The itchy and scratchy show:

association between co-occurring parasites and exploratory behaviour in mice

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Thesis submitted to the University of Ottawa in partial fulfillment of the requirements for the

MSc degree at the Ottawa-Carleton Institute of Biology

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Aknowledgments

I would like to begin by thanking Professor Vincent Careau for allowing me to work on a system which still boggles my mind in its complexity, for keeping me on course, for answering countless panicked texts when the equipment decided to stop working, for going over countless drafts and always providing insightful comments, and for always being available to discuss things or provide guidance. Thanks to both of my committee members, Dr. Gabriel Blouin-Demers and Dr. Mark Forbes, for pointing out blatant blind spots in my research and providing the resources to patch them up.

I would also like to extend my thanks to my lab mates who helped me in innumerable ways and whose company and discussions made time fly much too fast.

Special thanks to Alyssa Fiedler for showing me the ropes in the field, to Natalie Kermany for making sure I was still alive during long dark nights trapping alone, and to Phoenix Sandrock and Alyssa Pogson, the best field assistants anyone could ask for, thank you for your zealousness in the thankless job of collecting ticks.

Thanks to the Queen’s University Biological Research Station, for providing us with comfortable accommodations and allowing us to continue our research through the pandemic.

Finally, thanks to my parents, who supported me and encouraged me to pursue this in the first place.

Abstract

Host-parasite interactions are complex, involving host behaviour, host condition, and possible antagonism or mutualism between co-occurring parasites. The white-footed mouse (*Peromyscus leucopus*) is a small, abundant, generalist rodent, and the primary host for the black-legged tick (*Ixodes scapularis*) in its larval stage. Since larval ticks do not actively pursue their hosts (they wait for possible hosts to come to them), individual differences in mouse exploratory behaviour might be expected to correlate with tick burden. However, mice may also prevent tick attachment with defensive behaviour such as grooming. Yet, the relationships between tick parasitism and host exploratory and grooming behaviours have never been assessed. I analysed data from a capture-mark-recapture study conducted over 5 summers (2016-2020) in eastern Ontario, Canada, in which 1,035 mice were captured a total of 4,333 times. The probability of tick parasitism occurring and being more intense was significantly higher when the mouse was also parasitized by fleas, suggesting co-occurrence of these two parasites on host mice. A total of 510 mice were subjected to an open-field test to quantify exploratory and grooming behaviours. Exploration had a negative relationship with tick presence and a positive relation with flea presence. Most interestingly, there was a significant "tick × flea" interaction on exploratory behaviour such that fleas were positively associated with exploration only when ticks were absent. Surprisingly, there was no relationship between grooming behaviour in the open-field test and parasite presence, although grooming increased with mouse activity. This study shows that co-occurring parasite species (ticks and fleas) may interact to affect their host's exploratory behaviour. Alternatively, individual differences in exploratory behaviour of hosts may differentially affect their susceptibility to being infested with ticks, fleas, or both. Future manipulative studies should use experimental design to determine the causality of the parasitism-behaviour relationships observed.

Résumé

Les interactions hôte-parasite sont complexes, impliquant le comportement de l'hôte, la condition de l'hôte et les interactions (antagonisme ou mutualisme) entre les parasites co-occurrents. La souris à pattes blanches (*Peromyscus leucopus*) est un petit rongeur généraliste abondant et l'hôte principal de la tique à pattes noires (*Ixodes scapularis*) au stade larvaire. Étant donné que les larves de tiques ne poursuivent pas activement leurs hôtes (elles attendent que des hôtes potentiels viennent à elles), les différences individuelles dans le comportement d'exploration des souris pourrait être corrélé avec leurs charge de tiques. Cependant, les souris peuvent également empêcher l'attachement des tiques avec un comportement défensif tel que le toilettage. Pourtant, les relations entre le parasitisme des tiques et les comportements d'exploration et de toilettage de l'hôte n'ont jamais été évaluées. J'ai analysé des données d'une étude de capture-marquage-recapture poursuite durant 5 été (2016-2020) à l'est de l'Ontario, au Canada. Les données consistaient de 1 035 souris capturées un total de 4 333 fois. La probabilité que le parasitisme par les tiques se produise et soit plus intense était significativement plus élevée lorsque la souris était également parasitée par des puces, suggérant la co-occurrence de ces deux parasites sur les souris hôtes. Au total, 510 souris ont été soumises à un test d'environnement nouveau pour quantifier les comportements d'exploration et de toilettage. L'exploration avait une relation négative avec la présence de tiques et une relation positive avec la présence de puces. Plus intéressant encore, il y avait une interaction significative « tique × puce » sur le comportement d'exploration, de sorte que les puces n'étaient positivement associées à l'exploration que lorsque les tiques étaient absentes. Étonnamment, il n'y avait aucune relation entre le comportement de toilettage durant le test d'environnement nouveau et la présence de parasites, bien que le toilettage augmente avec l'activité de souris. Cette étude démontre que les taxons de parasites co-occurrentes (tiques et puces) peuvent interagir pour affecter le comportement d'exploration de leur hôte. Alternativement, les différences individuelles dans le comportement exploratoire des hôtes peuvent affecter différemment leur susceptibilité à être infesté de tiques, de puces ou des deux. Les futures études de manipulation devraient utiliser un plan expérimental pour déterminer la causalité des relations parasitisme-comportement observées.

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Introduction

Host behaviour and "sit and wait" parasites

By definition, parasites incur a cost to their hosts (Albery et al. 2021). Therefore, hosts are expected to minimise the chance of acquiring parasites. Acquisition of parasitic arthropods is dependent on the hunting strategy used by the parasite. While some parasites, such as mosquitoes, actively seek out their hosts, other parasites sit and wait in ambush. As the acquisition of "sit and wait" parasites most likely occurs when the host is active, such parasites are more dependant on host habitat choice and behaviour. While hosts need to be active and explore their environment to increase their chances of finding resources and potential mates, such activities likely expose them to clusters of waiting parasites. In Eastern chipmunks (*Tamias striatus*) for example, behaviours associated with boldness such as increased exploration and activity have been associated with larger parasitic burdens (Boyer et al. 2010, Bohn et al. 2017, Paquette et al. 2020).

Host behaviour might not only influence the rate of encounter with sit and wait parasites, but also the probability of acquiring them after an encounter. Indeed, hosts actively prevent parasites from feeding by removing them with defensive behaviour such as grooming. Presumably, parasites that are found on hosts have already successfully evaded the hosts defensive behaviour. Moreover, host behaviour might also be changed by the acquisition of parasites. Once parasites are feeding, their hosts may change their behaviour to compensate for the energetic cost of bearing parasites, such as decreasing the use of costly locomotion when highly parasitized (Finnerty et al. 2018, Hicks et al. 2018). These changes in behaviour may be one of the many factors that could make parasitized hosts more vulnerable to other parasites.

Black-Legged Ticks and White-footed mice

In Eastern Canada, the black-legged tick (*Ixodes scapularis*) is the primary vector for *Borrelia burgdorferi*, the pathogen responsible for Lyme disease. The most important reservoirs for the maintenance of *B. burgdorferi* in black-legged tick populations is the white-footed mouse (*Peromyscus leucopus*; hereafter referred to as mice). Black-legged ticks have three active life stages (adult, nymph, and larval stages), each with different host preferences and behaviour. Larvae and nymphs feed once before molting to the next life stage. Once they molt into adults, ticks can feed multiple times to reproduce. Adult ticks prefer large mammals, while nymphs have no preference, and larvae mostly feed on smaller animals such as birds or rodents. Tick larvae acquire *B. burgdorferi* from infected mice and may then transmit the bacterium to other mice (and humans) once they molt into nymphs, continuing the infection cycle. Adult black-legged ticks only actively seek out hosts within a few meters, while their larvae are even less active, making black-legged tick larvae a prime example of a “sit and wait” parasite (Falco and Fish 1991, Stafford 1992).

In the mouse-tick study system, the role of mouse habitat choice and activity on parasite acquisition has been relatively well studied in the wild. While the relationship between grooming behaviour and parasitic burden has been studied in other wild animals (Eads et al. 2017, Heine et al. 2017), it has been rarely studied in mice. This is due to the difficulty in observing grooming behaviour in wild mouse populations, since the mice are secretive and only active at night. Most studies on wild mice are limited to directly observing the animals while handling them or applying a treatment, which prevents them from measuring grooming (Ostfeld et al. 1996, Hersh et al. 2014, Gaitan and Millien 2016, Larson et al. 2018). Although one study quantified the number of ticks removed by grooming in wild-caught individuals within a laboratory setting, it did not measure individual grooming behaviour (Keesing et al. 2003). Observing the grooming behaviour of mice captured from a wild population in an artificial environment, such as an open-field arena, is the closest we can currently get to quantifying the animal's anti-parasitic behaviour. Here, my main objective was to test for a relationship between exploratory and grooming behaviours quantified during an open-field test and parasitism of mice by larval ticks.

Extrinsic factors

The number of ticks found on mice is a function of many extrinsic factors, including the abundance of active ticks in the environment, which fluctuates over time. The abundance of reproductive adult ticks reaches its peak in spring and autumn, with eggs being laid in May, resulting in two larval tick peaks; a peak in early summer from larval ticks that overwintered and a peak in late summer from larval ticks that hatched that year (Lindsay et al. 1999). Before finding a suitable host, larval ticks may die from predation by arthropods, cold, dehydration, drowning, or starvation, all of which can influence larval tick abundance (Samish and Alekseev 2001, Leal et al. 2020). For tick populations to maintain themselves, spring and autumn temperatures must remain above zero long enough for ticks to reach their next life stage (Clow et al. 2017). When ticks become dehydrated, they must retreat to a humid environment to rehydrate. In temperatures above 30°C or in low humidity, *Ixodes scapularis* are at risk of dehydration and spend less time active, which also increases their chances of starving (Eisen et al. 2016). Although precipitation maintains a humid environment, which is beneficial to ticks, it may also flood the understory and drown the ticks (Ostfeld et al. 2006, Eisen et al. 2016). Accordingly, years of middling temperatures and precipitation have been associated with higher larval tick burdens, while mouse host characteristics contributed very little (Mowry et al. 2019). On a day-to-day basis and at a highly localized scale, variations in humidity and temperature should be related to tick activity. It is possible that daily temperature may enhance a tick's host acquisition success, either by giving the larvae more time available to quest, or by altering mouse behaviour so that ticks are more likely to encounter them.

