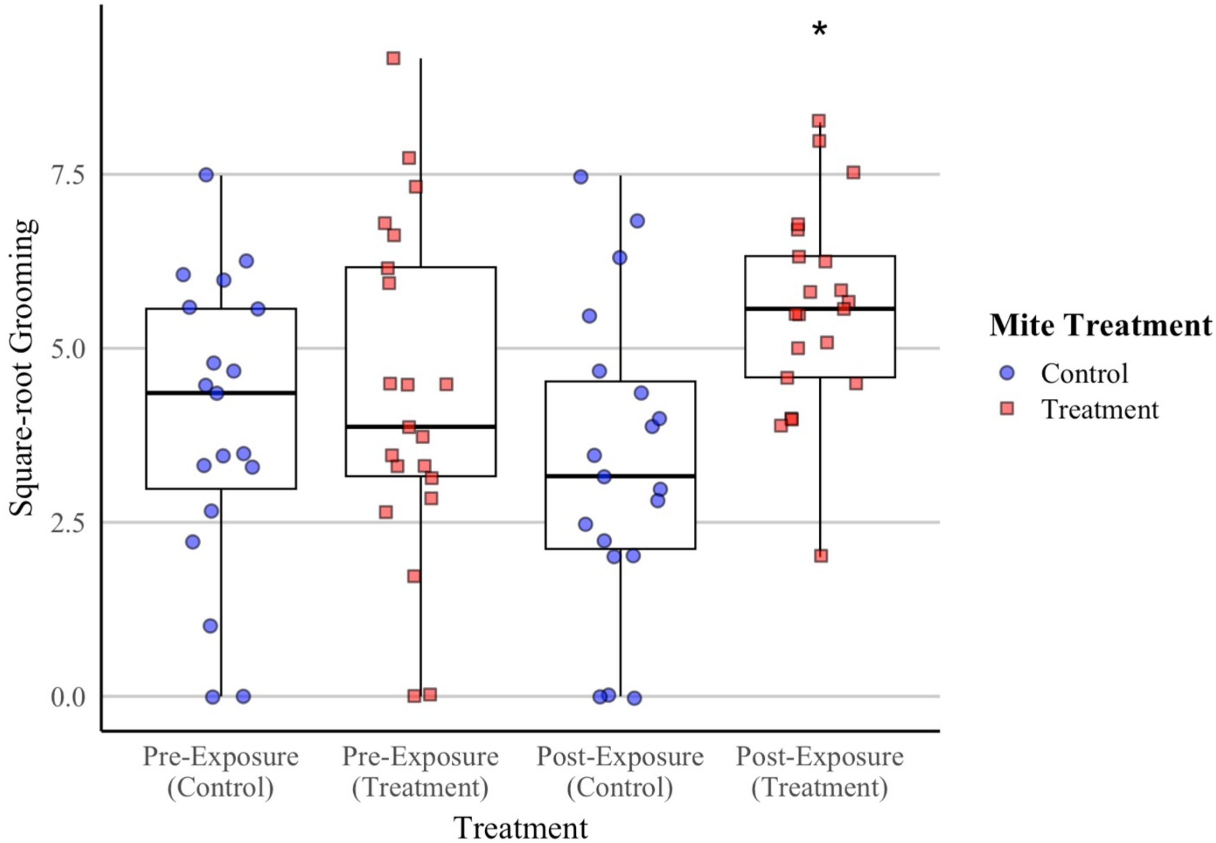

Figure A4. *Nicrophorus orbicollis* beetles increased grooming behaviours in response to exposure to *Poecilochirus carabi* mites. Grooming values are square-root transformed (y-axis), with higher values indicating more frequent grooming behaviours. The x-axis shows the treatment each beetle received and time period of observations (pre- or post-exposure). Points represent individual beetles and are jittered along the x-axis for better visualization. Blue circles represent control beetles; red squares represent mite-exposed beetles. Boxes represent the interquartile range (25^{th} – 75^{th} percentile), with the median shown as a bold line. Whiskers extend to the most extreme values within 1.5x the interquartile range. The asterisk denotes a statistically significant difference from all other groups (*emmeans* $p < 0.05$).

