Community ecology of Neotropical ticks, hosts, and associated pathogens

Helen J. Esser

Propositions

1. Host species richness is a poor measure of tick-borne disease risk.
   (this thesis)

2. Biodiversity conservation should include parasites.
   (this thesis)

3. The lack of universal rules in taxonomy hampers biodiversity conservation.

4. Biological illustration improves biological knowledge and observational skills.

5. The proposal for "controlled contact with isolated tribes" (Walker & Hill 2015, Science 348(6239): 1061-1061) violates the right of indigenous peoples to self-determination.

6. Nationalism is more divisive than unifying.

7. Limiting reproduction is the most impactful way of reducing one's carbon footprint.

Propositions belonging to the thesis, entitled
'Community ecology of Neotropical ticks, hosts, and associated pathogens'.

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Contents

Chapter 1

General introduction

Biodiversity and human health

The global biodiversity crisis is one of the most critical environmental issues of our time (Laurance 2007). With a sixth mass extinction event well under way (Ceballos et al. 2015), an urgent question for society is how biodiversity loss will affect the functioning of ecosystems and the benefits that humans obtain from their natural environment (Chapin et al. 2000, Díaz et al. 2006). Biodiversity provides important ecosystem services for human well-being, either as a regulator of fundamental ecosystem processes, as a final service itself, or as a good (Mace et al. 2012). Loss of biodiversity adversely affects these services in a number of ways, with important implications for human health (Chivian 2002, Cardinale et al. 2012).

Control of infectious diseases is increasingly promoted as a historically underappreciated, yet highly valuable ecosystem service that is likely to be affected by biodiversity loss (Ostfeld and Keesing 2000, LoGiudice et al. 2003, Pongsiri et al. 2009, Civitello et al. 2015). Biodiversity conservation could therefore offer a compelling approach to reduce disease risk (Keesing et al. 2010, Kilpatrick et al. 2017). The hypothesis that biodiversity protects against infectious diseases has recently attracted a substantial amount of attention, but its generality remains controversial (Randolph and Dobson 2012, Salkeld et al. 2013, Wood et al. 2014, Huang et al. 2016).

Disease ecology

Infectious diseases are emerging and re-emerging worldwide, imposing a significant burden on local economies and public health (Morens et al. 2004, Smith et al. 2014). Many of these diseases are vector-borne, i.e. they are transmitted between hosts by an infected organism, most often via the bite of blood-feeding arthropods, such as mosquitoes, sandflies, or ticks (Gubler 1998, Gratz 1999). When transmission occurs between an animal and human host, the disease is called a zoonosis. Wildlife appears to be the main source of emerging zoonoses (Cleaveland et al. 2001, McFarlane et al. 2012). Moreover, risk of exposure to vector-borne zoonoses has particularly increased in biodiversity "hotspot" regions that are progressively affected by habitat loss, illegal hunting, and demographic changes (Jones et al. 2008). The possibility of a causal link between these concurrent patterns has been a major focus of a relatively new discipline: disease ecology.

In its broadest sense, disease ecology focusses on understanding the ecological interactions between hosts, vectors, and pathogens in the context of their environment and evolution (Kilpatrick and Altizer 2010). It is a multidisciplinary field that borrows heavily from epidemiology, parasitology, and community ecology, to name just a few. Using a combination of quantitative methods, including field studies, molecular techniques, and modelling, disease ecologists have started to elucidate pathogen transmission dynamics in relation to community structure and environmental change (Johnson et al. 2015a). For example, "spillover" often occurs when anthropogenic changes of wildlife habitat disrupt complex interactions between hosts, vectors, and associated pathogens (Daszak et al. 2001). However, how frequently biodiversity loss increases disease risk and the underlying mechanisms remain subject of contentious debate (Randolph and Dobson 2012, Lafferty and Wood 2013, Ostfeld and Keesing 2013, Salkeld et al. 2013, Wood and Lafferty 2013, Wood et al. 2014, Huang et al. 2016, Levi et al. 2016, Wood et al. 2016).

Box 1: Glossary

Biodiversity: variety and variability of life on Earth, but used here to more specifically refer to diversity among vertebrate hosts at the species-level
Disease risk: the number of infected vectors in the environment, which is a function of vector infection prevalence and vector abundance
Ecosystem service: benefits provided by ecosystems that contribute to human well-being
High quality host: host on which an arthropod vector successfully feeds or develops
Infection prevalence: the proportion of infected vectors or hosts
Parasite: broadly defined to include bacteria, protozoa, viruses, helminths and arthropods
Reproduction host: host species used by the adult, reproductive stage of a parasite
Reservoir competence: ability of an infected host to maintain and transmit pathogens
Vector-borne disease: disease that is transmitted between hosts by another organism, most often a blood-feeding arthropod
Vector microbiome: the assemblage of commensal, symbiotic, and pathogenic micro-organisms that can be found in the vector body
Zoonosis: disease that can be transmitted between humans and animals

Dilution or amplification?

Paradoxically, biodiversity can either *increase* (amplify) or *decrease* (dilute) disease risk through a variety of mechanisms that are context- and scale-dependent (Keesing et al. 2006, Johnson et al. 2015b, Hofmeester 2016). For vector-borne zoonoses, a "dilution effect" may occur if several conditions are met: (1) the vector has generalized feeding habits, (2) host species vary in reservoir competence for pathogens and/or host quality for vectors, (3), high-quality hosts dominate in low-diversity communities, and (4) host taxa that are added as biodiversity increases reduce either encounter rates between high-quality hosts and vectors or the density of high-quality hosts. If these conditions are reversed, an amplification effect may arise (Keesing et al. 2006, Ostfeld and Keesing 2012). Although evidence exists for both phenomena, two recent meta-analyses concluded that the dilution effect was widespread among a diversity of disease systems (Civitello et al. 2015, Huang et al. 2017).

The suggested underlying mechanism of the dilution effect is that the most competent host species (i.e. those that maintain and transmit pathogens most effectively) have fast life histories: they are abundant, widespread, and invest minimally in certain aspects of adaptive immunity, making them ideal for parasites and pathogens to exploit (Johnson et al. 2012, Huang et al. 2013, Han et al. 2015). Species with fast life histories also tend to be smaller and hence less vulnerable to extinction (Cardillo 2003). Thus, host competence and host resilience to ecosystem perturbation are hypothesized to be closely coupled, such that more impoverished communities are dominated by hosts that support greater pathogen and/or vector abundance (Keesing et al. 2010, Ostfeld and Keesing 2012). However, the link between resilience and competence has only occasionally been tested and remains equivocal (McFarlane et al. 2012, Joseph et al. 2013, Young et al. 2013, Young et al. 2017). An implicit assumption of the dilution effect is that any change in host diversity is independent of host density, and should therefore not affect vector densities (Wood et al. 2014). The reasoning is that in less diverse communities, loss of host species results in density compensation by remaining species, such that total host abundance changes little with biodiversity (Keesing et al. 2010, Levi et al. 2016). However, if host species that dilute pathogen prevalence in more diverse communities (e.g. by diverting vector bites away from more competent host species) also function as important reproduction hosts for adult vectors, these individual host species may amplify vector abundance to such an extent that they ultimately increase disease risk

(Wood and Lafferty 2013, Hofmeester 2016). Most field studies have not controlled for increases in the density of individual host species that can result from changes in host diversity, so that it is empirically difficult to separate a dilution effect from a simple density effect (Begon 2008, Keesing et al. 2010, but see Hofmeester 2016). Identifying the conditions under which biodiversity loss either increases or decreases disease risk thus remains a challenge, particularly in multi-host, multi-pathogen systems (Johnson et al. 2015b).

Current knowledge gaps

A major limitation of current biodiversity-disease studies is that they typically consider only a single pathogen and/or vector (Johnson et al. 2015a, Kilpatrick et al. 2017). In natural ecosystems however, zoonotic pathogens are often sustained and transmitted by multiple hosts and vectors, with each species contributing differently to the pathogen's survival and reproduction (Cleaveland et al. 2001). In turn, these vectors and hosts support a range of other pathogenic, commensal and/or symbiotic microbial species that interact among each other (Clay et al. 2006). Empirical or theoretical studies focusing on single species of hosts, vectors and pathogens are therefore less appropriate, yet studies that consider broader host-vector-pathogen communities are rare (Hofmeester 2016, Young et al. 2017). Likewise, potential effects of biodiversity loss on vector microbiome remain unexplored (Gottdenker et al. 2014).

The ubiquity of parasites – broadly defined to include bacteria, protozoa, viruses, helminths and arthropods, following Anderson and May (1979) – in nature forms the basis of arguably the most important critique of the dilution effect: high host diversity comes with high parasite diversity ("diversity begets diversity", Hechinger and Lafferty 2005). Indeed, the number of human parasitic and infectious diseases increases strongly as one moves towards the equator, where biodiversity is highest (Guernier et al. 2004, Dunn et al. 2010). However, high parasite richness is not necessarily equivalent to high disease risk: they represent different ecological processes (colonization among vs. transmission within communities) that may respond differently to biodiversity loss (Johnson et al. 2015b). Only two studies have considered this, and both found that biodiversity loss can simultaneously decrease parasite richness while increasing the risk and severity of infectious disease outbreaks (Morand et al. 2014, Rottstock et al. 2014). Moreover, while parasite richness increases with biodiversity on a global scale, species interactions and hence disease transmission dynamics occur on a local

scale, e.g. within forest patches (Cohen et al. 2016). It is this local scale that is most relevant when assessing the risk of acquiring parasites across a biodiversity gradient (Kilpatrick et al. 2017).

Although our understanding of local disease transmission dynamics has substantially increased over the last decades, there remains a great deal of uncertainty regarding the direction, magnitude and mechanisms of the biodiversity-disease relationship in biodiversity hotspots (Gottdenker et al. 2014). Studies that examined how tropical forest degradation and associated biodiversity loss influence disease risk have shown mixed patterns (Gottdenker et al. 2014, Tucker Lima et al. 2017). Such contrasting findings may be caused, at least in part, by differences in definitions and/or inadequate measures of disease risk and biodiversity loss. For example, many studies use the proportion of infected vectors (pathogen prevalence) as a measure of vector-borne disease risk, yet transmission potential depends on the number of infected vectors, which is a function of pathogen prevalence and vector density (Randolph and Dobson 2012). Likewise, conventional measures of biodiversity loss, such as host species richness or forest fragment size, are less appropriate than those that take species identity into account (LoGiudice et al. 2008, Randolph and Dobson 2012). In order to move forward, comprehensive assessments of the composition and structure of host, vector, and microbial communities are needed, linked with appropriate measures of disease risk across a gradient of anthropogenic environmental change in biodiversity hotspots (Levi et al. 2016).

Objectives

The ultimate objective of my research is to contribute to a better understanding of how tropical biodiversity loss may impact parasite diversity and disease risk. I specifically focused on communities of wildlife, ticks, and tick-borne pathogens in Panama, part of the world's second largest 'megadiversity hotspot' (Myers et al. 2000). Achieving my objective required a thorough examination of tick diversity and distribution across vertebrate host species, which is a function of tick feeding preferences, host biological and ecological traits, and abiotic conditions (Randolph 2004). For example, host specificity is a key determinant of pathogen transmission and tick population dynamics (McCoy et al. 2013), and the probability of host-tick coextinction (Lafferty 2012). However, the degree to which the ticks in Panama are host-specific and hence sensitive to wildlife diversity loss has never been adequately quantified. Likewise, host body

size is considered to be an important determinant of parasite species richness (Kamiya et al. 2014a) as well as host extinction risk (Purvis et al. 2000). If larger host species harbour more diverse tick communities, wildlife diversity loss should have strong cascade effects on tick species diversity and abundance. Neither of these hypotheses have been empirically tested.

Testing these and other hypotheses has largely been hampered by difficulties in the identification of Neotropical ticks. While taxonomic keys exist for the adult stages, identifying immature ticks based on morphological characteristics is notoriously difficult. As a result, host-use patterns of larvae and nymphs are barely known for most tick species in Panama, while such data are indispensable for understanding the biodiversity-disease risk relationship. For example, the dilution effect hypothesis requires ticks to be generalist vectors that feed proportionally more from small vertebrates (disease reservoir hosts) in disturbed landscapes, thereby facilitating pathogen transmission. It is currently unknown whether these assumptions hold for ticks in tropical forests. Therefore, I formulated the following sub-objectives:

1) Quantifying host-specificity for the ticks of Panama
2) Developing a DNA barcode reference library for the identification of immature ticks
3) Identifying host and environmental determinants of:
    a. densities of questing ticks
    b. tick prevalence and diversity across a wide range of host species
    c. pathogen prevalence and microbial richness in ticks

These sub-objectives are essential for understanding how anthropogenic alterations of wildlife communities may affect the abundance and diversity of ticks, their microbial communities, and ultimately, tick-borne disease risk.

Study system

My study system was located in the Isthmus of Panama (Fig 1.1). By connecting two continents, this isthmus functions as a land bridge, a melting pot of species exchange (Webb 2006). It is also a global centre for marine commerce and a site of migratory bird activity, allowing for potential transfer of vectors and pathogens. Like other tropical regions, Panama's

exceptional biodiversity is threatened by widespread deforestation, illegal hunting and mining activities (Moreno 1993, Condit et al. 2001). The landscape of central Panama is characterized by a mosaic of old growth and secondary forests surrounded by pastures, scrubland, and human settlements (Condit 2001). The short distances between these relatively intact and heavily disturbed forest fragments offer ideal circumstances for studying host-tick-pathogen interactions across a biodiversity gradient.

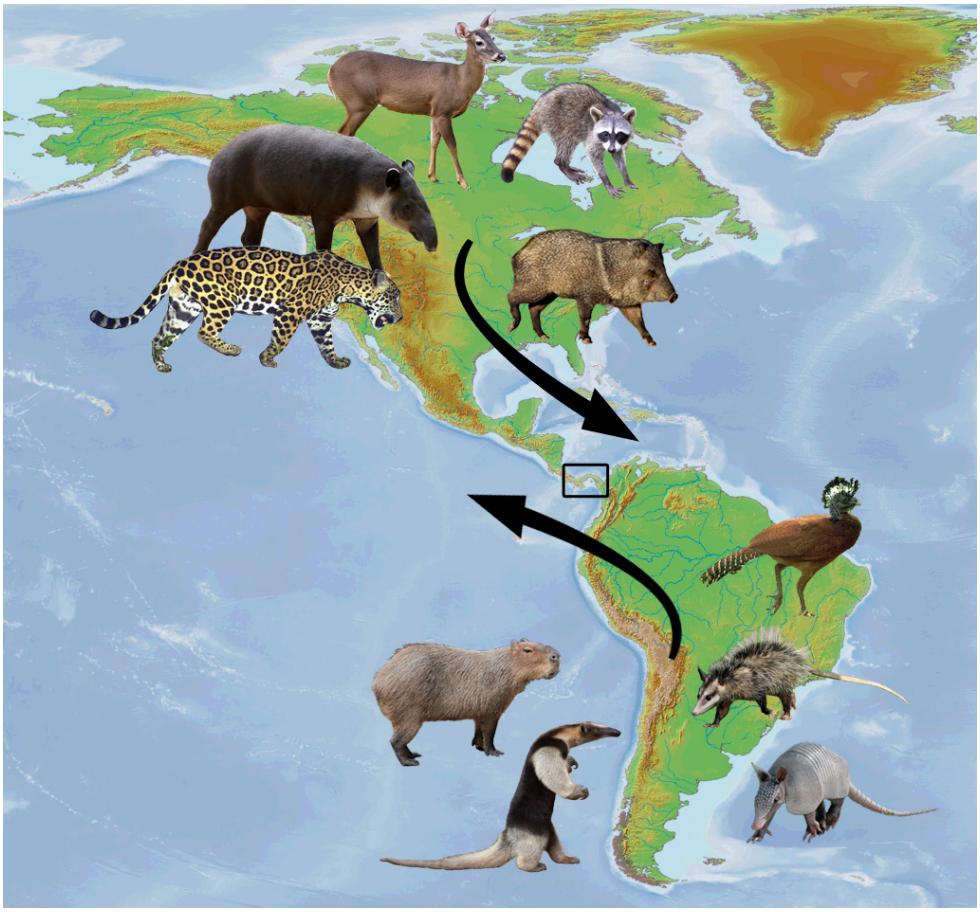

Figure 1.1 When the volcanic Isthmus of Panama (shown in square) rose up from the ocean floor it connected the North and South American continents, allowing for a major species migration event known as the Great American Biotic Interchange, which peaked ca. 3 million years ago. Species shown are all extant in Panama and are important hosts for ticks.

In addition to a rich wildlife, nearly 50 species of ticks from eight genera and two families have been described for Panama (Fairchild et al. 1966, Apanaskevich and Bermúdez 2013, Nava et al. 2014, Bermúdez et al. 2015b). Despite their medical importance, the ticks of Panama have been largely neglected by health-care providers and the scientific community (Bermúdez et al. 2017). The first accounts of tick-borne disease in Panama date back to the early 1900s, when hundreds of cases of relapsing fever (*Borrelia* sp.) were reported in the former Canal Zone (Dunn and Clark 1933). In the 1950s, Rocky Mountain spotted fever (*Rickettsia rickettsii*) appeared, one of the most virulent human infections ever identified (Calero et al. 1952). After half a century without any case reports, the disease re-emerged with 15 new cases between 2004 and 2017. Of these, 11 were fatal (*Sergio Bermúdez, personal communication*). In addition, there are records of *Candidatus* "Rickettsia amblyommii", a potential pathogen (Apperson et al. 2008, Rivas et al. 2015), as well as *Anaplasma* sp., *Babesia* sp. and *Ehrlichia* sp. in ticks, domestic animals, and wildlife (Darling 1913, Clark 1918, Bermúdez et al. 2009, Eremeeva et al. 2009, Santamaria et al. 2014, Bermúdez et al. 2016, Bermúdez et al. 2017). Increased focus on other infectious diseases has likely led to underreporting of tick-borne diseases and it remains unclear what other potential pathogens circulate in this region. Finally, recent accounts of four field biologists that developed serious red-meat allergy after tick bites further highlight the need for a better understanding of host-tick-pathogen interactions in Panama (Wickner 2014).

Thesis outline

Low host specificity of vectors is a prerequisite for the dilution effect to occur. However, given the large diversity of wildlife hosts and tick species in tropical forests, theory suggest that host specificity should be high in the ticks of Panama (MacArthur 1972). In Chapter 2, I tested this hypothesis for adult ticks by using quantitative network analyses in combination with phylogenetic tools and null model comparisons. I considered three important aspects of host specificity: (i) the relative ecological importance of each host species (structural specificity), (ii) the relatedness among host species (phylogenetic specificity), and (iii) spatial-scale dependence of host specificity (geographical specificity).

A lack of morphological identification keys for the immature ticks of Panama impedes studies on host-tick-pathogen interactions and the effect of biodiversity loss thereupon. Therefore, in Chapter 3, I describe the development of a DNA barcode reference library for the

identification of immature ticks. This library was created using adult ticks that I first identified to species based on taxonomic keys, after which their mtDNA COI barcode fragment was sequenced. The effectiveness of the library was then tested on larvae and nymphs collected from birds. In addition, I explore which avian ecological traits are associated with tick parasitism, and discuss the potential role of birds in tick-borne disease transmission.

One assumption of the dilution effect hypothesis is that low diversity communities are dominated by smaller-bodied vertebrates with fast life histories and lower immunocompetence, making them ideal hosts for parasites and pathogens to exploit (Huang et al. 2013). However, no study has actually tested whether smaller-bodied host species carry more diverse tick assemblages. In Chapter 4, I examined the relationship between tick diversity and host body size across a large number of host taxa that ranged in body size by three orders of magnitude. Thanks to the DNA barcode library, I was able to analyse adult and immature tick assemblages separately, as different life stages may show different feeding patterns.

According to the "diversity begets diversity" hypothesis, species richness of host and parasites should be coupled. Thus, if host diversity declines, so should parasite diversity. Parasites with complex host life cycles that sequentially use different host species should be even more sensitive to host extinction. In Chapter 5, I tested this hypothesis by comparing the diversity and abundance of tick and vertebrate host communities across 12 previously connected islands and forest fragments in the Panama Canal that ranged 1000-fold in size. I used camera trapping to survey wildlife and drag sampling to collect ticks.

In Chapter 6, I determined how changes in wildlife community composition affected tick abundance, species richness, and prevalence on small mammals, and how this in turn affected the diversity of prokaryotic communities in ticks, tick-borne pathogen prevalence, and ultimately, tick-borne disease risk. I used a combination of camera trapping, drag sampling, and live trapping of small mammals in 21 forest fragments across a gradient of anthropogenic disturbance. Ticks were identified to species using the DNA barcode library and their microbial community composition was characterized using 16S rRNA sequencing technologies.

Finally, in Chapter 7, I present a synthesis of the main findings and discuss how they contribute to our understanding of host-tick-pathogen interactions. I evaluate how Neotropical biodiversity loss impacts parasite diversity and disease risk, and discuss a different perspective on the role of parasites in biodiversity conservation.

Chapter 2

Host specificity in a diverse Neotropical tick community: an assessment using quantitative network analysis and host phylogeny

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Abstract

Host specificity is a fundamental determinant of tick population and pathogen transmission dynamics, and therefore has important implications for human health. Tick host specificity is expected to be particularly high in the tropics, where communities of ticks, hosts and pathogens are most diverse. Yet the degree to which tropical tick species are host-specific remains poorly understood. Combining new field data with published records, we assessed the specificity of tick-host associations in Panama, a diverse Neotropical region. The resulting dataset includes 5,298 adult ticks belonging to 41 species of eight genera that were directly collected from 68 vertebrate host species of 17 orders. We considered three important aspects of tick host specificity: (i) the relative ecological importance of each host species (structural specificity); (ii) relatedness among host species (phylogenetic specificity); and (iii) spatial scale-dependence of tick-host relationships (geographical specificity). Applying quantitative network analyses and phylogenetic tools with null model comparisons, we assessed the structural and phylogenetic specificity across three spatial scales, ranging from central Panama to countrywide. Further, we tested whether species-rich tick genera parasitized a wider variety of hosts than species-poor genera, as expected when ticks specialize on different host species. Most tick species showed high structural and/or phylogenetic specificity in the adult stage. However, after correcting for sampling effort, we found little support for geographical specificity. Across the three scales, adult ticks tended to be specific to a limited number of host species that were phylogenetically closely related. These host species in turn, were parasitized by tick species from distinct genera, suggesting switching among distantly related hosts is common at evolutionary timescales. Further, there was a strong positive relationship between the taxonomic richness of the tick genera and that of their hosts, consistent with distinct tick species being relatively specific to different host species. Our results indicate that in the adult stage, most ticks in the diverse Neotropical community studied are host specialists. This contrasts with earlier assessments, but agrees with findings from other host-parasite systems. High host specificity in adult ticks implies high susceptibility to local tick-host coextinction, limited ability to colonize new habitats and limited potential for interspecific pathogen transmission.

Introduction

Host specificity is a fundamental life history trait of parasites that is likely to play a major role in generating and maintaining parasite biodiversity (Poulin 2011, Dietrich et al. 2014). The degree to which parasites are host-specific is a key determinant of their ability to colonize new host species (Holt et al. 2003), their geographical range size and local abundance (Krasnov et al. 2004b, Krasnov et al. 2005), the probability of parasite-host coextinction (Koh et al. 2004, Lafferty 2012) and the potential routes by which pathogens can be transmitted across vertebrate host taxa, including humans (McCoy et al. 2013). Hence, quantifying host specificity will help elucidate the ecological and co-evolutionary relationships between parasite and host species that are relevant for human and veterinary medicine, as well as for biodiversity conservation (Daszak 2000, Krasnov et al. 2008a).

A group of organisms in which the question of host specificity is particularly important, are ticks (Acari: Ixodida). Ticks are obligatory hematophagous ectoparasites that feed on every class of terrestrial vertebrates throughout the world (Sonenshine 1991). They are major vectors of diseases to both humans and livestock, imposing a significant burden on public health and livestock producers (Jongejan and Uilenberg 2004). Ticks are especially abundant in the tropical regions, both in species and in numbers (Guglielmone et al. 2014). The tropics are also hotspots for vertebrate diversity (Myers et al. 2000) and hence are rich in potential host species for ticks. Resource specialization has been suggested as an important factor driving the remarkable species richness in these systems (MacArthur 1972, May 1973, Chesson 2000). Indeed, empirical studies of other host-parasite systems have shown that parasites tend to be more specific in richer host faunas (Poulin 1997, Krasnov et al. 2008b). Further, several features of ticks are predicted to limit their host ranges and select for host specificity (see Magalhães et al. 2007, McCoy et al. 2013, Dietrich et al. 2014 for a review) and host specificity is therefore expected to be high for tropical tick species.

Relatively few empirical studies have tested this hypothesis, none of which found conclusive evidence that high host specificity in tropical tick species is common. Cumming (1998) analysed a large dataset on African tick-host associations (Ixodidae and Argasidae) and concluded that these ticks showed a continuum in their degree of host specificity, ranging from specialists at the host species-, family-, or order-level to broad host generalists of a wide variety of vertebrate orders. A more recent study on ixodid ticks of mammals in South Africa found,

depending on the specificity index used, that ticks showed either very low or a wide diversity of specificity in all life stages (Espinaze et al. 2015). Using the same index, Nava & Guglielmone (2013) performed a meta-analysis on Neotropical ixodid ticks and argued that while some tick species are specific at the host genus- or family-level, strict host specificity is uncommon. These previous studies, however, did not correct for host availability or for the likelihood of observing the recorded tick host-use patterns. After accounting for these biases, Wells et al. (2013) found little evidence for host specificity in ixodid ticks of small mammals in Borneo. But because host associations of adults, nymphs and larvae were not analysed independently, stage-specific host specificity could have been missed in that study. Indeed, Espinaze et al. (2015) and Nava & Guglielmone (2013) found that host specificity differed among life stages, with immature ticks typically being more generalist than their adult conspecifics. Hence, the degree to which different life stages of tropical ticks are host-specific remains poorly understood and further studies are warranted.

The complexity of the tick-host interface requires consideration of at least three different aspects when measuring host specificity. First, structural differences in the distribution of tick populations across vertebrate hosts reflect the relative ecological importance of each exploited host species (Poulin et al. 2011). For example, two tick species that exploit the same number of host species may differ greatly in the extent to which they use each of these hosts (McCoy et al. 2013, Nava and Guglielmone 2013). Secondly, phylogenetic relatedness among host species is another important determinant of evolutionary specialization that is not always considered (Jorge et al. 2014). Preferred, more frequently parasitized host species may be more closely related to one another than sporadically parasitized host species (Poulin et al. 2011). Finally, specificity can also be measured as the consistency in host-use across a changing host landscape (Poulin et al. 2011). A growing number of studies suggest that host specificity in ticks may be spatially scale-dependent; with ticks tending to be host specialists at local scales and host generalists at larger geographical scales (reviewed by McCoy et al. 2013). These different aspects of host specificity are known as *structural specificity*, *phylogenetic specificity* and *geographical specificity*, respectively (Poulin et al. 2011), and they may vary markedly among the different life stages of a tick species (Nava and Guglielmone 2013, Guglielmone et al. 2014). To our knowledge, no study has so far considered all these aspects of specificity in tropical tick-host communities.

Here, we investigate the degree to which adult ticks are host specific in Panama, a diverse Neotropical region supporting over 40 species of ticks. Focusing on adult ticks, we assessed (i) the structural specificity of ticks at both the species- and community-level using quantitative network analyses that control for host availability; (ii) the phylogenetic specificity of ticks by estimating the standardized effect size of the mean pairwise phylogenetic distance of exploited host species; and (iii) the geographical specificity by comparing structural and phylogenetic specificity across three nested spatial scales that ranged from local (central Panama) to countrywide. We applied rarefaction to account for variation in the number of potential host species across the three spatial scales, and used null model comparisons to evaluate the likelihood of observing the recorded tick-host associations. We also tested whether species-rich tick genera parasitized a wider variety of hosts than species-poor genera, as would be expected if tick species have specialized on different host taxa. Lastly, we discuss the associations between ticks and domestic animals as these potentially include new relationships formed over relatively short evolutionary time periods.