Host density is another extrinsic factor determining tick acquisition by mice. In oak-dominated forests, most biotic interactions are driven by the synchronised acorn production of oak trees (fall masting). Each fall masting event causes a huge increase in food availability for overwintering animals and is followed by a drastic increase in rodent and tick larvae populations (Jones et al. 1998, Ostfeld et al. 2001). Since there are more mice after mast years, they are restrained to smaller territories due to conspecific aggression and must forage through their smaller territories more intensely. Because the mouse population is sampling more of the environment that could be inhabited by ticks, ticks are more likely to encounter a mouse and have higher feeding success on mice after masting years, whereas individual mice have lower tick burdens since the ticks are spread out over a larger population of hosts (Mowry et al. 2019). By contrast, when mouse density is low, ticks aggregate in high numbers on a few individuals (Mowry et al. 2019).

Another extrinsic factor potentially determining tick acquisition is the competition with other parasite species. Since hosts have a limited amount of space and resources that can be exploited by parasites without heavily impacting the host, parasites with similar feeding sites or food sources will compete against one another; in other words, the more similar parasites are to each other, the more likely they are to compete (Gobbin et al. 2021). When two or more species of parasites find themselves competing or interfering with one another, they will be less likely to co-occur, while if one of the parasites is beneficial to another parasite, then the abundance of the second parasite will increase with the presence of the first (Lello et al. 2004, 2008). Co-occurrence may also be reduced via the host immune system. Ticks inject a cocktail of different molecules to facilitate feeding and supress the inflammatory response in the host and make them more permissive to their presence (Anderson et al. 2017). However, hosts eventually acquire immunity against parasites, which might have a negative impact on the future feeding of both the same parasite and other parasites that use similar compounds (Lello et al. 2004, Rellstab et al. 2013).

Host intrinsic factors

Parasitic burden also depends on a series of factors intrinsic to the hosts, such as age, sex, reproductive status, and body mass, the inclusion of which has led to weak or mixed results in mouse-tick models (Moore and Wilson 2002, Halliday et al. 2014, Mowry et al. 2019, Butler et al. 2020). Age may have differing effects on the parasitic burdens of hosts and there have been examples of both the oldest and youngest of a host species harboring the most parasites (see below). Young rodents need to spend more energy on growth, are less efficient at removing parasites, and have a naïve immune system, all of which contribute to being more vulnerable to parasites (Hawlena et al. 2007). In some cases, adult mammals have been found to have lower parasitic burdens than juveniles, due to adaptive behaviour to avoid the parasites or acquired immunity (Hämäläinen et al. 2015). Alternatively, there have been cases when older individuals were more likely to harbor larger parasite burdens; in pythons, for example, ageing decreased immune response and rendered the host more vulnerable to parasites (Ujvari and Madsen 2006).

In mice, older individuals were observed to have higher larval tick burdens (Brunner and Ostfeld 2008, Mowry et al. 2019).

Parasites may be more prone to feed on hosts of a particular sex. In mammals, compared to their female conspecifics, adult males usually have more parasites, larger home ranges, and greater levels of testosterone which may adversely impact immune response (Schalk and Forbes 1997, Klein 2004). Following this trend, male mice have been found to harbor more tick larvae than females (Brunner et al. 2008, Brunner and Ostfeld 2008, Devevey and Brisson 2012, Ostfeld et al. 2018, Mowry et al. 2019). Both the sex and age trends found may be partly due to male and older mice being larger. Larger animals have more resources and space for parasites to use, and may be investing more into growth than into their defense against parasites (Moore and Wilson 2002). Supporting this, studies have shown that larger mice are more likely to have higher tick burdens (Brunner and Ostfeld 2008, Devevey and Brisson 2012, Mowry et al. 2019). Given that many extrinsic and intrinsic factors are potentially confounded (e.g., sex, age, and body mass), further attempts at identifying the factors affecting tick parasitism in mice must include an exhaustive evaluation of the relative importance of each factor while controlling for the other.

Objectives

In this study, my goal was to determine the influence of various extrinsic and intrinsic factors on tick burdens in mice, using data on 1,035 mice captured a total of 4,333 times as part of a capture-mark-recapture study conducted over 5 summers (2016-2020) in eastern Ontario, Canada. The extrinsic factors examined were mast years, daily mouse capture number, season, precipitation, temperature, and the presence of other parasites (mites, fleas, and botfly larva). The intrinsic factors examined were sex, mass, age, and reproductive status. A second goal was to test for a relationship between exploratory and grooming behaviours of mice and tick parasitism. It has been shown that mice with larger tick burdens have smaller home ranges (Gaitan and Millien 2016), a relationship that may be mediated through exploratory behaviour. Moreover, individual mice may consistently differ in the amount of time they groom themselves to remove parasites. However, grooming itself has not been studied or quantified in the tick-mouse system, which is surprising given that grooming behaviour is often cited as an important factor that might mitigate parasite feeding success (Keesing et al. 2003, Calabrese et al. 2011, Ostfeld et al. 2018, Mowry et al. 2019).

Methods

Study site

The study was conducted on the grounds of the Queens University Biological Research Station (QUBS) in Chaffey's Lock, Ontario, Canada (44°33'5"N; 76°19'27"W), and data collection for this project was authorized by the Ontario Ministry of Natural Resources. There were three trapping grids, all covered by deciduous forest, populated primarily with sugar maple (*Acer saccharum*) and oak trees (*Quercus rubra* and *Q. alba*). Each trapping grid consisted of a network of trapping sites and nest boxes laid out in a checkboard pattern (alternating trap and nest boxes) in a 15 × 15 m mesh. The first trapping grid was established in 2016 and contains 110 trapping sites and 117 nest box sites. Another trapping grid was established in 2016 and contains 63 trapping sites and 61 nest box sites. A third trapping grid was established in 2019 and contains 50 trapping sites and 20 nest box sites. Finally, mice were also sometimes captured outside the systematic grids using traps that were set at specific locations around the laboratory (see below).

Captures

Trapping occurred from early May to late August, using Longworth traps set at sundown and checked at sunrise. Each night, traps were baited with sunflower seeds and diced apples, with cotton batting added for nesting material. Grid sampling alternated, such that grids were usually not sampled more than two nights in a row. Nest boxes were checked every two weeks from April to October, weather permitting. All mice were captured and handled in accordance to protocols approved by the University of Ottawa Animal Care Committee and the Queen's University Animal Care Committee. Newly captured mice had permanent ear tags installed for individual identification. For each capture, the following information was noted: sex, age, body mass, and reproductive status. Sex was determined based on the distance between the genitals and anus, with males having a larger distance than females. Age was determined based on pelage colour: while adults were completely brown above the middorsal molt line, juveniles exhibit brown and grey pelage above the molt line (Collins 1923). Body mass was measured with a 50 g Pesola (±0.3%). Reproductive status was categorized as active or not depending on external sexual characteristics.

Individuals were also checked for parasites (mites, botflies, fleas, and ticks) by close inspection of the ears, face, tail, shoulders, toes, genitals, and by softly blowing through the fur on their backs and bellies. Ticks were not removed from heavily pregnant mice to prevent additional handling stress. All other ticks found on mice that could be removed in a timely manner were collected with fine typed tweezers and preserved in 90% alcohol for future studies.

A total of 112 ticks collected over 3 years (2016 to 2018) were identified; 37.5% were *Ixodes scapularis*, 1% were *Dermacentor variabilis*, and 61.5% were *Ixodes angustus.*

After the standard manipulation steps were completed at the capture site, all mice caught in a nest box were placed back in and left in their nest box. Individuals caught in a trap were either released or placed back in their trap and transported to a laboratory for behavioural measurement. Up to 16 individuals per day were brought to the laboratory, prioritizing mice that were caught less often, and avoiding testing individuals that had been tested within 7 days prior. Moreover, mice that were below 14 g, lactating, or pregnant were released. All mice were released on site.

Behavioural tests

In the laboratory, mice were kept in their trap until it was their turn for behavioural testing, which consisted of a 10-min open-field test. The test involved placing a mouse in a circular arena, which consisted of a white plastic tank with 1.4 meters in diameter and 86 cm walls to prevent animals from jumping out. The arena was surrounded by black curtains to prevent any disturbance from the surroundings and standardise the environment. The mouse to be tested was held by the scruff and tail and was then placed into the arena along the wall, facing away from the handler. Noise was kept to a minimum while the test was running. The arena was cleaned before and after each test with Accel and then rinsed with water. Each test was recorded using a camera (Basler ace IR) positioned above the arena and connected to the EthovisionXT software. Tracking immediately began when the mouse was detected inside the arena, and continued for 10 minutes at which point the test ended. During the whole test, an observer watched the video recording on the screen, manually documenting the beginning and end of all events of grooming directly in EthovisionXT. Tracking files were analysed in EthovisionXT to extract the total distance moved and total time spent grooming while in the arena. The animal was then weighed using a precision scale (Mettler Toledo, Model ML1602T/00). Ticks found during these manipulations were also removed. Once all open-field tests were completed, mice were given seeds and peanut butter and released 14 hours at most from when they were removed from their traps at their trapping site.