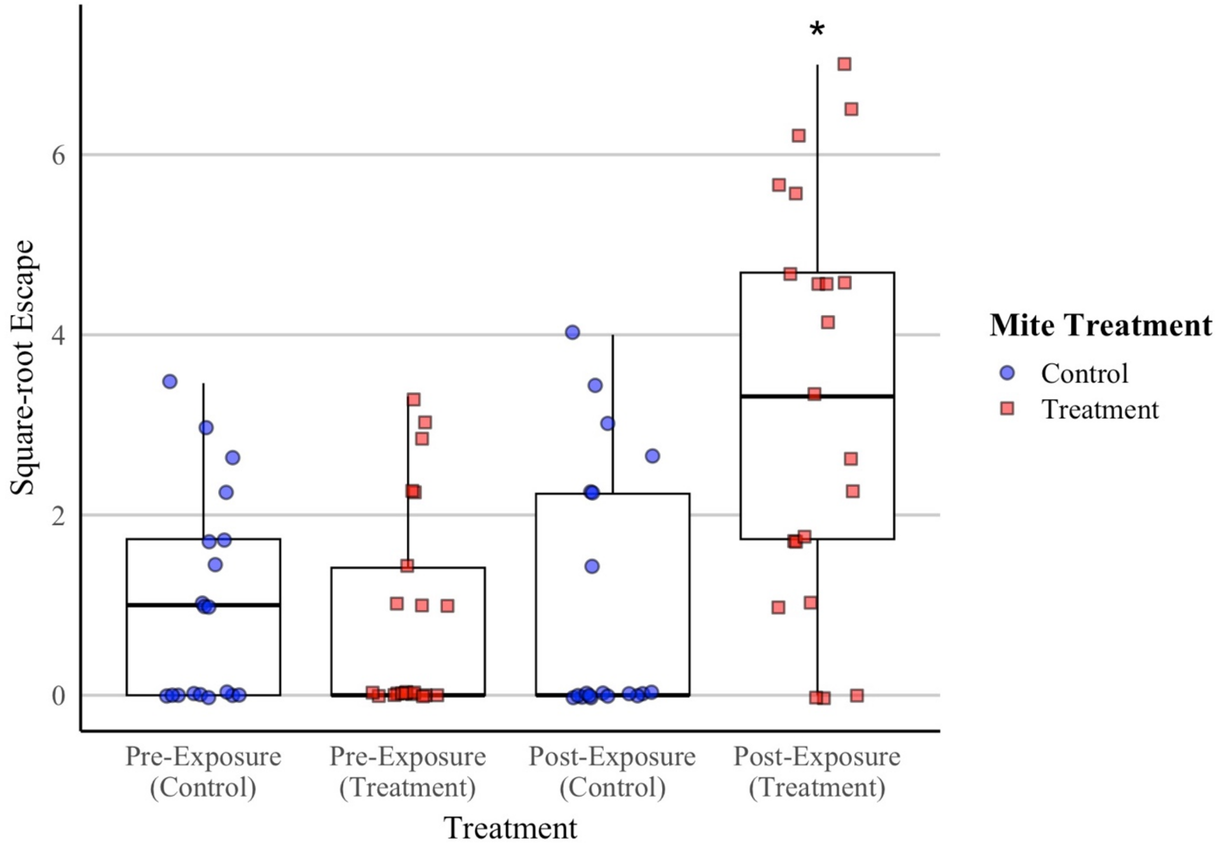

Figure A5. *Nicrophorus orbicollis* beetles increased escape behaviours in response to exposure to *Poecilochirus carabi* mites. Escape behaviour values are square-root transformed (y-axis), with higher values indicating more frequent escape behaviours. The x-axis shows the treatment each beetle received and time period of observations (pre- or post-exposure). Points represent individual beetles and are jittered along the x-axis for better visualization. Blue circles represent control beetles; red squares represent mite-exposed beetles. Boxes represent the interquartile range (25^{th} – 75^{th} percentile), with the median shown as a bold line. Whiskers extend to the most extreme values within 1.5x the interquartile range. The asterisk denotes a statistically significant difference from all other groups (*emmeans* $p < 0.05$).

