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Exploring the effects of pathogen infection on tick behaviour from individuals to populations

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Ticks vector a greater variety of pathogens than any other arthropod group, making them a significant threat to the public, companion animals and wildlife. Surveying wildlife habitats and areas commonly used for recreation for the presence of ticks and the pathogens that they carry is essential to the prevention of tick-borne diseases. Tick behaviour also plays an active role in transmission dynamics. We set out to study the host-seeking behaviour of *Amblyomma americanum*, the lone star tick, at the population level and at the individual level by collecting ticks in public parks in Gainesville, Florida, U.S.A., and by conducting behavioural assays on host-seeking behaviour (questing). We assayed a subset of the collected ticks for 3 consecutive days to determine whether ticks displayed repeatable, individual level differences in questing behaviour. Afterward, we screened ticks for two human pathogens, *Rickettsia* spp. and *Ehrlichia* spp. While we did not find differences in pathogen prevalence among the populations, we found that nymphal ticks infected with *Rickettsia amblyommatis* spent less time questing than uninfected nymphs. However, across all populations, we found a greater prevalence of *R. amblyommatis* in adult ticks. Additionally, nymphs were more likely to engage in questing behaviours than adults. Lastly, we found significant repeatability in the average height that a tick quests and the questing likelihood of a tick. These findings could fill a gap in the knowledge about trends in disease dynamics.

Vector-borne infections are a leading cause of human and wildlife infectious diseases. Ticks in particular pose a significant threat to public health, transmitting a greater variety of pathogens than any other arthropod group (Garira & Chirove, 2020; Jongejan & Uilenberg, 2004). Ticks are responsible for transmitting the majority of vector-borne pathogens to wildlife and domestic animals and are second only to mosquitoes in transmitting human diseases (de la Fuente et al., 2008). As emerging tick-borne diseases are discovered, and as ticks expand their ranges, it becomes essential to study host-seeking behaviours in response to pathogen infection and track pathogen prevalance across tick populations. Recent investigations in modelling vector-borne diseases have highlighted the importance of multiscale perspectives (e.g. multiple spatial scales or multiple scales of biological complexity) on disease dynamics (Garabed et al., 2020; Garira & Chirove, 2020). Using a multiscale perspective is essential while assessing behaviour as ignoring an aspect such as individual variation could lead to inaccurate predictions of transmission dynamics. Studying infectious diseases at the level of the individual and population can reveal patterns of within-population dynamics that affect between-population differences and potentially the spread of disease across a landscape (Garabed et al., 2020). However, very few studies address the behaviour of vectors at both the individual and population level.

Among-population differences in vector behaviour (e.g. oviposition, host-seeking behaviour, etc.) are expected to occur due to variation in local plant composition (Conley et al., 2011), habitat complexity (Lane et al., 2007; Richardson et al., 2021) and differences in the use of deterrents and pesticides (Overgaard et al., 2015). For example, studies have found that habitat type, and specifically temperature and relative humidity, can have an impact on the duration and height at which ticks engage in host-seeking behaviours (‘questing’) (Beck & Orozco, 2015; Benelli, 2020; Lees & Milne, 1951; Mejlon & Jaenson, 1997; Richardson et al., 2021; Schulze et al., 2001; Semtner & Hair, 1973; Semtner et al., 1971; Vail & Smith, 1998). Population differences in disease prevalence can also generate among-population differences in vector host-seeking

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behaviour based on infection-driven changes in behaviour (Benelli, 2020). Therefore, studies on how pathogen infection changes vector behaviour will be most impactful if they include individual level and population level assessments.