Statistical analysis

The statistical analysis was conducted in R version 4.03, and involved two models. The first model was based on all capture data. The dependent variable was the number of ticks counted on each capture. A Hurdle mixed model was fit with the glmmTMB package (Brooks et al. 2017) to estimate the effects of a series of extrinsic and intrinsic factors on tick burdens. Hurdle models are usually used to account for zero-inflation, and include two component models: a binary model that treats the data as zeroes or non-zeroes which accounts for zero-inflation, and a zero-truncated negative binomial distributed model fitting only the non-zero values (Zuur et al. 2009). While the data set is not zero-inflated, a hurdle model was fit to account for both the intensity (with the zero-truncated model) and prevalence (with the binary model) of ticks in the same model, while still being able to interpret either separately from the other (Markle et al. 2020, Obiegala et al. 2021). Prevalence is the proportion of the host sample population that has a parasite present, while intensity is the mean of parasites found on the infested portion of the host sample population. In this study the prevalence and intensity were for any tick spp. Since the zero-inflation component model is predicting the odds of obtaining a zero, negative estimates represent an increase in the odds of ticks being present on mice. Two Hurdle models were fitted:

one with a Poisson distribution and another with negative binomial distribution. The negative binomial distribution was retained because it had a less negative log likelihood than the Poisson model, an overdispersion parameter larger than 1, and resulted in changes in significance, which is indicative of some overdispersion (Zuur et al. 2009).

The two component models had the same independent variables, which included yearly mouse density (high or low), month, daily temperature, daily precipitation, body mass, daily mouse capture numbers, sex, age, reproductive status, and abundance of other parasites (i.e., number of fleas, botfly larvae, or mites). While age and reproductive status were moderately collinear with mass (Figure 1), the removal of mass from the model did not change their non-significance, and they were left in the model to illustrate that they had no effect on tick burden. Mouse density of year was near moderately collinear with daily number of mouse captures (Figure 1). For month, since March and April only had 2 and 5 captures with ticks respectively, both months were combined under April. I also included a dummy variable coding for whether or not the individual had been previously captured within the last 3 days to take into account the removal of ticks from the mice (3 days is the minimum amount of time necessary for larval ticks to feed and drop off from their host (Nuss et al. 2017)). All continuous variables (temperature, precipitation, body mass, daily mouse capture numbers, and flea/botfly/mite abundance) were scaled to a mean of zero and a variance of 1. Categorical factors with two categories were treated as centered continuous variables (i.e., captured <4 days: no = -0.5, yes = 0.5; mouse density for the year: low = -0.5, high = 0.5; sex: female = -0.5, male = 0.5; age: juvenile = -0.5, adult = 0.5; reproductive status: no = -0.5, yes = 0.5). Daily temperature and precipitation were taken from the nearby Lyndhurst Shawmere weather station, with missing data filled with data from the

Grenadier weather station (data from Government of Canada's past weather and climate historical data).

Three random effects were included in the model: year, handler identity, and mouse identity. Year was included to take into account differences between years, which can vary greatly, for instance, the trapping season was delayed by a month in 2020. The identity of the handler was also included as a random effect to account for possible differences in observation between handlers for parasite counts. Since many individual mice were repeatedly captured, mouse identity was included as a random effect to account for this source of non-independence and quantify among-individual variance in tick parasitism. Since the presence of random effects modify residuals, the "Diagnostics for HierArchical Regression Models" package (DHARMa) was used to access goodness of fit using a quantile-quantile plot of simulated residuals (Hartig 2020). Using DHARMa, overdispersion and zero-inflation were tested and found not to be significant. Since it is currently unknown how repeatability can be accounted for in zero-inflated models, individual repeatability was calculated using the rptBinary package only for the zero-inflation component (there is no specific function for zero truncated negative binomial distribution). rptBinary is based on work by Nakagawa and Schielzeth (2010). The trigamma function from the r.squaredGLMM package was used to calculate marginal and conditional pseudo R^2 for the zero-inflation component model (Nakagawa and Schielzeth 2013). The marginal pseudo R^2 only includes the fixed effects, while the conditional pseudo R^2 also includes the random effects in its calculation. Both are summaries of the variance explained in a model. For the conditional model component, no pseudo R^2 could be found since it is currently unknown how to calculate the index within zero-truncated negative binomial distributions.

The second part of the analysis was restricted to a subset of captures for which the mouse was brought back to the laboratory for behavioural testing. Two separate linear mixed models were run using the lme4 package in R 4.03, with distance moved or time spent grooming during the open-field test as the dependent variable. Distance moved was square root transformed and time spent grooming was transformed with a 0.6 exponent. After transformation, both variables were scaled to a mean of zero and a variance of 1. The fixed effects were mouse density of the year (high/low), month, test sequence, time of day, daily mouse capture numbers, mouse mass, sex, age, reproductive status, and tick/flea/botfly/mite presence. Because results from the first part of the analysis suggested that ticks and fleas co-occur (see below), we also included an interaction between tick and flea presence. Mass, test sequence, and time of day were scaled to a mean of zero. The presence of each parasite was centered by setting absence/presence to -0.5/0.5. Mouse identity and year were included as random effects.

Finally, the same model was re-run for both distance moved and time spent grooming, but I replaced the presence/absence of parasites by the actual parasite count separated into among-individual and within-individual effects using within-subject centring (van de Pol and Wright 2009). For the individual mean parasite count, the average was calculated for every individual and included as a variable that captures the among-individual relationship between parasite count and behaviour. For the within-individual effect, the individual mean parasite count was subtracted from the parasite count on each observation, and this variable was then included in the model to estimate the within-individual relationship between parasite count and behaviour.

Results

Descriptive statistics

From 2016 to 2020, weekly summer trapping sessions resulted in 4,333 captures on 1,035 (523 males, 512 females) mice (see Figure 2 for mouse captures and individual mice per year). The number of trap nights in 2016, 2017, 2018, 2019, and 2020 were respectively 5337, 6258, 4723, 4537, and 3920. Of the individual mice caught, 602 were caught as subadults, 701 were caught as adults, and among them, 268 were captured as both subadults and adults. Ticks were detected on 778 (18%) of the captures for a total of 4,105 ticks. Most ticks were counted and collected in the last two years of monitoring, with 497 and 3,439 ticks collected in 2019 and 2020, respectively (Figure 3). For fleas, there were a total of 350 fleas on 206 of the captures. There was a total of 310 mites counted on 152 of the captures, most infested wild mice had 10-20 mites at a time. There was a total of 23 botfly larvae counted on 16 captures. The range, grand mean, intensity, and mode for each parasite on the mice can be found in Table 1. The intensity was calculated from the mean parasite burden among infested mice for each parasite taxon.

Extrinsic and intrinsic variables affecting tick parasitism

Among the fixed effects for the Hurdle conditional component model (which included only mice that had at least one tick), yearly mouse density, month, temperature, reproductive status, sex, flea burden, botfly larvae burden, and mite burden were not significant (Table 2a). Juvenile and male mice tended to have more ticks, but the effect was not significant (Table 2a). The effect of being reproductively active was also non-significant and had a small effect. Heavier mice had more ticks (Table 2a). Mice had fewer ticks if they had already been captured within the last 3 days, ticks found on mice after three days were most likely newly acquired rather than individuals that had been missed during previous handling (Table 2a). When more mice were captured in a day, fewer ticks were observed on individual mice (Table 2a). When more fleas were present, more ticks were also observed on individual mice.

Among the fixed effects for the Hurdle zero-inflation component model (for which the tick burdens were considered zero or non-zero values), yearly mouse density, month, temperature, precipitation, sex, age, reproductive status, botfly larvae burden, and mite burden were all non-significant (Table 2b). It is more likely for a mouse to have a tick present if it was not captured previously within the last 3 days (Table 2b). Heavier mice and mice with more fleas had greater odds of a tick being present (Table 2b). The odds of there being no ticks on a mouse increased with the number of mice captured on that day (Table 2b). The repeatability on the link-scale for mouse identity in the zero-inflation part of the model was 0.02 (p5% = 0.01 confidence intervals: 0.005, 0.044), indicating that tick presence was not repeatable in mice. The marginal delta pseudo R^2 for the zero-inflation component was 0.06, while the conditional deltapseudo R^2 was 0.35, so most of the variability explained by the model was due to the random factors, but much variability was unexplained.

Relationship between tick parasitism and behaviour

A total of 510 individuals (280 males, 230 females) were brought back to the laboratory and subjected to the open-field test, on 1,123 separate captures. Among the fixed effects included in the linear mixed model for distance moved, the mouse density of the year, month, sex, reproductive status, mite presence, and botfly larvae presence were not significant (Table 3). Mice covered less distance in the open-field test when they were heavier (body mass), with every subsequent trial (test sequence), and when it was later in the day (Table 3). Moreover, adult mice covered less distance than juveniles (Table 3). The presence of ticks and fleas both had a significant effect on distance moved, with less distance covered when ticks were present, but more distance covered when fleas were present (see Table 3 and Figure 4). Interestingly, the "tick × flea" interaction was significant, revealing that the negative effect of ticks on distance covered occurred only when fleas were co-occurring (Figure 4). Repeatability of distance moved was 0.32 (95% confidence intervals: 0.251, 0.412),. The marginal trigamma pseudo R^2 was 0.25, while the conditional trigamma pseudo R^2 was 0.53, so a little over half of the variance was due to random effects.

In the version of the model with the abundance of parasites mean centered, individuals with higher mean flea burdens covered more distance (estimate = 0.274, $P = 0.038$). Within individual flea burdens, among and within tick burdens, and interactions between flea burden and tick burden were not significant. All other results for variables were similar to those from the presence absence model.