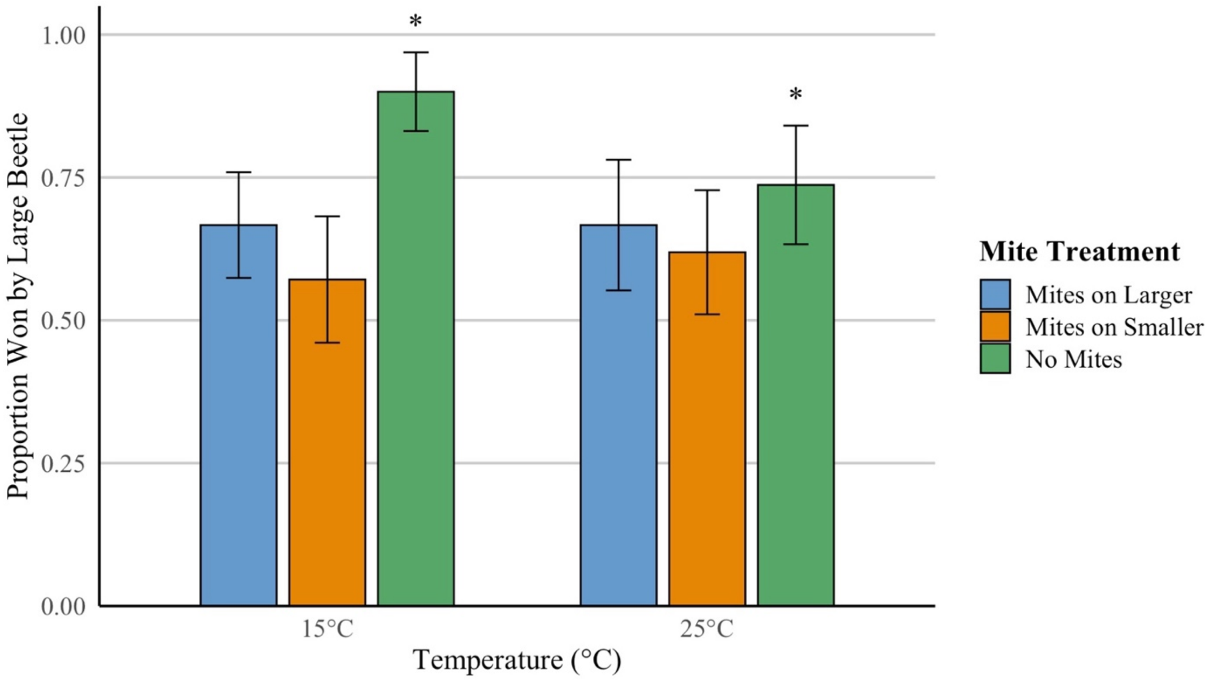

Figure A6. Proportion of contests won by the larger *Nicrophorus orbicollis* competitor across mite and temperature treatments. Trials were conducted in three treatment groups: the smaller beetle carried mites (orange bars), the larger beetle carried mites (blue bars), or no mites were present (green bars), under two temperature conditions (15°C and 25°C). Competitive outcomes did not differ based on which beetle initially carried mites, and temperature did not affect outcome. Larger beetles won more often in the absence of mites than in groups with mites present. The y-axis shows the proportion of contests won by the larger individual (1 = all contests won by the larger beetle, 0 = all contests won by the smaller beetle). Error bars show ± 1 SE. Asterisks indicate statistically significant differences between the control group and both mite treatment groups at each temperature.

APPENDIX B: PRONOTUM WIDTH REPEATABILITY MEASUREMENTS

Table A1. Representative subset of beetle measurements from one practice session used to assess repeatability of pronotum width measurements in *Nicrophorus orbicollis.* Each beetle was measured three times using digital calipers (± 0.01cm, N = 35) to assess measurement error in these body size estimates. Measurements were taken blindly, with beetles stripped of identifying labels and shuffled between each round to avoid bias. See main text for estimates of measurement error and repeatability.