Methods

Study area

Data were collected throughout Panama, part of the world's second largest 'megadiversity hotspot' for endemic vertebrates (Myers et al. 2000). Over forty species of ticks have been reported from Panama, divided over eight genera and two families (Fairchild et al. 1966, Apanaskevich and Bermúdez 2013, Nava et al. 2014, Bermúdez et al. 2015b). Panama also has a wide variety of environmental conditions and habitats, ranging from mangrove swamps to tropical forests and from savannahs to the páramo. Elevation ranges from *c*.0–3,500 m. Panama has a tropical moist weather pattern with an average diurnal temperature of 27 °C. Average temperature and humidity are high throughout most of the country, but considerably milder at elevations > 600 m. Rainfall varies both regionally (*c*.1,750–4,000 mm) and temporally, with a pronounced dry season in the lowlands from January to April (Ridgely and Gwynne 1989).

Data collection

We collected data on host feeding relationships of ticks (Ixodidae and Argasidae) from January 2009 until May 2014. Sampled hosts included wild animals, either live-captured or found as

road kills, as well as humans and domestic animals from different environments throughout Panama. We searched the entire body of hosts but only ticks found firmly attached were considered in further analyses. Ticks were preserved in 95 % ethanol and later identified using the taxonomic keys provided by Fairchild et al. (1966) and Onofrio et al. (2006). We used the taxonomic criteria of Nava et al. (2014) for the *Amblyomma cajennense* species complex, which is represented by *A. mixtum* in Panama. Additional data on ticks and their vertebrate hosts were obtained from published regional monographs (see Appendix 2: Table A2.1).

Most tick species of the family Ixodidae are characterized by a three-host life-cycle, in which the larvae, nymphs and adults feed from different host individuals that may belong to distinct species (Guglielmone et al. 2014). Hence, pooling data on host associations of different tick life stages could confound potential patterns of stage-specific host specificity and such data should therefore be analysed separately. Unfortunately, the larvae and nymphs of the three-host ticks in Panama (35 out of 37 species of Ixodidae) are notoriously difficult to identify, making earlier records unreliable. Moreover, the immature life stages of several tick species in our dataset remain undescribed (Guglielmone et al. 2014). We therefore limited our study to adult ticks and included species of both Ixodidae and Argasidae; the species of the latter family are also generally characterized by possessing multi-host life-cycles (Hoogstraal and Aeschlimann 1982).

The overall dataset included adult tick-host associations from a wide variety of habitats and altitudes collected in over 54 locations throughout the country (Fig 2.1). The true coverage is much larger but the description of many collection localities retrieved from the literature did not allow for a specific allocation on the map, even though they could be used for the analysis of geographical specificity (see below). We followed the consensus list of valid tick names as compiled by Guglielmone et al. (2010), which recognizes three genera of Argasidae (i.e. *Antricola*, *Argas* and *Ornithodoros*) and five genera of Ixodidae (i.e. *Amblyomma*, *Dermacentor*, *Haemaphysalis*, *Ixodes* and *Rhipicephalus*) for Panama.

Structural specificity

Indices of host specificity that consider both the number of host species and the relative frequency with which they are exploited, such as those based on the widely used Shannon index in ecology, are excellent for measuring structural host specificity (Poulin et al. 2011). Here, we used two such metrics: H_2' and d_i' (Blüthgen et al. 2006). These metrics were developed for the

analysis of bipartite networks, a standardized framework for the quantification of ecological specialization (Blüthgen et al. 2008, Poulin 2010). Bipartite networks represent associations (links) between species (nodes) of two trophic levels and are either based on weighted (quantitative) or unweighted (binary) links. The two metrics used here are based on weighted links, i.e. they were calculated using the relative frequencies with which tick-host associations occur (see Appendix 2: Table A2.2 for formulas). By accounting for variation in the "strength" of the interactions, they provide an ecologically more meaningful measure of host specificity than do metrics based on unweighted links, i.e. presence/absence data (Bersier et al. 2002, Blüthgen et al. 2006). Both indices were calculated using the network-level (Dormann et al. 2009) and species-level analyses (Dormann 2011) tools in the R package 'bipartite' (Dormann et al. 2008).

The H_2' index, the standardized two-dimensional Shannon entropy, is a measure of structural specificity of the entire network, henceforward community-level (Blüthgen et al. 2006). Values range from 0 for the most generalist community to 1 for the most specialist community. The index increases with deviations of the network's observed frequency distribution of species interactions from their expected probability distribution. This null distribution of interactions reflects a situation where all species interact with their partners in proportion to their observed frequency totals (Blüthgen et al. 2006).

The d_i' index, the standardized Kullback-Leibler distance, is a measure of structural specificity for each individual node, henceforward species-level (Blüthgen et al. 2006). Like H_2', values range from 0 for the most generalist to 1 for the most specialist species. For species *i*, the value of d_i' increases with deviations of the observed frequency distributions from a null distribution that assumes that the interactions with species *i* are proportional to overall partner availability. Thus, d_i' increases with reciprocal specificity between two partners and hence reflects the "exclusiveness" of species interactions (Blüthgen et al. 2006).

These two indices take into account what many other host specificity indices do not: resource availability. If not accounted for, estimates of host specificity of ticks that occur in only a few samples will be biased, with rare species being systematically classified as more specific (Cumming 2004, Devictor et al. 2010). The H_2' and d_i' indices do not suffer from this classical artefact since the use of rare resources (i.e. host species) is not given the same weight as the use of common ones. Thus, these indices are able to discriminate species with strong host preferences from those using available host species simply in proportion to their occurrence in the environment.

However, Dormann et al. (2009 and references therein) showed that most metrics, including those based on weighted links, are affected by network dimensions (i.e. number of species) and sampling intensity (i.e. total observation records per species). Observed metric estimates should therefore be evaluated against expectations based on null models that control for these network properties (Blüthgen et al. 2008, Dormann et al. 2009). Here, we used two such null models, each with 1,000 replicates, to test whether the observed estimates deviated significantly from what would be expected by chance.

Null model I was based on an algorithm developed by Patefield (1981), which randomly redistributes the interactions across all species in the matrix while maintaining column and row totals identical to those of the observed matrix. This algorithm is analogous to most re-sampling-based contingency table tests such as χ^2 or Fisher's exact test (Dormann et al. 2009) and is implemented in the R package 'bipartite' as function 'r2dtable' (Dormann et al. 2008). By constraining the marginal sums, this null model corrects for uneven numbers of species observation records (Blüthgen et al. 2008).

Null model II was based on an algorithm developed by Vázquez et al. (Vázquez et al. 2007), which redistributes the interactions only across those species that were actually observed to interact, thereby maintaining connectance. This algorithm is implemented in the R package 'bipartite' as 'vaznull' (Dormann et al. 2008). By constraining the realized links of the original network, it takes into account that unrealized connections between certain tick and host species may in fact represent life-history restrictions, i.e. 'forbidden links'. These forbidden links may arise from a lack of host availability, such as non-overlap of tick and host habitat in space or time, but may also result from host avoidance. Hence, null model II can be regarded as very constrained in comparison with null model I.

Phylogenetic specificity

Closely related species tend to share similar biological, behavioural and physiological traits (Jorge et al. 2014). Hence, the more phylogenetically related a given set of host species, the more likely they should be to share the same parasite species. In comparative analysis, this is similar to the problem of non-independence of species (Felsenstein 1985). We used a widely employed method to assess relatedness among host species in each tick species' diet: the Mean Phylogenetic Distance (MPD) between each pair of parasitized host species (Webb et al. 2002, Vellend et al. 2011). We used a taxonomic classification with 19 hierarchical levels above

species (see Appendix 2: Fig A2.1). Branch lengths were set to unity and we weighted the MPD by the number of tick-host associations. This method is fairly independent from species richness and therefore from sampling effort (Vellend et al. 2011, Jorge et al. 2014).

However, the extent to which parasitized host species represent a non-random selection from the total host community cannot generally be assessed using raw MPD values (Vellend et al. 2011). We therefore calculated standardized effect sizes of the MPD values (SES_{MPD}) to evaluate whether observed host relationships deviated from what would be expected based on the relatedness of the available host species. The SES_{MPD} is basically a *Z*-score, which describes the difference between the observed MPD and the MPD expected under a null model, divided by the standard deviation of the MPD in the null data. This approach is equivalent to -1 times the Net Relatedness or Nearest Relative Index (NRI) that is widely used in community ecology and has a similar interpretation (Kembel et al. 2010, Jorge et al. 2014). The null model that we used here randomizes the names of the host species on the terminal branches of the phylogeny, so that the distribution of the branches remains intact. This null model is implemented in the R package 'picante' as "taxa.labels" (Kembel et al. 2010). Positive SES values indicate greater phylogenetic distance among parasitized host species than expected by chance, whereas negative SES values indicate small phylogenetic distances, i.e. high phylogenetic specificity.

Geographical specificity

To assess structural and phylogenetic host specificity in geographical space, we subsetted the total dataset twice, yielding separate datasets on tick-host associations for three scales: (i) the entire country of Panama (*c*.74,340 km^2), including a wide variety of natural and anthropogenic habitats ranging from lowlands to highlands up to 3,000 m; (ii) the lowlands of Panama (*c*.59,710 km^2), including a variety of natural and anthropogenic habitats up to 600 m; and (iii) central Panama (*c*.2,178 km^2), including an area of 20 km on either side of the Panama Canal, most of which lies below 300 m with a uniform temperature and humidity (Harmon 2005). Henceforward, these three spatial scales will be referred to as "large", "intermediate" and "small", respectively (see Appendix 2: Table A2.1 for more details).

While we used null models to compare the patterns within the species data matrix, we need to consider for our comparison across the three spatial scales that the local dataset is nested in the regional one, and the regional is nested in the nation-wide data. Hence, our tick-

host community matrices are additive, so that resource potential increases with scale. If not corrected for this sampling bias, a decline in host specificity with increasing spatial scale (*sensu* McCoy et al. 2013) may simply arise due to a larger number of available host species (Devictor et al. 2010). For a meaningful comparison of structural and phylogenetic specificity across the three scales, we therefore rarefied the largest two matrices so that their total number of interactions was identical to the smallest matrix. Using the 'sample' command in R with 1,000 randomizations, we resampled the entries of the matrix with a probability for sampling each link given by the proportion of its link strength (see Appendix 2: Table A2.2). All analyses were carried out with the R statistical software, version 3.2.4 (R Core Team 2017).

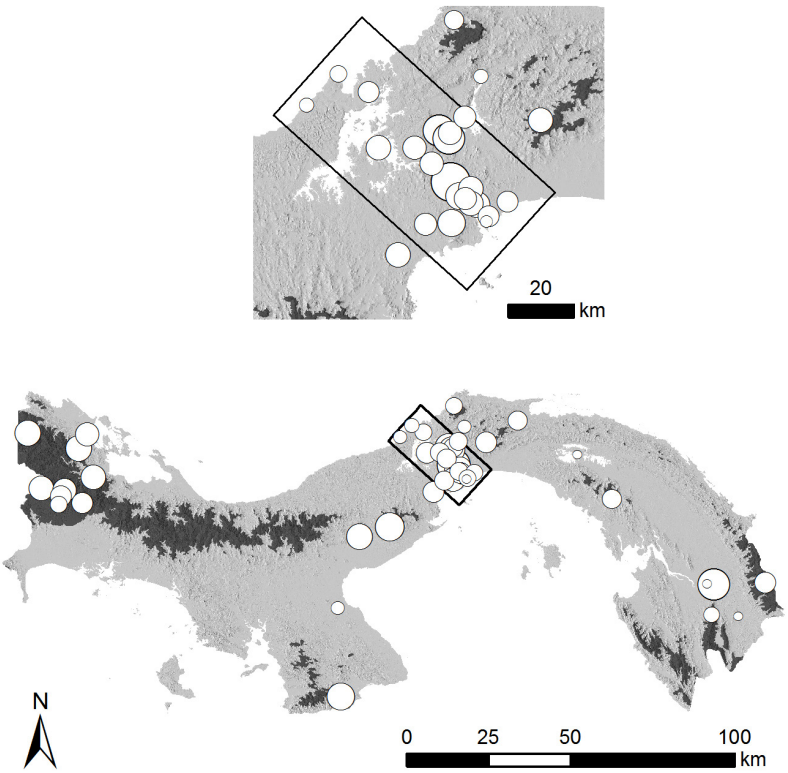

Figure 2.1 Map of Panama showing the sampling locations across the three spatial scales: large (entire country), intermediate (light grey areas), and small (black box inset). These sampling locations show the minimum coverage as the description of many collection localities retrieved from literature did not allow for a specific placement on the map.

Richness relationships

If tick species specialize on different host taxa, then more species-rich tick genera should parasitize a wider variety of hosts than species-poor genera. However, the observed number of host species is likely to be an underestimate since species richness is strongly affected by sampling effort. We corrected for biases arising from the undersampling of rare host species by computing the Chao1 index, an abundance-based estimator for asymptotic species richness (Chao 1984), using EstimateS version 9.1.0 (Colwell 2013). We used Spearman's rho (ρ) to test the prediction that a positive relationship exists between generic tick species richness and generic Chao1 estimates of total host species richness.

Because d_i' and MPD are more sophisticated measures of host specificity than the basic number of host species, we also tested for the relationship between these two indices and generic tick species richness. If most tick species show high structural specificity towards different species of hosts, then generic d_i' estimates should be higher for species-poor tick genera than for species-rich tick genera. Hence, we expected d_i' to decline with generic tick species richness. In contrast, if most tick species show high phylogenetic specificity towards different species of hosts, then MPD estimates should be lower for species-poor tick genera than for species-rich tick genera. Hence, we expected MPD estimates to increase with generic tick species richness.

Results

Structural specificity

Structural specificity of the entire network was high for each spatial scale (large: H_2' = 0.74, intermediate: H_2' = 0.75, small: H_2' = 0.77). Significance was assessed by determining the proportion of randomized estimates (n = 1,000) that was equal to or greater than the observed H_2' estimate. For each spatial scale, the observed H_2' estimate was significantly larger than predicted by each of the two null models (P = 0), indicating high structural specificity of tick-host communities (Fig 2.2a).

Structural specificity values at the species-level (d_i') ranged from 0.22–1.00 (median 0.76) at the large scale, from 0.26–1.00 (median 0.77) at the intermediate scale, and from 0.33–1.00 (median 0.73) at the small scale (Fig 2.2b). Significance was assessed for each tick species by determining the proportion of randomized estimates (n = 1,000) that was equal to

or greater than the observed d_i' estimate. With a single exception, all observed d_i' estimates were significantly higher than predicted by null model I for each spatial scale (Table 2.1). Compared to the more constrained null model II however, observed d_i' estimates were significantly higher for 30 out of 41 tick species at the large scale, 21 out of 28 tick species at the intermediate scale, and 15 out of 25 tick species at the small scale.

While comparisons with null model I provide an upper bound estimate of the number of specialist tick species, comparisons with null model II provide a lower bound estimate. This is because null model I assumes that all host species in the dataset are available to each tick species, whereas null model II assumes that any unrealized connection between a tick and host species represents a forbidden link. Since some forbidden links may actually reflect host avoidance rather than a lack of host availability, part of the tick species that appear to be host generalists under null model II are in fact host specialists that do not discriminate among the, sometimes quite limited, number of host species they do parasitize. This may be true for several tick species that were almost exclusively collected from a single host species (e.g. *Amblyomma coelebs* and *Dermacentor latus* on Baird's tapir, *Amblyomma naponense* and *Dermacentor imitans* on collared peccary, *Dermacentor nitens* on horse), or that were abundant on only a small number of host species (e.g. *Amblyomma calcaratum* on anteaters, *Amblyomma. varium* on sloths). Overall, these results suggest high structural specificity at the host species-, family-, or order-level during the adult life stage of most tick species in Panama.

Phylogenetic specificity

For 29 out of 41 tick species, over 90% of the collection records came from a single vertebrate order, and 12 tick species were each associated with a single vertebrate species. This suggests that many tick species in Panama are associated with phylogenetically closely related host species during the adult life stage. Indeed, the SES_{MPD} estimates showed that at the large scale, 33 out of 41 species were phylogenetically more host-specific than expected by chance. At the intermediate scale this was true for 23 out of 28 tick species, and at the small scale, 18 out of 25 species of ticks showed significant phylogenetic specificity (Table 2.1). Phylogenetic specificity was found at the level of host species (e.g. *Haemaphysalis leporispalustris* on forest rabbit), host family (e.g. *Amblyomma nodosum* on anteaters), and host order (e.g. *Ixodes rubidus* on carnivores), although some tick species parasitized several host orders (e.g. *Amblyomma dissimile* on amphibians and reptiles, *Haemaphysalis juxtakochi* on odd- and even-

toed ungulates). Interestingly, while most ticks tended to feed from phylogenetically closely-related host species, these hosts themselves were parasitized by tick species from distinct genera (Fig 2.3).

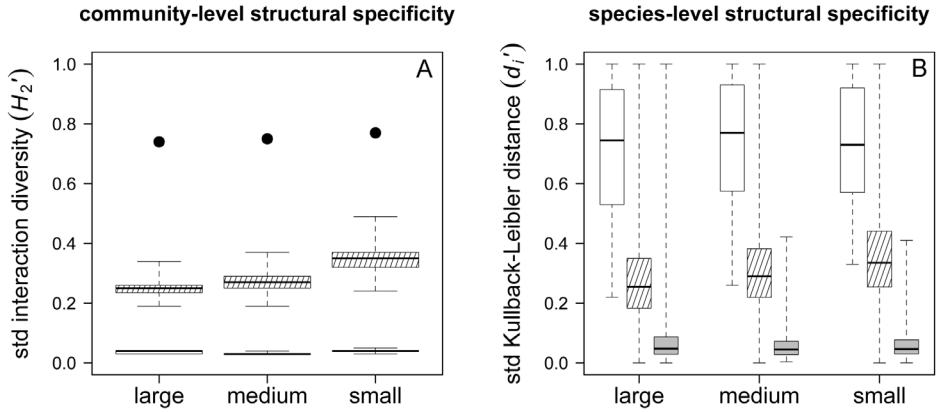

Figure 2.2 Observed *vs* null model estimates of structural host specificity. Observed estimates for **(a)** community-level specialization H_2' (*black dots*) and **(b)** species-level specialization d_i' (*white box plots*) are much larger than estimates predicted by null model I (*grey box plots*) and null model II (*dashed box plots*) for each spatial scale (large, intermediate, small). Plot whiskers extend from minimum to maximum estimates.

Geographical specificity

Overall, we did not find strong evidence for scaling of host specificity with geographical space. While structural specificity at the community-level (H_2') declined marginally with increasing scale, it remained high for each spatial scale and these values were not affected by rarefaction. Similarly, structural specificity at the species-level (d_i') was high for each spatial scale, with negligible effects of rarefaction and no clear trend across the three spatial scales. Four tick species that showed structural specificity at larger scales, did not do so at smaller spatial scales when compared to null model II values. One tick species (*Ornithodoros puertoricensis*) showed the opposite trend (Table 2.1). As a result, the proportion of structural specialists was slightly lower at the smallest scale. Finally, no major changes were observed for phylogenetic specificity across the three spatial scales. With only three exceptions, tick species whose MPD

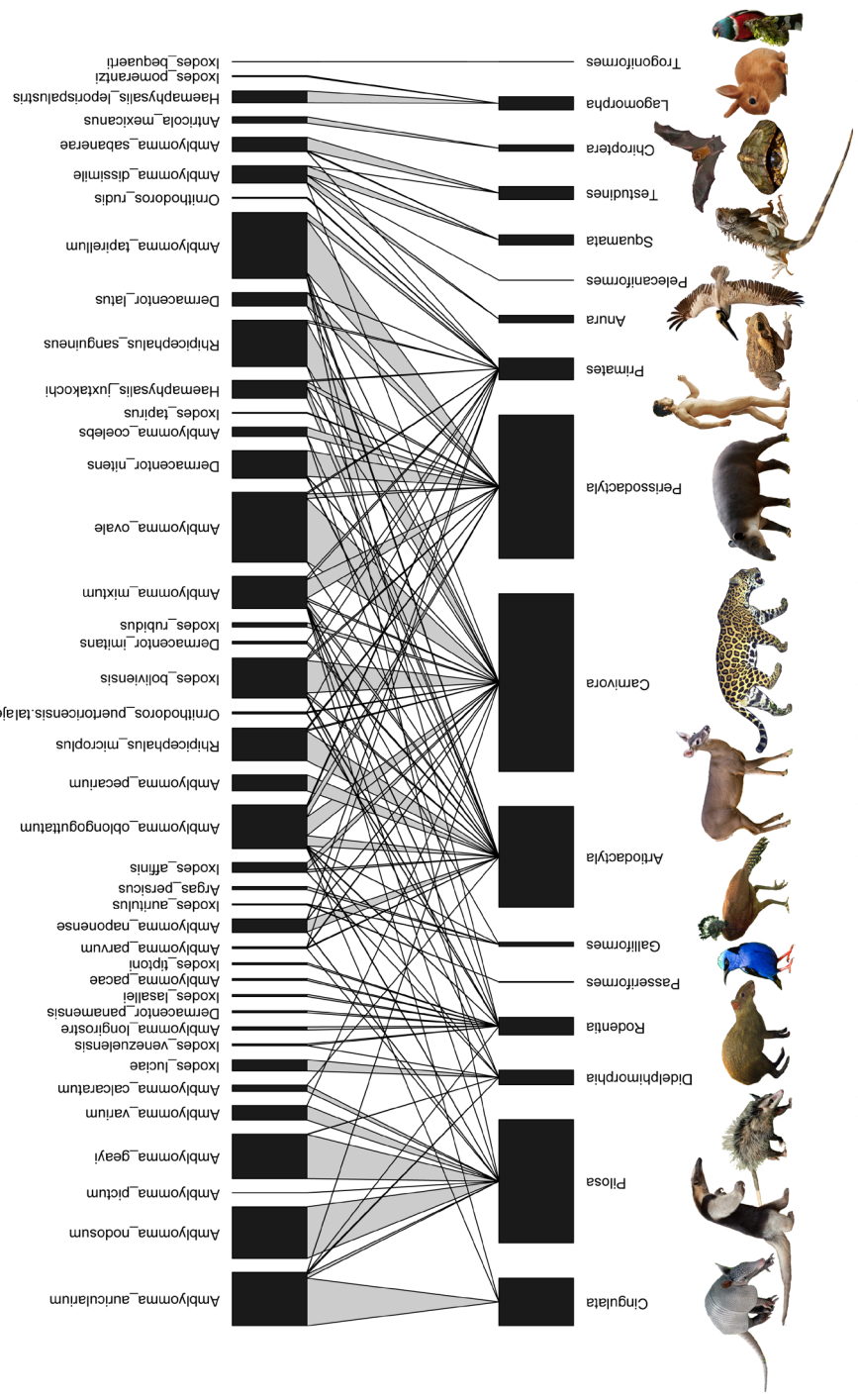

Figure 2.3 Quantitative tick-host interaction network. Relationships between the tick species of Panama and their vertebrate hosts as visualized by a bipartite network. Host species are pooled to the taxonomic level of vertebrate order for clarity. Nodes (*black*) represent species and links (*grey*) correspond to species interactions. Variation in interaction frequencies are reflected by the width of the links. The network is arranged such that it shows minimal crossings of interactions, which allows for easier interpretation.

values were significant at larger scales, were also significant at smaller spatial scales when recorded. The proportion of phylogenetic specialists was slightly lower at the smallest scale.

Richness relationships

There was a strong, positive correlation between the number of species within each tick genus and the estimated total number of host species (Chao1) parasitized by that genus (Spearman's $\rho = 0.93$, $P = 0.001$). Likewise, we found a significant positive correlation between generic MPD (phylogenetic specificity at the tick genus-level) and generic tick species richness (Spearman's $\rho = 0.83$, $P = 0.011$). There was a significant negative correlation between generic d_i' (structural specificity at the tick genus-level) and generic tick species richness (Spearman's $\rho = 0.95$, $P < 0.0001$). These results suggest that different tick species within the same genus are specific to different host species, both structurally and phylogenetically.

Relationships with domestic animals

A total of 14 different tick species were recorded from 7 species of domestic animals. The tick species most often associated with poultry, horse, cattle and dog are globally recognized as economically important pests of these host species, i.e. *Argas persicus* (poultry tick), *Dermacentor nitens* (tropical horse tick), *Rhipicephalus microplus* (southern cattle tick), and *R. sanguineus* (brown dog tick), respectively (Fig 2.4). Other ticks that were commonly found on domestic animals include *Amblyomma mixtum* (part of the *A. cajennense* species complex), which was predominantly collected from horses, *Amblyomma ovale* and *Ixodes boliviensis*, which were most abundant on dogs, and *Amblyomma oblongoguttatum*, a more generalist tick that was found on all domestic animals except poultry. The remaining tick species (*Amblyomma auricularium*, *Amblyomma coelebs*, *Amblyomma parvum*, *Amblyomma tapirellum*, *Dermacentor latus*, *Ixodes affinis*) were infrequently collected from domestic animals and their records may represent incidental infestations. In addition, a total of 15 different tick species parasitized humans, of which *Amblyomma tapirellum* was most often involved (Fig 2.4).