For the time spent grooming in the arena with parasite presence/absence, only the time of day and the daily number of mouse captures were significant, with mice grooming more later in the day and when there were more mice caught on that day (see Table 4). When parasite burdens were standardized by the individual's mean, mice groomed less during high density years and individuals with higher mean botfly larvae burden groomed more than other individuals. The time of day and daily number of mouse captures were still significant. In the version of the model with the abundance of parasites mean centered, within individual variation of tick burdens was positively associated with increased grooming (estimate = 0.0162, $P = 0.046$), while among individuals, those with higher-than-average botfly burdens groomed more (estimate = 0.52, $P = 0.054$). The repeatability of grooming behaviour was 0.18 (95% confidence intervals: 0.114, 0.267). The marginal trigamma pseudo R^2 was 0.06, while the conditional trigamma pseudo R^2 was 0.30, so most of the explained variance was due to random effects, but much variance remains unexplained by the model.

Discussion

I set out to determine the influence of intrinsic and extrinsic factors on mouse tick burdens and examine the relationship between tick parasitism and mouse behaviour in a standardised open-field test. Of all intrinsic factors, the only significant one was body mass, such that larger mice had more chance of being parasitized by a tick than smaller mice. The significant extrinsic factors were daily mouse and flea abundance, such that higher levels of tick parasitism and tick presence were observed on days when fewer mice were caught and on mice that were also parasitized by fleas. Interestingly, the interaction between tick and flea parasitism significantly influenced the exploratory behaviour of mice within the open-field test. To my knowledge, this is the first study showing an association between co-occurring parasites and host behaviour.

Intrinsic factors: age

The effect of age on tick parasitism is somewhat mixed in the *P. leucopus*/tick system. Some studies that identified and used larval *I. scapularis* counts in their analysis found no effect of age on the tick burdens of mice (Bouchard et al. 2011, Devevey and Brisson 2012, Larson et al.

2018). One study found significant results similar to the higher tick parasitism in juveniles that was found in this study (Butler et al. 2020). Analyses of a long-term data set (20 years) in Dutchess County, New York, revealed that subadults were more likely to have ticks than juveniles, while being an adult had little relationship with parasite burden (Brunner and Ostfeld 2008, Mowry et al. 2019). In our study, we did not differentiate subadults from juveniles, such that higher tick intensity in subadults may have been masked since they were classified together with juveniles. Based on these other studies, the increase in tick intensity on juvenile (or subadult) mice may be due to less efficient maintenance behaviour, underdeveloped immune system, increased movement and activity, and differences in habitat use compared to adults.

Intrinsic factors: body mass

I found that body mass was not a significant predictor of tick intensity but was for tick presence. While directionally it is similar, this result is different than those usually found in the literature since mass usually results in greater tick burdens. In a long-term data set that has been analyzed multiple times, of all intrinsic factors considered, body mass had the greatest impact on tick burden, with heavier mice being more likely to have more ticks (Brunner and Ostfeld 2008, Mowry et al. 2019). A shorter study also found that larval tick burdens increased with mass in both sexes (Schmidt et al. 1999). Another short study, however, found that body mass had no effect on tick burden (Devevey and Brisson 2012). In general, when it comes to parasites, heavier animals tend to have greater parasitic burdens. There could be multiple reasons why heavier mice are more likely to have parasites present. In the *P. leucopus*/tick system, larger mice may be more likely to have ticks since they may have larger home ranges and invest more in growth.

Intrinsic factors: sex

Studies on *P. leucopus* have found that males have more ticks than females (Brunner and Ostfeld 2008, Devevey and Brisson 2012, Mowry et al. 2019). In my study, although the sex difference was not significant for the intensity and prevalence of ticks, the observed sex bias in parasite burdens tended to be towards male hosts. It has been suggested that the increased parasitism in male hosts might be related to increased activity and movement (which both change in this system with mouse density, see below), or levels of testosterone (Moore and Wilson 2002). Another explanation would be that males generally have more parasites because male hosts are larger than females in many species. This possibility, however, can be disregarded in my study because body mass was included as a covariate in the model.

Intrinsic factors: reproductive status

Although the reproductive status variable was not significant for intensity and prevalence, the observed trend was towards higher tick intensity and prevalence in reproductive mice, when compared to their nonreproductive counterparts. While reproductive males are more active than nonreproductive males, classifying reproductive status as a two-level categorical variable ("yes or no") may have been an oversimplification for females, since their behaviour (and tick burden) may change when they transition from pregnant to lactating. For example, Mowry et al. (2019) found that pregnant females had slightly more ticks and lactating females had decreased tick burdens than non-reproductive females (while still detecting a sex effect). In our study, when reproductive status was classified as a 5-levels categorical variable (i.e., non-reproductive, scrotal, swollen vulva, pregnant, and lactating; see Table S1), the level of tick parasitism was significantly lower in lactating females than in scrotal males (estimate = -0.679, *P* = 0.024) and females with swollen vulva (estimate = -1.401, *P* = 0.029). Overall, the results obtained in the *P. leucopus*/tick system so far suggest that increased activity associated with mating (in scrotal males and females with swollen vulva) may result in higher tick parasitism, whereas decreased activity (lactating female) may result in lower tick parasitism.

Extrinsic factors: temperature and precipitation

Daily mean temperature and total precipitation had a very small and nonsignificant effect on mouse tick intensity and tick prevalence. The lack of effect from temperature and precipitation may be because the ticks did not attach themselves on the same day as they were observed on mice. Therefore, daily averages may be too coarse to successfully predict larval tick burdens on individual mice. Other studies had more success using yearly weather measures; larval tick burdens were greater during years of middling temperatures and precipitation (Mowry et al. 2019), and the average larval burden per mouse was at its lowest following drought years (Jones and Kitron 2000). The mice are also affected by weather variation and their numbers plummet during drought years (Jones and Kitron 2000, Dhawan et al. 2018). In fact, the impact of both water availability and cumulative degree days on tick burdens is stronger when mouse density is low (Mowry et al. 2019). In my study, the variability for this effect was most likely included in the year effect (included in the model as a random effect). This may be why the tick prevalence component of the Hurdle model explained so little variance when the random effects were not accounted for. A longer time series than is considered here is needed to study yearly variation effectively.

Extrinsic factors: mouse density

The daily number of mice captured provides a daily, local estimate of mouse activity and is related to mouse density. Mouse populations reach high densities in years following acorn masts (Clotfelter et al. 2007), but the number of mice present at a given site is not constant throughout the year. Indeed, mouse reproduction may start over winter, but continues during spring and over the summer, which can cause an exponential increase in population size. Therefore, the daily number of mice captured probably better represents density (and activity) than the yearly number of mice captured. When mouse density is high, mice have smaller tick burdens due to dilution effects (Ostfeld et al. 1996), while when mouse density is low, higher tick burdens are predicted (Mowry et al. 2019). It was expected in this study that there would be a reduction in tick intensity when there are more daily captures and an increase in the prevalence of infested mice. Although the yearly mouse density was not significant, we found that the daily number of mice captured was negatively correlated with both prevalence and intensity. Hurdle models can be used to model intensity and prevalence when it is known that there are no false negative zeroes (as was the case in Obiegala et al., 2021). However, both true zeroes and false zeroes (due to observer error) are treated as being the same in a Hurdle model, and this may have a significant impact on certain fixed effects within the zero-inflation component model; when there are more mice, observers may grow tired and become less efficient at detecting tick larvae. To confirm that captures truly had no ticks, each capture would have had to be housed for six days, so that feeding ticks would drop off from their host and be collected, which would be unfeasible considering that keeping the mice captive for such extended periods might affect wild mouse behaviour. Another explanation is that mouse density is not related to the tick burdens of mice, and tick larvae are instead drawn away from mice because of the increase in chipmunks, who also reach high population densities following acorn masts (Schmidt et al. 1999). Yet another explanation is that mouse behaviour, such as their movement, changes at high densities and it is because of this change in behaviour that the mice then have fewer ticks (Ostfeld et al. 1996). Higher densities might also make mice more likely to be infested with other parasites, such as fleas.

Extrinsic factors: fleas

My analysis revealed that ticks and fleas might be co-occurring on host mice. It is possible that most of the collected ticks, which have not yet been identified, are *I. angustus*, which like fleas, are nidicolous (both may complete their entire life cycles while remaining within the nest of their host) and interact with their hosts in similarly ways. Likewise, certain mouse behaviours may favor both ticks and fleas at the same time or make acquiring both at the same time more likely. While the abundance of fleas had a significant effect on the intensity of ticks on mice, it had a small effect relative to the effect of body mass or the number of mice captured. The abundance of fleas had a much larger effect on the likelihood of tick presence. If it is the presence of ticks that is influencing the flea burdens of mice, it may be due to the immunosuppressive qualities of tick saliva. In mice, *I. scapularis* does not illicit an inflammatory response after repeat feeding, making mice very permissive to their presence (Anderson et al. 2017). *I. scapularis* saliva contains both anti-inflammatory and pain suppressing proteins, which decreases the likelihood of the mouse removing the tick after attachment since the tick bite would not cause discomfort (Ribeiro et al. 1985). Perhaps the compounds injected by *I. scapularis* also decrease the discomfort experienced by mice from the presence of fleas. In future studies, researchers may want to explore the effect of *I. scapularis* saliva on the feeding success of other parasites.

Exploratory behaviour

Irrespective of the mechanism for the observed co-occurrence of ticks and fleas on mouse hosts, interestingly, I found a significant interaction between ticks and fleas on exploratory behaviour (controlling for various covariates, see Table 3). When fleas were absent, distance moved in the open-field test was not different between tick-free vs tick-infested mice. When fleas were present, however, tick-free mice covered significantly more distance than tick-infested mice. Considering that this study is observational, it is impossible to determine the causality of the relationship between the co-occurrence of ticks and fleas and mice exploratory behaviour. On one hand, ticks and fleas may interact to affect their host's exploratory behaviour. For example, the discomfort caused by fleas might be absent when mice are also infested with ticks, which in turn might cause differences in the distance moved in the open-field test (note: this effect is not observed on grooming behaviour). On the other hand, individual differences in exploratory behaviour of mice may affect their susceptibility to being differentially infested with ticks and fleas, but it remains unclear why more exploratory mice would be more likely to be infested with fleas but not ticks. Future manipulative studies should use experimental designs to determine the causality of the parasitism-behaviour relationships observed.