Individual differences in behaviour, which are consistent over time and across different ecological contexts (often referred to as 'animal personalities') are widespread in a diversity of animals (Kralj-Fišer & Schuett, 2014). The role of consistent individual differences in behaviour in host–parasite dynamics are nearly always assessed from the perspective of host behaviour (Barber & Dingemanse, 2010; Kortet et al., 2010). However, some recent studies have focused on individual variation in host-seeking behaviours in ectoparasites. For example, Marliére et al. (2020) discovered individual differences in locomotory behaviour in the kissing bug *Rhodnius prolixus*, the primary vector of Chagas disease, and Benelli (2018) used repeated assays to describe individual left–right behavioural asymmetries in the questing behaviour of *Ixodes ricinus* (Benelli et al., 2018). The facultatively ectoparasitic mite *Macrocheles muscadomesticae* also exhibits individual differences in host attachment propensity, and the consistency of host attachment behaviour differs between populations (Durkin et al., 2020). Understanding individual level variation in a disease vector behaviour, especially in medically important vectors, could give us a better understanding of disease dynamics, transmissibility and impacts on public health (Cator et al., 2020). Despite a scarcity of empirical investigations, the value of understanding individual variation in vector behaviour was demonstrated by Van Es et al. (1999) with ticks. Individual *I. ricinus* ticks were observed in field enclosures to quantify substantial interindividual differences in questing activity as the basis for stochastic models of questing behaviour at the population level (Van Es et al., 1999). Although the impact of individual variation in tick behaviour on disease transmission has not yet been formally tested, it is predicted that differences among individuals in host-seeking behaviour, especially if driven by infection status, will play an important role in disease dynamics.

Here, we studied host-seeking behaviour (questing likelihood, questing duration and questing height) in the lone star tick, *Amblyomma americanum*, at the individual and population level. The lone star tick is an aggressive human-biting tick that is a competent vector of many pathogens that pose threats to public health, including Rocky Mountain spotted fever (RMSF), human monocytic ehrlichiosis, tularemia and heartland virus (Childs & Paddock, 2003). The lone star tick is also predicted to expand its geographical range northward and westward from the south and central United States with changing climatic conditions (Sagurova et al., 2019), making it crucial to understand its host-seeking behaviour within and among populations. After behavioural analyses, ticks were screened for two bacterial human pathogens: *Rickettsia* spp. and *Ehrlichia* spp. *Rickettsia amblyommatis* is of particular interest because it is highly prevalent in some lone star tick populations but exhibits high among-population differences in prevalence (De Jesus et al., 2019; Mixson et al., 2006; Richardson et al., 2021). By repeatedly measuring a subset of individuals from three of our four focal tick populations, we aimed to study tick host-seeking behaviour at multiple scales.

METHODS

Study Sites and Tick Collection

We collected a total of 788 adult and nymph *A. americanum* ticks at four different sites in north-central Florida. We removed 21 ticks from analyses due to poor sequencing during the pathogen screening stage. Ticks were collected at four city parks in Gainesville, Florida, U.S.A. that included hiking trails open to the public: Split Rock Conservation Area ($N = 169$), Clear Lake Nature Park ($N = 70$), San Felasco Hammock Preserve State Park ($N = 137$) and Morningside Nature Center ($N = 215$). Since Split Rock Conservation Area and Clear Lake Nature Park are within the same forest area and collection sites were ~1.5 km apart, we considered individuals from these two sites to be one population ($N = 239$). From a previous study, we also collected 176 ticks from Ordway Swisher Biological Station (OSBS), a University of Florida controlled research station with unaltered natural habitat that is inaccessible to the public. Data focussing on questing behaviour across different habitat types was previously published using this subset of ticks from Ordway Swisher (Richardson et al., 2021). In the present analysis, we compared ticks across the three Gainesville city parks (average ~14 km distance between each site) and the rural OSBS (~40 km from Gainesville; see Supplementary material for map and exact distances). Rural collections occurred in July–August 2020 and city park collections occurred in May–July 2020. Collections could not occur simultaneously due to COVID-19 limitations.

Temperature, humidity and wind speed were recorded before each collection with a Kestrel 3000 Wind Meter. Ticks were collected in the late morning to early afternoon as this has been suggested as a peak questing time for *A. americanum* ticks (Schulze et al., 2001). Ticks were collected from each site twice a month for 3 months from May to August 2020. The collection method used was a tick drag, which is a white canvas sheet that is dragged behind the researcher to which questing ticks attach. Approximately 100 drags were completed per collection and drags were checked for ticks approximately every 30 m. In the city parks, drags were conducted on and around the hiking trails being sure to stay no more than 6 m off the trails. In OSBS, drags were conducted through unaltered terrain. Ticks were collected in glass vials with open tops, with mesh in place to ensure airflow.