Table 2.1 Observed values for structural (d_i') and phylogenetic (SES_{MPD}) specificity at the species-level. Values are shown for each spatial scale. Significance was assessed by the Patefield null model (NM I), the Vaznull null model (NM II), and randomization of taxa labels (NM III) and is given as ***P < 0.001; **P < 0.01; *P < 0.05, or ns (not significant)

	Large					Intermediate					Small				
	d_i'	NM I	NM II	SES_{MP}	NM III	d_i'	NM I	NM II	SES_{MPD}	NM III	d_i'	NM I	NM II	SES_{MPD}	NM III
Amblyomma **spp.**															
A. auricularium	0.88	***	***	-2.02	*	0.89	***	***	-1.91	*	0.97	***	**	-1.83	ns
A. calcaratum	0.45	***	ns	-2.32	*	0.41	***	ns	-2.00	*	0.66	***	ns	-2.02	*
A. coelebs	0.42	***	ns	-2.63	**	0.41	***	ns	0	***	0.49	***	ns	0	***
A. dissimile	0.95	***	**	-2.40	**	0.94	***	***	-2.40	**	0.94	***	***	-2.59	**
A. geayi	0.87	***	***	-2.60	***	0.87	***	***	-2.58	***	0.85	***	***	-2.48	***
A. longirostre	1.00	***	*	0	***	1.00	***	*	0	***	1.00	***	*	0	***
A. mixtum	0.40	***	ns	-2.67	**	0.42	***	ns	-2.48	*	0.40	***	ns	-2.75	**
A. naponense	0.61	***	*	-0.72	ns	0.61	***	*	-0.64	ns	0.57	***	ns	-0.72	ns
A. nodosum	0.92	***	***	-2.58	**	0.92	***	***	-2.48	**	0.87	***	*	0	***
A. oblongoguttatum	0.22	***	ns	-3.24	**	0.26	***	ns	-2.89	**	0.33	***	ns	-2.59	**
A. ovale	0.58	***	***	-2.04	*	0.65	***	***	-2.08	**	0.67	***	**	-1.84	*
A. pacae	0.82	***	*	-2.36	*	1.00	***	*	0	***	1.00	***	*	0	***
A. parvum	0.37	***	ns	-1.58	ns	0.36	***	ns	-1.49	ns	0.37	***	ns	-0.35	ns
A. pecarium	0.76	***	**	0	***	0.76	***	*	0	***	0.81	***	*	0	***
A. pictum	0.54	*	ns	0	***	–	–	–	–	–	–	–	–	–	–
A. sabanerae	0.94	***	**	-2.65	**	0.94	***	**	-2.64	**	0.93	***	**	-2.68	**
A. tapirellum	0.62	***	***	-2.21	*	0.67	***	***	-2.18	**	0.62	***	*	-1.40	ns
A. varium	0.64	***	*	-2.46	*	0.61	***	*	-2.45	**	0.56	***	ns	-2.34	*
Antricola **spp.**															
A. mexicanus	1.00	***	*	0	***	1.00	***	*	0	***	–	–	–	–	–
Argas **spp.**															
A. persicus	0.99	***	**	0	***	1.00	***	*	0	***	–	–	–	–	–

Table 2.1 Continued

Dermacentor **spp.**															
D. imitans	0.47	***	ns	-0.73	ns	–	–	–	–	–	–	–	–	–	–
D. latus	0.47	***	ns	-1.17	ns	–	–	–	–	–	–	–	–	–	–
D. nitens	0.73	***	**	-1.69	*	0.58	***	ns	-1.61	*	0.60	***	ns	-1.71	*
D. panamensis	0.91	***	*	0	***	–	–	–	–	–	–	–	–	–	–
Haemaphysalis **spp.**															
H. juxtakochi	0.57	***	*	-1.97	*	0.57	***	*	-2.08	**	0.59	***	ns	-2.12	*
H. leporispalustris	0.98	***	**	0	***	1.00	***	*	0	***	–	–	–	–	–
Ixodes **spp.**															
I. affinis	0.79	***	*	-1.90	*	0.78	***	*	-1.32	ns	0.80	***	*	-1.12	ns
I. auritulus	1.00	***	*	-1.39	ns	–	–	–	–	–	–	–	–	–	–
I. bequearti	1.00	***	**		***	–	–	–	–	–	–	–	–	–	–
I. boliviensis	0.46	***	*	-1.52	ns	–	–	–	–	–	–	–	–	–	–
I. lasallei	0.83	***	*	-2.36	**	–	–	–	–	–	–	–	–	–	–
I. luciae	0.94	***	**	-3.04	***	0.89	***	*	-2.80	***	0.96	***	*	-2.84	***
I. pomerantzi	0.58	***	ns	0	***	–	–	–	–	–	–	–	–	–	–
I. rubidus	0.83	***	*	-3.74	***	–	–	–	–	–	–	–	–	–	–
I. tapirus	0.22	ns	ns	0	***	–	–	–	–	–	–	–	–	–	–
I. tiptoni	1.00	***	*	0	***	–	–	–	–	–	–	–	–	–	–
I. venezuelensis	1.00	***	*	-2.42	**	–	–	–	–	–	–	–	–	–	–
Ornithodoros **spp.**															
O. puertoricensis	0.70	***	ns	0.65	ns	0.79	***	*	0.69	ns	0.92	***	*	0.65	ns
O. rudis	0.52	***	ns	0	***	0.52	***	ns	0	***	0.51	***	ns	0	***
Rhipicephalus **spp.**															
R. microplus	0.79	***	**	-2.69	**	0.84	***	***	-2.60	**	0.85	***	***	-2.69	**
R. sanguineus	0.65	***	***	-1.05	ns	0.72	***	***	-0.78	ns	0.73	***	**	-0.45	ns

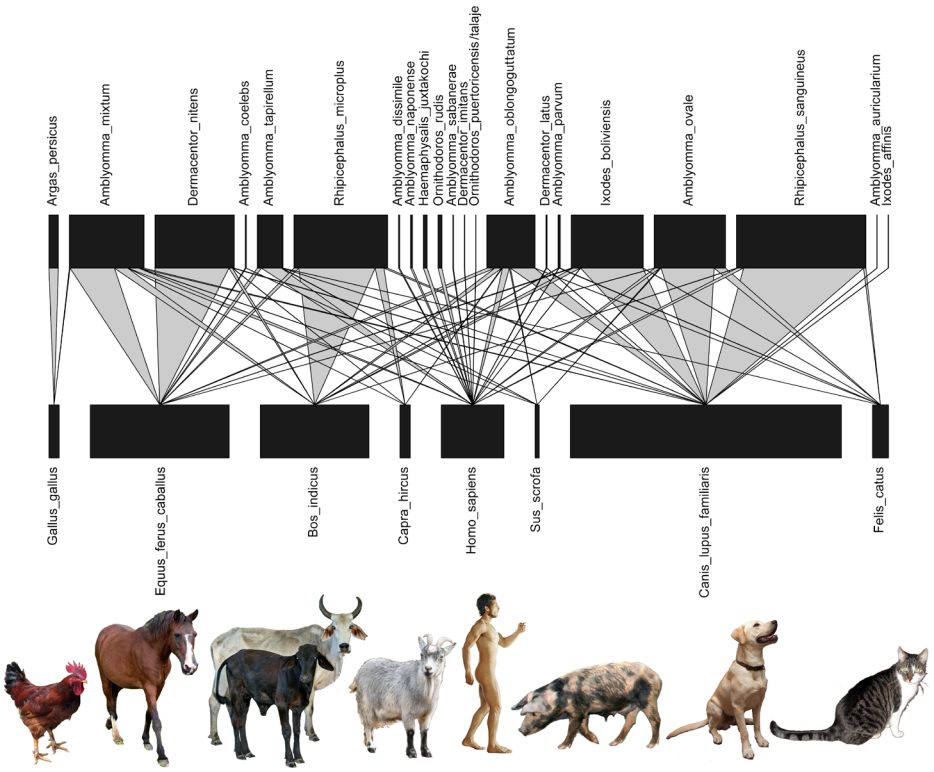

Figure 2.4 Host associations of ticks with domestic animals and humans, visualized by a bipartite network. Nodes (*black*) represent species and links (*grey*) correspond to species interactions. Variation in interaction frequencies are reflected by the width of the links. The network is arranged such that it shows minimal crossings of interactions, which allows for easier interpretation.

Discussion

Our results indicate that the majority of tick species in our study system showed significant structural and/or phylogenetic specificity during the adult life stage, regardless of the spatial scale considered. Thus, adult ticks used some host species disproportionally more than others, and host species tended to be phylogenetically closely related. This specificity was found at the host species-, family- and order-level, with only few tick species having substantial adult tick records from multiple host orders. Moreover, more diverse tick genera parasitized more diverse host species, suggesting that distinct tick species have specialized on different host species.

While most tick species were specialists of phylogenetically closely related host species, these host species in turn were parasitized by ticks from different genera, resulting in asymmetric tick-host phylogenetic interactions.

Our findings are consistent with empirical studies of other host-parasite systems, including helminths, chewing lice and fleas parasitic on small mammals. These studies indicated that most parasites are highly host-specific to a limited number of host species (Poulin et al. 2006), and that host specificity tends to be phylogenetically constrained (Krasnov et al. 2004a, Krasnov et al. 2008b) and geographically scale-invariant (Krasnov et al. 2008a). A recent analysis of flea-mammal networks showed that closely related host species shared the same flea species, but that these fleas belonged to different lineages (Krasnov et al. 2012b). This pattern is similar to that observed in our study and can be explained by processes such as host-switching, ecological fitting and/or co-evolutionary alternation (Thompson 2005, Brooks and Hoberg 2007, Krasnov et al. 2012b). Further, McCoy et al. (2013) reported a positive correlation between the number of African tick species within a given genus and their recorded number of hosts, as we did here for Neotropical ticks. The high specificity of parasite-host associations is likely a product of the continual coevolution of host defences and parasite counter-defences that should select for reciprocal specialization (Thompson 2005).

Several empirical studies have suggested that most tick species tend to be host generalists (Klompen et al. 1996, Cumming 1999, Nava and Guglielmone 2013, Wells et al. 2013, Espinaze et al. 2015, but see Hoogstraal and Aeschlimann 1982). However, almost all of these studies also recognize that ticks show a continuous spectrum in specificity, ranging from the host species- to beyond the host order-level. In those cases, where tick species are not at either end of this spectrum, their classification as either host specialist or generalist can be somewhat subjective (Cumming 1998). For example, Hoogstraal & Aeschlimann (1982) considered tick species that feed exclusively from the class Reptilia (tortoises, snakes and lizards) to be strictly host-specific. In contrast, Nava & Guglielmone (2013) classified ticks that feed on different host families and orders as generalists. This highlights the need for null models to evaluate whether obtained estimates of host specificity are significantly different from those predicted by the tick species' expected probability distribution across its host species.

Although we did not find any spatial scaling of host specificity, such pattern may still exist across larger geographical scales. Most tick species in our study system have geographical distributions that extend beyond Panama (Guglielmone et al. 2014), and our

results can therefore not be generalized across the entire range of these species. For example, tick species that were either exclusively (i.e. *A. longirostre*, *A. pecarium* and *H. leporispalustris*) or primarily (i.e. *A. coelebs* and *D. nitens*) associated with one particular host species in Panama, were shown to feed from a variety of host species and families across their entire range (Nava and Guglielmone 2013). Likewise, tick species that exclusively (i.e. *A. nodosum*) or primarily (i.e. *A. auricularium*, *A. calcaratum* and *A. pacae*) parasitized one particular host family in Panama, were specific at the host order-level across their entire range (Nava and Guglielmone 2013). Thus, whereas we found high phylogenetic specificity within a relatively small portion of their range, Nava & Guglielmone (2013) found that these tick species exhibited much lower phylogenetic specificity across their Neotropical distribution, throughout which the spectrum of potential host species is much larger. The hypothesis that ticks may be "local specialists but global generalists" (McCoy et al. 2013), may therefore still hold for these species. However, recent discoveries of cryptic species among tick populations from different geographical areas (e.g. *A. cajennense* (Nava et al. 2014), *A. parvum* (Lado et al. 2016), *R. microplus* (Burger et al. 2014) and *R. sanguineus* (Moraes-Filho et al. 2011)), as well as experimental evidence for host-associated genetic races (Poulin and Keeney 2008, Dietrich et al. 2014, Araya-Anchetta et al. 2015), stresses the need for considering tick population genetic structure in future studies, particularly when large geographical areas are considered.

Very few tick species in our study system can be considered host generalists in the broadest sense, i.e. by using host species in proportion to their availability (lack of structural specificity) while at the same time feeding from distantly related host species (lack of phylogenetic specificity). For example, *A. mixtum* (part of the *A. cajennense* species complex) parasitized 16 species of wild and domestic hosts in natural and anthropogenic environments, yet nearly half of our records involve horses. Hence, host specificity in this species was structurally low, but phylogenetically high. Other empirical studies have also revealed that apparent generalist tick species may show local host preferences (Poulin and Keeney 2008, McCoy et al. 2013, Araya-Anchetta et al. 2015), which illustrates the complementarity and importance of considering both structural and phylogenetic aspects of host specificity (Poulin et al. 2011).

Domestic animals were principally parasitized by tick species that are globally recognized as important economic pests and which were able to spread to Panama following the introduction of their domestic hosts (Jongejan and Uilenberg 2004). Only few native tick

species were frequently collected from domestic animals. Perhaps not surprisingly, these ticks are known to feed from a wide variety of natural host species (Guglielmone et al. 2014), although they too tended to show a structural and phylogenetic bias. Specifically, *A. ovale* and *I. boliviensis* infested dogs in high numbers but were principally associated with wild carnivores. Likewise, *A. mixtum* and *A. oblongoguttatum* parasitized no less than nine different host orders, the largest number for all tick species in our dataset, but the former was most often found on odd-toed ungulates (particularly horses) while the latter chiefly fed from carnivores (particularly canids) and, to a lesser extent, ungulates (including horses and cattle). Overall, our results suggest that, probably with the exception of *A. mixtum*, domestic animals are not important host species for most of the native tick species in Panama.

Highly specific tick-host relationships as observed in our study have implications for tick-borne disease transmission. On the one hand, high host specificity limits the potential routes for interspecific pathogen transmission, thereby decreasing the risk for emerging infectious diseases. On the other hand, our findings that closely related hosts are parasitized by distantly related ticks, suggest that host switching events frequently occurred throughout the life history of these ticks. Previous empirical studies have shown that ticks can switch hosts under changing environmental conditions, such as climate change, host availability, or even acaricide use (Bermúdez et al. 2010, McCoy et al. 2013, Dietrich et al. 2014). In fact, host switching has been suggested to be ubiquitous for many parasites at both evolutionary and ecological time scales (Brooks and Hoberg 2007). Current ecological perturbations and human activities should only facilitate the potential for host switching, which in turn may increase the risk for tick-borne pathogen transmission between hosts, including livestock, pets and humans (Brooks and Hoberg 2007).

Despite the robustness of the specificity indices we used, our analyses and inferences do have limitations that are inherent to all studies based on field observations and published datasets. First, more intensive sampling would likely provide new tick-host associations, potentially lowering host specificity estimates for some tick species. However, as we corrected for differences in sampling effort, we do not expect the overall conclusions to be profoundly affected. Moreover, using four additional network indices to measure structural specificity, the results remained the same: host specificity is high for the adult ticks in Panama (see Appendix 2: Supplementary analyses, Fig A2.2, Table A2.2). Secondly, studies based on field collections are usually unable to differentiate between failed and successful feeding events. Experiments

are needed that assess differential tick performance on various host species to support field-based evidence for host specificity (Dietrich et al. 2014, Van Oosten et al. 2016). Thirdly, with the continual discovery of species complexes there is a need for genetic data to determine whether perceived "generalists" may in fact consist of multiple cryptic "specialist" species (McCoy et al. 2013, Araya-Anchetta et al. 2015).

Another important aspect to consider is the potential differences in feeding relationships between larvae, nymphs and adult ticks. While larvae and nymphs only feed from vertebrates for their development, the adults of many tick species also search for a mating partner on a host, which may drive specificity in adults but generality in immature stages (Espinaze et al. 2015). Unfortunately, the host associations and in some cases morphological descriptions of immature ticks are poorly documented for Panama, so that we had to limit our study to adult ticks. Empirical studies from elsewhere in the Neotropics suggest that the immature forms of three-host ixodid ticks may feed from entirely different host groups (Guglielmone et al. 2014) and that they tend to be less host-specific (Nava and Guglielmone 2013) than their adult counterparts. It thus seems reasonable to expect that the host-use patterns of immature ticks in Panama differ from those of the adult stage. An important question is whether these larvae and nymphs are true host generalists, or rather specific to different groups of host species compared to the adult stage. This knowledge is imperative for predicting environmental impacts, such as cascade effects of biodiversity loss on tick populations and/or disease transmission. More complex life-cycles in combination with high host specificity increase the risk of local parasite-host coextirpation (Lafferty 2012). Thus, if different life stages are specific to different host species, loss of host diversity should cause stronger bottleneck events compared to a situation where only the adult life stage is host-specific. Future studies that focus on ontogenetic shifts in tick-host relationships are therefore warranted.

It is important to stress that our results do not rule out the possibility that some tick species are more constrained by adaptations to environmental conditions than by host adaptation. Many tick species spend the majority of their life-cycle off-host, so that both abiotic (climatic) and biotic (host) factors determine tick distribution, abundance and host relationships (Randolph 2004). Cumming (1999) already showed that the range limits of most African tick species are not limited by their host species' distribution, suggesting that environmental factors may be more important. In Panama, environmental specificity of ticks

plays an important role in the life history of the Argasidae. These so-called "endophilic" tick species are confined to caves, burrows, roosts and other habitats where host species gather in large numbers and/or regularly return to (Klompen et al. 1996). Indeed, the *Ornithodoros* ticks in our study were among the least host-specific species. Some of the ixodid ticks in Panama also show clear environmental preferences. For example, certain species of *Dermacentor* and *Ixodes* seem to be restricted to wetter, montane environments (Fairchild et al. 1966, Bermúdez et al. 2015b). On the other hand, these particular habitats are characterized by extraordinary vertebrate diversity, yet the adult ticks of these species still predominantly feed from a limited number of closely related host species. This suggests that both environmental adaptations and host adaptations may act in concert to shape the specific tick-host relations observed in Panama.

Future experimental studies may reveal the relative importance of environmental conditions *versus* host suitability for explaining the highly specific tick-host relationships that we found in our study. Specifically, to what extent do the realized host relationships that were observed match potential host relationships if abiotic factors were irrelevant? Experimental studies have so far demonstrated that many tick species are able to complete their life-cycles on laboratory animals that are phylogenetically distant from their natural host species (Troughton and Levin 2007, Olegário et al. 2011, Ramirez et al. 2016). This suggests that the potential host specificity of ticks may be lower than their realized specificity. However, a substantial body of evidence also suggests that tick physiological processes, such as molting, engorgement, hatching, oviposition, and even survival, are negatively affected when ticks are fed on unnatural host species (Randolph 1979, Fielden et al. 1992, Labruna et al. 2000, Labruna et al. 2002, McCoy et al. 2002, Labruna et al. 2009, Olegário et al. 2011, Dietrich et al. 2014, Van Oosten et al. 2016). These studies also showed that tick fitness was higher on laboratory animals that were phylogenetically more closely related to the tick species' natural host species. Clearly, there is a need for better integration of both field-based and experimental studies to increase our understanding of tick-host specificity (Poulin and Keeney 2008, Van Oosten et al. 2016).

Conclusions

Our findings indicate that most tick species in Panama are scale-invariant host specialists during the adult life stage. This implies high vulnerability to local tick-host coextirpation (Lafferty 2012) so that any reduction of host diversity will lead to impoverished tick communities that are dominated by generalist tick species (Devictor et al. 2010). These persistent generalist species may be instrumental in tick-borne disease dynamics as they bear the highest potential for widespread pathogen transmission across host species in local communities (Randolph 1998, Brooks and Hoberg 2007). Host extinction may, therefore, more likely increase rather than limit the risk of tick-borne disease outbreaks (Brooks and Hoberg 2007). Future studies should investigate how alterations of tick-host network properties due to anthropogenic disturbances affect disease dynamics, particularly in tropical regions where wildlife diversity is rapidly eroding (Myers et al. 2000).

Appendix 2

Table A2.1 Summary of data on ticks, their vertebrate hosts and the area for each spatial scale. Abbreviations: TS, number of tick species; HS, number of host species; THA, number of tick-host associations; SL, number of species links (non-zero entries in the matrix)

	TS	HS	THA	SL	Area (km^2)
Spatial scale					
Large	41	68	5,298	207	74,340
Medium	28	57	4,037	153	59,710
Small	25	44	2,666	111	2,178
Data source					
Unpublished	20	21	1,159	59	–
Published*	40	66	4,139	191	–

* (Dunn 1923, Dunn 1934, Fairchild 1943, Fairchild et al. 1966, Bermúdez et al. 2009, Bermúdez et al. 2010, Bermúdez et al. 2011, Bermúdez et al. 2012a, Bermúdez et al. 2012b, Apanaskevich and Bermúdez 2013, García et al. 2014, Bermúdez et al. 2015a, Bermúdez et al. 2015b)

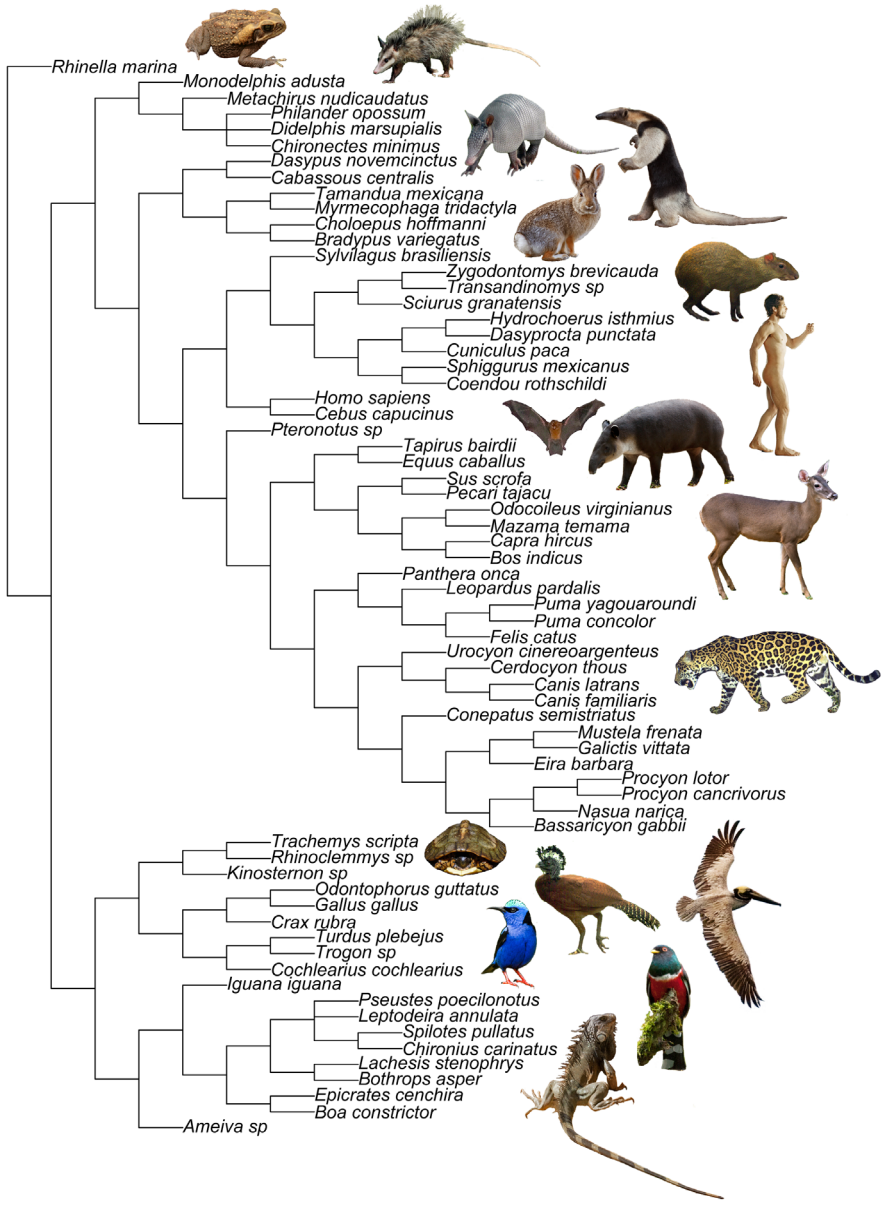

Figure A2.1 Phylogenetic tree of vertebrate host species in our dataset. We used Wilson and Reeder (2005) as taxonomic reference for Mammalia, supplemented by Voss and Jansa (2009) for Didelphidae, Huchon and Douzery (2001) for hystricognath rodents, Weksler (2006) for muroid rodents, Johnson et al. (2006) for Felidae, Bardeleben et al. (2005) for Canidae, Koepfli et al. (2007, 2008) for Procyonidae and Mustelidae, Fry et al. (2006), for Squamata, Lee (2013) for Testudines and Jarvis et al. (2014) for Aves.

Table A2.2 Summary of the network metrics considered in this study

Level	Metric	Definition	Formula	Reference
Species				
d_i'	Standardized Kullback-Leibler distance	Degree of interaction specialization of species *i*	$d_i = \sum_{j=1}^{J}\left(p_{ij}' \cdot \ln\frac{p_{ij}'}{q_j}\right)$, which is normalized to $d_i' = \frac{d_i - d_{min}}{d_{max} - d_{min}}$	(Blüthgen et al. 2006)
Network				
H_2'	Standardized Interaction Diversity	Degree of interaction specialization among ticks and hosts of the entire network	$H_2 = -\sum_{i=1}^{I}\sum_{j=1}^{J}(p_{ij} \cdot \ln p_{ij})$, which is normalized to $H_2' = \frac{H_{2max} - H_2}{H_{2max} - H_{2min}}$	(Blüthgen et al. 2006)
E_2	Interaction Evenness	Evenness of interactions in the network	$E_2 = H_2 / \ln IJ$	(Dormann et al. 2009)
G_{qw}	Generality	Weighted mean effective no. of host species per tick species	$G_{qw} = \sum_{j=1}^{J}\frac{A_j}{N}e^{H_j}$	(Bersier et al. 2002)
V_{qw}	Vulnerability	Weighted mean effective no. of tick species per host species	$V_{qw} = \sum_{i=1}^{I}\frac{A_i}{N}e^{H_i}$	(Bersier et al. 2002)
Q	Modularity	Degree of network partitioning into modules of highly connected species	$Q = \frac{1}{2N}\sum_{ij}(A_{ij} - K_{ij})\delta(m_i, m_j)$	(Dormann and Strauss 2014)

Note: metrics were calculated over contingency tables with *I* rows of host species and *J* columns of tick species; the number of observed interactions between host species *i* and tick species *j* was defined as a_{ij}; *N* is the total number of observed interactions for the entire web; *L* is the number of all realized links; A_i and A_j are respectively the total number of interactions in which *i* and *j* were involved (i.e. the respective row and column totals); p_{ij} is defined as a_{ij} in relation to *N*; p_{ij}' is the proportion of a_{ij} in relation to A_i; q_i and q_j was defined as respectively A_i and A_j in relation to N; A_{ij} and K_{ij} are respectively the normalized interaction matrix and null model matrix, where the sum of all link strengths equals 1; m_i and m_j are the modules to which species *i* and *j* are respectively assigned; the indicator function $\delta(m_i, m_j)$ equals 1 when $m_i = m_j$ and is otherwise equal to zero

Supplementary analyses

In addition to the network-level (H_2') and species-level (d_i') specificity indices, we used four other quantitative metrics (Table A2.2) to test whether the structural specificity of tick-host communities in Panama is significantly higher than predicted by null models I and II:

(1) Generality (G_{qw}); defined as the reciprocal of the Shannon diversity of links for the highest trophic level, this index reflects the effective mean number of host species per tick species, weighted by their marginal totals. The higher G_{qw} for tick species *i*, the less specific it is, i.e. the more host species it parasitizes. Originally developed by Bersier et al. (2002), who used base 2 logarithms in their equations, we based equations on ln.

(2) Vulnerability (V_{qw}); Like G_{qw} but now for the lowest trophic level, this index reflects the effective mean number of tick species per host species, weighted by their marginal totals (Bersier et al. 2002). The higher V_{qw} for host species *j*, the less specific it is, i.e. the more tick species it supports and thus the larger its importance for the overall tick community.

(3) Interaction Evenness (E_2); based on the Shannon diversity of all possible links, this index expresses how homogeneously the tick and host species are connected (Blüthgen et al. 2008). Networks whose distribution of observed interaction frequencies is highly heterogeneous will have E_2 values close to 0. In contrast, E_2 values close to 1 imply well-connected networks in which tick species interact with the available host species with similar frequencies. Rather than using the sum of only the realized links (Tylianakis et al. 2007), we used the product of the matrix dimensions (i.e. all potential links) to determine E_2 (Dormann et al. 2009). For more details, see the manual of the package 'bipartite', version February 19, 2015 (Dormann et al. 2008).

(4) Modularity (*Q*); this index quantifies the presence of cohesive groups called "modules", in which species are linked better within than across modules. To estimate the level of modularity and the number of modules within the network, we used the QuaBiMo-algorithm, which allows for the use of weighted (quantitative) links and computes modularity *Q* using a Markov Chain Monte Carlo approach (Dormann and Strauss 2014). A total of 10^8 MCMC steps were used with a tolerance level of 10^{-10}. *Q* ranges from 0 for random networks to 1 for perfectly modular networks. High modularity suggests specificity of ticks for certain groups of hosts that in turn do not support many other species of ticks.

We found that generality, vulnerability and interaction evenness were significantly lower, and that modularity was significantly higher, than predicted by both null models (*P* =

0.000, Fig A2.2). Rarefaction had negligible effects on each of the metrics' values and there was no clear trend among rarefied values of Q, E_2, G_{qw} and V_{qw} across the spatial scales (Table A2.3). The high Q estimates suggest high structural specificity towards particular groups of hosts and that these "modules" of interacting tick and host species had few connections to other modules. The low E_2 estimates reveal high heterogeneity in interaction frequencies and low connectivity between species of ticks and vertebrate hosts. The low G_{qw} and V_{qw} estimates indicate that ticks parasitized very few host species and hosts were parasitized by very few tick species compared to null-model expectations. These results are in agreement with the estimates of H_2' and d_i', corroborating that the host associations of adult ticks in Panama tend to be highly specific.

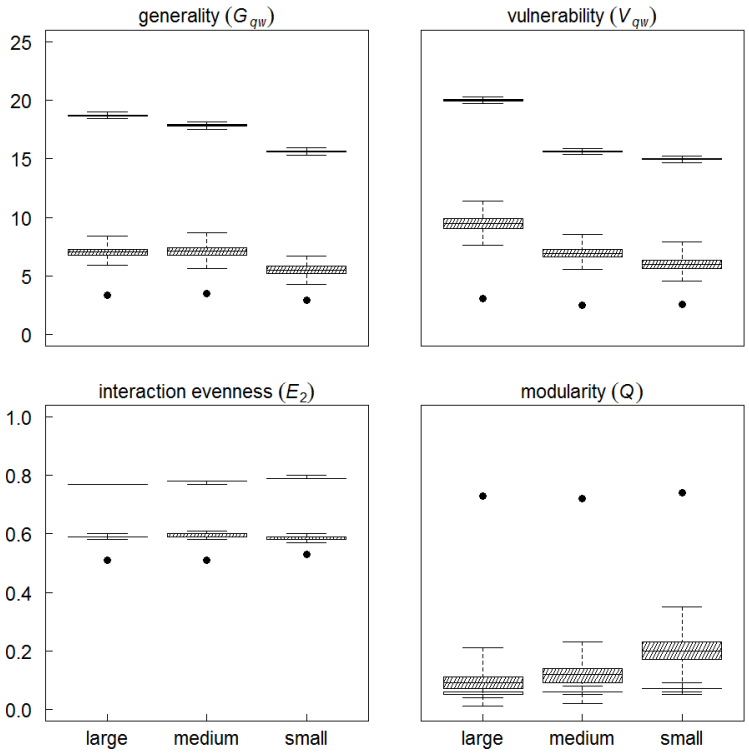

Figure A2.2 Estimates of generality, vulnerability, interaction evenness and modularity (*black dots*) of tick-host interaction networks were significantly different from those predicted by null model I (*grey boxplots*) and null model II (*dashed boxplots*) at each spatial scale.