Although parasites, by definition, should have a cost, studies on *P. leucopus* generally report no such cost for tick parasitism. Mouse survival (Hersh et al. 2014) and stress (Gaitan and Millien 2016) do not seem to be affected by larval tick burden. In fact, the opposite seems to apply, as mice with the largest burdens are also the most likely to survive, perhaps because they are less susceptible to predation while using a habitat that also promotes the acquisition of ticks (Ostfeld et al. 1996, Hersh et al. 2014). It has been suggested that mice might be compensating for the cost of bearing ticks by diminishing their movement (Gaitan and Millien 2016), which should cause a reduction in movement observable at the within-individual level. At the same time, one could also expect that mice that consistently display higher levels of exploratory behaviour are more likely to harbor more ticks than the average mouse, which should cause a positive relationship at the among-individual level. Results from the within-subject centering approach revealed that within-individual changes in tick parasitism did not influence exploratory behaviour (estimate = -0.008, $P = 0.21$). Among-individual variation in tick burdens was weakly and positively correlated with distance moved, such that individuals that moved more had slightly higher average tick burdens over their capture history (estimate = 0.021; $P = 0.093$). The lack of among- and within-individual difference in exploration with greater tick burdens could indicate that the cost of bearing ticks does not impact exploratory behaviour.

However, since mice that moved more had higher average flea burdens than other mice (estimate = 0.267; $P = 0.044$) but did not have significantly higher movement when they had more fleas (estimate = -0.009; $P = 0.84$), it is likely that increased flea burdens are associated with more explorative mice. Considering that fleas spend most of their time either on the mouse or in the mouse's nest, these mice could be more explorative because of more frequent feeding by the fleas occupying their nest. In an experimental setting in which another rodent species was exposed to fleas, they found that depending on age, the rodent responded differently to compensate for the energetic cost of the parasite burden; juveniles harboring fleas had reduced growth rates, while adults increased their energy consumption (Garrido et al. 2016). In the wild, adult animals could need to increase their movement rate to find more food to compensate for the presence of fleas. Likewise, it might be that individual mice have higher lifetime flea burdens because they are more explorative. It could be the case that more explorative mice also have more social interactions. During non-hostile interactions, mice might groom one another, resulting in the removal of ticks, while also propagating fleas from one mouse to the other, increasing their chances of carrying fleas.

Grooming behaviour

Although many studies have suggested that grooming behaviour is important to consider in the *P. leucopus*/tick system (Calabrese et al. 2011, Ostfeld et al. 2018, Mowry et al. 2019), none have quantified grooming behaviour in this system. Here, I quantified time spent grooming during open-field tests, but found that it had a slight effect on tick parasitism. Individuals who on average groom more did not have fewer ticks than individuals that groom less, which is contrary to the expectation that grooming results in the removal of parasites. Within individuals, however, there was a slight, yet not significant increase in grooming when there were more ticks (estimate = 0.015, P*P* = 0.056), suggesting that increased tick intensity may result in increased grooming. Although a previous study found that the presence of ticks did not result in increased stress for the mouse based on the size of the adrenal gland, no such comparison was made for changes in the intensity of ticks within the same mouse (Gaitan and Millien 2016). In rodents, the time spent grooming in the open-field test is associated with increased stress (Zhang et al. 2019). While it is weak and not a direct association, this increased grooming with greater intensity of ticks within individuals may be an indication that increased tick burdens on mice can increase the stress of the host.

It may be that the number of parasites on a capture is not directly related to the individual's capacity for parasite removal, but more strongly determined by an individual's encounter rate with the parasites. When a mouse encounters ticks more often than usual, it might also groom more often than usual. It could also be that the time spent grooming is not related to the quality of grooming behaviour; hosts that are bad at removing parasites through grooming may not groom more or less than hosts who are more likely to remove parasites from grooming.

In this study, mice with overall higher mean botfly burdens groomed more (estimate = 0.535, $P = 0.0416$). It should be noted that, in the case of botfly parasitism, grooming does not result in the removal of the parasite. In *Peromyscus maniculatus*, the time spent on maintenance activities, which included grooming, increased with the presence of botfly larvae in a laboratory setting (Smith 1978). This may have been preventive behaviour to stop further parasitic infections, or a result of irritation. Given the association between stress and grooming behaviour during open-field tests, increased grooming could also be caused by higher stress in mice infested with botflies. However, Brown and Fuller (2006) found that the presence of botfly larvae was not associated with higher corticosterone levels in other mouse populations. This could be because the mice compensate for botfly infection by changing to a high protein diet or by increasing their consumption of high energy meals, resulting in larger stores of fat, but also committing to riskier behaviour to do so (Cramer and Cameron 2006). Further studies with paired measures of grooming and corticosterone are necessary to test whether the increased grooming in mice parasitized with botflies is a result of increased stress or an independent response.

The relationship between increased grooming and the increase in mouse captures in a day might also be because of increased stress due to increased conspecific aggression. In rats, grooming behaviour in arenas increases in frequency and complexity as rats habituate to the environment, so animals that habituate faster to the arena would also spend more time grooming (Rojas-Carvajal et al. 2018). As the animal habituates, it would transition from short grooming sessions of the head and hands to more complex grooming of the body and tail. Splitting the grooming behaviour into stress induced head grooming and full body grooming may yield better results in the future. It should also be noted that, while these measures were taken from a wild population, observations were made in an artificial setting. With the continued advancement in accelerometers, it could become possible to install them on wild mice and capture grooming behaviour under natural conditions. With this we might be able to find how much time mice spend grooming in the wild on a daily basis and differentiate between causes of grooming.

Conclusions

Among the intrinsic factors considered, body mass was consistent with results in the literature, with large mice being the most at risk of high tick burdens, although in this study it was only found that larger mice were more likely to have ticks present. The relationships with reproductive status and age, however, are weaker and less consistent since certain categories of both differ in their relationship with tick burdens, and the way both are defined or categorised is not consistent across studies. Among the extrinsic factors, tick parasitism seems to be affected by the number of mice in the environment, which also affected exploratory behaviour within a novel environment. Another correspondence between levels of tick parasitism and exploratory behaviour was the interaction with flea parasitism. Indeed, fleas and ticks co-occurred on their mouse hosts, and their interaction was significantly associated with mouse exploratory behaviour. However, a few limitations should be kept in mind when interpreting my results. First, as mentioned above, my results are correlative, such that I cannot distinguish causality (i.e., are fleas and ticks causing changes in mouse behaviour, or mouse behaviour causing tick and fleas to co-occur). Second, an exclusion experiment would have to be performed to truly see if there is co-occurrence between fleas and ticks (Lutermann et al. 2015, Veitch et al. 2020). Furthermore, as none of the parasites observed were identified to life stage and species, it may be that the patterns observed are only true at the taxon level or that they are patterns due to a dominant parasite species. Nevertheless, my study is the first to establish a link between tick and flea co-occurrence and host exploratory behaviour, which certainly warrants further research. While we found no relationship between grooming time in the open-field test and parasite presence, grooming did increase with higher average botfly burden and slightly with increased tick burdens within individuals, indicating that in this case, grooming may be a response to irritation. It remains to be seen which portion of grooming behaviour is truly associated with parasite removal and how such grooming might be observed in wild populations. One way forward in either case, would be the use of accelerometers, which not only can yield an accurate measure of activity, but potentially of their grooming behaviour in their natural habitat.

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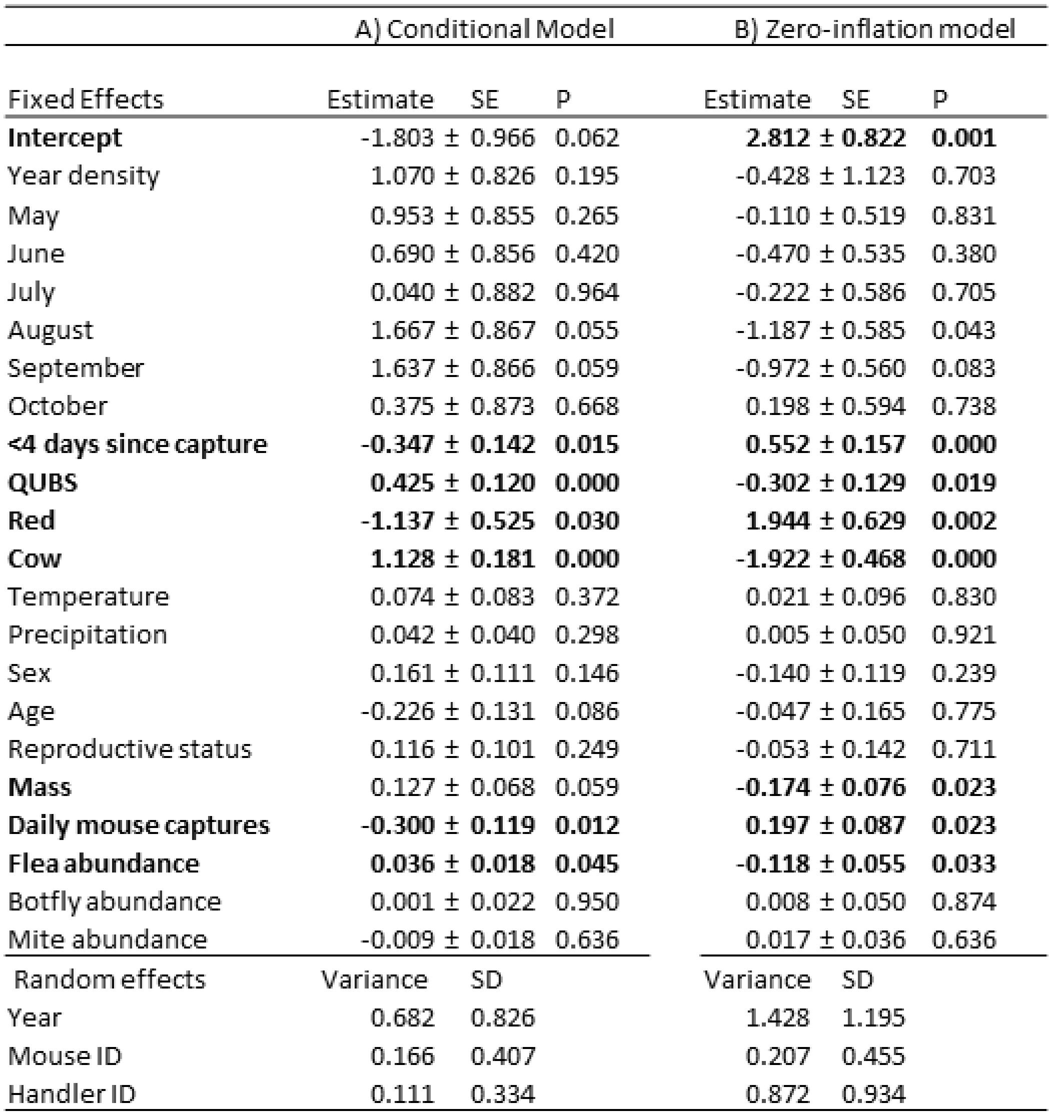
Tables and Figures