Beetle ID	1st Measurement (cm)	2nd Measurement (cm)	3rd Measurement (cm)
5222	0.49	0.48	0.48
5201	0.67	0.67	0.67
5213	0.63	0.63	0.63
5166	0.65	0.65	0.64
5024	0.63	0.62	0.62
5137	0.64	0.64	0.64
5221	0.50	0.50	0.48
5023	0.59	0.59	0.59
5015	0.58	0.58	0.58
5031	0.54	0.54	0.54
5070	0.60	0.60	0.59
5008	0.65	0.65	0.65
5059	0.64	0.64	0.64
5147	0.72	0.71	0.71
5032	0.71	0.71	0.71
4997	0.57	0.57	0.57
5144	0.67	0.67	0.67
5065	0.55	0.55	0.55
5094	0.68	0.68	0.68
5178	0.65	0.65	0.65
5051	0.60	0.59	0.60
5053	0.65	0.65	0.65
5118	0.67	0.67	0.68
5131	0.64	0.64	0.64
5132	0.70	0.70	0.70
5012	0.61	0.61	0.60
5037	0.61	0.61	0.61
5109	0.57	0.57	0.57
5173	0.80	0.79	0.79
4996	0.61	0.61	0.61
5079	0.57	0.57	0.57
5190	0.65	0.65	0.65
4995	0.56	0.56	0.56
5098	0.73	0.73	0.73
5036	0.54	0.54	0.54

APPENDIX C: SUPPLEMENTARY STATISTICAL ANALYSES AND MODEL RESULTS

Table A2. Results from the best performing model[1] explaining variation in competition outcome as a function of pronotum difference (proxy for beetle size difference) and sex.
N = 35 trials

Variable	**β [95% CI]**	***z*-value**	***p*-value**
(Intercept)	1.16 [-0.54, 3.21]	1.28	0.201
Pronotum Difference[2]	21.19 [-1.24, 56.26]	1.50	0.134
Sex[3] (M)	-1.78 [-3.93, -0.03]	-1.85	0.064
*Global model: Winner ~ Pronotum difference * Sex, family = binomial			

[1]Top model identified through AICc ranking of candidate models derived from the global model.
[2]Pronotum width difference in cm between the two competing beetles.
[3]Sex of competing individuals (male or female). Intercept value set as female.

Table A3. Top-performing models explaining variation in competition outcome as a function of pronotum difference (proxy for beetle size difference) and sex.
N = 35 trials

Model Rank[1]	**Fixed Effects**	**df**	**logLik[2]**	**AICc[3]**	**ΔAICc[4]**	**Weight[5]**
1	Pronotum difference + Sex	3	-15.64	38.05	0.00	0.356
2	Sex	2	-17.32	39.01	0.96	0.221
3	Pronotum difference	2	-17.62	39.61	1.56	0.163
4	Null model	1	-18.81	39.75	1.70	0.152
*Global model: Winner ~ Pronotum difference * Sex, family = binomial						

[1]Models ranked by AICc; those within 2 AICc units of the top-ranked model are shown, along with the null model.
[2]Log-likelihood of the fitted model; higher values indicate better fit but do not determine model ranking, which is based on AICc and incorporates a penalty for model complexity.
[3]Akaike Information Criterion (corrected for small sample size); lower values indicate better model support.
[4]Difference in AICc between the best performing model and the model being compared; smaller values indicate stronger support.
[5]Relative likelihood that the model is the best one among the candidate set, given the data. Weights sum to 1 across all recombinant models (including those not shown here).

Table A4. Results from the best performing model[1] explaining variation in competition outcome as a function of mite treatment, pronotum difference, temperature, and sex. Effects with $p < 0.05$ are highlighted in bold.
N* = *126

Variable	**β [95% CI]**	***z*-value**	***p*-value**
(Intercept)	**1.21 [0.37, 2.17]**	**2.68**	**0.007**
Mites Started on Larger[2]	-0.81 [-1.90, 0.20]	-1.54	0.124
Mites Started on Smaller[3]	**-1.20 [-2.30, -0.19]**	**-2.26**	**0.024**
Sex[4] (M)	0.68 [-0.10, 1.49]	1.69	0.091
*Global model: Outcome ~ Mite treatment + Sex + Pronotum difference + Temperature + Mite treatment*Pronotum difference*Temperature*Sex, family = binomial			

[1]Top model identified through AICc ranking of candidate models derived from the global model.
[2]Trials where the mites started on the larger beetle. Intercept value set as mites absent.
[3]Trials where mites started on the smaller beetle. Intercept value set as mites absent.
[4]Sex of competing individuals (male or female). Intercept value set as female.