Questing Assays

'Questing' refers to a series of behaviours where ticks climb tall grasses and other vegetation, stop and stretch out their forearms, and wait to attach to a nearby host (Holderman & Kaufman, 2013; McClure & Diuk-Wasser, 2019). Questing assays were conducted outdoors in a standard location in a screened-in porch approximately 30–60 min after collection. Questing assays were conducted in clear plastic plant saucers (12 cm height; 18.5 diameter) with a 55 cm wooden dowel (with 1 cm demarcations) in the middle of the saucer secured with putty. The glass vial top was removed, human breath was used to activate/stimulate the tick (Vassallo, 2002) and the vial was placed near the putty so that upon leaving the vial the tick would access the dowel for questing. Ticks were given 10 min inside the questing arena, during which time we recorded the height and duration of each questing event (the tick halted movement and raised/extended its forelegs off the dowel). Following the assays, ticks were placed in a microcentrifuge tube and stored in a −80 °C freezer.

Repeatability of Questing Behaviour

We tested a subset of the ticks collected in the city parks ($N = 283$) for three consecutive days. The repeated questing assays followed the same protocol as the other questing assays but had two additional testing points at 24 h and 48 h after the initial assay. Repeatability is a colloquial term for consistent individual differences in behaviour, estimated by the amount of behavioural variation that is due to differences between individuals rather than differences within individuals across time (Bell et al., 2009). Larger repeatability values suggest that individuals differ from one another in the behaviour of interest, and that those behavioural differences are consistent over time. Although a larger number of measurements per individual tick can provide more precise estimates of repeatability (Wolak et al., 2012), we were concerned that housing ticks individually in plastic containers would alter their behaviour as time passed, and thus we chose to use three measurements with a sufficiently large sample size (Wolak et al., 2012).

DNA Extraction and Pathogen Screening

We extracted DNA from all 788 ticks using a Qiagen DNeasy Tissue and Blood extraction kit (Qiagen Inc., Valencia, CA, U.S.A.), following the tissue protocol. Prior to the extraction each vial was placed inside liquid nitrogen, then each tick was quickly crushed with a pestle. All ticks were individually screened for both *Rickettsia* spp. and *Ehrlichia* spp. using conventional PCR with a pan-genus primer pair. The molecular techniques were based on Tucker (2017), and the primers were developed by Doyle et al. (2005) and Kidd et al. (2008) (see Table 1, Supplementary material).

Statistical Analyses

Population level analyses

To analyse *R. amblyommatis* infection prevalence, we used a binary logistic regression with infection status (positive/negative) as a response variable and tick life stage (adult/nymph) population identity (ID) as independent variables. To analyse whether or not ticks engaged in questing behaviour during our assays, we used a binary logistic regression with infection questing (yes/no) as a response variable and tick life stage (adult/nymph), infection status (positive/negative) and population ID as independent variables. To analyse questing height and duration, we excluded all cases where the tick did not quest within the 10 min period (440 cases, $N = 323$ ticks remaining in the analysis) and analysed total time spent questing using a general linear model with site ID, infection status (positive/negative), tick life stage (adult/nymph) and an interaction between life stage and infection status as independent variables. Time spent questing was log-transformed to meet model assumptions for normally distributed residuals. For the average height at which ticks quested, model assumptions for parametric tests were not met, so we used nonparametric Wilcoxon signed-rank tests to assess the effects of life stage and infection status on questing height and a nonparametric Kruskal–Wallis test to assess the effect of population ID on questing height. To account for type I error associated with multiple testing for the nonparametric tests, we used a Bonferroni-adjusted α value of 0.017.

Individual level analyses

To test for among-individual differences in questing behaviour, we evaluated three aspects of questing behaviour: likelihood to engage in questing during the assay (yes/no), the total time spent questing and the average height quested. Statistical analyses were conducted in RStudio v.4.0.3 (RStudio Team, 2020). For all binary response variable models, we used generalized linear mixed effects models fitted by restricted maximum likelihood (REML) with a logit link function (nboot = 1000). The linear mixed effects models had the following independent variables: life stage (nymph/adult), infection status (positive/negative) and the day that the assay occurred (day 1, 2 or 3), with tick ID as a random effect. To perform these analyses, we used the R package 'rptR' for repeatability estimation of Gaussian and non-Gaussian data (Stoffel et al., 2017). We followed the methods of Roth et al. (2021) to analyse the repeatability of the total time spent questing due to a continuous, non-Gaussian distribution (R code can be found in Appendix 2 of Roth et al., 2021).