Table A2.3 Network properties of tick-host associations after network rarefaction of the large and intermediate scales. Standard errors (SE) of rarefied values were all smaller than 0.006

	Geographic scale		
Metric	Large	Intermediate	Small
Interaction evenness (E_2)	0.52	0.52	0.53
Vulnerability (V_{qw})	2.96	2.44	2.55
Generality (G_{qw})	3.26	3.37	2.89
Modularity (Q)	0.70	0.70	0.74

Chapter 3

Molecular ecological insights into Neotropical bird–tick interactions

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Abstract

In the tropics, ticks parasitize many classes of vertebrate hosts. However, because many tropical tick species are only identifiable in the adult stage, and these adults usually parasitize mammals, most attention on the ecology of tick-host interactions has focused on mammalian hosts. In contrast, immature Neotropical ticks are often found on wild birds, yet difficulties in identifying immatures hinder studies of birds' role in tropical tick ecology and tick-borne disease transmission. In Panama, we found immature ticks on 227 out of 3,498 individually–sampled birds representing 93 host species (24% of the bird species sampled, and 13% of the Panamanian land bird fauna). Tick parasitism rates did not vary with rainfall or temperature, but did vary significantly with several host ecological traits. Likewise, Neotropical–Nearctic migratory birds were significantly less likely to be infested than resident species. Using a molecular library developed from morphologically–identified adult ticks specifically for this study, we identified eleven tick species parasitizing birds, indicating that a substantial portion of the Panamanian avian species pool is parasitized by a diversity of tick species. Tick species that most commonly parasitized birds had the widest diversity of avian hosts, suggesting that immature tick species are opportunistic bird parasites. Although certain avian ecological traits are positively associated with parasitism, we found no evidence that individual tick species show specificity to particular avian host ecological traits. Finally, our data suggest that the four principal vectors of Rocky Mountain spotted fever in the Neotropics rarely, if ever, parasitize Panamanian birds. However, other tick species that harbour newly–discovered rickettsial parasites of unknown pathogenicity are frequently found on these birds. Given our discovery of broad interaction between Panamanian tick and avian biodiversity, future work on tick ecology and the dynamics of emerging tropical tick-borne pathogens should explicitly consider wild bird as hosts.

Introduction

Wild birds are increasingly recognized as playing an important role in human and animal health. Emerging zoonotic diseases such as avian influenza, West Nile Virus, and Lyme disease often have wild birds in their transmission cycle (Rappole and Hubalek 2003, Kilpatrick et al. 2006, Ogden et al. 2008), and wild birds can act as reservoir hosts in endemic areas (Ginsberg et al. 2005). Birds also have the potential to introduce diseases into previously naïve populations by spreading pathogens and/or their vectors over large distances via migratory flyways (Hamer et al. 2012, Lindeborg et al. 2012). Hard ticks (Ixodidae) are haematophagous ectoparasites regularly found on wild birds, providing them habitat and blood-meal resources, and vector more human pathogens than any other arthropod group (Jongejan and Uilenberg 2004). Migratory birds captured in temperate regions upon their return from wintering grounds have been observed infected with larval and nymphal stages of tropical tick species (Hamer et al. 2012). The ability of migratory birds to move tropical ticks over long distances, in concert with global climate change (Léger et al. 2013), potentially exposes extra-tropical regions to novel tropical tick-borne pathogens and vice versa (Jongejan and Uilenberg 2004, Parola et al. 2008, Randolph 2010). Therefore, empirical studies are needed that evaluate which tick species are frequently involved in bird parasitism and which ecological characteristics of bird species are related to increased tick infestation levels.

In the New World tropics, the greatest risk of tick-borne disease for human health comes from *Rickettsia rickettsii*, the etiological agent of Rocky Mountain Spotted Fever (RMSF), which has a current fatality rate of 20–40% (Parola et al. 2013). In the Neotropics, the principle vector of RMSF are members of the *Amblyomma cajennense* species complex, although other species of ticks have been shown to harbour *R. rickettsii* (Dantas-Torres 2007, Labruna 2009). In Panama, four confirmed RMSF vectors occur: *A. mixtum*, which is the local taxon in the *A. cajennense* species complex (Nava et al. 2014), along with three other species which are widely found in the Neotropical region: *Dermacentor nitens*, *Haemaphysalis leporispalustris*, and *Rhipicephalus sanguineus* s.l. (Parola et al. 2013). Although the first clinical cases of RMSF were reported in Panama in the 1950s, the disease went unreported for over 50 years until a fatal case in 2004 (Estripeaut et al. 2007). In the past decade, additional fatal cases have been reported in Panama (Tribaldos et al. 2011) as well as adjacent Costa Rica (Argüello et al. 2012) and Colombia (Hidalgo et al. 2011). Improvements in diagnosis are clearly responsible for at

least part of the surge in recent cases, although climate change, habitat modification, and/or increases in human–wildlife contact may also be responsible (Eremeeva and Dasch 2015). While adults of the genus *Amblyomma* and several other Neotropical tick species typically exploit mammals, or reptiles and amphibians to a lesser degree (Fairchild et al. 1966), immature forms are routinely found on birds (Fairchild et al. 1966, Jongejan and Uilenberg 2004, Labruna et al. 2007, Ogrzewalska et al. 2009, Ogrzewalska et al. 2010, Ogrzewalska et al. 2011, Ogrzewalska et al. 2012, Pacheco et al. 2012, Ogrzewalska et al. 2013) including, rarely, nymphs of the *A. cajennense* species complex (Labruna et al. 2007).

At the same time, improvements in molecular detection of tick endosymbionts has uncovered a diversity of novel *Rickettsia* strains of unknown pathogenicity in Neotropical ticks (Labruna et al. 2004, Labruna et al. 2011, Ogrzewalska et al. 2013), and wider distributions of other *Rickettsia* species known to cause RMSF-like symptoms. Recently, *R. rickettsii* has been found in a variety of tick species throughout the Americas and is believed to have caused many overlooked cases of rickettsial disease in South America (Eremeeva and Dasch 2015). Rickettsial bacteria have been found in several species of ticks recovered from Neotropical birds (Ogrzewalska et al. 2012, Pacheco et al. 2012, Ogrzewalska et al. 2013).

Our understanding of Neotropical bird-tick associations and hence their role in disease transmission has been hampered by species identification problems; most immature Neotropical ticks, especially those of the genus *Amblyomma*, are not readily identifiable to species by morphology alone. *Amblyomma* species diversity peaks in the Neotropics, where taxonomic keys serve to identify the nymphal stage of few *Amblyomma* species (Martins et al. 2010). For example, in a recent survey of ticks found parasitizing humans in Panama, only 38% of the recovered specimens of *Amblyomma* could be identified to species (Bermúdez et al. 2012b). Similarly, in a study documenting tick infestation patterns in wild birds from south-eastern Brazil, nearly 48% of the specimens of *Amblyomma* could not be identified to species (Labruna et al. 2007). That study and others (Marini et al. 1996, Ogrzewalska et al. 2013) demonstrate that new tools and approaches are essential to properly assess the role of wild birds in tick ecology and tick-borne disease transmission in the Neotropics.

Museum specimens are often valuable resources for broad studies of ecological patterns (Rocha et al. 2014). Here, we exploit seven years of extensive bird specimen collection across Panama to clarify the ecological interactions between the diverse tick and bird fauna of this Neotropical country. The collecting program of the STRI Bird Collection visited over 100

field sites, sampling nearly 3500 terrestrial birds from 384 species, almost half of the roughly 800 non-aquatic bird species recorded from Panama. Our study provides an unparalleled insight into the ecological relationships between birds and ticks in a Neotropical setting. Specifically, our goals were to: 1) identify how avian ecological traits influence the frequency of tick parasitism of Panamanian bird species; 2) produce a robust DNA barcode library capable of identifying most of the commonly encountered immature ticks in Panama; and 3) use the DNA barcode library to identify to species immature ticks collected off of Panamanian wild birds to determine host specificity patterns and the role of parasite ecological filtering in shaping bird-tick associations in Panama. Our results should provide insights into the relation between avian and ixodid biodiversity that may better inform our understanding of Neotropical tick ecology and may provide insights for our understanding of emerging tropical tick-borne diseases.

Methods

Bird and tick specimen collection

All bird records come from the vouchered collecting program of the STRI Bird Collection, conducted between 2008 and 2012. Collecting occurred year round; however as our collection strategy during this period was the developed as part of a larger program on the ecology of avian-mediated zoonoses, collecting was balanced at most sites between the rainy (May–December) and dry (January–April) season, as well as across the migratory seasons of Nearctic–Neotropical migrants. Full specimen metadata are available in S1 Table (online) and in the STRI Collections portal (http://stricollections.org).

Our field procedures begin with capturing birds in mist nets (or occasionally collecting with shotgun). Per STRIBC field protocols, wild birds were euthanized in the field and flash frozen on solid CO_2 in individual freezer bags to eliminate the risk of cross-contamination of ectoparasites prior to transportation to the lab. There, the entire ectoparasite assembly is recovered as the first step in the ornithological specimen preparation process via whole body ruffling following (Clayton and Drown 2001) who demonstrated that post-mortem ruffling is superior for estimating ectoparasite abundances compared to visual inspection and other live-bird sampling strategies. The number of sampling locations was more than 100, however we combined locations that were within 5 kilometres of each other, resulting in a total of 43 sampling locations throughout Panama (Fig 3.1) for the purpose of this study (S2 Table, Online).

EA separated ticks from other ectoparasites and identified all to age class and sex when possible. Ticks are stored in the STRI Cryological collections in single tubes per avian specimen in 95% ethanol and maintained at -20°C. All specimens collected by the STRI Bird Collection was done with the prior approval of ANAM, Panama's environmental authority (permit numbers: E/A-60- 10, SE/A-137-10, SE/A-96-09, SE/A-44-10, SE/A-66-11, SE/ A-2-12), and collecting methods have been approved by the Smithsonian Tropical Research Institute's Institutional Animal Care and Use Committee (IACUC permits: 2007–03-03-15-07, 2011-0927-2014-03). Complete ornithological specimen metadata is available at: http://stricollections.org.

Ecological patterns of tick parasitism on birds

We generated a tick parasitism data matrix (presence or absence of hard ticks: Ixodidae). Non-land birds from marsh, aquatic, and riverine habitats (e.g. Anseriformes, Charadriiformes, Alcedinidae) were excluded from the analysis, as well as aerial foragers (e.g. Apodidae, Hirundinidae), resulting in 3498 specimen records (S1 Table, Online), from 384 avian species. Using this matrix, we analysed whether key traits of the host species were correlated with tick parasitism using contingency table analysis. We considered the relationship of eight traits on tick parasitism, some of which have previously been correlated with tick parasitism in birds: residents vs. non-breeding migrants, females vs. males, terrestrial foraging vs. arboreal, ground cavity nesting, tree hole nesting, lowland vs. montane habitats, bark insectivory, forest vs. non-forest habitats. We evaluated the influence of sex and migratory status on the entire dataset, however because ecological traits for many migratory birds are more labile away from their breeding grounds and classifications based on temperate zone ecology may not reflect behaviour in Panama, we evaluated the relationship between the remaining six traits and tick parasitism only on resident birds (i.e. those species that breed in Panama). Montane species were defined as those found almost exclusively 600 meters above sea level. Terrestrial foraging species include those species that forage primarily, but not exclusively, on the ground or within 15 centimetres of the ground. Forest inhabitants live in or require mature tropical forests; most edge species were classified as non-forest inhabitants. We evaluated all possible combinations of ecological characters using logistic regression models.

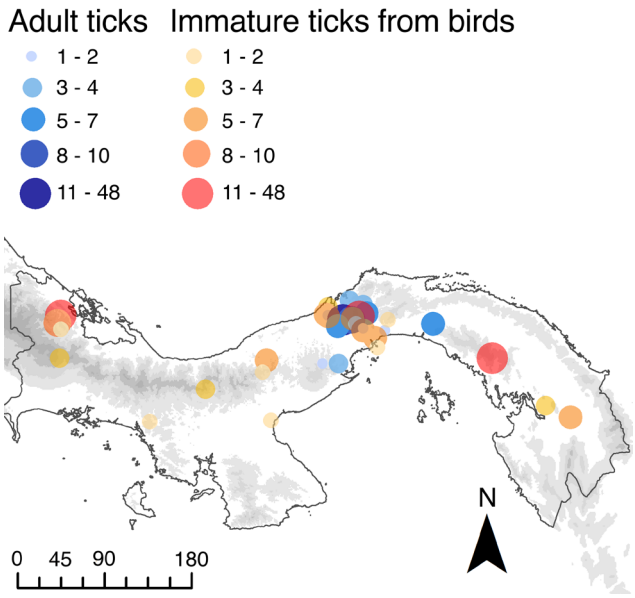

Figure 3.1 Map of collecting areas for adult and immature ticks. Blue circles represent areas where adult ticks were sampled for the reference library; orange circles represent locations where immature ticks were sampled from birds.

Adult DNA Barcode Reference Library

We generated a DNA barcode reference library for the ticks of central Panama using morphologically pre-identified adult ticks collected as part of on-going research programs (HJE and JRL) on the tick-host interactions in the area surrounding the Panama Canal, and also in a few cases from ethanol preserved museum specimens of adult ticks (see Appendix 3: Fig A3.1). Field collections were accomplished either by removal of ticks from hosts (live-captured animals, road kill, livestock, and pets) or by collecting questing ticks from the free environment via flagging, i.e. sweeping a white cotton cloth along vegetation and leaf litter and harvesting accumulated ticks. Adults were identified using morphological characters and existing taxonomic keys (Fairchild et al. 1966, Onofrio et al. 2006), and were stored in 95% ethanol and frozen at -20°C prior to molecular analysis (see next section). Our adult reference library included 96 individuals (S3 Table, Online) that were morphologically assigned to 19 of the approximately 37 species of hard ticks recognized for the Republic of Panama, including 14 of 18 species of *Amblyomma* (Fairchild et al. 1966). Unless already part of a museum collection,

after DNA extraction adult reference ticks were stored in 95% ethanol and are maintained as voucher specimens in the ectoparasite collection of the STRIBC. A public dataset for these 96 specimens, including geographic details of collection, specimen photographs, and museum voucher information can be found on the BOLD data portal v3 (Ratnasingham and Hebert 2007) under the name: DS-TICKA (dx.doi.org/10.5883/DS-TICKA).

Immature Ticks from Panamanian Wild Birds

We selected 186 immature ticks from the pool of immature ticks collected from birds for molecular species-level identification using the adult reference DNA barcode library as the basis for identification (S1 Table, Online). We obtained usable DNA barcode sequences for 130 (see Results). Sample details for immature ticks can be found on the BOLD data portal under the name: DS-TICKI (dx.doi.org/10.5883/DS-TICKI).

Molecular methods

To allow for the preservation of museum vouchers, DNA was extracted from adult specimens from either two legs removed from the specimen, or from a rear quarter section of the abdomen cut from the body, done under an entomological dissecting microscope. We used the entire body of immature ticks for DNA extraction. In all cases, the material being extracted was frozen in a 2 ml tube suspended in liquid nitrogen and pulverized using a sterile micro-pestle to improve DNA yield. We initially obtained poor DNA yield after attempted DNA extractions using DNAeasy spin columns (Qiagen, Valencia, CA), following the manufacturer's instructions (except that we reduced the final elution volume to 50 µl). Subsequently, we switched to the QIAamp DNA Micro kit (Qiagen), which uses similar spin column technology but is optimized for smaller samples and resulted in superior DNA yields.

Amplification of the DNA barcoding region (5' region of the COI mitochondrial gene, 43) was accomplished using the standard invertebrate primers (LCO1490 and HCO2198; 44) following (Kumar et al. 2007), except that we halved the reaction volume (i.e. 25 µL) and raised DNA to 4 µL; we used Qiagen taq and buffers. Positive and negative controls were run in every reaction. Amplifications were visualized on a low-melting agarose gel from which a single PCR product was extracted using a sterilized scalpel blade, and sequenced at the Naos Molecular Laboratory, Smithsonian Tropical Research Institute. DNA sequences and tracefiles can be

examined in BOLD under the DS-TICKA database and DS-TICKI database. Sequences have also been deposited in GenBank under accession numbers KF200076 –KF200171.

Tree building and barcode distance analysis

In order to understand species limits and confirm morphological identification among our adult reference ticks (N = 96), we generated a neighbour-joining tree in MEGA v.5.1 (Tamura et al. 2011) using Kimura-2 parameter (K2P) distances. We assessed branch support by bootstrapping the topology with 500 replicates. We examined the K2P distance matrix and resulting topology for evidence of genetic divergence among our adult reference library that might provide evidence for the presence of cryptic species (Hebert et al. 2004) using both a standard genetic distance approach (3% K2P) as well as looking for the assignation of multiple Barcode Index Numbers (BINs) to a given species. The Barcode Index Number is an alternative, numerical taxonomy that clusters taxa into interim operational taxonomic units using a stage process to employ single linkage clustering (Ratnasingham and Hebert 2013). BINs are assigned automatically in the BOLD database portal based on the global dataset of DNA barcode sequences (i.e. including samples not generated in this study). We repeated all tree-building, genetic distance, and clustering analyses for a second, expanded dataset that combined the 96 adult reference sequences with 130 sequences from immature ticks collected from birds.

Species-level associations between ticks and wild birds in Panama

We used the identifications of immature ticks from STRIBC bird specimens to further examine species-specific bird-tick associations in Panama. First, we assessed host-specificity by examining the correlation between the frequency of occurrence of a given tick species (number of birds infested by that species) and the diversity of avian host species (number of host species) for all tick species recovered in our dataset; if immature ticks are non-host specific, this correlation should be strong. While this approach provides one measurement of host–tick specificity, it potentially overlooks the role of ecological filtering by hosts for particular parasite species (Combes 2001), which we tested for by examining differences in the frequency of parasitism by host ecological traits for each tick species identified using COI barcodes using G-tests of independence. We visualized the interaction between immature tick species and specific avian host species via quantitative interaction networks created using the bipartite

package following the authors' instructions (Dormann et al. 2009) in the R statistical application (R Core Team 2017). For those birds that had multiple ticks identified by DNA barcoding we scored each tick species separately but only once in the data matrix.

Finally, to estimate the proportion and distribution of the total Panamanian tick species pool that might depend on birds as host vertebrates for immature life stages, we estimated the total species richness of ticks that parasitize wild birds in Panama through species accumulation curves generated in the EstimateS software package (Colwell 2013). When sampling is exhaustive, the species accumulation curve should reach an asymptote. However, even non-exhaustive sampling can still yield sufficient data to provide a reasonable estimate of the true species richness, which can be assessed by observing an asymptote in the statistical estimate of species richness (Colwell and Coddington 1994). We generated species-accumulation curves (SACs) for the 130 ticks where we were successfully able to generate DNA barcodes using both the adult reference library cluster-indicated taxonomy, and using the BIN numerical taxonomy generated in the BOLD database. In both cases, we used the Chao1 species richness estimator (Chao 1987), which attempts to non-parametrically correct the observed species richness as a function of the proportion of species observed exactly once or twice in the dataset. Mean Chao1 values were obtained from 100 reshuffles of our data set with replacement in EstimateS; sampling with replacement being critical in order to account for sampling error.

Results

Ecological traits of Panamanian wild birds parasitized by ticks

We evaluated patterns of tick parasitism in 3,498 bird specimens from the STRI bird collection, representing 384 species, i.e. nearly half of the roughly 800 non-aquatic bird species recorded from Panama. Ticks parasitized a total of 227 specimens of Panamanian birds (6.5% of all individuals; S1 Table, Online) representing 93 avian species and 24 families. Among the 227 infested birds, both the median and modal number of ticks recovered was 1. However, 15% of the infested birds had 4 or more ticks; one bird had 101 ticks, which was the most recovered from any bird in the study. Resident birds (i.e. species that breed in Panama) were 3.8 times as likely to be parasitized by ticks compared to non-breeding Nearctic–Neotropical migratory species (6.8% vs. 1.8%; Fisher's exact test, $P = 0.001$). 90 of 343 resident species had at least

one bird sampled infested with ticks, whereas only 3 of 40 Nearctic–Neotropical migratory species had an infested individual, although sample sizes were lower for migratory species relative to resident species (mean $N_{resident}$ = 9.5, mean $N_{migratory}$ = 5.4; two-tailed unequal variance t-test, P < 0.001).

Among avian families with at least 25 specimens evaluated, the families with the greatest proportion (%) of specimens parasitized by ticks were: Thamnophilidae (antbirds: 18%; 12 of 20 species); Furnariidae (ovenbirds and woodcreepers: 15%; 11 of 20 species); Polioptilidae (gnatcatchers: 15%; 1 of 3 species); Turdidae (thrushes: 15%; 7 of 15 species); and Troglodytidae (wrens: 14%; 9 of 16 species). Because of behavioural differences between males and females (including time spent in the nest, where infestation may be more likely), we anticipated a difference in infestation rates between males and females. Although females had a slightly higher prevalence of tick parasitism than males (7% vs. 6%), the difference was not significant (Fisher's exact test, *P* = 0.23). Among resident birds, a logistic model indicated that only forest habitation, terrestrial foraging, bark insectivory, and lowland residency were significantly positively associated with tick parasitism (Table 3.1).

Among 26 locations where we sampled a minimum of 20 birds, we found no relationship between the frequency of tick parasitism and annual mean temperature, temperature seasonality, annual precipitation, or precipitation seasonality. However we did recover an effect of taxonomic composition, specifically, the proportion of the sampled avifauna belonging to the five families most frequently parasitized by ticks (S1 Table, Online).

DNA identifications of adult ticks agree with morphological taxonomy

The 96 individuals in the adult reference library of morphologically identified ticks from central Panama formed 20 clusters with pairwise Kimura-2 parameter (K2P) genetic distances greater than 5% (see Appendix 3: Fig A3.1). All 96 could be placed in clusters in agreement with the original morphological identification of the voucher with bootstrap support values of at least 99%. Among the 20 species—clusters, average nearest-neighbour K2P distance to another cluster was 15.6% and the minimum nearest-neighbour K2P distance was 12.5% (range: 12.5%– 20.5%).

Two species contained DNA barcode sub-clusters with between-cluster sequence divergence well below the 12.5–20.5% difference observed between named species, but greater than 3.0%. *Haemaphysalis juxtakochi* comprised two clusters that differed by 3.0% pairwise K2P

distance, with each cluster supported by 99% bootstrap support, while *Amblyomma ovale* contained one cluster of four individuals supported by 97% bootstrap and a fifth individual that varied by an average K2P distance of 3.2%. In both cases, individuals from both clusters were collected at a shared location, suggesting that these might represent cryptic biological species. As a consequence, a numerical DNA barcoding taxonomy (BIN barcode identification number) based on genetic distances among barcodes clusters recovered 22 unique BINs in our adult dataset; representing the 20 clusters that agree with our morphological named species as described above, as well as second BINs for both *H. juxtakochi* and *A. ovale*.

Table 3.1 Ecological traits associated with ticks among Panamanian resident wild birds (N = 3274).

Ecological Trait	Odds ratio	P value (Wald's)
Bark insectivores*	8.3	0.004
Terrestrial foragers*	4.0	$<10^{-15}$
Forest vs. non-forest dwellers*	3.3	$<10^{-9}$
Tree hole nesters	2.5	0.20
Ground cavity nesters	2.0	0.14
Lowland vs. montane*	1.7	0.01

Traits significant for a positive association with tick parasitism marked with (*). Significance determined by multiple logistic regression.

DNA identifications of immature ticks from birds

We generated useable DNA barcode sequences from 130 immature ticks out of a total of 172 samples attempted (76% success rate). Two failures were due to double peaks in the electropheragram recovered in multiple amplification and sequencing attempts (MJM2941-T01 and MJM4264-T01); we removed these individuals from further analyses. One individual (MJM7015) amplified its avian host (*Poecilotriccus sylvia*) DNA. The remaining 39 individuals ailed in either the PCR or the sequencing step (S1 Table).

Sequences from the immature ticks formed 13 DNA barcode clusters. When we merged the immature barcode dataset with the adult reference library (Fig 3.2), 122 of 130 (94%) taxa formed 11 DNA barcode clusters which included an adult reference. Thus, we can confirm that immature ticks of the following species parasitize wild birds in Panama: *H. juxtakochi, A. dissimile, A. ovale, A. longirostre, A. geayi, A. sabanerae, A. varium, A. calcaratum*, and *A. nodosum* (S1 Table, Online). Our data establish that at least 8 of the 18 species of

Amblyomma ticks found in Panama parasitize birds. This includes the third global record of *A. dissimile* – a reptile and anuran specialist – parasitizing a wild bird (Mangrove Cuckoo, *Coccyzus minor*, (Fairchild et al. 1966, Scott and Durden 2015)). In the case of *H. juxtakochi*, which is represented in the adult reference library by two DNA barcode clusters (and two unique BINs in the molecular taxonomy), we recovered both clusters from bird samples. The two remaining clusters of immature ticks did not include an adult reference, so they can only be identified using molecular taxonomy (see below).

Using the BIN molecular taxonomy, our sample of immature ticks clustered into 13 BINs, including 11 of the 22 BINs recovered in the adult reference library. Hence, we were able to assign a BIN to 100% of the samples that provided DNA sequences and to 76% (130 of 172) of all samples for which we attempted DNA barcoding. Eight other immature ticks (6%) formed two novel clusters on our phylogenetic trees; and for these we were only able to assign a BIN numerical taxonomic identification. The first BIN (ACC9360) was formed by just one immature tick that had a nearest neighbour-distance of 14.4% to the reference library cluster of *Ixodes affinis*. A second cluster (BIN: ACC9045) was formed by seven immatures whose nearest-neighbour cluster on the BOLD database did not include ticks from this study. Instead, they were most closely related to *H. leporispalustris* collected in Canada, with a nearest-neighbour distance of 6.0%. *H. leporispalustris* also occurs in Panama, but without a Panamanian adult reference sequence and given the large sequence variation, we are unable to determine whether our sample represents a genetically-divergent Panamanian *H. leporispalustris* population or distinct species of *Haemaphysalis* yet to be recorded in Panama. Thus, we refer to these two taxa as probable members of *Ixodes* and *Haemaphysalis* genera, respectively.

Using either BIN or traditional taxonomy, species accumulation curves for our sample of immature ticks recovered from wild birds were essentially asymptotic, as were species richness estimators designed to account for unobserved species (Fig 3.3). Using the BIN taxonomy, the Chao1 species richness estimate was 13.3 (95% confidence interval: 13.0–19.0), compared to an observed IN species richness of 13 (Fig 3.3). Likewise, the species accumulation curve using the traditional taxonomy recovered a mean Chao1 estimate of 11.5 (95% confidence interval: 11.0–19.3), compared to an observed species richness of 11 (see Appendix 3: Fig A3.2). These results suggest that at most only a few more tick species would be recovered from wild birds in Panama given considerably greater sampling effort, and that their occurrence on wild birds would be exceedingly rare.