Table A5. Top-performing models explaining variation in competition outcome as a function of mite treatment (mites on larger or smaller beetle, or absent), pronotum difference, temperature, and sex.
N* = *126

Model Rank[1]	Fixed Effects	df	logLik[2]	AICc[3]	ΔAICc[4]	Weight[5]
1	Mite treatment + Sex	4	-73.89	156.10	0.00	0.122
2	Mite treatment	3	-75.34	156.88	0.78	0.082
3	Sex	2	-76.69	157.48	1.38	0.061
4	Mite treatment + Sex + Temperature	5	-73.70	157.89	1.79	0.050
5	Null model	1	-77.96	157.95	1.84	0.048
*Global model: Outcome ~ Mite treatment + Sex + Pronotum difference + Temperature + Mite treatment*Pronotum difference*Temperature*Sex, family = binomial						

[1]Models ranked by AICc; those within 2 AICc units of the top-ranked model are shown, along with the null model.
[2]Log-likelihood of the fitted model; higher values indicate better fit but do not determine model ranking, which is based on AICc and incorporates a penalty for model complexity.
[3]Akaike Information Criterion (corrected for small sample size); lower values indicate better model support.
[4]Difference in AICc between the best performing model and the model being compared; smaller values indicate stronger support.
[5]Relative likelihood that the model is the best one among the candidate set, given the data. Weights sum to 1 across all recombinant models (including those not shown here).

Table A6. Results from the best performing model[1] explaining variation in competition outcome as a function of mite presence, pronotum difference, temperature, and sex. Effects with $p < 0.05$ are highlighted in bold.
N = 126

Variable	**β [95% CI]**	***z*-value**	***p*-value**
(Intercept)	**1.22 [0.39, 2.18]**	**2.71**	**0.007**
Mites Present[2]	**-1.00 [-2.00, -0.11]**	**-2.10**	**0.036**
Sex[3] (M)	0.64 [-0.13, 1.44]	1.62	0.106
*Global model: Outcome ~ Mites + Sex + Pronotum difference + Temperature + Mites*Sex*Pronotum difference*Temperature, family = binomial			

[1]Top model identified through AICc ranking of candidate models derived from the global model.
[2]Trials with mites present (regardless of which beetle they began on). Intercept value set as mites absent.
[3]Sex of competing individuals (male or female). Intercept value set as female.

Table A7. Top-performing models explaining variation in competition outcome as a function of mite presence, pronotum difference, temperature, and sex.
N = 126

Model Rank[1]	Fixed Effects	df	logLik[2]	AICc[3]	ΔAICc[4]	Weight[5]
1	Mites + Sex	3	-74.25	154.70	0.00	0.085
2	Mites	2	-75.58	155.26	0.56	0.064
3	Mites + Pronotum difference + Sex + Mites*Pronotum difference	5	-72.85	156.20	1.50	0.040
4	Mites + Sex + Temperature	4	-74.02	156.38	1.67	0.037
5	Mites + Pronotum difference + Sex + Mites*Pronotum difference + Sex*Pronotum difference	6	-71.91	156.52	1.82	0.034
6	Mites + Pronotum difference + Sex + Sex*Pronotum difference	5	-73.10	156.69	1.99	0.032
18	Null model	1	-77.96	157.96	3.24	0.006
*Global model: Outcome ~ Mites + Sex + Pronotum difference + Temperature + Mites*Sex*Pronotum difference*Temperature, family = binomial						

[1]Models ranked by AICc; those within 2 AICc units of the top-ranked model are shown, along with the null model.
[2]Log-likelihood of the fitted model; higher values indicate better fit but do not determine model ranking, which is based on AICc and incorporates a penalty for model complexity.
[3]Akaike Information Criterion (corrected for small sample size); lower values indicate better model support.
[4]Difference in AICc between the best performing model and the model being compared; smaller values indicate stronger support.
[5]Relative likelihood that the model is the best one among the candidate set, given the data. Weights sum to 1 across all recombinant models (including those not shown here).