RESULTS

Population Level Analyses

Only two ticks tested positive for *Ehrlichia* infection, both were adult lone star ticks from the same collection site in Gainesville (Split Rock). Thus, we removed them from further analysis. We found no evidence for population level differences in *R. amblyommatis* infection prevalence ($\chi^2_3 = 0.93$, $P = 0.82$; Table 2). However, across all populations, we found a greater prevalence of *R. amblyommatis* in adult ticks (0.386) compared to nymphs (0.280) ($\chi^2_1 = 6.52$, $P = 0.01$; Fig. 1a, Table 2). We found no evidence for among-population differences in tick's likelihood to engage in questing behaviour ($\chi^2_3 = 2.66$, $P = 0.45$; Table 2). However, we found that nymphs were more likely to engage in questing during the 10 min assay compared to adult ticks ($\chi^2_1 = 6.03$, $P = 0.01$; Fig. 1b). Questing likelihood was not influenced by *R. amblyommatis* infection ($P = 0.99$). Although the duration of ticks' questing bouts was not influenced by life stage, infection status or population ID (Table 2), there was a significant interaction between tick life stage and infection status ($F_{1,322} = 4.36$, $P = 0.03$). Uninfected nymphs spent significantly

Table 2
Results of statistical models assessing population level effects of infection status on tick questing behaviour

	Test statistic	*P*
***Rickettsia* prevalence**		
Independent variable		
Population ID	$\chi^2_3 = 0.93$	0.82
Life stage	$\chi^2_1 = 6.52$	**0.01**
Questing likelihood		
Independent variable		
Population ID	$\chi^2_3 = 2.66$	0.45
Life stage	$\chi^2_1 = 6.03$	**0.01**
Infection status	$\chi^2_1 = 0$	0.99
Questing duration		
Independent variable		
Life stage	$F_{1,316} = 1.02$	0.31
Infection status	$F_{1,316} = 1.11$	0.29
Population ID	$F_{3,316} = 0.09$	0.96
Life stage*PCR gel	$F_{1,316} = 4.36$	0.03
Questing height		
Independent variable		
Life stage	$\chi^2_1 = 27.6$	**0.001**
Infection status	$\chi^2_1 = 0.04$	0.83
Population ID	$\chi^2_3 = 30.6$	**0.001**

Significant *P* values are shown in bold.

Table 1
The primers used for pathogen screening of collected ticks

Pathogen	Primer	Direction	Sequence	Reference
Rickettsia spp.	107F	Forward	5'-GCT TTA TTC ACC ACC TCA AC-3'	Kidd et al. (2008)
	299R	Reverse	5'-TRA TCA CCA CCG TAA GTA AAT-3'	
Ehrlichia spp.	DSB-321	Forward	5'-TTG CAA AAT GAT GTC TGA AGA TAT GAA ACA-3'	Doyle et al. (2005)
	DSB-671	Reverse	5'-GCT GCT CCA CCA ATA AAT GTA TCY CCT A-3'	

more time questing compared to infected nymphs (103 s versus 65.3 s; post hoc Student's *t* test: $t = 1.97$, $P < 0.05$, 95% CI = 0.039, 0.45), whereas adult questing time did not differ based on infection status (95% CI = −0.15, 0.31; Fig. 2). Finally, the height at which ticks quested was not influenced by infection status ($\chi^2_1 = 0.04$, $P = 0.83$), but nymphs quested approximately 7 cm higher than adult ticks (41.6 cm versus 33.9 cm, respectively; $\chi^2_1 = 27.6$, $P < 0.001$; Table 2). Finally, ticks collected in the rural population (OSBS) quested between 7 cm and 12 cm higher on average compared to ticks collected at the three city park sites ($\chi^2_3 = 30.6$, $P < 0.001$; Fig. 3).