Table 1. Metrics describing parasitism of 1,035 white-footed mice (*Peromyscus leucopus*) caught a total of 4,333 times in eastern Ontario, Canada, 2016-2020, where mice are parasitized by ticks, fleas, mites, and botflies. Prevalence is the percentage of captures in which the mouse was infested with each parasite. Intensity is the average number of parasites on infested mice. Both prevalence and intensity were calculated from all years and sites combined.

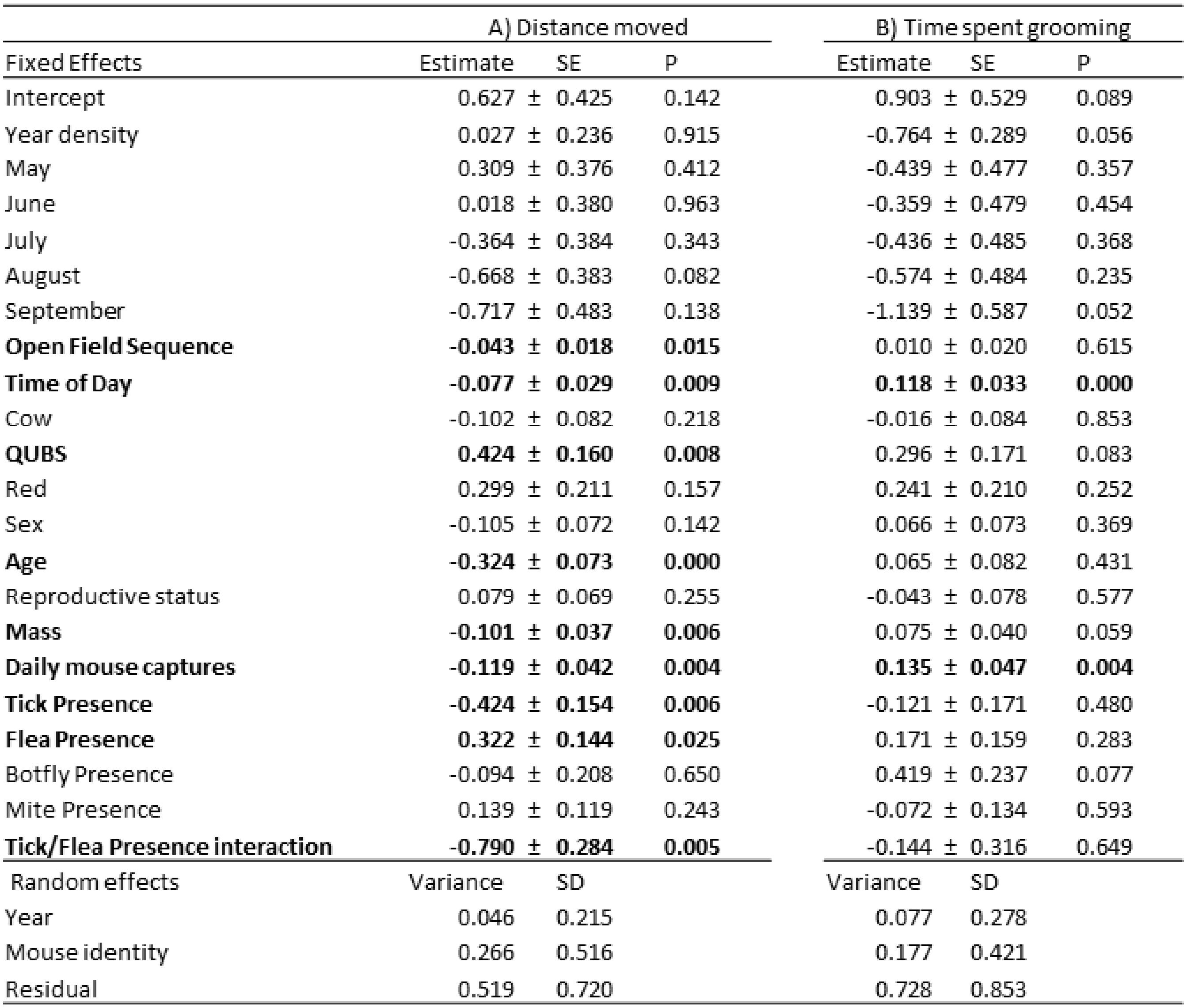
	Tick	Flea	Mite	Botfly
Prevalence	17.9%	4.7%	3.5%	1.1%
Max burden	69	15	40	4
Intensity	5.27	1.7	2.04	1.25
Mean on all mice	0.945	0.081	0.071	0.014
Mode in infested mice	1	1	1	1

Table 2. The parameter estimates and variance of the random effects from the A) zero truncated negative binomial distributed portion and the B) zero-inflation binary portion of a hurdle mixed model of tick abundance counted on 1,035 white-footed mice (*Peromyscus leucopus*) captured a total of 4,333 times in eastern Ontario, Canada, 2016-2020. The months of tick collection were from April (reference level) to October. Temperature, precipitation, mass, daily number of mice captured, and flea/botfly/mite abundance were scaled. Sex, age, and reproductive status were centered at -0.5 and 0.5. The random effects were year, mouse identity, and handler.



	A) Conditional Model		B) Zero-inflation model	
Fixed Effects	Estimate SE	P	Estimate SE	P
Intercept	-1.803 ± 0.966	0.062	**2.812 ± 0.822**	**0.001**
Year density	1.070 ± 0.826	0.195	-0.428 ± 1.123	0.703
May	0.953 ± 0.855	0.265	-0.110 ± 0.519	0.831
June	0.690 ± 0.856	0.420	-0.470 ± 0.535	0.380
July	0.040 ± 0.882	0.964	-0.222 ± 0.586	0.705
August	1.667 ± 0.867	0.055	-1.187 ± 0.585	0.043
September	1.637 ± 0.866	0.059	-0.972 ± 0.560	0.083
October	0.375 ± 0.873	0.668	0.198 ± 0.594	0.738
<4 days since capture	**-0.347 ± 0.142**	**0.015**	**0.552 ± 0.157**	**0.000**
QUBS	**0.425 ± 0.120**	**0.000**	**-0.302 ± 0.129**	**0.019**
Red	**-1.137 ± 0.525**	**0.030**	**1.944 ± 0.629**	**0.002**
Cow	**1.128 ± 0.181**	**0.000**	**-1.922 ± 0.468**	**0.000**
Temperature	0.074 ± 0.083	0.372	0.021 ± 0.096	0.830
Precipitation	0.042 ± 0.040	0.298	0.005 ± 0.050	0.921
Sex	0.161 ± 0.111	0.146	-0.140 ± 0.119	0.239
Age	-0.226 ± 0.131	0.086	-0.047 ± 0.165	0.775
Reproductive status	0.116 ± 0.101	0.249	-0.053 ± 0.142	0.711
Mass	0.127 ± 0.068	0.059	**-0.174 ± 0.076**	**0.023**
Daily mouse captures	**-0.300 ± 0.119**	**0.012**	**0.197 ± 0.087**	**0.023**
Flea abundance	**0.036 ± 0.018**	**0.045**	**-0.118 ± 0.055**	**0.033**
Botfly abundance	0.001 ± 0.022	0.950	0.008 ± 0.050	0.874
Mite abundance	-0.009 ± 0.018	0.636	0.017 ± 0.036	0.636
Random effects	Variance SD		Variance SD	
Year	0.682 0.826		1.428 1.195	
Mouse ID	0.166 0.407		0.207 0.455	
Handler ID	0.111 0.334		0.872 0.934	

Table 3. Fixed and random effect parameter estimates from a linear mixed model of A) distance moved and of B) time spent grooming in white-footed mice (*Peromyscus leucopus*) during a 10-min open-field test in eastern Ontario, Canada, 2016-2020. A total of 510 individuals were tested 1,123 times. Random effects included were year and mouse identity.