Table A8. Results from the best performing model[1] explaining variation in the proportion of mites transferred as a function of mite treatment, sex, pronotum difference, and competition outcome. Effects with $p < 0.05$ are highlighted in bold.
N = 87

Variable	**β [95% CI]**	***z*-value**	***p*-value**
(Intercept)	**-2.36 [-2.80, -1.95]**	**-10.92**	**< 0.001**
Mites Started on Smaller[2]	0.21 [-0.13, 0.57]	1.20	0.232
Sex[3] (M)	**0.50 [0.28, 0.72]**	**4.42**	**< 0.001**
Pronotum Difference[4]	6.41 [-0.17, 12.96]	1.91	0.056
Outcome[5]	**-0.39 [-0.73, -0.05]**	**-2.24**	**0.025**
Mite Started on Smaller:Outcome	**0.61 [0.17, 1.06]**	**2.72**	**0.006**
*Global model: Proportion transferred ~ (Mite treatment + Sex + Pronotum difference + Outcome)^2, family = binomial, weights = Mites before trial			

[1]Top model identified through AICc ranking of candidate models derived from the global model.
[2]Trials where mites started on the smaller beetle. Intercept value set as mites absent.
[3]Sex of competing individuals (male or female). Intercept value set as female.
[4]Pronotum width difference in cm between the two competing beetles.
[5]Outcome of the trial (whether the larger or smaller beetle won). Intercept value set as “smaller beetle won”.

Table A9. Pairwise comparisons of the proportion of mites transferred from the starting beetle among mite treatments and outcome combinations for competitive trials: mites started on the larger beetle; mites started on the smaller beetle; no mites. Outcomes refer to whether the larger or smaller beetle won the contest. Comparisons are based on estimated marginal means from the top model, using Tukey adjustment for multiple testing.

Contrast[1]	Estimate[2]	SE[3]	z-ratio[4]	p-value[5]
Started on larger, smaller won vs. Started on smaller, smaller won	-0.21	0.18	-1.20	0.629
Started on larger, smaller won vs. Started on larger, larger won	0.39	0.18	2.25	0.111
Started on larger, smaller won vs. Started on smaller, larger won	-0.44	0.16	-2.63	**0.042**
Started on smaller, smaller won vs. Started on larger, larger won	0.61	0.16	3.90	**0.001**
Started on smaller, smaller won vs. Started on smaller, larger won	-0.22	0.14	-1.54	0.413
Started on larger, larger won vs. Started on smaller, larger won	-0.83	0.14	-5.97	**<0.001**

[1]Specified factor levels whose estimated marginal means are being compared.
[2]Estimated difference in marginal means between the levels being compared; positive values indicate that the first level listed has a higher mean than the second.
[3]Standard Error of the estimated difference; smaller values indicate greater precision in the estimate.
[4]Test statistic calculated as the estimate divided by its standard error; used to assess whether the observed difference is significantly different from 0.
[5]Probability of observing a z-ratio as extreme as the one calculated, under the null hypothesis of no difference between levels; lower values ($p < 0.05$) indicate a statistically significant contrast.

Table A10. Top-performing models explaining variation in the proportion of mites transferred as a function of mite treatment (beetle that started with mites), sex, pronotum difference, and competition outcome.
N = 87

Model Rank[1]	Fixed Effects	df	logLik[2]	AICc[3]	ΔAICc[4]	Weight[5]
1	Mite treatment + Outcome + Pronotum difference + Sex + Outcome*Mite treatment	6	-260.00	533.05	0.00	0.149
2	Mite treatment + Outcome + Sex + Outcome*Mite treatment	5	-261.86	534.48	1.43	0.073
3	Mite treatment + Outcome + Pronotum difference + Sex + Outcome*Mite treatment + Outcome*Pronotum difference	7	-259.59	534.61	1.56	0.068
112	Null model	1	-294.06	590.16	57.12	0
*Global model: Proportion transferred ~ (Mite treatment + Sex + Pronotum difference + Outcome)^2, family = binomial, weights = Mites before trial						

[1]Models ranked by AICc; those within 2 AICc units of the top-ranked model are shown, along with the null model.
[2]Log-likelihood of the fitted model; higher values indicate better fit but do not determine model ranking, which is based on AICc and incorporates a penalty for model complexity.
[3]Akaike Information Criterion (corrected for small sample size); lower values indicate better model support.
[4]Difference in AICc between the best performing model and the model being compared; smaller values indicate stronger support.
[5]Relative likelihood that the model is the best one among the candidate set, given the data. Weights sum to 1 across all recombinant models (including those not shown here).