Individual Level Analyses

We found significant repeatability (i.e. consistent differences among individuals) in tick questing behaviour. Questing height was significantly repeatable, with a repeatability value of 0.238 ($P = 0.00108$, CI = [0.09, 0.401]; Fig. 4), and was predicted by infection status, life stage and the day that the assay occurred. The total time a tick spent questing was not repeatable (CI = [−0.0709, 0.165]; Fig. 4). The likelihood to quest was significantly repeatable ($R = 0.172$, $P = 5.35\text{e-}06$, CI = [0.051, 0.22]; Fig. 4) and was predicted by infection status, life stage and the day that the assay occurred. Significant repeatability in questing likelihood suggests that in the populations we tested, some ticks consistently did not engage in questing when presented with the opportunity, and some ticks consistently chose to quest during the assay. Additionally, the likelihood to quest was more repeatable in infected ticks than in uninfected ticks (infected ticks: $R = 0.215$, $P = 0.000338$,

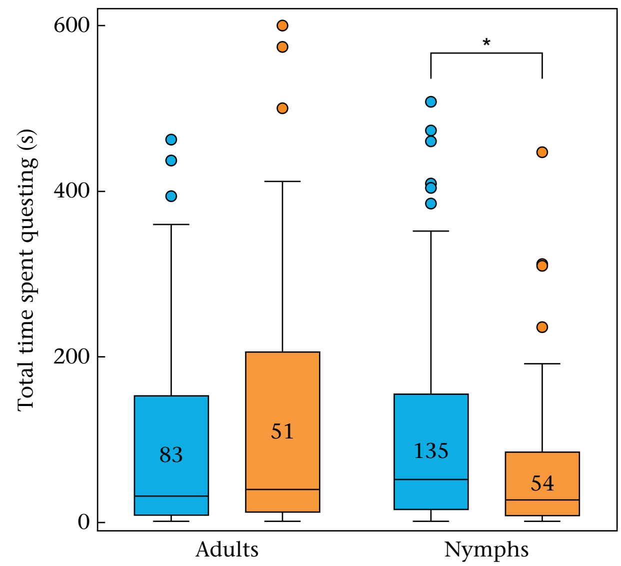

Figure 2. Total time spent questing for adult and nymph ticks with the infection status (blue = negative; orange = positive) of *Rickettsia amblyommatis*. Numbers inside the boxes represent the sample sizes. Boxes represent Tukey box plots, which extend to the 25th and 75th percentiles, the inner line denotes the median. *$P < 0.05$.

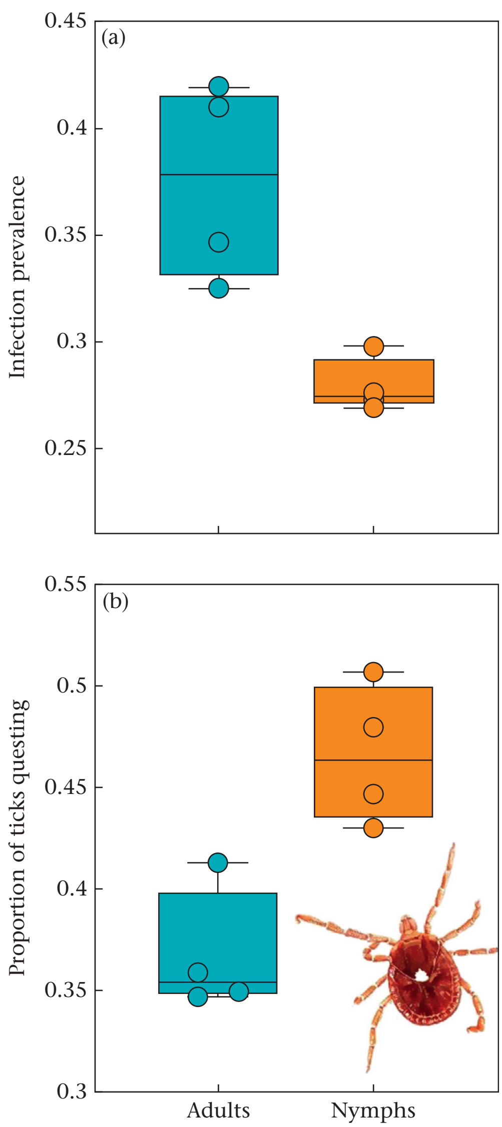

Figure 1. (a) Infection prevalence in adult and nymph ticks and (b) proportion of adult and nymph ticks questing. Each point represents the average of each collection site. Image inlay in (b) represents a lone star tick in typical questing stance with forelegs outstretched. Artwork by Özgür Uğuz.