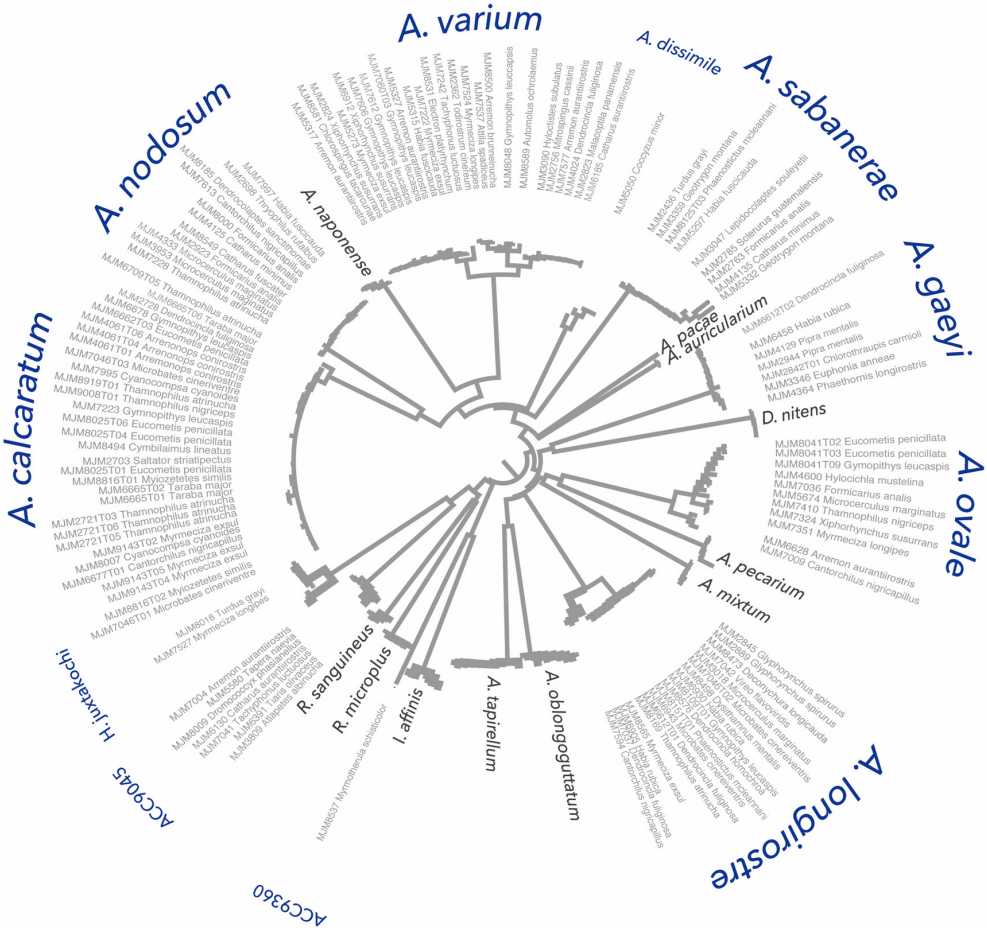

Figure 3.2 DNA barcoding neighbour-joining tree of combined data matrix of immature ticks and adult reference library ticks. Thin grey tip labels refer to specimen number and host species. Clade labels in blue refer to tick species recovered from birds, clade labels in grey refer to ticks unobserved on birds. Two clades where not represented in the adult reference library so they are labelled with their molecular taxonomy Barcode Identification Number (BIN). The distribution of Panamanian ticks recovered from wild birds is unbalanced towards certain species of *Amblyomma*. Importantly, we recovered no immature ticks on birds from species known to vector *Rickettsia rickettsii*, the causative agent of Rocky Mountain spotted fever (Parola et al. 2013).

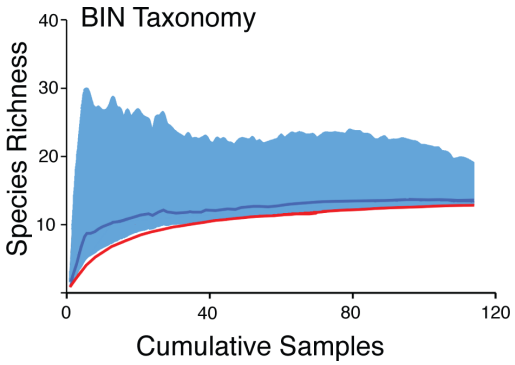

Figure 3.3 Species accumulation curve (SAC) for immature ticks recovered from Panamanian wild birds based on BIN numerical taxonomy (see Appendix 3: Fig A3.2 for SAC based on traditional tick taxonomy). Red line = S, mean observed species richness; dark blue line = $\hat{S}$, mean Chao1 S estimate; filled blue area defines 95% upper and lower confidence limits (CI) for $\hat{S}$. The convergence of the S and $\hat{S}$ curves, as well as the asymptotic nature of these curves and the CI curves, suggests that at most only a few more tick species would be recovered from wild birds in Panama, and that their occurrence would be rare.

Immature ticks show no species-level host specificity or ecological filtering of avian hosts

Immature ticks showed no measurable avian species host specificity. Tick species collected from at least two different bird individuals always occurred on at least two different avian host species, and most frequently occurring tick species recovered from wild birds in our samples had the greatest number of avian host species (Pearson's rho = 0.984, $P < 0.0000001$). In 5 of 11 birds from which we sequenced multiple individual ticks, we found more than one species or more than one haplotype of tick, suggesting multiple independent colonisations of the host.

In addition to the lack of species-level host specificity, we observed little evidence that individual tick species show preferences towards host ecological traits. Instead, networks of specific tick and avian host species demonstrated broad and varied interactions by the ecological traits that were previously shown to influence rates of bird parasitism (Fig 3.4). We found no difference in the frequency of forest versus non-forest hosts among our 13 tick BIN species ($G = 12.37$, d.f. = 12, $P = 0.42$, Fig 3.4a), nor in the frequency of arboreal-foraging hosts ($G = 22.18$, d.f. = 12, $P = 0.036$, Bonferonni-corrected $\alpha = 0.013$, Fig 3.4b). Likewise, neither the proportion of bark insectivorous hosts ($G = 10.287$, d.f. = 12, $P = 0.59$, Fig 3.4c) nor the proportion of montane hosts ($G = 17.996$, d.f. = 12, $P = 0.12$, Fig 3.4d) varied among tick species. We found equivalent results using the traditional tick taxonomy.

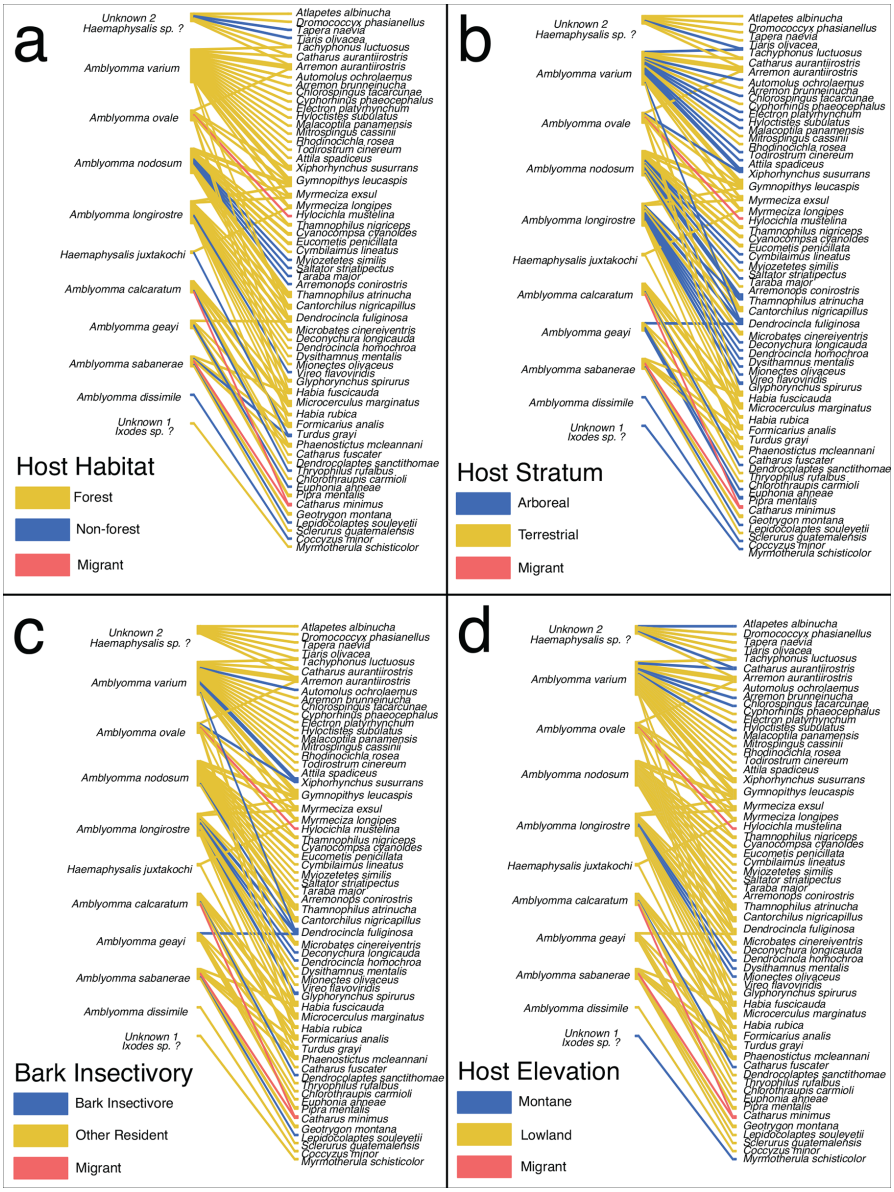

Figure 3.4 Bird–immature tick quantitative interaction networks. **(a)** Blue: interactions involving non-forest bird species, yellow: forest inhabiting bird species; **(b)** yellow: terrestrial-foraging bird species; blue: arboreal bird species; **(c)** blue: bark insectivores; yellow: other species; **(d)** blue: montane bird species, yellow: lowland species; **(a-d)** pink: non-breeding migrant bird species. The frequency of hosts among these four ecological traits was not significant among the 11 sampled tick species (see Results for details).

Discussion

Patterns of tick parasitism on Panamanian wild birds

Although only a minority of Panamanian land birds are infested by ticks, the broad participation in bird–tick interactions by a diversity of bird and tick taxa indicates that wild birds may play an important role in the life history of many Neotropical tick species and have the potential to play a role in the transmission of tick-borne diseases. While only 6.5% of all birds examined carried ticks, parasitism by ticks includes a diverse taxonomic array of avian hosts. We observed ticks on 93 of the 384 species we examined, which represent 24 avian families. When examining parasitism rates for passerines (11.2%), the Panamanian parasitism rate is in line with rates (9–17%) from other studies that focused almost exclusively on passerine birds (Marini et al. 1996, Ogrzewalska et al. 2010, Ogrzewalska et al. 2015).

We found no evidence that tick parasitism by site varied by either absolute or seasonal differences in temperature or rainfall. This indicates that, at the macro-scale, climatological patterns in the Neotropics likely have little influence on the parasitism frequency. Nonetheless, differences in ecological traits among Panamanian birds appear to modulate tick parasitism frequency. We found that lowland, forest inhabiting, ground and bark foraging birds were significantly more likely to be infested with ticks than birds with other ecologies.

Lack of host specificity and ecological filtering in bird-tick interactions

A previous study indicated that the adult life stages of Neotropical ticks show high levels of structural and phylogenetic host specificity (Esser et al. 2016b). The degree of host specificity in immature Neotropical ticks however, remains largely unexplored because of the difficulty of their morphological identification to species. Using our molecular identifications, we found no evidence for strong host specificity in Panamanian tick–bird interactions. Instead, the number of host species increased with the number of sampling events for that tick species, demonstrating that immature Neotropical ticks parasitize a wide variety of birds from diverse families, with no clear preference for particular host species.

Our finding of limited or no host specificity tracks a more general shift in our understanding of the specificity of immature tick–host relationships. Researchers applying modern statistical approaches generally have found lower levels of host specificity in immature ticks than was assumed in the historical literature (McCoy et al. 2013, Nava and Guglielmone

2013, Wells et al. 2013, Madinah et al. 2014). Instead of absolute host specificity, research suggests that ecological correlates between potential hosts and ticks may determine the host diversity of tick species (McCoy et al. 2013). This is a restatement of Combe's "ecological filter" concept of parasitism (Combes 2001, Poulin 2011). Our finding of four key ecological traits that are positively correlated with tick parasitism rates in birds allows us to test the ecological filter concept in bird–immature tick interactions.

Here, we found no evidence that certain tick species have ecological preferences to bird species based on any of these four traits. The result was robust using both traditional tick taxonomy and the molecular taxonomy. This latter result is notable because one of the key ways that cryptic tick species might co-occur is by having cryptic host ecological specificities among immature stages (Hebert et al. 2004). Whether such ecological filtering occurs between adult ticks and mammals in Panama awaits further study. Our finding of neither strict-host specificity, nor the more relaxed ecological filtering type of specificity in bird–tick interactions, coupled with the diversity of bird species involved and the potentially greater dispersal ability of birds, indicates that bird–tick interactions will have meaningful implications for the demographics of Neotropical ticks and also the ecology of tick-borne pathogens (McCoy et al. 2013). Thus, tick-borne disease transmission models based on patterns of host specificity of adult ticks may require re-examination in order to incorporate the more labile ecology of immature ticks.

DNA barcoding of adult and immature ticks

Our findings demonstrate that DNA barcodes are a reliable method to identify Panamanian hard ticks (Ixodidae) to species, and can overcome the frequently-cited difficulties in identifying immature forms of Neotropical ticks to species using only morphological characters (Ogrzewalska et al. 2009, Bermúdez et al. 2010, Ogrzewalska et al. 2010). Alternatives to molecular identification of immature ticks include rearing immatures to life stages that can be reliably identified to species (Labruna et al. 2007), but this is time consuming, requires special laboratory conditions that vary among species, and most immature ticks die before reaching an identifiable life stage (Labruna et al. 2007, Ogrzewalska et al. 2009). DNA barcoding appears to yield a much greater percentage of successful species identifications. We were able to identify ~70% of immature ticks, whereas a rearing study from Brazil was able to identify only 12% of the immatures (Ogrzewalska et al. 2009).

The role of wild birds in the transmission ecology of tick-borne pathogens

Our ability to identify immature ticks from Panamanian birds permits insight to the potential role of wild birds in the transmission ecology of Neotropical ticks and tick-borne diseases. We found no evidence that wild birds are involved in the transmission ecology of Rocky Mountain Spotted Fever (RMSF). RMSF is the most virulent tick-borne disease known in the Western Hemisphere and is caused by infection from *Rickettsia rickettsii*. In Panama, RMSF was first reported over 60 years ago (de Rodaniche and Rodaniche 1950), although it remained unreported again until 2004 (Estripeaut et al. 2007), when it resulted in a fatal case in western Panama. Since 2004, RMSF has been regularly reported in central and western Panama (Bermúdez et al. 2011). Several species of ixodid ticks are confirmed vectors of *R. rickettsii*, including four species of Panamanian ticks: *Dermacentor nitens, Rhipicephalus sanguineus* s.l.*, Haemaphysalis leporispalustris,* and *Amblyomma (cajennense) mixtum*. However, members of the *A. cajennense* species complex are considered the primary vector of *R. rickettsii* in tropical America (Dantas-Torres 2007), and in Panama *R. rickettsii* has been detected principally in *A. mixtum* (de Rodaniche 1953, Estrada-Peña et al. 2004) with a single record in *D. nitens* (Bermúdez et al. 2009). We found no examples of *A. mixtum, D. nitens*, *or R. sanguineus* s.l. parasitizing birds in our study, although we did find five birds infested with *Haemaphysalis* sp. ticks. Our species accumulation curves for tick species found on wild birds suggest that at best only a few, rare, species are yet to be recovered from resident wild land birds in Panama. While a couple of studies from other Neotropical regions have reported that immature forms of species in the *A. cajennense* complex parasitize various wild bird species, Labruna et al. (2007) challenged the morphological identifications in these cases. Our findings along with others (Ogrzewalska et al. 2009, Ogrzewalska et al. 2010, Pacheco et al. 2012, Ogrzewalska et al. 2015) demonstrate that RMSF vectors are at best rare parasites of wild birds, and collectively suggest a relatively negligible role for wild birds in RMSF transmission in Panama and elsewhere in the Neotropics.

On the other hand, it is likely that immature ticks on wild bird in Panama harbour a diversity of other rickettsial pathogens. Although we did not attempt to isolate *Rickettsia* from sampled ticks, other studies have demonstrated that 10 of the 11 tick species that we recovered from Panamanian wild bird harbour *Rickettsia*. *Rickettsia amblyommii* has been recovered as a parasite of *A. ovale* in Panama (Bermúdez et al. 2009, Eremeeva et al. 2009) as well as *A. geayi* and *A. longirostre* in Brazil (Ogrzewalska et al. 2010, Labruna et al. 2011) and several species of

ticks recovered from Neotropical–Nearctic migrant birds in south Texas, USA (Cohen et al. 2015). *Rickettsia amblyommii* has been suggested, but not confirmed, to be a cause of spotted fever-like disease in North America (Apperson et al. 2008). *Rickettsia parkeri*, which was recently identified as the cause of human spotted fever rickettsiosis in south-eastern USA as well as in Brazil, Uruguay and Argentina was recovered from Brazilian samples of *A. nodosum* and *A. ovale* (Labruna et al. 2011). Other *Rickettsia* species, not yet known to cause human disease, have been recovered from *A. calcaratum* (Ogrzewalska et al. 2013), *A. dissimile* (Miranda et al. 2012), *A. varium* (Romer et al. 2011) and *H. juxtakochi* (Labruna et al. 2011). Work in the Neotropical regions concerning the pathogenicity of *R. amblyommii* and *R. parkeri* and other rickettsiales is in its infancy. Likewise, spotted fever group rickettsioses are often under-detected, especially in Middle and South America (Romer et al. 2011). While the rate of infestation of ticks on wild birds in Panama is relatively modest (~6.5%), as a recent study from south Texas demonstrates, even low relative frequencies of parasitism may have regionally–important consequences for tick and emerging disease ecology given the absolute numbers of wild birds involved (Cohen et al. 2015). Assuming typical avian densities recorded for Panama (Robinson et al. 2000), as many as 96,000,000 Panamanian wild birds might be infested with ticks. Thus, our finding of a pervasive and diverse relationship between birds and immature ticks in Panama suggests that further consideration of the role of wild birds in the ecology of Neotropical ticks and the pathogens they vector is warranted.

Appendix 3

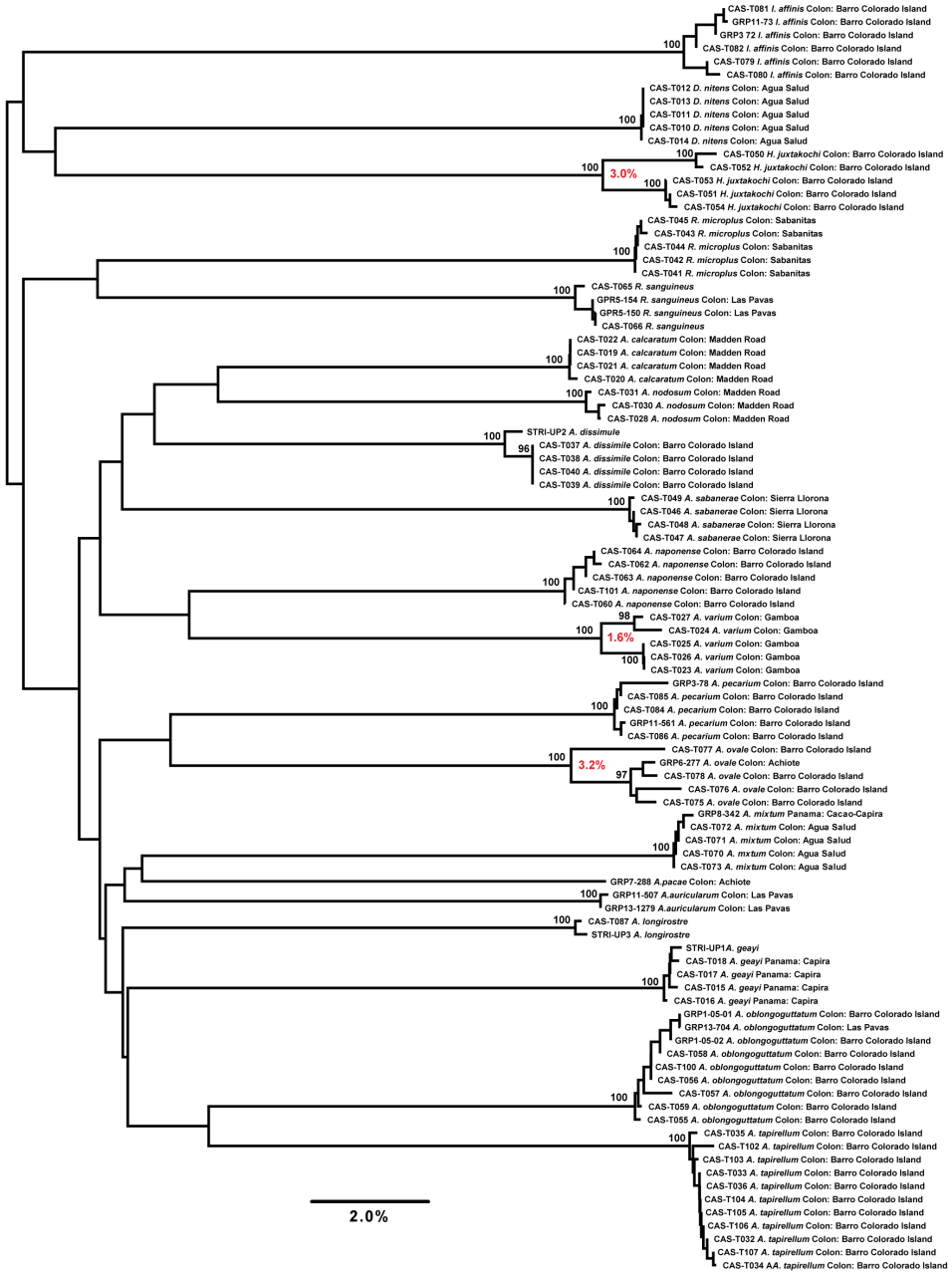

Figure A3.1 Neighbour-joining tree of 96 adult ticks based on COI DNA barcode codes.

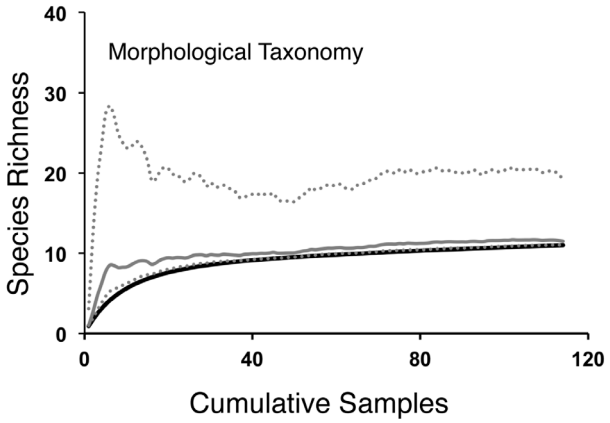

Figure A3.2 Species accumulation curve (SAC) for immature ticks recovered from Panamanian wild birds based on BIN numerical taxonomy. Black line = S, mean observed species richness; solid grey line = Ŝ, mean Chao1 S estimate; dotted grey lines = 95% upper and lower confidence limits (CI) for Ŝ. As Chao1 is downward biased, the 95% lower CI is probably not useful. Fairchild et al. (1966) estimated that 37 species of hard ticks occur in Panama.

Chapter 4

Host body size and the diversity of tick assemblages on Neotropical vertebrates

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Abstract

Identifying the factors that influence the species diversity and distribution of ticks (Acari: Ixodida) across vertebrate host taxa is of fundamental ecological and medical importance. Host body size is considered one of the most important determinants of tick abundance, with larger hosts having higher tick burdens. The species diversity of tick assemblages should also be greater on larger-bodied host species, but empirical studies testing this hypothesis are lacking. Here, we evaluate this relationship using a comparative dataset of feeding associations from Panama between 45 tick species and 171 host species that range in body size by three orders of magnitude. We found that tick species diversity increased with host body size for adult ticks but not for immature ticks. We also found that closely related host species tended to have similar tick species diversity, but correcting for host phylogeny did not alter the relationships between host body size and tick species diversity. The distribution of tick species was highly aggregated, with approximately 20% of the host species harbouring 80% of all tick species, following the Pareto principle or 20/80 rule. Thus, the aggregated pattern commonly observed for tick burdens and disease transmission also holds for patterns of tick species richness. Our finding that the adult ticks in this system preferentially parasitize large-bodied host species suggests that the ongoing anthropogenic loss of large-bodied vertebrates is likely to result in host-tick coextinction events, even when immature stages feed opportunistically. As parasites play critical roles in ecological and evolutionary processes, such losses may profoundly affect ecosystem functioning and services.

Introduction

Parasites are an important component of natural communities, in which host species are habitat to a wide range of microparasites (e.g. bacteria and protozoa) and macroparasites (e.g. helminths and arthropods) (Nunn et al. 2003). Parasite species richness and abundance varies both among and within host taxa, suggesting that some host species are more likely to be parasitized than others (Wilson et al. 2002). As parasites are able to profoundly affect host survival, fecundity and population dynamics, identifying which host traits explain the non-random pattern in which parasites are distributed across host lineages is highly relevant for human and veterinary medicine, as well as wildlife conservation (Morand and Poulin 1998, Altizer et al. 2003, Nunn et al. 2003, Ezenwa et al. 2006b, Huang et al. 2014). Knowing which host traits increase the likelihood of parasite host-switching to livestock, humans, or re-introduced wildlife, and predicting which parasites are present in understudied host species, will allow assessing which host species are at greatest risk from infectious diseases by identifying 'problematic' parasites such as host generalists before they emerge (Huang et al. 2014).

Body size is the host trait most often invoked to explain the structure of parasite assemblages (Poulin 2004). Larger hosts have larger external surface areas and thus represent larger "habitats" that provide more space and resources for parasites to exploit (Kuris et al. 1980, Poulin 1995). Larger hosts also have larger home ranges, travel longer distances, and may visit more diverse habitats than smaller species, all of which increases their likelihood of acquiring a diverse parasite fauna (Nunn et al. 2003, Krasnov et al. 2004c). On the other hand, body size is negatively correlated with population density (Blackburn et al. 1993, Arneberg 2002). Less abundant hosts have lower probabilities of contacting parasites and should therefore accumulate fewer species and individuals of parasites than hosts living at higher densities (Anderson and May 1978, Morand and Poulin 1998). Large-bodied hosts also tend to have slower life-history strategies, which is considered a trade-off for higher immunocompetence (Lee 2006), so that larger hosts may be more resistant to tick parasitism. Yet a recent meta-analysis identified host body size as a key universal determinant of parasite species richness across host and parasite taxa (Kamiya et al. 2014a). However, one group of parasitic organisms that have rarely been considered in these studies, but which are of considerable medical and veterinary concern, are ticks.

Ticks (Acari: Ixodida) are excellent models for studies on the ecology and evolution of host-parasite associations as they are obligatory blood-feeding arthropods that parasitize every class of terrestrial vertebrates around the world (Sonenshine 1991). Like other parasites, ticks are found on only a subset of all apparently suitable hosts (Randolph 2004), and which host characteristics drive ecological patterns in tick parasitism remains poorly resolved. While some studies have found clear relationships between intraspecific host traits and tick burdens (Tälleklint and Jaenson 1997, Hughes and Randolph 2001, Harrison et al. 2010, Vor et al. 2010, Kiffner et al. 2011a, Kiffner et al. 2011b, Anderson et al. 2013, Heylen et al. 2013), others have not (Brunner and Ostfeld 2008, Pollock et al. 2012). These earlier studies, however, largely focused on a single tick species, mostly from the *Ixodes ricinus* species complex, and its distribution across one or two host species. In contrast, only few studies have examined host determinants of tick parasitism for a broader range of host taxa (Gallivan and Horak 1997, Marsot et al. 2012, Hofmeester 2016, Miller et al. 2016) and the question of how the species diversity of tick assemblages (i.e. the "tick fauna" of a host species, *sensu* Poulin (2004)) covaries with interspecific host traits such as body size remains unresolved.

Species-rich communities of ticks and hosts, such as those found in the New World tropics, provide a great opportunity to tackle this question. Here, we used comparative analyses to assess whether and how tick species richness, diversity, and proportional similarity (henceforward tick assemblage structure) were related to host body size across a wide range of vertebrate host groups in Panama. We show that the results are dependent on tick life stage, with positive relationships of tick assemblage structure with host body size for adult ticks, but a lack of any relationship for immature ticks. We provide possible explanations for this difference and discuss the implications of our findings.

Methods

Study area

We compiled data on tick-host associations from Panama, a country that supports a large diversity of vertebrates, many of which are endemic to the Neotropics (Patterson and Costa 2012) Panama is also rich in ticks, both in species and in numbers (Fairchild et al. 1966). Over forty species of ticks from seven genera and two families have been reported so far, and new species continue to be described (Fairchild et al. 1966, Apanaskevich and Bermúdez 2013, Nava

et al. 2014, Bermúdez et al. 2015b). Tick-host associations in Panama have been recorded from a wide variety of environmental conditions and habitats, ranging from mangrove swamps to tropical forests and from savannahs to high-altitude cloud forests (Fairchild et al. 1966). Panama has tropical moist weather with an average diurnal temperature of 27 °C. Average temperature and humidity are high throughout most of the country, but considerably milder at elevations >600 m. Elevation ranges from ca. 0 – 3,500 m. Rainfall varies both regionally (ca. 1,750-4,000 mm) and temporally, with a pronounced dry season in the lowlands from January to April (Ridgely and Gwynne 1989).