	A) Distance moved				B) Time spent grooming			
Fixed Effects	Estimate		SE	P	Estimate		SE	P
Intercept	0.627	±	0.425	0.142	0.903	±	0.529	0.089
Year density	0.027	±	0.236	0.915	-0.764	±	0.289	0.056
May	0.309	±	0.376	0.412	-0.439	±	0.477	0.357
June	0.018	±	0.380	0.963	-0.359	±	0.479	0.454
July	-0.364	±	0.384	0.343	-0.436	±	0.485	0.368
August	-0.668	±	0.383	0.082	-0.574	±	0.484	0.235
September	-0.717	±	0.483	0.138	-1.139	±	0.587	0.052
Open Field Sequence	**-0.043**	**±**	**0.018**	**0.015**	0.010	±	0.020	0.615
Time of Day	**-0.077**	**±**	**0.029**	**0.009**	**0.118**	**±**	**0.033**	**0.000**
Cow	-0.102	±	0.082	0.218	-0.016	±	0.084	0.853
QUBS	**0.424**	**±**	**0.160**	**0.008**	0.296	±	0.171	0.083
Red	0.299	±	0.211	0.157	0.241	±	0.210	0.252
Sex	-0.105	±	0.072	0.142	0.066	±	0.073	0.369
Age	**-0.324**	**±**	**0.073**	**0.000**	0.065	±	0.082	0.431
Reproductive status	0.079	±	0.069	0.255	-0.043	±	0.078	0.577
Mass	**-0.101**	**±**	**0.037**	**0.006**	0.075	±	0.040	0.059
Daily mouse captures	**-0.119**	**±**	**0.042**	**0.004**	**0.135**	**±**	**0.047**	**0.004**
Tick Presence	**-0.424**	**±**	**0.154**	**0.006**	-0.121	±	0.171	0.480
Flea Presence	**0.322**	**±**	**0.144**	**0.025**	0.171	±	0.159	0.283
Botfly Presence	-0.094	±	0.208	0.650	0.419	±	0.237	0.077
Mite Presence	0.139	±	0.119	0.243	-0.072	±	0.134	0.593
Tick/Flea Presence interaction	**-0.790**	**±**	**0.284**	**0.005**	-0.144	±	0.316	0.649
Random effects	Variance		SD		Variance		SD	
Year	0.046		0.215		0.077		0.278	
Mouse identity	0.266		0.516		0.177		0.421	
Residual	0.519		0.720		0.728		0.853	

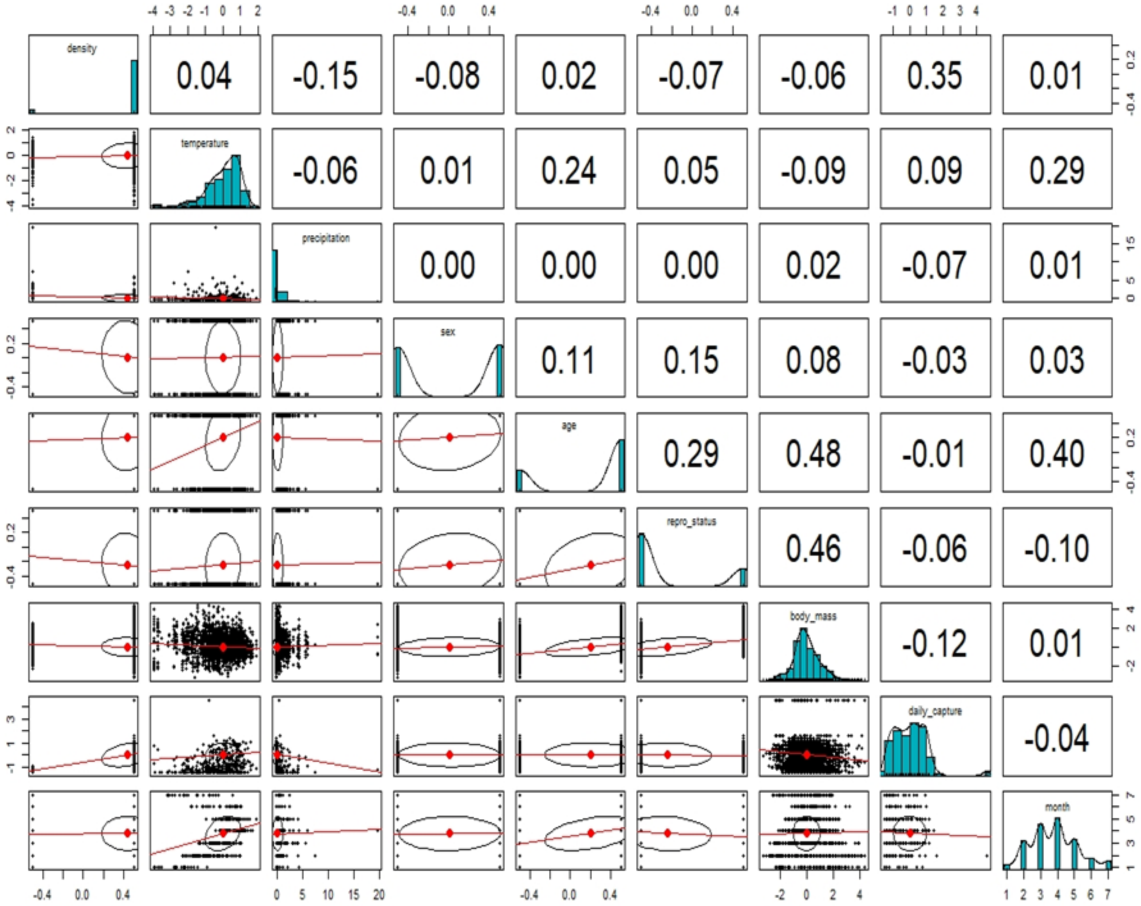


Figure 1. Correlation matrix for all pairs of independent variables. Panels in the lower triangle display scatter plots and regression lines for every possible pairing. Panels in the upper diagonal give the corresponding Pearson correlation coefficient, with text size proportional to its absolute value.

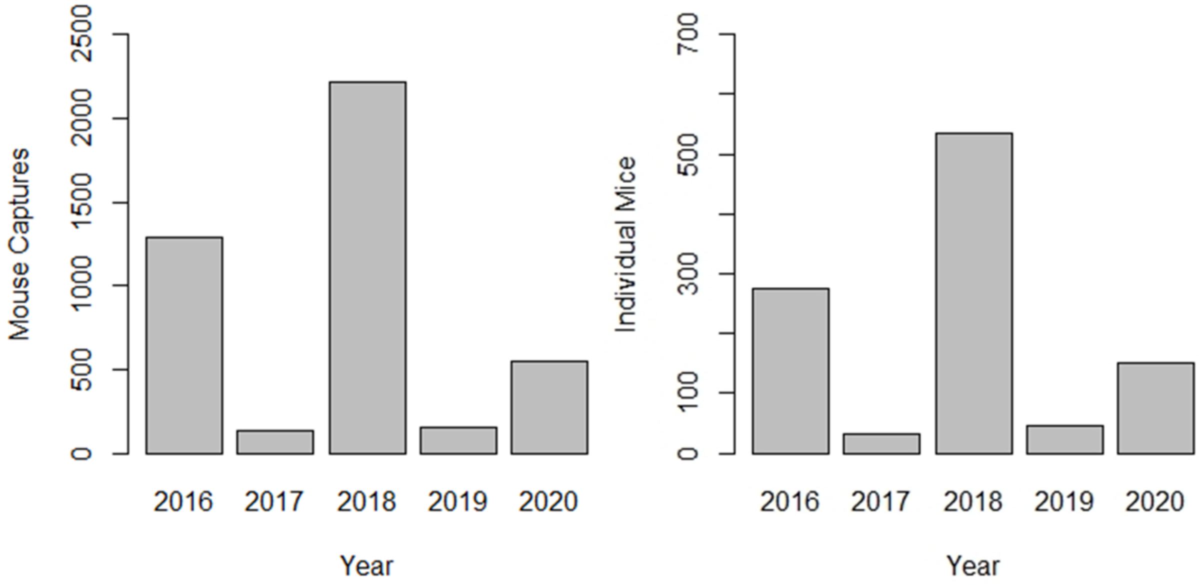


Figure 2. Number of white-footed mice (*Peromyscus leucopus*) (A) captured and (B) individuals caught per year in eastern Ontario, Canada, 2016-2020. From these figures, years 2016, 2018, and 2020 were qualifies as high-density years, whereas 2017 and 2019 were qualified as low-density years.

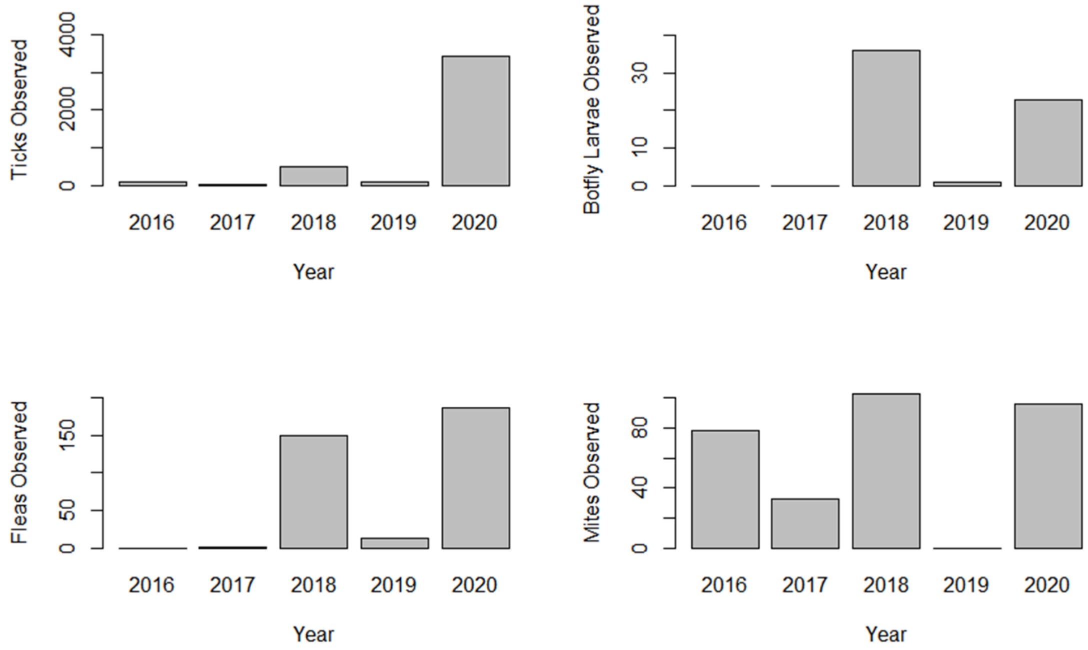


Figure 3. Total number (per year) of (A) ticks, (B) botfly larvae, (C) fleas, and (D) mites counted during 4,333 captures of white-footed mice (*Peromyscus leucopus*) in eastern Ontario, Canada, 2016-2020.