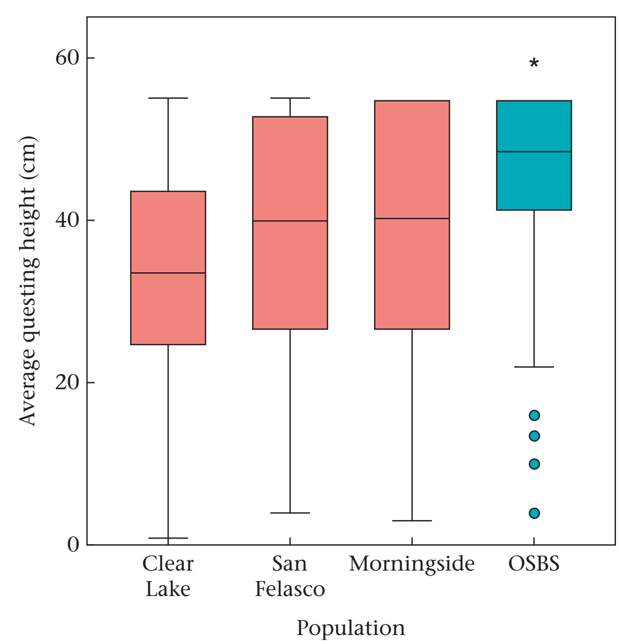

Figure 3. The average height quested across all the collection sites. The collection sites include three city parks (Clear Lake Nature Park, San Felasco Hammock State Park, Morning Side Nature Center) and one rural site (Ordway Swisher Biological Station; OSBS). Boxes represent Tukey box plots, which extend to the 25th and 75th percentiles, the inner line denotes the median. *$P < 0.001$.

CI = [0.058, 0.302]; uninfected ticks: $R = 0.134$, $P = 0.00216$, CI = [0.018, 0.211]; Fig. 4).

DISCUSSION

The host-seeking behaviours of vectors play an important role in the transmission dynamics of vector-borne diseases. Host-seeking behaviours, like questing behaviour in ticks, are driven by intrinsic and extrinsic factors such as environmental variables and pathogen infection status, and simultaneously pose a risk of desiccation (Needham & Teel, 1991; Portugal et al., 2020). Awareness of prevalent tick-borne diseases in locations that the public frequents, such as parks and hiking trails, is necessary for prevention strategies. With this in mind, we conducted a tick collection survey at local parks in Gainesville, Florida as well as behavioural assays to assess relationships between infection status and behaviour. Comparing host-seeking behaviours in ticks on an individual level and at a population level can disclose information that could potentially allow for the creation of management strategies that lower the risk of transmission of tick-borne diseases to the public.

Population Level Differences in Questing Behaviour

Knowledge of the host-seeking behaviours of tick populations as well as the pathogen prevalence at public parks can be crucial to determining the disease risk of an area. At a population level, we did not find any differences between the locations with infection prevalence of *R. amblyommatis*, nor did we find among-populations differences in questing behaviour. However, we did find that adult ticks were more likely to be infected with *R. amblyommatis* than nymphal ticks (Fig. 1a). The higher infection rates in adults compared to nymphs has been seen previously in *A. americanum* and *I. ricinus* with *Rickettsia*, *Ehrlichia* and *Borrelia* infections (Klitgaard et al., 2019; Mejlon & Jaenson, 1993; Steiert & Gilfoy, 2002). This pattern could be occurring because these are three-host ticks, meaning adults could have been exposed to one additional host as compared to the nymphal tick, giving them a higher chance of picking up a pathogen from an infected host (Anderson & Magnarelli, 2008). While it is unclear what effects *R. amblyommatis* could have on survivability of *A. americanum*, past studies have shown that parasitism can cause changes to the infected host's life history traits (Agnew et al., 2000). For example *Ixodes scapularis* infected with *Anaplasma phagocytophilum* develop tolerance to cold temperatures, an elevated fat content and increased resistance to desication (Neelakanta et al., 2010).