Data compilation

We used data from Dunn (1923), Fairchild et al. (1966), Bermúdez et al. (2009, 2010, 2011, 2013, 2015b), Apanaskevich and Bermúdez (2013), Murgas et al. (2013), García et al. (2014), Esser et al. (2016b), and Miller et al. (2016). In addition, we collected larvae and nymphs from vertebrate hosts between 2008 and 2014, including humans, domestic animals, and wildlife (mammals, amphibians, and reptiles), the latter of which were either found as road kills or sampled during live-capture studies. We searched the entire body of hosts, but only collected ticks found firmly attached, and preserved these in 95% ethanol. Larvae and nymphs of the genus *Rhipicephalus* (*Boophilus*) and nymphs of *Haemaphysalis* were identified using the taxonomic keys provided by Fairchild et al. (1966). Nymphs of *Amblyomma ovale* were identified using the taxonomic keys of Martins et al. (2010). *Ornithodoros puertoricensis* larvae were identified by morphological descriptions in Venzal et al. (2008) and later confirmed by 16S rDNA sequencing. Larvae and/or nymphs of *Amblyomma*, *Haemaphysalis,* and *Ixodes* ticks that could not be identified based on morphology were sequenced using 16S rDNA, or using the mtDNA COI barcoding fragment, following Miller et al. (2016).

Given the ectoparasitic nature of ticks, host body size is best reflected by the skin surface area of each host species. Since such data is not readily available, we used the allometric scaling relationship between body mass *M* and skin surface area *A* as a measure of host body size, where $A \propto M^{2/3}$ (West et al. 1999). Data on host body mass (average for males and females, in grams) for each species were obtained from various sources (Eisenberg 1989, Smith et al. 2003, Dunning 2007, Greer et al. 2007, De Magalhães and Costa 2009, Reid 2009, Meiri 2010, Arner 2012, Feldman and Meiri 2013) and hence raised to a 2/3 power prior to analyses.

Characterization of tick assemblage structure

We used non-parametric methods that consider differences in species abundance (i.e. the number of ticks collected per host species) to compute three indices that have been widely used in ecology: estimated total species richness, true diversity, and proportional similarity. Each of these indices characterizes a different aspect of the tick assemblage structure across vertebrate host species. We used the Chao1 index (Chao 1984), an asymptotic estimator of species richness, to compute the number of tick species per host species that would be expected under exhaustive sampling, using the EstimateS software package version 9.1.0 (Colwell 2013). Estimation of Chao1 is based on the concept that the number of species that remain undetected in a sample can be estimated from the number of rare species observed within that sample;

$$\hat{S}_{Chao1} = S_{obs} + \left(\frac{n-1}{n}\right)\left(\frac{F_1(F_1-1)}{2(F_2+1)}\right),$$

where $\hat{S}_{Chao1}$ is the estimated total tick species richness, S_{obs} is the observed tick species richness, *n* is the number of individual ticks collected, and F_1 and F_2 are respectively the number of tick species observed only once (singletons) or twice (doubletons) on a host species. Values of $\hat{S}_{Chao1}$ approach S_{obs} when the accumulation of tick species on a host has reached an asymptote.

We used the exponential of Shannon entropy (Shannon 1948, MacArthur 1965) to estimate the "true diversity" (rather than simply species richness) of the tick assemblage for each host species, following Jost (2006). Adapted to species-level analyses, Shannon entropy is based on both the number and relative abundance of tick species on each host species, thereby taking into account that some ticks are more common than others (Shannon 1948). Exponential transformation linearizes the index and allows for diversity to be measured in units of "effective number of species", i.e. numbers of equivalent, equally abundant species. In contrast to Shannon entropy, true diversity obeys the "doubling principle", so that a host species with twice as many equally abundant tick species is twice as diverse (Jost 2006). We calculated true diversity over contingency tables with *I* rows of host species and *J* columns of tick species, using the R package 'bipartite' (Dormann et al. 2008);

$$\exp(H_i') = \exp\left(-\sum_{j=1}^{S_{obs}} p_{ij} * \ln p_{ij}\right),$$

where H_i' is the Shannon entropy of the tick assemblage on host species *i*, and p_{ij} is the proportion of tick species *j* on host species *i*. Values of true diversity are equal to S_{obs} for tick assemblages that are perfectly even, i.e. when all tick species are equally abundant.

We used Czekanowski's proportional similarity index (Schoener 1968, Feinsinger et al. 1981) to quantify the proportion of the total tick population that is supported by each host species, again using the R package 'bipartite' (Dormann et al. 2008). When adapted to species-level analysis, this index measures the area of overlap between the frequency distribution of the tick assemblage on host species *i* with that of the total tick population across all hosts (Feinsinger et al. 1981);

$$PS_i = 1 - 0.5 \sum_j |p_{ij} q_j| ,$$

where PS_i is the proportional similarity index for host species *i*, and q_j is the proportion of tick species *j* in the total tick population. Values of PS_i range from q_j for host species that harbor only one tick species *j* to 1 for host species that harbour tick assemblages in direct proportion to the tick population as a whole.

Statistical analysis

Because singletons (i.e. tick species observed only once) were relatively common among the host species in our dataset, we first explored whether the three indices used were truly independent of sampling effort (i.e. number of host individuals examined). To test for this we performed multiple regressions of the three indices on host body size and sampling effort. To meet underlying statistical assumptions (i.e. linearity of relationship, statistical independence of errors, homoscedasticity of errors, and normality of error distribution), we $\log_{10}$-transformed host body size, sampling effort, and $\hat{S}_{Chao1}$ estimates prior to analyses. We used a logit-transformation for the PS_i index as these values represent proportions. For the adult tick fauna, estimated total species richness ($\hat{S}_{Chao1}$) and true diversity ($e^{H_i'}$) were positively related to sample size, but proportional similarity (PS_i) was not. For the immature tick fauna, all three indices were positively related to sample size (see Appendix 4: Table A4.1). To account for this bias, we substituted each index – with the exception of PS_i for adult ticks – by its residual deviation from a linear regression on sample size, following Poulin (1995) and others (Morand and Poulin 1998, Nunn et al. 2003, Krasnov et al. 2004c, Ezenwa et al. 2006b). Since these

residuals reflect the deviation of expected values under the regression model, they are independent of sample size and can be used as corrected estimates of the three indices (Poulin 1995).

Another confounding factor in comparative analyses is that closely related host species may have similar parasite assemblages and/or may share host traits such as body size through phylogenetic inertia (Felsenstein 1985). That is, host species are expected to co-vary in proportion to the amount of time they share in evolutionary history and have only been evolving independently since they diverged from their most recent common ancestor. Treating host species that share much of their phylogenetic history as statistically independent observations is therefore inappropriate, leading to pseudoreplication and higher Type I error rates (Felsenstein 1985, Harvey and Pagel 1991, Poulin 1995). We tested whether phylogenetic correction was needed by estimating Pagel's λ statistic for host body size and each index of the tick assemblage structure using the R package 'phytools' (Pagel 1999, Revell 2012). Pagel's λ is a scaling parameter that expresses the similarity of the covariances among species relative to the covariances expected under a Brownian motion model of trait evolution. Values range from λ = 0 (no phylogenetic association of traits) to λ = 1 (strong phylogenetic dependence) (Münkemüller et al. 2012). We used log-likelihood ratio tests (LRT) based on Chi-Squared distributions with 1 degree of freedom to determine whether maximum likelihood estimates of λ values for each trait were significantly different from zero (Freckleton et al. 2002). The LRT indicated that more closely related host species tended to have similar body sizes and tick assemblage structures, indicating that phylogenetic correction was necessary (see Appendix 4: Table A4.2).

We corrected for dependence of data points by using Felsenstein's comparative method of phylogenetically independent contrasts (Felsenstein 1985, Pagel 1992, Felsenstein 2008). Assuming a Brownian motion model of trait evolution, this method uses phylogenetic information to contrast the values of all pairs of sister host taxa. Original tip data (i.e. the mean values for a set of host species) are thereby transformed into values that are statistically independent and identically distributed. We computed contrasts using the R package 'ape' (Paradis et al. 2004). Specifically, we implemented the recently extended version of Felsenstein's method, in which an orthonormal transformation is applied to compute contrasts with standardized coefficients (Felsenstein 2008). Phylogenies of the vertebrate species in our data set follow the taxonomy of Wilson and Reeder (2005) with additional resolution derived

from several sources (see Appendix 4: Fig A4.1). The resulting host phylogenies for the adult and immature tick datasets had respectively 19 and 22 taxonomic levels above species. Accurate branch length information were not available for the whole phylogenetic trees and we therefore set all branch lengths to unity. Because the method of independent contrasts requires a fully bifurcating tree, we randomly resolved polytomies into series of bifurcations with zero length branches, following Purvis and Garland (1993). Previous studies have shown that both Pagel's λ statistic and the independent contrasts method are robust to polytomies, missing branch length information, and evolutionary models different from Brownian motion (Diaz-Uriarte and Garland 1998, Münkemüller et al. 2012). Relationships between contrasts of host body size and tick assemblage structure were tested using linear models forced through the origin, following Garland et al. (1992). To evaluate the influence of phylogenetically controlled analyses, we also ran non-phylogenetic analyses using the actual species traits. All analyses were carried out with the R statistical software, version 3.2.4 (R Core Team 2017).

Results

The final dataset for adult ticks included 5,494 records, comprising 211 unique tick-host combinations among 41 tick species from 8 genera and 71 host species from 18 orders. The final dataset for immature ticks included 2,476 records, comprising 246 unique tick-host combinations among 30 tick species from 8 genera and 130 host species from 18 orders. Both datasets contained tick species from the same genera, (i.e. *Amblyomma*, *Antricola*, *Argas*, *Dermacentor*, *Haemaphysalis*, *Ixodes*, *Ornithodoros*, and *Rhipicephalus*) as well as representatives from a diverse range of vertebrate host taxa (i.e. birds, mammals, reptiles, and amphibians), and are available as supplementary file 2 (Online). In the adult tick dataset, mammals were the best represented group in terms of taxonomic diversity of both host and tick species, as well as in having the largest number of records (Table 4.1). In the immature tick dataset, birds were the taxonomically most diverse host group, but mammals had the highest diversity of tick species and the largest number of records (Table 4.1).

More than half (54%) of the records for immature ticks (562 larvae and 776 nymphs) consisted of unpublished data, including a considerable number of tick-host associations that have never been recorded in the literature (Table 4.2). This is mostly due to the fact that the larvae and nymphs of several tick species in our dataset (e.g. *Amblyomma pecarium*, *A.*

sabanerae, *A. tapirellum*) remain as of yet undescribed morphologically, while many others have been described only recently (see Guglielmone et al. 2014). The use of DNA sequencing allowed us to identify the immature stages of these tick species and their respective host associations. New host records include Didelphidae (Didelphimorphia) for larvae of *A. geayi*, *A. longirostre*, *A. naponense*, *A. pacae*, *A. sabanerae*, and for nymphs of *A. oblongoguttatum, A. tapirellum*, *A. sabanerae*; Echimyidae (Rodentia) for larvae of *A. geayi, A. pacae*, *A. naponense, A. sabanerae*, *A. varium*, and for nymphs of *A. naponense, A. sabanerae*, *A. varium;* Dasyproctidae (Rodentia) for nymphs of *A. naponense*, *A. oblongoguttatum*, *A. tapirellum*; Tayassuidae (Artiodactyla) for nymphs of *A. pecarium*, *A. tapirellum*; Atelidae (Primates) for nymphs of *A. geayi*; Equidae (Perissodactyla) for nymphs of *A. oblongoguttatum*; Colubridae (Squamata) and Cricetidae (Rodentia) for larvae and nymphs of *A. sabanerae*; and Teiidae (Squamata) for larvae and nymphs of *Ixodes affinis*. We also report, for the first time, human infestation with nymphs of *A. geayi* and *A. varium* (Table 4.2).

Estimates of total species richness ($\hat{S}_{Chao1}$), true diversity ($e^{H'_i}$), proportional similarity (PS_i), and host body size were positively-skewed and shared similar ranges for both adult and immature ticks faunas. However, the medians were significantly different (Table 4.3). For adults, for example, the 21% 'richest host species' (for which $\hat{S}_{Chao1} \geq 5$) together harboured 71% of all adult tick species. Likewise, for immature ticks, the 18% 'richest host species' (for which $\hat{S}_{Chao1} \geq 3$) together harbored 80% of all immature tick species. Thus, a small proportion of the host species sustained a large proportion of the tick species.

Table 4.1 Summary of the tick-host datasets: number of host orders and species that were sampled, number of observed tick species, and number of records (i.e. tick-host associations) per host group

	Adult ticks dataset				Immature ticks dataset			
	# host orders	# host species	# tick species	# records	# host orders	# host species	# tick species	# tick records
Birds	5	8	7	41	7	68	12	175
Mammals	10	51	38	5215	9	55	27	2252
Reptiles	2	13	2	182	1	5	3	45
Amphibians	1	1	1	56	1	2	3	4

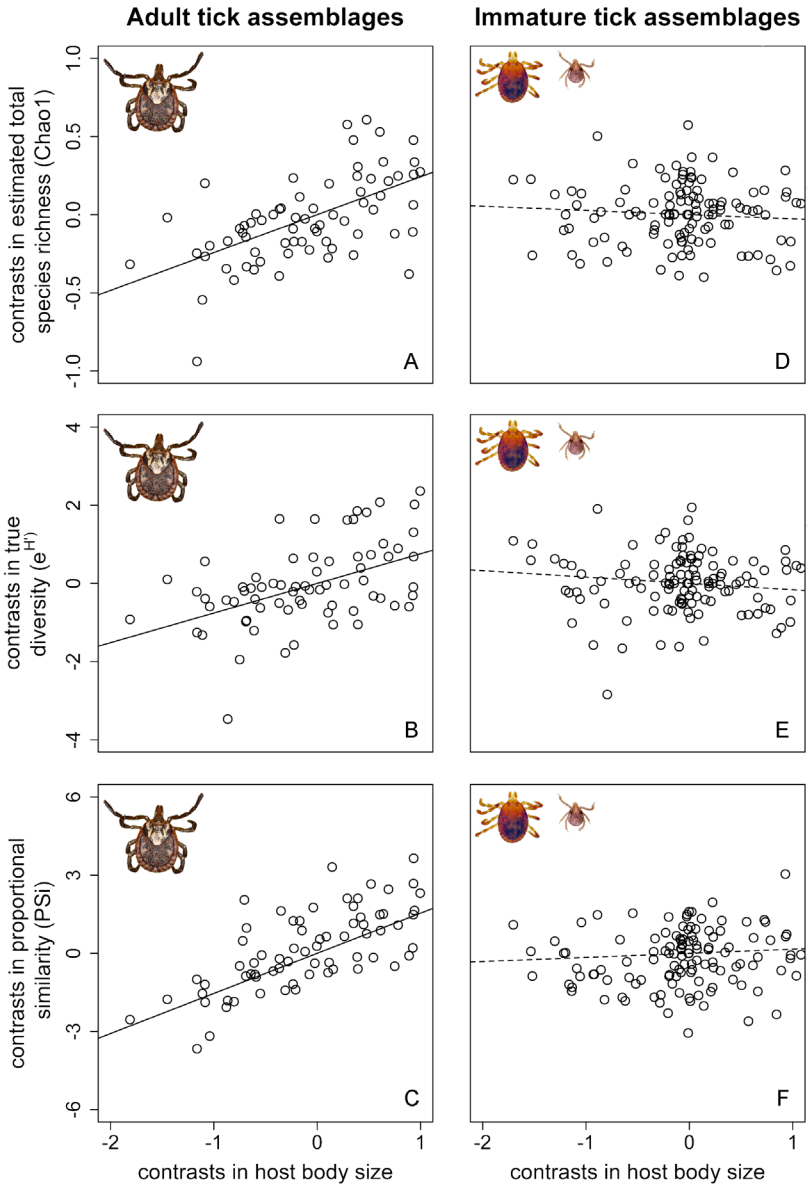


Figure 4.1 Relationship between host body size and the structure of adult and immature tick assemblages. Estimated total species richness **(a)**, true diversity **(b)**, and proportional similarity **(c)** of adult tick assemblages were significantly related to host body size, but no such relationships were found for immature tick assemblages **(d-f)**. Plotted values are phylogenetically independent contrasts. Regressions were run through the origin and corrected for sampling effort.

Table 4.2 Unpublished tick-host associations used in this study.

	Amblyomma auricularium	*Amblyomma dissimile*	*Amblyomma geayi*	*Amblyomma longirostre*	*Amblyomma mixtum*	*Amblyomma naponense*	*Amblyomma oblongoguttatum*	*Amblyomma ovale*	*Amblyomma pacae*	*Amblyomma parvum*	*Amblyomma pecarium*	*Amblyomma sabanerae*	*Amblyomma tapirellum*	*Amblyomma varium*	*Haemaphysalis juxtakochi*	*Ixodes affinis*	*Ornithodoros puertoricensis*	*Rhipicephalus microplus*	*Rhipicephalus sanguineus*
Host species (larvae)																			
Artiodactyla																			
Bos indicus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	0
Carnivora																			
Canis familiaris	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9
Didelphimorphia																			
Didelphis marsupialis	15	15	19	1	11	1	0	6	31	0	0	81	0	4	71	0	0	0	0
Marmosa robinsoni	0	0	1	0	0	0	0	0	0	0	0	3	0	0	0	1	0	0	0
Metachirus nudicaudatus	0	0	0	0	0	0	0	0	1	0	0	19	0	0	0	0	0	0	0
Philander opossum	4	0	0	0	0	0	0	0	1	0	0	24	0	0	0	0	0	0	0
Rodentia																			
Hoplomys gymnurus	2	0	0	0	0	1	0	5	4	0	0	26	0	0	3	0	0	0	0
Proechimys semispinosus	7	16	8	0	0	2	0	15	1	0	0	16	0	5	57	0	52	0	0
Transandinomys talamancae	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0
Squamata																			
Ameiva sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11	0	0	0
Total	28	31	28	1	11	4	0	26	38	0	0	172	0	9	131	12	52	10	9

Table 4.2 Continued

Hosts species (nymphs)																			
Anura																			
Rhinella alata	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Rhinella marina	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Artiodactyla																			
Bos indicus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	18	3
Pecari tajacu	0	0	0	0	0	2	2	0	0	0	6	0	6	0	4	0	0	0	0
Carnivora																			
Canis familiaris	3	0	0	0	4	0	7	14	0	1	0	0	0	0	0	0	0	0	121
Nasua narica	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0	0
Didelphimorphia																			
Didelphis marsupialis	37	2	3	0	31	0	1	18	7	0	0	71	1	0	23	0	0	0	0
Metachirus nudicaudatus	0	0	0	0	0	0	0	0	0	0	0	9	0	0	4	0	0	0	0
Philander opossum	7	1	0	0	0	0	0	1	2	0	0	9	0	0	0	0	0	0	0
Perissodactyla																			
Equus ferus caballus	0	0	0	0	34	0	3	0	0	0	0	0	0	0	0	0	0	0	0
Pilosa																			
Tamandua mexicana	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Primates																			
Alouatta palliata	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Homo sapiens	0	0	0	0	0	0	0	0	0	0	1	0	20	1	10	0	0	0	0
Rodentia																			
Dasyprocta punctata	0	0	0	0	0	13	3	1	0	0	0	0	5	0	40	0	0	0	0

Table 4.2 Continued

Hoplomys gymnurus	2	0	0	0	1	0	0	18	0	0	0	1	0	0	6	0	0	0	0
Hydrochoerus isthmius	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Melanomys caliginosus	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Proechimys semispinosus	9	0	0	0	1	1	0	54	0	0	0	1	0	0	70	0	1	0	0
Transandinomys bolivari	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0
Transandinomys talamancae	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0	1	0	0	0
Squamata																			
Ameiva sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15	0	0	0
Basiliscus basiliscus	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Boa constrictor	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Iguana iguana	0	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leptophis depressirostris	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Total	58	22	4	0	72	16	16	119	9	1	7	93	32	1	165	16	1	18	124

Table 4.3 Summary statistics for the assemblage structure of adult and immature tick fauna and host body size

Variables	Adult ticks			Immature ticks			Mann-Whitney test*	
	range	median	skewness	range	median	skewness	*U*	*P*-value
Tick assemblage structure								
Estimated total species richness	1 – 16.99	2	2.28	1 – 18.99	1	4.46	5614.5	0.005
True diversity	1 – 8.09	1.30	2.29	1 – 6.85	1	2.46	5375	0.034
Proportional similarity	~0.00 – 0.46	0.06	1.18	~0.00 – 0.54	0.04	2.10	5239	0.113
Host body size	4 – 7917.86	208.01	4.26	2.52 – 7917.86	13.12	7.36	7666.5	<0.001

* Mann-Whitney U tests are for differences in the medians between adult and immature tick faunas.

Table 4.4 Results of linear regression between host body size and tick assemblage structure. Results are separated for non-phylogenetic (species values) and phylogenetically controlled (independent contrasts) analyses. Both analyses were controlled for sampling effort.

Tick assemblage structure	Species values				Independent Contrasts			
	F ratio (df)	B	*P*	R^2_{adj}	*F* ratio (df)	β	*P*	R^2_{adj}
Adult tick fauna	(1, 69)				(1, 69)			
Estimated total species richness	37.43	0.24	<0.001	0.34	36.88	0.24	<0.001	0.34
True diversity	21.62	0.75	<0.001	0.23	21.97	0.76	<0.001	0.23
Proportional similarity	77.31	1.77	<0.001	0.52	59.91	1.54	<0.001	0.46
Immature tick fauna	(1, 128)				(1, 128)			
Estimated total species richness	2.89	-0.04	0.092	0.01	0.34	-0.01	0.559	-0.01
True diversity	3.33	-0.19	0.070	0.02	1.59	-0.12	0.210	<0.01
Proportional similarity	0.08	0.04	0.782	-0.01	2.08	0.19	0.152	0.01

Host body size was positively related to the estimated total species richness (Fig 4.1a), true diversity (Fig 4.1b), and proportional similarity (Fig 4.1c) of adult tick assemblages after controlling for the effects of sampling effort and host phylogeny. No such relationship was found for immature tick assemblages (Fig 4.1d-f, Table 4.4). Similar results with only marginal differences were obtained when we did not control for host phylogeny (Table 4.4). When we repeated the analyses with only those host species for which at least 10 individuals were examined, the positive relationships for adult ticks became even stronger, whereas any relationship between host body size and tick assemblage structure was still lacking for immature ticks (see Appendix 4: Table A4.3).

Discussion

Our study provides the first empirical evidence for an increase of species richness and diversity of adult tick assemblages with host body size across a wide range of vertebrate host taxa. The proportional similarity of adult tick assemblages also increased with host body size, indicating that larger host species sustained a larger portion of the total adult tick fauna than smaller host species. In contrast, no such relationships were found for immature ticks, indicating that their assemblage structure is independent of host body size. Correcting for host phylogeny did not alter these relationships. These findings suggest that the adult ticks in this highly diverse system preferentially parasitize larger-bodied host species, whereas the immature ticks may potentially have a more opportunistic feeding strategy.

The contrasting results for adult and immature ticks stresses the importance of analysing their respective host associations independently. Lumping different transmission stages for parasites with complex life cycles may cloud any stage-specific relationship of parasite richness with host body size, particularly if those species are indirectly transmitted (Arneberg 2002). This is clearly demonstrated by our study, in which we found strong relationships of host body size with tick assemblage structure for adult ticks, but not for immature ticks. When host associations for adult and immature ticks were combined, all relationships became non-significant (results not shown). Such lumping could also explain the discrepancies among comparative studies of other parasite groups across a range of birds, mammals, reptiles, amphibians, and fish. While the host body size – parasite species richness relationship was positive in some studies (Gregory et al. 1996, Arneberg 2002, Ezenwa et al.

2006b, Huang et al. 2014), it was less consistent (Poulin 1995, Hughes and Page 2007, Kiffner et al. 2014) or even absent in others (Feliu et al. 1997, Morand and Poulin 1998, Clayton and Walther 2001, Stanko et al. 2002, Nunn et al. 2003, Krasnov et al. 2004c) after host phylogeny was controlled for. Future comparative studies should therefore account for potential variation among different transmission stages by analysing their host associations separately.

What might explain the discrepancy between the host body size – tick diversity patterns between adult and immature ticks? A plausible explanation is higher host specificity in adult ticks for species with a multi-host life cycle. Most of the tick species in our dataset follow a three-host life cycle, in which larvae, nymphs and adults feed from different host individuals that may belong to distinct species (Guglielmone et al. 2014). While immature ticks use these hosts only for feeding, the adults of many tick species also use hosts for finding a mating partner. This difference might drive selection for more host-specific feeding, apparently for larger-bodied species, in adult ticks and more opportunistic feeding in immature ticks (Espinaze et al. 2015). In addition, several morphological and physiological characteristics of adult ticks may facilitate specificity for larger-bodied host species. Adult ticks have larger surface area to volume ratios and are therefore less sensitive to water stress, a major cause of mortality, than the smaller immature ticks (Randolph and Storey 1999). This difference in desiccation risk is reflected by the vertical position at which adult and immature ticks quest for a host: immature ticks tend to stay closer to the more moist conditions at the base of the vegetation, thereby having access to host species of all body sizes, whereas adult ticks quest in higher vegetation layers, where they may miss the smaller host species (Randolph and Storey 1999). Adult ticks also have higher fat reserves and a slower metabolic rate than immature ticks, allowing them to quest and survive for longer periods of time, which in turn increases their chances of encountering large-bodied host species that typically have lower population densities (Randolph 2004).

A meta-analysis on host specificity in Neotropical hard ticks indeed showed that immature ticks tend to be less host-specific than their adult counterparts (Nava and Guglielmone 2013). One hypothesis put forward by the authors is that larvae and nymphs may have greater adaptive plasticity than adult ticks. However, a recent experimental test showed that while immature ticks were less discriminating in their host choice than adults, their engorgement success and survival rates dropped drastically when feeding on atypical host species (Dietrich et al. 2014). Thus, there should be at least some selection for host use during

the immature life stages as well, so that one would expect a relationship, albeit weak, between the assemblage structure of immature ticks and host traits such as body size. Variation in the quality of our dataset could partially account for the lack of such relationship. Over half of our immature tick data originate from published sources that had different objectives and therefore different sampling protocols. Further, while adult ticks are easily spotted and identified morphologically, immature ticks are more easily missed and, in our study region, notoriously difficult to identify based on morphology. As a result, most published studies typically report only a small number of identified immature ticks. Future studies that aim to identify much larger samples of the immature tick fauna on a given host species are much needed if we are to elucidate which host traits determine the assemblage structure of immature ticks.

Alternatively, the assemblage structure of immature ticks may be related to specific host traits that vary *among* individuals of a host species rather than *across* host species. For example, physiological and/or behavioural differences between male and female hosts may explain why some studies found sex-biased parasitism, usually towards male hosts, in both ixodid and argasid immature ticks (Krasnov et al. 2012a). Tick assemblage structure may also show temporal and/or spatial variability, but few studies have examined these variations in the tropics (Lareschi and Krasnov 2010). Future comparative studies in tropical regions should target specific host species for which detailed information can be collected through space and time, including individual host traits (e.g. sex, age, reproductive status) and environmental conditions (e.g. relative humidity, ambient temperature, season). Such studies are then able to test for the independent effects of both intrinsic (host-related) and extrinsic (environmental) factors on the structure of tick assemblages.

Finally, our findings provide additional confirmation that tick-host interactions follow the Pareto principle (Woolhouse et al. 1997), i.e. that a minority of host species harbour a majority of tick species. The highly skewed distribution in which parasites and pathogens are dispersed over their hosts has previously been demonstrated for tick burdens (Kiffner et al. 2011b, Marsot et al. 2012) and tick-borne disease transmission (Perkins et al. 2003). Our results indicate that the same principle also applies to tick species richness, with approximately 20% of the host species harbouring 80% of all tick species, a pattern also known as the '20/80' rule (Woolhouse et al. 1997).