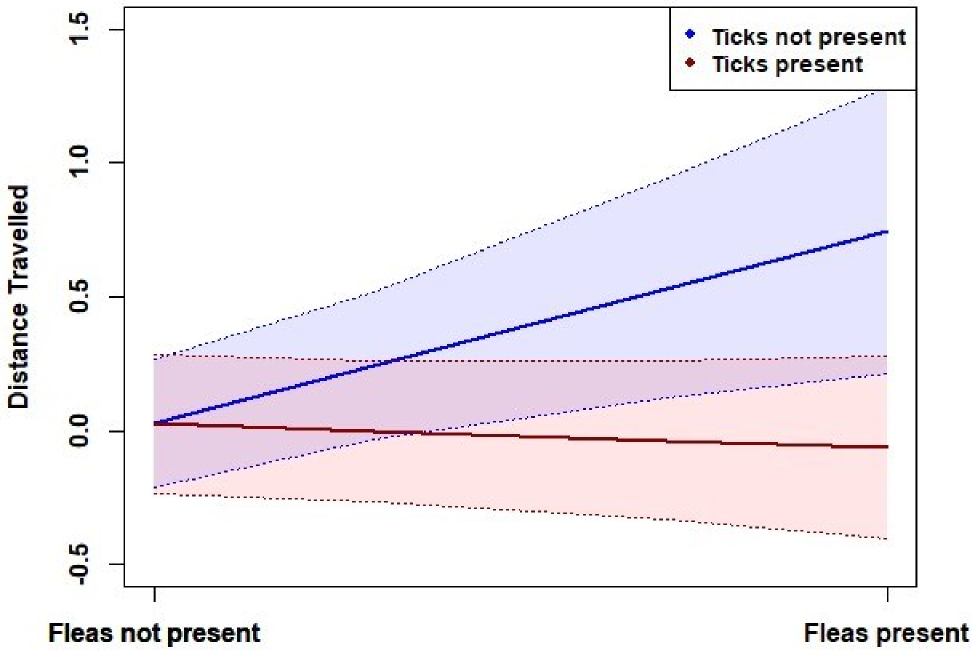
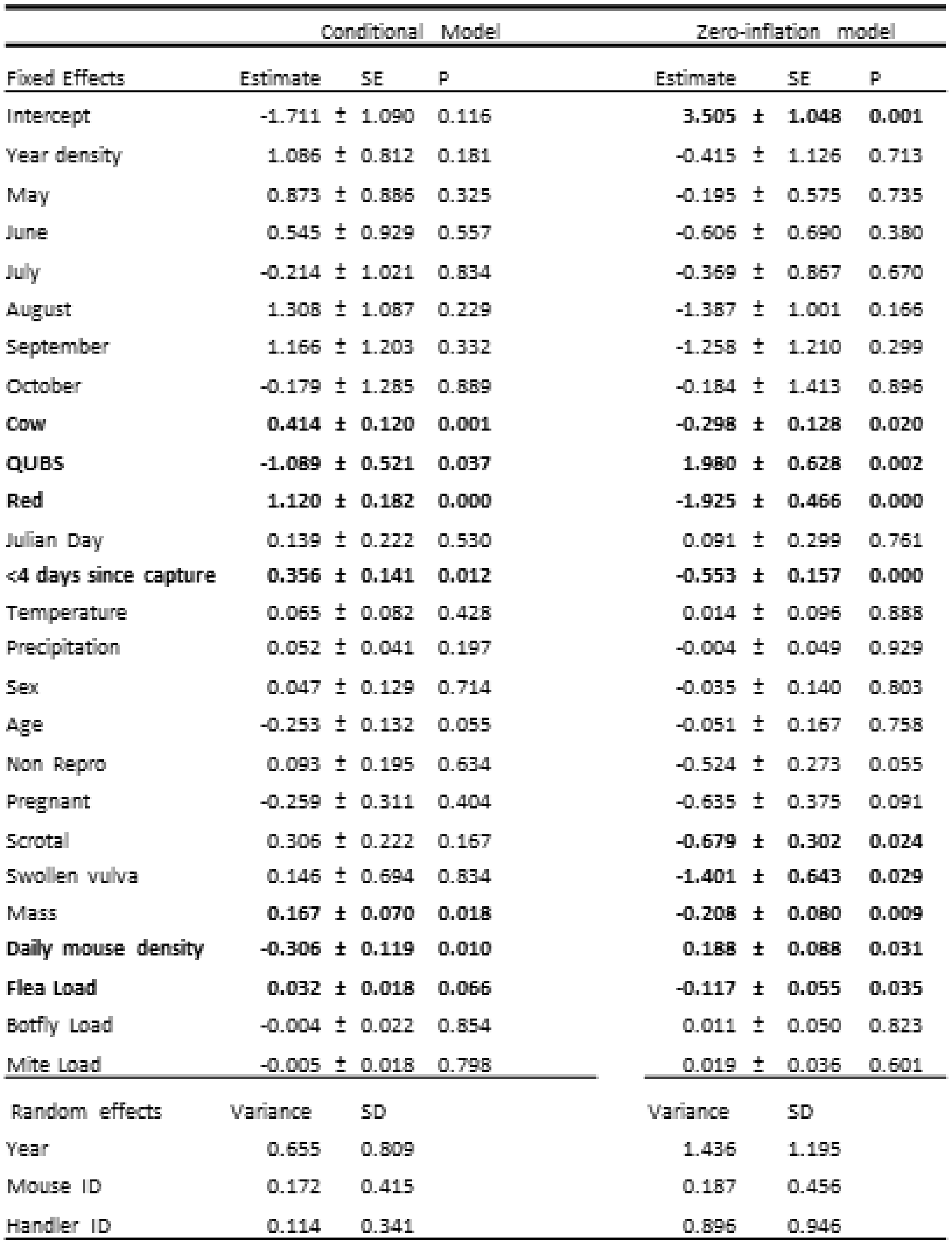


Figure 4. Distance moved (square-root transformed and scaled to a mean of zero and a variance of 1) in 510 white-footed mice (*Peromyscus leucopus*) during a 10-min open-field test (1,123 tests in total), showing how the co-occurrence of fleas and tick affect mouse exploratory behaviour. Tick and flea presence were treated as centered continuous variables. The pale coloured areas indicate upper and lower 95% confidence intervals.

Supplementary Table

Table S1. Same model as in Table 2, but with reproductive status entered as a categorical factor with five levels (non-reproductive, scrotal, swollen vulva, pregnant, and lactating) instead of a “yes or no” factor. The reference level was “lactating”.



	Conditional Model				Zero-inflation model			
Fixed Effects	Estimate		SE	P	Estimate		SE	P
Intercept	-1.711	±	1.090	0.116	**3.505**	**±**	**1.048**	**0.001**
Year density	1.086	±	0.812	0.181	-0.415	±	1.126	0.713
May	0.873	±	0.886	0.325	-0.195	±	0.575	0.735
June	0.545	±	0.929	0.557	-0.606	±	0.690	0.380
July	-0.214	±	1.021	0.834	-0.369	±	0.867	0.670
August	1.308	±	1.087	0.229	-1.387	±	1.001	0.166
September	1.166	±	1.203	0.332	-1.258	±	1.210	0.299
October	-0.179	±	1.285	0.889	-0.184	±	1.413	0.896
Cow	**0.414**	**±**	**0.120**	**0.001**	**-0.298**	**±**	**0.128**	**0.020**
QUBS	**-1.089**	**±**	**0.521**	**0.037**	**1.980**	**±**	**0.628**	**0.002**
Red	**1.120**	**±**	**0.182**	**0.000**	**-1.925**	**±**	**0.466**	**0.000**
Julian Day	0.139	±	0.222	0.530	0.091	±	0.299	0.761
<4 days since capture	**0.356**	**±**	**0.141**	**0.012**	**-0.553**	**±**	**0.157**	**0.000**
Temperature	0.065	±	0.082	0.428	0.014	±	0.096	0.888
Precipitation	0.052	±	0.041	0.197	-0.004	±	0.049	0.929
Sex	0.047	±	0.129	0.714	-0.035	±	0.140	0.803
Age	-0.253	±	0.132	0.055	-0.051	±	0.167	0.758
Non Repro	0.093	±	0.195	0.634	-0.524	±	0.273	0.055
Pregnant	-0.259	±	0.311	0.404	-0.635	±	0.375	0.091
Scrotal	0.306	±	0.222	0.167	**-0.679**	**±**	**0.302**	**0.024**
Swollen vulva	0.146	±	0.694	0.834	**-1.401**	**±**	**0.643**	**0.029**
Mass	**0.167**	**±**	**0.070**	**0.018**	**-0.208**	**±**	**0.080**	**0.009**
Daily mouse density	**-0.306**	**±**	**0.119**	**0.010**	**0.188**	**±**	**0.088**	**0.031**
Flea Load	**0.032**	**±**	**0.018**	**0.066**	**-0.117**	**±**	**0.055**	**0.035**
Botfly Load	-0.004	±	0.022	0.854	0.011	±	0.050	0.823
Mite Load	-0.005	±	0.018	0.798	0.019	±	0.036	0.601
Random effects	Variance		SD		Variance		SD	
Year	0.655		0.809		1.436		1.195	
Mouse ID	0.172		0.415		0.187		0.456	
Handler ID	0.114		0.341		0.896		0.946	