We found no evidence for population differences in the propensity to initiate questing behaviour. Past studies have found that *Ixodes* nymphal ticks collected in the southern United States are less likely to emerge from leaf litter to quest than ticks collected from

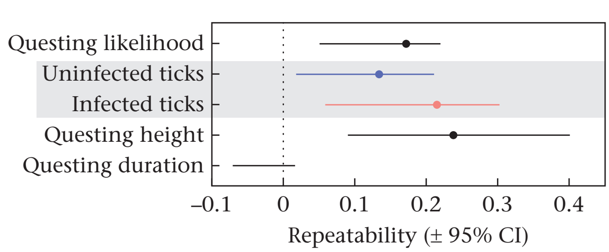

Figure 4. Repeatability estimates for three questing traits measured three times in repeated questing assays. The two values in the grey bar represent the repeatability of questing likelihood estimated for uninfected and infected ticks separately. Points represent repeatability, error bars represent 95% confidence intervals, and intervals that overlap zero represent nonrepeatable behaviours.

the northern United States (Arsnoe et al., 2015). Additionally, a study completed in Florida collected *A. americanum* ticks from different habitat types and found that ticks from xeric hammock habitats spent up to twice as much time questing as ticks from successional hardwood forests (Richardson et al., 2021). For the present study, collection sites at city parks were an average of ~14 km away from each other and all had similar habitat types, so it is possible that our collection sites were too close to each other to see differences in questing behaviours among populations.

We found that nymphs were more likely to engage in questing during our assays than adult ticks. This contrasts with results of previous studies of *I. ricinus*, where at any point in the year, the proportion of adults questing was significantly higher than the proportion of nymphs questing (Perret et al., 2004). These contrasting results could be due to species differences, as the duration and height at which ticks quest can be due to host preference, which can be species specific (Loye & Lane, 1988; Mejlon & Jaenson, 1997; Randolph, 2004; Randolph & Storey, 1999). However, the risk of desiccation has been correlated with smaller size in ticks, making nymphal ticks more at risk of desiccation than adults (Schmidt-Nielsen, 1984; Stafford, 1994; Yoder & Spielman, 1992). This may lead nymphal ticks to engage in more questing bouts that are shorter in duration to avoid desiccating as opposed to adults who are able to quest for longer periods. In addition, adult ticks have been found to live for years without taking a blood meal (Hair & Howell, 1970; Jaworski et al., 1984), suggesting that nymphs may have a shorter window to find a bloodmeal and may be less risk averse while questing.

Lastly, we found that ticks collected at the rural biological station quested at greater heights than ticks collected in city parks. There has been an abundance of literature indicating that environmental factors such as temperature and humidity can have a significant effect on the host-seeking behaviour of ticks (Lees & Milne, 1951; Semtner et al., 1971; Semtner & Hair, 1973; Semtner et al., 1971; Vail & Smith, 1998, 2002). However, our collection sites shared similar environmental characteristics, with habitat types consisting of successional hardwood forests and xeric hammocks dispersed throughout. We are unsure whether differences in temperature and humidity were affecting questing behaviours in our study, as we measured environmental variables at chest height whereas ticks may be experiencing microclimates in the leaf litter that influence questing behaviour (Civitello et l., 2008; Randolph & Storey, 1999). Schulze (2001) found more questing *A. americanum* per sampling drag at an ambient temperature of 22 °C compared to 27 °C, so we cannot rule out environmental effects on questing behaviour (Schulze et al., 2001). Our rural site had an average temperature of 32.57 °C and 61.18% relative humidity (RH) while our city parks had an average of 28.87 °C and 65.09% RH during the collection periods, where on average, the ticks collected in the rural population (OSBS) quested between 7 cm and 12 cm higher than the ticks collected at the three city park sites. This pattern could be due to differences in host availability in rural areas compared to city parks. Large hosts such as deer were prevalent in the rural site but uncommon in the city parks, while small mammals such as rodents and dogs were the majority of possible hosts in city parks. Additionally, it has been seen that tick life stage plays a role in host preference, with adult ticks preferring larger mammals such as deer and nymphs showing preference for smaller mammals (Fujimoto et al., 1986; Loye & Lane, 1988; Randolph & Storey, 1999).

Individual Level Differences in Questing Behaviour

Individual variation in tick host-seeking behaviour could have significant effects on transmission dynamics of tick-borne diseases. To determine whether tick questing behaviours were repeatable, we conducted questing assays for three consecutive days and found that average questing height and the propensity to initiate questing were repeatable behaviours while the total time that a tick spent questing was not repeatable. While there has been one study showing that individual ticks possess behavioural asymmetries while questing (Benelli et al., 2018), to our knowledge, ours is the first study to quantitatively assess the repeatability of tick behaviour. Questing behaviours in ticks are necessary for host attachment, feeding and future egg laying and play a vital role in the transmission of pathogens. Although we are unaware of the underlying mechanisms of the individual variation described here, these ticks were collected in the same habitats and tested under standardized conditions, which suggests that factors independent of environmental drivers like humidity influence tick questing behaviour. Furthermore, this suggests that environmental changes that are predicted to alter tick questing behaviour (Lees & Milne, 1951; Semtner et al., 1971; Semtner & Hair, 1973; Semtner et al., 1971; Vail & Smith, 1998, 2002) may not affect all ticks similarly.