Table A11. Results from the best performing model[1] explaining variation in behaviour (PC1 scores) as a function of time period, treatment, sex, and natural log-transformed pronotum width. Effects with $p < 0.05$ are highlighted in bold.
N = 80

Variable	β [95% CI]	df	*t*-value	*p*-value
(Intercept)	**-0.73 [-1.30, -0.16]**	**69.70**	**-2.49**	**0.015**
Treatment (Mites Present)[2]	**1.99 [1.21, 2.77]**	**69.70**	**4.92**	**< 0.001**
Time Period (Pre-Exposure)[3]	0.35 [-0.32, 1.03]	38.00	1.02	0.313
Treatment (Mites Present):Time Period (Pre-Exposure)	**-1.87 [-2.81, -0.94]**	**38.00**	**-3.92**	**< 0.001**
*Global model: Behaviour PC ~ (Treatment + Time Period + ln Pronotum Width + Sex)^3, random = 1 | Beetle ID				

[1]Model identified through AICc ranking of candidate models derived from the global model.
[2]Beetles exposed to mites during exposure phase. Intercept value set as control (no mites).
[3]Time period before exposure to treatment. Intercept value set as exposure phase.

Table A12. Top-performing models explaining variation in behaviour (PC1 scores) as a function of time period, treatment (mites vs. control), sex, and natural log-transformed pronotum width. ***N = 80***

Model Rank[1]	**Fixed Effects**	**df**	**logLik**[2]	**AICc**[3]	**ΔAICc**[4]	**Weight**[5]
1	Time period + Treatment + Treatment*Time period	6	-129.08	271.30	0.00	0.330
2	Time period + Treatment + Pronotum width (logtrans) + Treatment*Time period	7	-128.86	273.29	1.98	0.122
116	Null model	3	-143.17	292.66	21.35	0
*Global model: Behaviour PC ~ (Treatment + Time Period + ln Pronotum Width + Sex)^3, random = 1 | Beetle ID						

[1]Models ranked by AICc; those within 2 AICc units of the top-ranked model are shown, along with the null model.
[2]Log-likelihood of the fitted model; higher values indicate better fit but do not determine model ranking, which is based on AICc and incorporates a penalty for model complexity.
[3]Akaike Information Criterion (corrected for small sample size); lower values indicate better model support.
[4]Difference in AICc between the best performing model and the model being compared; smaller values indicate stronger support.
[5]Relative likelihood that the model is the best one among the candidate set, given the data. Weights sum to 1 across all recombinant models (including those not shown here).

Table A13. Pairwise comparisons of behavioural scores (PC values summarizing grooming, escape, and general behaviours) between control and mite-exposed treatments across time periods. Treatment: control or mite-exposed; Time period: pre-exposure or post-exposure. Comparisons are based on estimated marginal means from the top model, using Tukey adjustment for multiple testing.

Contrast[1]	Estimate[2]	SE[3]	df[4]	t-ratio[5]	p-value[6]
Control, Post-Exposure vs. Treatment Post-Exposure	-1.99	0.40	69.7	-4.92	**<0.001**
Control, Post-Exposure vs. Control Pre-Exposure	-0.35	035	38.0	-1.02	0.738
Control, Post-Exposure vs. Treatment Pre-Exposure	-0.47	0.40	69.7	-1.16	0.654
Treatment, Post-Exposure vs. Control Pre-Exposure	1.64	0.40	69.7	4.05	**0.001**
Treatment, Post-Exposure vs. Treatment Pre-Exposure	1.52	0.33	38.0	4.62	**<0.001**
Control Pre-Exposure vs. Treatment Pre-Exposure	-0.115	0.40	69.7	-0.28	0.992

[1]Specified factor levels whose estimated marginal means are being compared.
[2]Estimated difference in marginal means between the levels being compared; positive values indicate that the first level listed has a higher mean than the second.
[3]Standard Error of the estimated difference; smaller values indicate greater precision in the estimate.
[4]Degrees of freedom: the number of independent pieces of information used to estimate variability in the model; affects the shape of the t-distribution used for significance testing.
[5]Test statistic calculated as the estimate divided by its standard error; used to assess whether the observed difference is statistically significant.
[6]Probability of observing a t-ratio as extreme as the one calculated, under the null hypothesis of no difference between levels; lower values ($p < 0.05$) indicate a statistically significant contrast.