The findings of our study are relevant for human and veterinary health as well as biodiversity conservation. We show that adult ticks preferentially feed from larger-bodied host

species, which suggests that humans and livestock have an increased risk of acquiring tick bites from a wide range of species. On the other hand, large species of wildlife tend to be disproportionally affected by anthropogenic disturbances such as habitat loss and fragmentation (Cardillo et al. 2005). Loss of these species is therefore likely to result in host-tick coextinction events, even if immature stages feed opportunistically (Lafferty 2012). As parasites play critical roles in ecological and evolutionary processes, such losses may profoundly affect ecosystem functioning as well as the long-term persistence of vertebrate hosts by indirectly favouring generalist parasites and pathogens (Gómez and Nichols 2013).

Appendix 4

Table A4.1 Multiple regressions of tick assemblage structure on host body size and sampling effort

Tick assemblage structure	Host body size			Sampling effort		
	β	*t*	*P*	β	*t*	*P*
Adult tick fauna						
Estimated total species richness	0.28	6.90	<0.001	0.23	5.91	<0.001
True diversity	0.87	5.12	<0.001	0.59	3.65	<0.001
Proportional similarity	1.63	7.59	<0.001	0.35	1.72	0.09
Immature tick fauna						
Estimated total species richness	-0.08	-2.25	0.026	0.33	9.21	<0.001
True diversity	-0.33	-2.43	0.017	1.05	7.44	<0.001
Proportional similarity	0.11	0.62	0.535	1.10	5.98	<0.001

Table A4.2 Pagel's λ statistics for the detection of phylogenetic signal

Variables	λ	LRT	*P*
Adult tick fauna			
Host body size	0.51	-597.19	<0.001
Species richness	0.22	-189.51	0.006
True diversity	0.30	-117.88	0.003
Proportional similarity	0.43	-137.25	0.001
Immature tick fauna			
Host body size	0.99	-1068.47	<0.001
Species richness	0.99	-309.10	<0.001
True diversity	0.75	-192.01	<0.001
Proportional similarity	0.82	-232.61	<0.001

Lambda statistics indicate that all indices of tick assemblage structure and host body size showed significant phylogenetic signal (i.e. $\lambda > 0$). Values shown are maximum likelihood estimates for lambda (λ), and their associated log likelihood ratio test (LRT) and *P* values.

Table A4.3 Results of linear regression between host body size and tick assemblage structure. Host species for which fewer than 10 individuals were examined were excluded from the analyses. Results are separated for non-phylogenetic (species values) and phylogenetically controlled (independent contrasts) analyses. Both analyses were controlled for sampling effort.

Tick assemblage structure	Species values				Independent Contrasts			
	F ratio (df)	β	*P*	R^2_{adj}	*F* ratio (df)	β	*P*	R^2_{adj}
Adult tick fauna	(1, 29)				(1, 29)			
Estimated total species richness	42.93	0.34	<0.001	0.58	44.54	0.34	<0.001	0.59
True diversity	21.68	1.42	<0.001	0.41	21.60	1.68	<0.001	0.41
Proportional similarity	52.08	1.79	<0.001	0.63	55.15	1.91	<0.001	0.64
Immature tick fauna	(1, 24)				(1, 24)			
Estimated total species richness	0.24	-0.04	0.628	-0.03	1.36	-0.09	0.256	0.01
True diversity	0.63	-0.27	0.435	-0.02	1.12	-0.41	0.300	<0.01
Proportional similarity	0.16	-0.11	0.690	-0.03	0.01	-0.02	0.936	-0.04

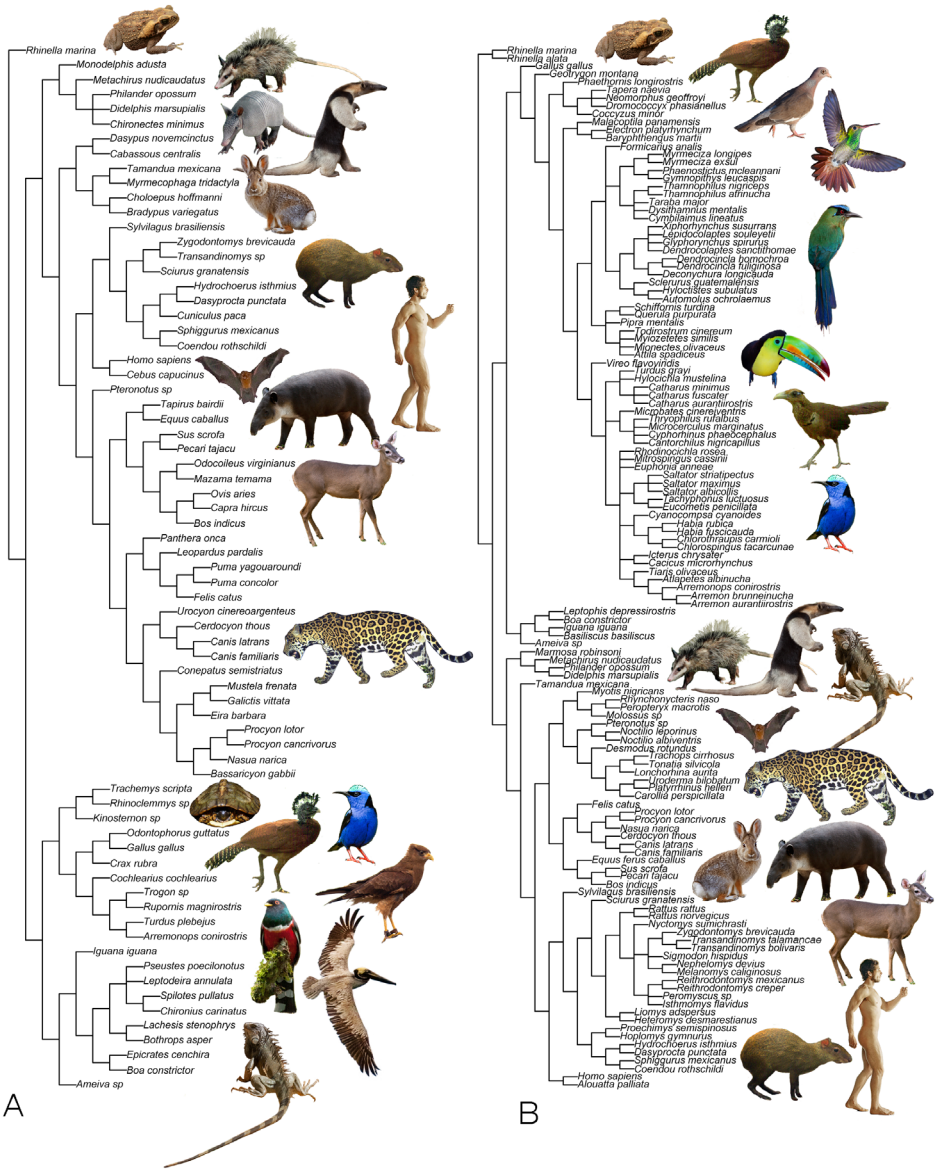
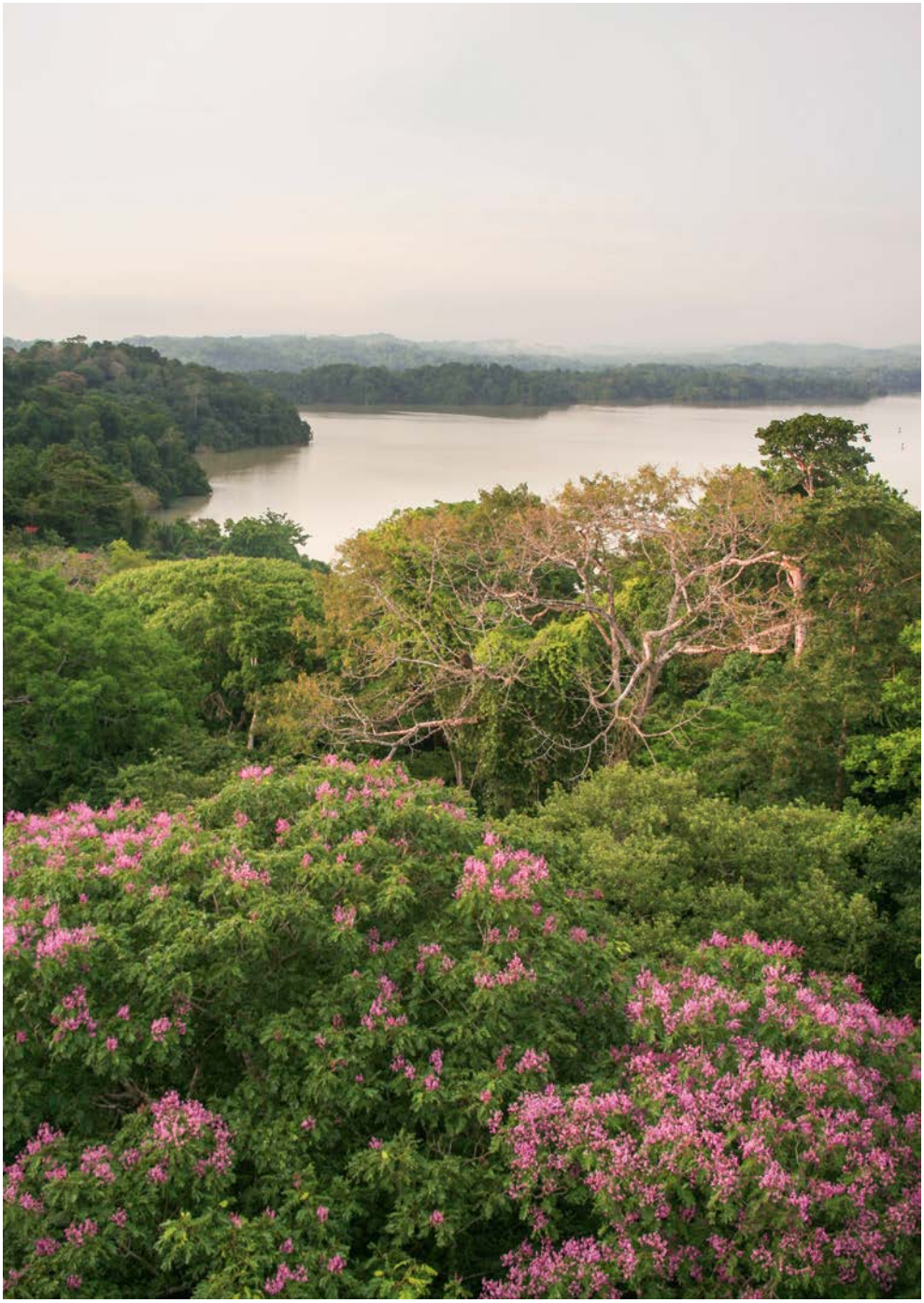


Figure A4.1 Phylogeny of vertebrate hosts for adult ticks **(a)** and immature ticks **(b)**. Branch lengths were set to 1 and thus do not represent actual time since divergence. Polytomies were randomly resolved. We used Wilson and Reeder (2005) as taxonomic reference for Mammalia, supplemented by Huchon and Douzery (2001) for hystricognath rodents, Bardeleben et al. (2005) for Canidae, Fry et al. (2006) for Squamata, Johnson et al. (2006) for Felidae, Weksler (2006) for muroid rodents, Koepfli et al. (2007, 2008) for Procyonidae and Mustelidae, Voss and Jansa (2009) for Didelphidae, Agnarsson et al. (2011) for Chiroptera, Lee (2013) for Testudines, and Jarvis et al. (2014) for Aves.



Chapter 5

Host–parasite coextinction: evidence from tick-host communities and implications for disease transmission

Helen J. Esser, Edward Allen Herre, Roland Kays, Yorick Liefting, and Patrick A. Jansen

Abstract

Theory predicts that the diversity and abundance of ticks and their hosts should be coupled, and that less diverse tick communities should be dominated by generalist species. Despite the relevance to the transmission of tick-borne pathogens, direct empirical tests of these hypotheses are lacking. Therefore, we surveyed the diversity and abundance of tick and vertebrate host communities across 12 previously connected forest fragments along the Panama Canal that ranged 1000-fold in size. We found that the abundance and species richness of ticks was positively related to that of wildlife. Moreover, specialist tick species were only present in fragments where their specific reproduction hosts were captured by camera traps, suggesting local host-tick coextirpation in fragments were both were absent. Further, less diverse tick communities were dominated by *A. oblongoguttatum*, a strong host generalist tick species. Our results indicate that loss of wildlife has cascading effects on tick communities through local host-parasite coextirpation. Dominance of generalist tick species that results from wildlife diversity loss could facilitate interspecies pathogen transmission.

Introduction

Extinction cascades form one of the most insidious, but often-ignored drivers of biodiversity loss (Dunn et al. 2009). By eliminating organisms that are essential to the survival of others, the initial loss of keystone or host species can catalyse secondary extinctions throughout ecological communities (Colwell et al. 2012). Parasites are expected to be particularly prone to local coextinction because they need minimum thresholds of host abundance in order to maintain viable populations (Dobson et al. 2008). This is especially likely for parasites that show strong host specificity and/or have complex life cycles involving multiple host species (Koh et al. 2004, Lafferty 2012). Thus, as host species are lost, parasite communities are expected to reach the point that only species with low host specificity remain (Dobson et al. 2008, Dunn et al. 2009, Lafferty 2012).

The existence and form of host-parasite coextinction events are highly relevant for disease risk, as alterations to parasite community composition will affect pathogens vectored by these parasites. Many pathogens – particularly those of medical and veterinary importance – are capable of infecting multiple host species (Woolhouse et al. 2001). If loss of host species (e.g. through habitat fragmentation or hunting) is accompanied by an increase in the abundance of generalist vectors, these pathogens may be facilitated. First, generalist vectors are able to exploit a multitude of host species per definition, thereby promoting interspecific pathogen transmission (Ostfeld and Keesing 2012). Second, generalist vectors may feed proportionally more from disease-reservoir hosts in degraded wildlife communities, further increasing the potential for disease emergence (Allan et al. 2003, Keesing et al. 2010, Gottdenker et al. 2012). Therefore, understanding how host species loss affects the species composition and functional properties of communities of parasitic vectors, and hence the opportunities for pathogen transmission, is critically important.

Here, we focus on local coextinction of ticks with their hosts across a size gradient of forest fragments. Ticks are obligatory ectoparasites of terrestrial vertebrates – mammals, birds, reptiles and sometimes amphibians – and are known to transmit a wide variety of pathogenic microorganisms (Jongejan and Uilenberg 2004). Their life cycle is characterized by multiple developmental stages and they are known to exhibit host specificity to various degrees, especially as adults (Esser et al. 2016b). Because of their reliance on vertebrates and high mortality when they fail to find a host, ticks should be strongly affected by local loss of wildlife

(Ostfeld and Keesing 2012). Thus, larger and more diverse host communities should also harbour larger and more diverse tick communities.

Past empirical studies often correlated variation in the abundance of questing ticks with forest fragment size as a proxy for host abundance and diversity (Allan et al. 2003, Brownstein et al. 2005, Ogrzewalska et al. 2011). These forest fragments were typically situated in agricultural or suburban landscapes, where abundances of some host species may be elevated by external nutrient subsidies, or conversely, species loss may be exacerbated by increased hunting pressure, hence distorting expected biodiversity patterns based on island biogeography theory (Mendenhall et al. 2014). Other studies correlated the abundance of questing ticks with that of only one or a few focal host species (Rand et al. 2003, Ostfeld et al. 2006, Tagliapietra et al. 2011, Kilpatrick et al. 2014), thereby ignoring how the abundances and diversities of other, non-focal species in the host community may affect tick communities. Only rarely have studies directly assessed the abundance and species composition of broader host communities in relation to tick abundance (Szabó et al. 2009, Hofmeester 2016). But to our knowledge, no empirical study so far has tested how the species diversity of tick communities is affected by loss of host diversity.

Here, we address these limitations by using drag sampling and camera trapping to directly assess the abundance and diversity of broader communities of both questing ticks and their hosts across 12 previously interconnected forest fragments adjacent to the Panama Canal. These fragments range a thousand fold in area, are largely surrounded by water and relatively well protected from poachers, providing an ideal opportunity for a natural experiment. We hypothesized that larger and more diverse host communities supported larger and more diverse tick communities, with less diverse communities dominated by generalist tick species.

Methods

Study site

Fieldwork was carried out in the Barro Colorado Nature Monument (BCNM, 9°10'N, 79°51'W), a nature reserve that comprises 5400 ha of forested islands and peninsulas in the Gatun Lake section of the Panama Canal, Panama. The fragments were part of a continuous forest until ~100 y ago, when damming of the Chagres River produced forest islands and peninsulas that range widely in size and thus the wildlife communities that they could support (Asquith et al.

1997). The eastern peninsulas are separated from the nearby Soberania National Park by narrow land bridges and the clearings of the Panama Canal Railroad, whereas the western peninsulas are surrounded by pastureland and *Tectona grandis* teak plantations, inhospitable to forest wildlife. All sites support semi-deciduous tropical moist forests and are characterized by a seasonally moist tropical weather pattern (Leigh 1999). The largest of the islands, the 1560 ha Barro Colorado Island (BCI), supports a rich fauna with abundant populations of medium- to large-sized mammals such as the lowland paca *Cuniculus paca*, Central American agouti *Dasyprocta punctata*, collared peccary *Pecari tajacu* and Central American red brocket deer *Mazama temama* (Meyer et al. 2015). In contrast, the smallest islands within the BCNM do not have permanent populations of large mammals and some are too small to support even medium-sized mammals (Asquith et al. 1997). Ticks are abundant in Panama, both in species and in numbers. For most species, tick-host associations are relatively well-documented (Chapter 2 – 4).

Sampling

We sampled 12 forest fragments that ranged in size from 2.6 to 2811 ha (Fig 5.1). The sampling period ran from Jan – March 2010, when tick abundance peaks in central Panama. Fragment size was calculated in ESRI ArcGIS 9.3.1 from a Digital Elevation map with a resolution of 10 m (central Panama GIS coverage DVD, Version 2, 2007-04-25, Panama Canal Monitoring Project, USAID, the Smithsonian Tropical Research Institute and USGS WEBB program). Depending on the size of the fragment, we established between one and six 1-ha sampling plots per fragment.

Wildlife surveys – We used arrays of camera traps (Kays et al. 2011) to estimate wildlife abundance and diversity for each plot. These cameras photograph animals that pass in front of a passive infrared sensor, and thus record species presence as well as the passage rate of species. Camera trapping is a reliable and non-invasive technique for the survey of medium- to large-sized terrestrial mammals and birds in all environmental conditions (Tobler et al. 2008, Kays et al. 2011). We used Reconyx RC55 (Reconyx Inc, Holmen, WI) camera traps with a built-in infrared flash, a passive infrared motion sensor, and a 1/5th second trigger speed. Cameras were active 24/7 and took sequences of ten consecutive pictures per trigger, with no delay between triggers. A 1-GB flash memory card stored the photographs with the time and dates of each event. At each of ten pre-generated random points across the sampling plots, we ran

one unbaited camera trap for at least 8 d. Cameras were attached to tree stems 30 cm above ground level and were spaced at least 10 m apart, pointed to the direction with the most open understorey. We used the walk test function of the cameras to measure the maximum detection distance for each camera as an estimate of the area surveyed. Detection distances ranged from 2-12.5 m (median: 5 m) among cameras, depending on vegetation density.

All photographs were uploaded to and processed in a custom-made database (Kays et al. 2011). Pictures were grouped such that each sequence represented the passage of one individual animal or a group of social animals. Animal identifications were based on Reid (2009). Birds, primates, and reptiles were excluded, except for the great tinamou *Tinamus major*. We used encounter rates as a proxy for relative host abundance. This measure reflects the rate at which ticks waiting in the understory encounter a potential host. Encounter rates were calculated for each species by dividing the number of captures by the length of camera deployment (in days) and the distance (in m) over which the species was effectively detected. This "effective detection distance" depends on body size (larger detection distances for larger species) and sampling season (shorter detection distances at high humidity)(Rowcliffe et al. 2011). Effective dry-season detection distance was calculated for each species as $d = 0.10\ BM + 2.84$, where BM is the body mass (cf Rowcliffe *et al.* 2011). Effective detection distances ranged from 2.8 m (for Robinson's mouse opossum *Marmosa robinsoni*) to 6.4 m (for white-tailed deer *Odocoileus virginianus*). If the maximum detection distance at a camera point was smaller than d due to vegetation blocking the view, we corrected for this by truncating d to the smaller distance.

Encounter rates were subsequently used to calculate wildlife biomass per plot (kg/m/d) by summarizing the product of encounter rate and average body mass per species in each plot. Wildlife biomass should explain changes in tick community composition better than host abundance, as it captures information on the presence of larger vertebrates, which are important reproduction hosts for many tick species (Chapter 4). Average body mass values for each species were obtained from Reid (2009) and Rowcliffe *et al*. (2011).

We used the first-order Jackknife (Jack1) index, an asymptotic estimator of species richness, to compute the number of species that would be expected under exhaustive sampling using EstimateS version 9.1.0 (Colwell 2013). This index is considered more suitable for camera trapping data than the Chao indices (Tobler et al. 2008). Jack1 values were estimated by lumping all camera traps per fragment, with each camera trap representing a sampling unit.

Outcomes were compared to the number of species that were actually observed to determine what fraction of the total wildlife species richness was captured by camera traps. Jack1 values that are equal to the observed species richness indicate that the accumulation of species has reached an asymptote (Colwell 2013).

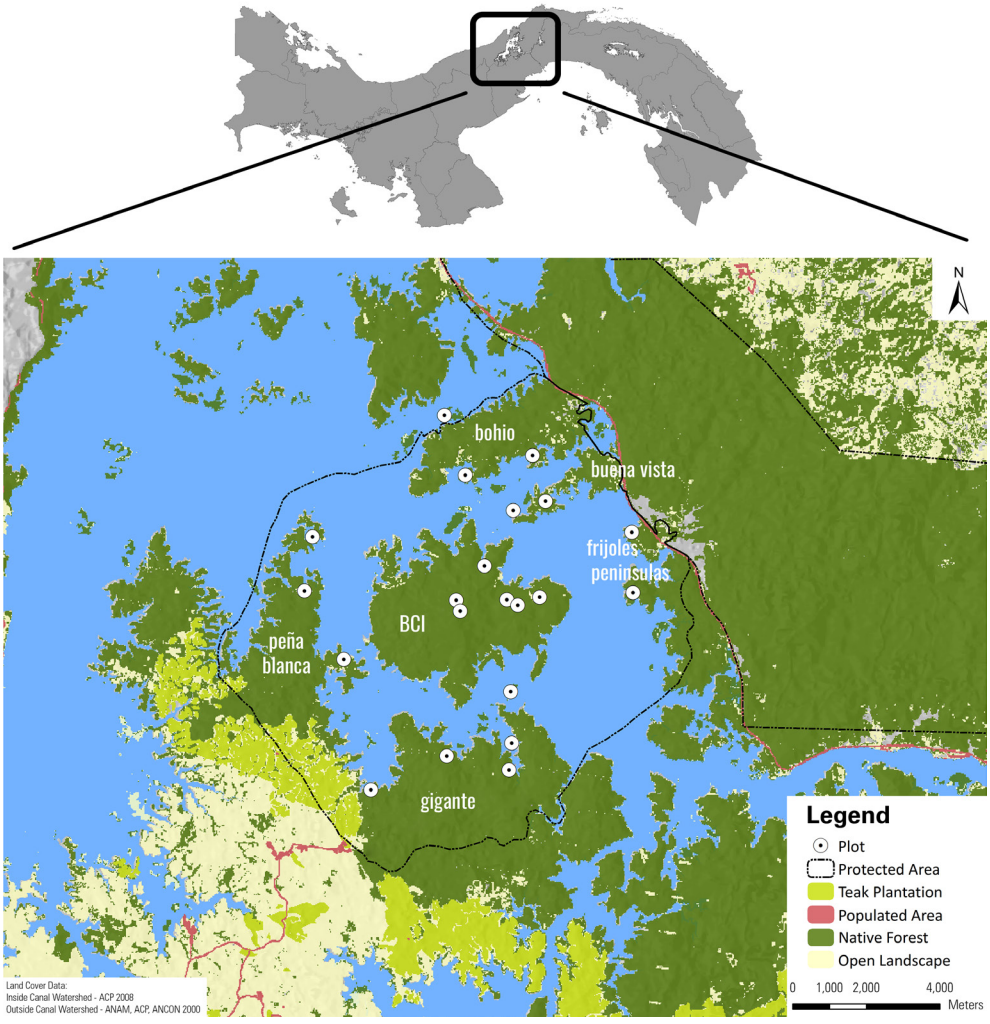


Figure 5.1 Map of the study area. A total of 21 plots across 12 forest fragments were sampled for ticks and wildlife in the Barro Colorado Nature Monument, Gatun Lake, Panama.

Tick surveys – We used drag sampling (Falco and Fish 1992) to estimate the community composition of questing ticks in each plot. This method involves pulling a white cotton cloth of 1 m wide over the ground and through the vegetation along a line transect. We sampled four 50-m transects in each 1-ha plot, totalling 200 m^2. Every 5 m, ticks were removed from the cloth with masking tape and stored in 97% ethanol. We collected all ticks between Jan 22 and Feb 1, which is during the dry season when tick numbers are particularly high. Ticks were identified at the Gorgas Memorial Institute in Panama City, using an extensive reference collection and taxonomic keys provided by Fairchild *et al.* (1966) and Onofrio *et al.* (2006). Tick species richness was determined after lumping all identified ticks per fragment.

Statistical analyses

We used a linear mixed model (LMM) as implemented in the *nlme* package (Pinheiro et al. 2017) to evaluate the relationship of wildlife biomass and forest fragment size. Forest fragment size was $\log_{10}$-transformed prior to analyses and fragment was entered as random factor with varying intercept. We used a linear model (LM) to evaluate the relationship between the estimated total wildlife species richness (Jack1) per fragment and $\log_{10}$-transformed fragment size, weighted by the number of plots in each fragment.

We used generalized linear mixed models (GLMM) with a negative binomial distribution and log-link function as implemented in the *glmmADMB* package (Fournier et al. 2012) to test how densities of adults, nymphs, and larvae were related to wildlife species richness and biomass. Wildlife species richness and biomass were standardized prior to analyses by extracting the mean and dividing by two standard deviations following Gelman (2008). Fragment was entered as random factor with varying intercept. We used a linear model (LM) to test how the total number of tick species in each fragment was related to wildlife species richness and biomass, weighted by the number of plots. Wildlife species richness and biomass were standardized prior to analyses by extracting the mean and dividing by two standard deviations (Gelman 2008).

We used a GLMM with a binomial distribution and logit link function to test how the proportional abundance of the only true generalist tick species in this study (*A. oblongoguttatum*, Chapter 2) was related to tick species richness. Fragment was entered as random factor with varying intercept. Finally, we used Mann-Whitney U tests to determine whether the abundance of specialist tick species (*A. naponense*, *A. tapirellum*, *H. juxtakochi*)

was constrained to fragments where their reproduction hosts (peccary, peccary, and deer, respectively) were present. All analyses were carried out in R 3.3.3. (R Core Team 2017).

Results

The total camera-trapping effort amounted to 1,717 camera-days. Camera traps captured 4,017 animal passages in total, of which 3,308 concerned semi-terrestrial birds and mammals that were retained in the analysis. The total number of species recorded was 21, among which agouti, peccary and paca were the most common (Table 5.1). There were clear differences in wildlife community among the fragments, with some species (e.g. the northern tamandua *Tamandua mexicana* and brocket deer) recorded mostly on the largest islands, and others (e.g. white-tailed deer, brown four-eyed opossum *Metachirus nudicaudatus*, and forest rabbit *Sylvilagus brasiliensis*) found exclusively on the mainland peninsulas (see Appendix 5: Table A5.1). No large mammals (>10 kg) or carnivores were recorded in the four smallest fragments, with the exception of tayra *Eira barbara* on one of these islands. At the smallest of the islands (2.6 ha), we photographed just two species: Tome's spiny rat *Proechimys semispinosus* and nine-banded armadillo *Dasypus novemcinctus* (see Appendix 5: Table A5.1).

The estimated total wildlife species richness (Jack1) ranged from 2 in the smallest fragment to 17.7 in the largest, and increased with fragment size (LM: $R^2 = 0.71$, $\beta = 3.89$, $P < 0.001$, Fig 5.2a). For most fragments, Jack1 estimates were equal or close to the actual observed number of species (Table 5.2). The cameras recorded 73 – 100% of the estimated total number of species present at each fragment. Wildlife biomass ranged more than 26-fold across fragments (0.3 to 7.9 kg $d^{-1}m^{-1}$) and increased with fragment size (LMM: $\beta = 2.04$, $P < 0.01$, Fig 5.2b).