Intrinsic factors such as pathogen infection can affect tick questing behaviour (Busby et al., 2012; Lefcort & Durden, 1996; Romashchenko et al., 2012). For example, Romashchenko et al. (2012) found that *Ixodes persulcatus* ticks infected with *Borrelia burgdorferi* quested at higher heights, and Lefcort and Durden (1996) found this same pattern in *I. scapularis* nymphs. However, ours is the first study to show that pathogen infection can alter the repeatability of questing behaviour. We found that ticks infected with *R. amblyommatis* exhibited increased repeatability in questing likelihood compared to uninfected ticks. A study conducted on the field cricket, *Gryllus integer*, found that pathogen exposure with *Serratia marcescens* increases among-individual behavioural variation and repeatability (DiRienzo et al., 2016). With regard to individual level behavioural variation during infection, it is difficult to know whether this variation is permanent and specific to the individual, or a temporary side-effect of infection (DiRienzo et al., 2016). It is possible that exposure to a pathogen may not have an obvious behavioural effect at the population level, but it may affect individuals (Coats et al., 2010; DiRienzo et al., 2016; Kekäläinen et al., 2014; Seaman & Briffa, 2015).

In addition to the life stage differences in questing behaviour described above, we also found that *R. amblyommatis* infection reduced questing time only in nymphal ticks. Ours is not the first study to find life stage differences in infection-driven changes in behaviour, as another study found that *I. scapularis* nymphs displayed increased questing heights when infected with *B. burgdorferi*, while infected adults avoided climbing to quest (Lefcort & Durden, 1996). However, we are unsure as to the degree to which the differences that we found (~40 s on average) are ecologically relevant, and thus should be investigated further, perhaps under more natural settings in mesocosms.

Conclusions

Tick-borne diseases are an increasingly important threat as climate change expands tick ranges into novel habitats. Population level differences in tick host-seeking behaviours may be driven mainly by environmental factors such as temperature, humidity and host availability. However, differences in infection within a population can show behavioural changes on average, but investigations solely at the population level cannot account for behavioural variation that already exists within the population. The presence of an infection in an individual could potentially alter behavioural types in a variety of ways. The present study is the first to discover repeatable individual variation in tick host-seeking behaviours. Behaviour plays a significant role in vector-borne transmission dynamics, so accounting for the degree of naturally occurring variation in vector populations and the effect of pathogen infection on the expression of host-seeking behaviours may become increasingly important in predicting transmission dynamics. We hope that the data presented here will provide vector-borne disease modellers a baseline to incorporate individual variation in tick behaviour into predictive models (e.g. building off of Van Es et al., 1999). Many of the methods used in the present study could be replicated for future studies with laboratory-reared ticks so that one could assess repeatability in questing behaviours in a controlled environment and with experimental infections. With this, one can control for factors such as infection status, blood feeding and environmental conditions and test individuals before and after infection.

Data Availability

The data associated with the manuscript can be found on Figshare (https://figshare.com/articles/dataset/Data_from_Richardson_et_al_-_Tick_questing_across_scales/16879897).

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Author Contributions

Elise A. Richardson: Conceptualization, data curation, methodology, investigation, formal analysis, Writing –original draft. **David J. Buttrick**: Data curation, investigation, Writing – review & editing. **Samantha A. Shablin**: Formal analysis, Writing – review & editing. **Brittney Jabot**: Data curation, visualization, Writing – review & editing. **Caitlin E. Taylor**: Data curation, investigation Writing – review & editing. **Estelle M. Martin**: Supervision, resources, methodology, Writing – review & editing. **Carl N. Keiser**: Conceptualization, resources, methodology, formal analysis, Writing – review & editing.

Conflicts of Interest

None.

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Supplementary Material

Supplementary material associated with this article is available, in the online version, at https://doi.org/10.1016/j.anbehav.2022.02.004.

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