A total of 21,262 ticks were collected from the 12 fragments, including 18,336 larvae, 2,596 nymphs, and 330 adults (Table 5.3). Immature stages of *Haemaphysalis* and *Amblyomma* formed the majority of all ticks captured (60% and 38% respectively). The total number of tick species ranged from 2 to 7 per fragment (Table 5.2) and increased with wildlife species richness and biomass (LM: $R^2 = 0.35$, wildlife richness: $\beta = 0.25$, $P < 0.05$, wildlife biomass: $\beta = 0.52$, $P < 0.05$; Fig 5.3a-b). Tick abundance varied among fragments and between life stages (Table 5.2), and increased with wildlife species richness for each developmental stage (GLMM: adults: $\beta = 1.12$, $P < 0.01$, nymphs: $\beta = 1.37$, $P < 0.05$, larvae: $\beta = 1.78$, $P < 0.01$, Fig 5.3c). Tick abundance

also increased with wildlife biomass, but this relationship was significant for nymphs only (GLMM: adults: $\beta = 0.47$, $P > 0.05$, nymphs: $\beta = 0.99$, $P < 0.05$, larvae: $\beta = 0.74$, $P > 0.05$, Fig 5.3d).

A total of six tick species from three genera were identified to the species level (Table 5.3). Of these species, five are known as strongly host specific during the adult stage, while only one species – *A. oblongoguttatum* – is a true generalist (Chapter 2). *A. oblongoguttatum* occurred even in one of the smallest fragments, where no other adult ticks were found. The proportion of *A. oblongoguttatum* in each fragment decreased with tick species richness (GLMM: odds ratio = 0.62, $P < 0.05$; Fig 5.4). In contrast, two of the five host-specific tick species occurred only in one or two large fragments. The other three host-specific species, which occurred in at least 5 different fragments, were found only where their reproduction host species were present (one-tailed Mann-Whitney U test, *H. juxtakochi*: U = 2, $P < 0.01$, *A. naponense*: U = 6, $P < 0.05$, *A. tapirellum*: U = 6, $P < 0.05$; Fig 5.5). Loss of specialists and dominance of generalist tick species with fragmentation agrees with the hypothesis.

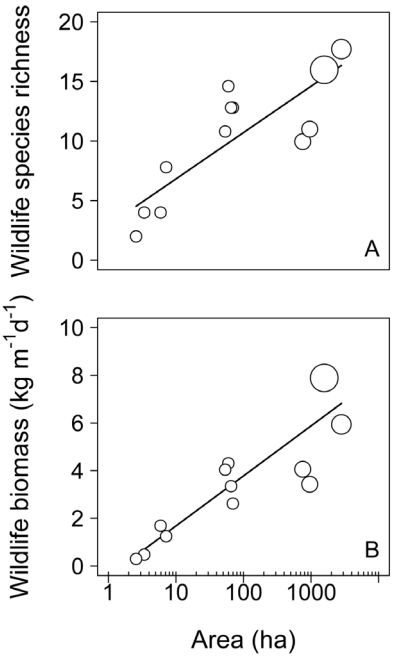


Figure 5.2 Species richness and biomass of wildlife communities across 12 different-sized forest fragments. Wildlife **(a)** total species richness and **(b)** biomass increased significantly with forest fragment size. Dot size is proportional to sampling intensity (i.e. number of plots).

Table 5.1 Wildlife recorded by arrays of non-baited camera traps across 12 forest fragments in the Barro Colorado Nature Monument, Panama.

Species	Common name	No. captures	% of all captures	No. fragments	Smallest fragment (ha)
Dasyprocta punctata	Central American agouti	1536	46.4	11	3.4
Pecari tajacu	collared peccary	670	20.3	8	65.5
Cuniculus paca	lowland paca	340	10.3	11	3.4
Nasua narica	white-nosed coati	203	6.1	8	65.5
Mazama temama	red brocket deer	119	3.6	3	69.6
Proechimys semispinosus	spiny rat	98	3.0	11	2.6
Odocoileus virginianus	white-tailed deer	97	2.9	6	65.5
Dasypus novemcinctus	nine-banded armadillo	51	1.5	10	2.6
Didelphis marsupialis	common opossum	39	1.2	9	7.2
Felis pardalis	ocelot	35	1.1	5	65.5
Tinamus major	great tinamou	32	1.0	5	7.6
Sciurus granatensis	red-tailed squirrel	27	0.8	5	53.6
Mouse sp.	unknown mouse sp.	15	0.5	2	753.1
Tamandua mexicana	northern tamandua	13	0.4	2	5.9
Metachirus nudicaudatus	brown four-eyed opossum	12	0.4	2	69.6
Eira barbara	tayra	9	0.3	4	3.4
Conepatus semistriatus	striped hog-nosed skunk	4	0.1	1	956.0
Cabassous centralis	northern naked-tailed armadillo	3	<0.1	2	69.6
Marmosa robinsoni	Robinson's mouse opossum	2	<0.1	1	1567.3
Sylvilagus brasiliensis	forest rabbit	2	<0.1	1	65.5
Puma yagouaroundi	jaguarundi	1	<0.1	1	65.5

* Nomenclature follows Wilson & Reeder (2005).

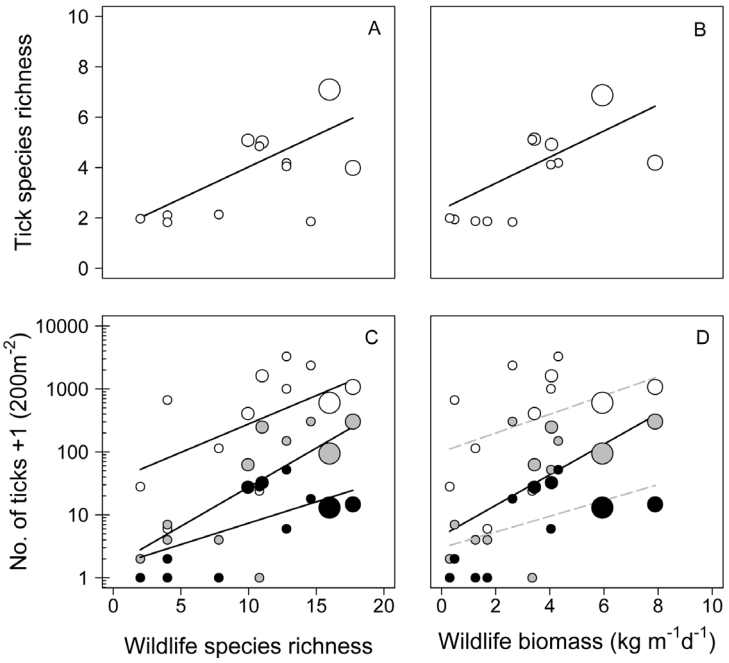


Figure 5.3 Tick species richness increased significantly with **(a)** wildlife species richness and **(b)** wildlife biomass. Abundances of questing adults (*black*), nymphs (*grey*) and larvae (*white*) also increased significantly with **(c)** wildlife species richness and with **(d)** wildlife biomass, although the latter relationship was only significant for nymphs. Dot size is proportional to sampling intensity (i.e. number of plots).

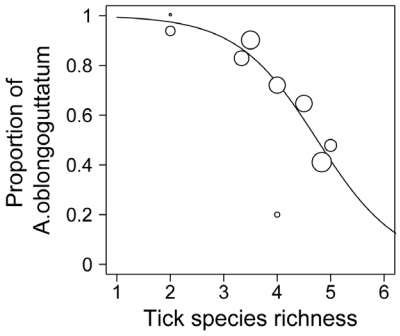


Figure 5.4 The proportional abundance of *A. oblongoguttatum*, the only true generalist tick species in this study, declined when tick communities became richer in species. Dot size is proportional to sample size (i.e. the number of adult ticks).

Table 5.2 Data on wildlife and tick communities across 12 different-sized forest fragments in the Barro Colorado Nature Monument, Panama.

			Wildlife			Questing ticks			
Fragment	No. plots	Area (ha)	No. observed species	No. estimated species (Jack1)	Biomass (kg $m^{-1}d^{-1}$)	Adult density (200 m^{-2})	Nymphal density (200 m^{-2})	Larval density (200 m^{-2})	No. species
Gigante	3	2811.3	13 (17.71)	17.71	5.94*	13.7*	301.3*	1072.3*	4
BCI	6	1567.3	15 (15.98)	15.98	7.89*	12*	93.3*	601.5*	7
Peña Blanca	2	956.0	11 (11)	11	3.43*	31.5*	247*	1615*	5
Bohio	2	753.1	9 (9.95)	9.95	4.06*	26.5*	61.5*	405.5*	5
Frijoles Point	1	69.6	11 (12.8)	12.8	2.62	51	148	3275	4
Frijoles Island	1	65.5	11 (12.8)	12.8	3.35	5	51	1000	4
Palenquilla	1	59.6	11 (14.6)	14.6	4.31	17	303	2361	2
Buena Vista	1	53.6	9 (10.8)	10.8	4.04	27	0	23	5
Palm Island	1	7.2	6 (7.8)	7.8	1.25	0	3	113	2
Mona Grita	1	5.9	4 (4)	4	1.69	0	3	5	2
Trap Island	1	3.4	4 (4)	4	0.48	1	6	665	2
Refuge Island	1	2.6	2 (2)	2	0.30	0	1	27	2

* Average across plots

Table 5.3 Ticks captured with standardized drag sampling across 12 forest fragments in the Barro Colorado Nature Monument, Panama.

Species	Larvae	Nymphs	Adults (male)	Adults (female)	% of all captures	No. fragments
Amblyomma	7450	612	-	-	37.9	12
A. naponense	-	-	14	14	0.1	6
A. oblongoguttatum	-	-	117	106	1.0	9
A. ovale	-	3	-	-	<0.1	1
A. pecarium	-	-	-	2	<0.01	2
A. tapirellum	-	-	31	26	0.3	5
Haemaphysalis						12
H. juxtakochi	10,849	1981	11	8	60.4	12
Ixodes	37	-	-	-	0.2	1
I. affinis	-	-	1	-	<0.01	1
Total	18,336	2596	174	156	100	12

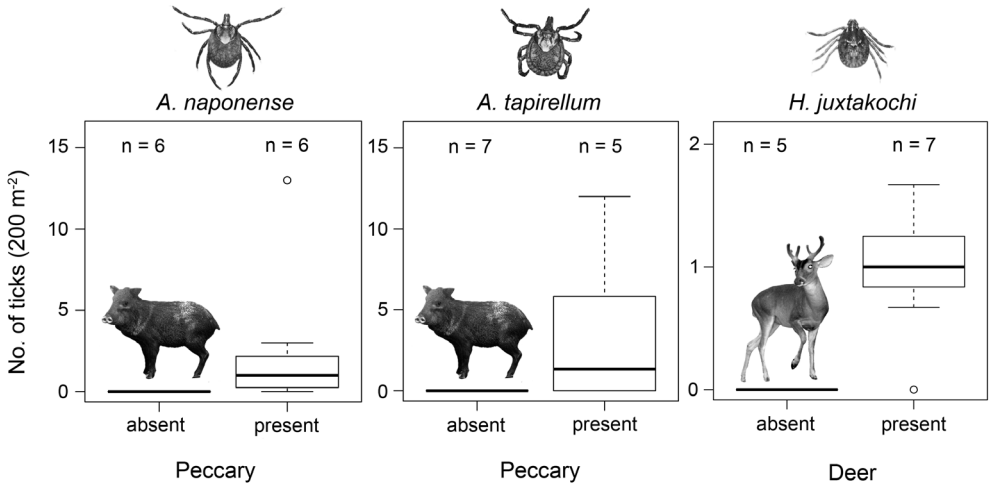


Figure 5.5 Median abundances of specialist tick species in fragments with and without their specific host species. Specialist tick species were only present when their reproduction host species was present. Deer includes both brocket deer and white-tailed deer.

Discussion

Our direct measures of the relative abundance and diversity of both wildlife and questing tick communities across 12 forest fragments in Panama allowed us to perform the first empirical tests for relationships between local host and tick coextirpation. Larger forest fragments had larger and more diverse wildlife communities. In turn, larger and more diverse wildlife communities supported larger and more diverse tick communities. Tick species known to exhibit high host-specificity as adults were absent in fragments in which their hosts were not recorded so that species-poor communities were dominated by a generalist tick species. Our results provide empirical evidence supporting these theoretical predictions and indicate that local loss of wildlife has cascading effects on tick communities through local host-parasite coextirpation.

Tick species richness

Theoretically, the presence of multiple life stages, strong host specificity, and higher extinction risk for hosts than non-hosts should all increase parasite coextinction risk (Lafferty 2012). For example, ticks that use large mammals as principal reproduction hosts should be more vulnerable to local coextirpation because these host species need sufficiently large habitat to maintain viable population sizes, and are thus the first to disappear from small and isolated fragments (Meyer et al. 2015).

Consistent with previous studies and island biogeography theory, wildlife species richness and biomass-weighted abundance increased with fragment size (Andrén 1994, Turner 1996, Chiarello 1999, Fahrig 2003, Kinnaird et al. 2003). In turn, tick species richness increased linearly with wildlife species richness and biomass. This is in agreement with theoretical predictions (Lafferty 2012), and consistent with expectations based on earlier studies that showed that adult ticks in this region are highly host specific (Esser et al. 2016b), and that larger hosts support more diverse adult tick communities (Esser et al. 2016a). Indeed, five out of seven tick species that were collected are known to have only one or two primary reproduction hosts (Esser et al. 2016b). We note that the observed loss of tick species emerged despite the potential for re-colonization of ticks via host movement among fragments. Similar patterns were found in a study that focussed on birds and their lice across forest fragments in southern

China (Bush et al. 2013). Clearly, the continuous presence of reproduction hosts is indispensable for sustaining tick populations.

Tick abundance

Most hosts have few ticks and only some have many, producing a roughly negative binomial distribution of ticks among their hosts (Brunner and Ostfeld 2008). Further, given that ticks depend on hosts for their development and survival, tick abundance is widely believed to increase with host abundance (Ostfeld and Keesing 2012). We found that tick abundance increased with both wildlife species richness and biomass, although the latter relationship was significant only for nymphs. For adults, increased abundance with wildlife species richness can be attributed to their strong host specificity, so that richer host communities can support more diverse and hence larger tick communities. For nymphs and larvae, which tend to be more opportunistic in host selection than adults (Hoogstraal and Aeschlimann 1982), this relationship can be explained in that a higher diversity of host species implies more feeding opportunities (Schmidt and Ostfeld 2001), possibly from higher-quality hosts (Keesing et al. 2009).

Our results are consistent with those of Ogrzewalska *et al.* (2011), who found that abundances of questing ticks increased with fragment size across 12 Brazilian forest fragments. Although primary reproduction hosts were reported to be absent from the smallest fragments in that study, it had no quantitative data on the broader terrestrial host communities to test for relationships between the community composition of ticks and that of their hosts across the fragments, which – in addition – were isolated by agricultural land instead of water (Ogrzewalska et al. 2011). Our results agree with and extend those previous findings by providing direct abundance and diversity estimates of hosts and ticks.

Implications for disease emergence

Our finding that less diverse communities were dominated by more generalist ticks may have implications for tick-borne pathogen transmission, as generalist vectors are more likely to transmit pathogens among different host species (Ostfeld and Keesing 2012). Specifically, we found that the relative abundance of *A. oblongoguttatum* was higher as the tick community became more depauperate. This species was the only true host generalist tick in this study and has been recorded from at least 27 species within 9 different orders, including humans,

livestock, pets, and wild animals, several of which are habitat generalists (Esser et al. 2016b). Flexibility in host selection apparently allows this species to persist in even the most degraded wildlife communities, where specialist ticks disappear. Although the potential role of *A. oblongoguttatum* as vector of human pathogens has been understudied, spotted fever group rickettsiae have been isolated from this species during a first analysis (Bermúdez et al. 2009). Moreover, generalist vectors are responsible for transmitting most infectious diseases (Ostfeld and Keesing 2012) and feed proportionally more from disease reservoir hosts in impoverished wildlife communities (Allan et al. 2003, Keesing et al. 2010, Gottdenker et al. 2012).

On the other hand, inference is complicated by the fact that the increasing dominance of generalist ticks was accompanied by a sharp reduction of tick abundance. Lower tick abundance reduces the rate at which individual hosts encounter ticks. Since disease risk is the product of the likelihood of tick encounter and the likelihood of tick infection (Randolph and Dobson 2012), the relative strength of each of the two responses (i.e. higher proportions of generalists vs lower tick abundance) will ultimately determine whether loss of wildlife either increases or decreases the risk to which individuals are exposed when crossing these fragments.

Future directions

The combined methods of tick drag sampling and camera trapping provide a new and informative community-wide approach for the study of tick-host relationships (Hofmeester 2016). However, it is important to also recognize limitations of our methods, and future studies should be designed with the following factors taken into consideration. First, our sampling focused on terrestrial mammals. Wild birds are known hosts for immature stages of Neotropical ticks (Miller et al. 2016), and we included only large terrestrial birds in our estimate of host species richness and abundance. In addition, birds may help recolonize diminishing tick populations on small islands and fragments by dispersing ticks from adjacent mainland sites. Second, complete tick-host associations can only be established by directly collecting ticks from individual host animals. Many species of ticks known to be common on the host species that we recorded with cameras were not present among the identified ticks, possibly because the drag sampling method is not as effective for collecting those tick species. Future studies should ideally use a combination of drag sampling and live trapping to collect ticks from both the free environment and directly from hosts.

Concluding remarks

We provide empirical evidence for a positive relationship between tick and wildlife abundance and diversity, as well as for local coextirpation of ticks with their hosts following forest fragmentation. Our finding that impoverished tick communities exhibited a higher relative abundance of generalists implies that opportunities for pathogen transmission may be higher in habitats with degraded wildlife communities, as generalist ticks feed proportionally more from disease reservoir hosts in degraded environments (Allan et al. 2003, Keesing et al. 2010, Gottdenker et al. 2012). If these patterns are general, then ticks may be useful bioindicators of ecosystem health, with low tick abundance and diversity reflecting low wildlife diversity (Ogrzewalska et al. 2011, Lafferty 2012, Bush et al. 2013) and a potentially elevated risk of interspecific disease transmission.

Appendix 5

Table A5.1 Encounter rates (in $m^{-1}d^{-1}$ x 100) and body weight (kg) of wildlife recorded by arrays of non-baited camera traps across 12 forest fragments in the Barro Colorado Nature Monument, Panama. Values shown are averages across fragment.

	Weight (kg)	Gigante	BCI	Peña Blanca	Bohio	Frijoles Point	Frijoles Island	Palenquilla	Buena Vista	Palm Island	Mona Grita	Trap Island	Refuge Island
Dasyprocta punctata	3.5	24.5	42.0	18.7	37.8	16.0	32.8	19.7	33.8	21.9	18.8	6.0	0.0
Cuniculus paca	8.5	3.8	4.0	2.0	16.5	4.1	11.1	5.5	2.7	5.2	11.8	2.5	0.0
Proechimys semispinosus	0.4	0.1	3.7	0.4	1.1	5.7	2.2	0.9	0.9	0.9	0.0	2.7	4.1
Pecari tajacu	19.0	11.7	18.1	6.1	3.4	4.4	1.6	5.4	7.9	0.0	0.0	0.0	0.0
Mazama temama	16.5	0.0	5.2	0.0	0.4	1.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Odocoileus virginianus	29.0	4.7	0.0	2.0	0.0	0.5	1.3	4.1	1.4	0.0	0.0	0.0	0.0
Felis pardalis	12.5	0.1	1.2	1.2	0.0	0.0	0.7	0.3	0.0	0.0	0.0	0.0	0.0
Puma yagouaroundi	6.5	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0
Eira barbara	4.5	0.2	0.1	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	1.6	0.0
Nasua narica	4.6	3.7	3.0	4.6	7.3	1.2	7.5	8.5	3.5	0.0	0.0	0.0	0.0
Dasypys novemcinctus	5.0	0.3	0.1	1.0	2.7	0.4	0.0	0.4	0.4	1.1	0.6	0.0	5.6
Cabassous centralis	3.0	0.3	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Didelphis marsupialis	1.5	0.9	0.6	0.6	1.8	0.8	1.7	0.4	1.7	0.4	0.0	0.0	0.0
Metachirus nudicaudatus	0.4	0.8	0.0	0.0	0.0	3.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Marmosa robinsoni	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mouse sp.	0.1	0.0	0.9	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sciurus granetensis	0.4	0.3	0.8	2.2	0.0	0.0	0.0	0.4	0.4	0.0	0.0	0.0	0.0

Table A5.1 Continued

Tamandua mexicana	6.2	0.0	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0
Sylvilagus brasiliensis	1.0	0.0	0.0	0.0	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.0	0.0
Conepatus semistriatus	2.5	0.0	0.0	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Tinamus major	1.2	0.8	1.3	0.0	0.0	0.0	1.3	1.7	0.0	0.4	0.0	0.0	0.0

Chapter 6

Host–tick–pathogen interactions across a Neotropical disturbance gradient

Helen J. Esser, Janet E. Foley, Yorick Liefting, Nicole Stephenson, Michael R. Miller, Edward Allen Herre, Frans J.J.M. Bongers, Herbert H.T. Prins, and Patrick A. Jansen

Abstract

Control of infectious diseases has been promoted as a service of biodiverse ecosystems, with high biodiversity buffering against disease risk. One recurrent critique of this so-called "dilution effect" is that higher biodiversity should also promote higher diversity of parasites and pathogens. However, high parasite diversity does not necessarily equate with high disease risk, and it remains poorly understood whether and how biodiversity loss affects disease risk in parasite-rich environments. Here, we show that total microbial and pathogen richness indeed increased with tick species richness, which in turn was positively related to wildlife species richness in tropical forests. In contrast, tick densities and infestation of small mammals as well as pathogen prevalence were dependent on the structure and composition of wildlife communities, rather than species richness *per se*. Wildlife community disassembly either diluted, amplified, or had no effect on infection prevalence in ticks, depending on the pathogen and degree of disturbance. However, hyperabundance of medium- to large-sized frugivores and herbivores in sites that lacked apex predators was related to exponential increases in tick density, negating any effect of reduced pathogen prevalence. Our study shows how anthropogenic disturbance of tropical forests can have cascading effects on tick communities and tick-borne disease risk, and highlights the importance of directly assessing the structure and composition of wildlife communities.

Introduction

The ongoing loss of global biodiversity is unprecedented in both magnitude and pace, raising urgent questions as to how this loss will affect ecosystem functioning and human well-being (Díaz et al. 2006, Bradshaw et al. 2009). Meanwhile, zoonotic infectious diseases are globally emerging, the majority of which have a wildlife origin (Woolhouse 2002). Risk of exposure to wildlife zoonoses has particularly increased in biodiversity 'hotspot' regions that are progressively affected by habitat loss, illegal hunting, and demographic changes (Jones et al. 2008). A growing body of literature suggests a causal link between these concurrent patterns, with species-rich communities buffering against disease risk: a phenomenon called "the dilution effect" (LoGiudice et al. 2003, Ezenwa et al. 2006a, Mills 2006, Pongsiri et al. 2009, Johnson et al. 2013b, Civitello et al. 2015). Control of infectious diseases has therefore been promoted as an important ecosystem service that is likely to be affected by biodiversity loss (Keesing et al. 2010). However, the generality of a negative biodiversity-disease relationship remains the subject of contentious debate (Randolph and Dobson 2012, Lafferty and Wood 2013, Ostfeld and Keesing 2013, Salkeld et al. 2013, Wood and Lafferty 2013, Huang 2014, Wood et al. 2014, Hofmeester 2016, Levi et al. 2016, Wood et al. 2016).

Paradoxically, biodiversity can either *increase* (amplify) or *decrease* (dilute) disease risk through a variety of mechanisms that are context- and scale-dependent (Keesing et al. 2006, Johnson et al. 2015b). This dual role of biodiversity in the emergence and transmission of infectious diseases lies at the heart of recent critiques. For a dilution effect to occur, species' reservoir competence should be closely coupled with resilience to biodiversity loss, such that more impoverished communities are dominated by competent hosts. Further, any changes in host diversity should be independent of host density, such that the addition of less competent host species to a community does not increase vector abundance. If these conditions are reversed, an amplification effect may arise (Keesing et al. 2006, Ostfeld and Keesing 2012). However, the link between resilience and competence remains equivocal, and it is empirically difficult to separate a dilution effect from a simple density effect (Begon 2008, Keesing et al. 2010, Wood and Lafferty 2013). Identifying the conditions under which biodiversity loss either increases or decreases disease risk therefore remains a challenge, particularly in multi-host, multi-pathogen systems (Johnson et al. 2015b).

A common argument in favour of an amplification effect is the 'diversity begets diversity' hypothesis (Hechinger and Lafferty 2005). Hosts function both as habitat and resource for parasites, and act as important dispersal agents. High host diversity and abundance should thus promote high parasite diversity and abundance (Hechinger and Lafferty 2005, Kamiya et al. 2014b). Indeed, species richness of human parasitic and infectious diseases increases strongly as one moves towards the equator (Guernier et al. 2004, Dunn et al. 2010). Conversely, both empirical and modelling studies have shown that biodiversity loss may decrease parasite richness and even lead to local parasite-host coextinctions, particularly for parasites with complex life cycles that sequentially use different host species (Koh et al. 2004, Lafferty 2012, Bush et al. 2013)(Chapter 5). However, high parasite richness does not necessarily equate with high disease risk: they represent different ecological processes (colonization among vs. transmission within communities) that may respond differently to biodiversity loss (Johnson et al. 2013a, Morand et al. 2014, Rottstock et al. 2014, Johnson et al. 2015b).

There is a clear need for empirical studies that examine whether and how biodiversity loss differentially affects parasite diversity and disease risk, particularly in species rich environments such as the tropical biome. Given that disease systems involve complex multi-trophic level interactions, such studies should consider broader communities of hosts, vectors, and pathogens (Wood et al. 2014, Johnson et al. 2015b). Finally, conventional measures of biodiversity loss such as host species richness are less appropriate than those that take species identity into account, so that directly measuring the composition and structure of host communities is crucial for the biodiversity-disease risk debate to move forward (LoGiudice et al. 2008, Randolph and Dobson 2012). Unfortunately, such studies are rare (Hofmeester 2016, Young et al. 2017).

Here, we studied communities of vertebrate hosts, ticks, and tick-borne bacterial microbes across an anthropogenic disturbance gradient in central Panama, a biodiversity hotspot. Using state of the art methods, we determined whether and how changes in wildlife community composition affected tick abundance, species richness, and prevalence on small mammals, and how this in turn affected the diversity of prokaryotic communities in ticks as well as tick-borne pathogen prevalence. We show that anthropogenic disturbances of wildlife communities had cascading effects on tick communities and disease risk, but that the strength

and direction of these effects were pathogen-specific and dependent on the degree of disturbance.

Methods

Study sites

We sampled 21 forest sites across central Panama (Fig 6.1), a region that supports a high species diversity of both wildlife (Patterson and Costa 2012) and ticks (Fairchild et al. 1966). However, ongoing deforestation has transformed the landscape of central Panama into a mosaic of old growth and secondary forests surrounded by pastures, scrubland, and human settlements (Condit et al. 2001). The 21 forest sites differed in size, isolation and protection, ranging from large national parks to small forest remnants scattered between the protected areas, and thus varied widely in their wildlife community composition (Meyer et al. 2015). The study region has seasonally moist tropical weather, with an average diurnal temperature of 27 °C and a pronounced dry season from January until May Rainfall varied among sites and years, ranging from ca. 1825 to 3975 mm yr^{-1} (ACP 2016). (Leigh 1999). Elevation at our 21 sites ranged between ca. 30 and 460 m (ASTER GDEM V2). All sampling took place during the dry seasons of 2012 to 2014.

Wildlife

We conducted camera trapping surveys to quantify the relative abundance and community composition of wildlife (Kays et al. 2011). At each site, camera traps (PC900, Reconyx Inc. Holmen, WI, USA) were placed at 16 computer-generated points in grids for at least 3 weeks. Sites were sampled again in a subsequent year if species accumulation curves did not approach an asymptote after the first survey. Cameras in large areas were placed in a subset (1 x 1 km) of the area, while cameras in small fragments were placed so as to sample the entire plot. Cameras were attached to tree trunks with the lens 20-30 cm above ground level with 30-333 m distance between cameras, depending on the size of the fragment. We used the walk test function of the cameras to measure the maximum detection distance for each camera as an estimate of the area surveyed. Detection distances ranged from 2.0-8.8 m (median: 3.6 m) among cameras, depending on vegetation density. Cameras were continuously active and took

sequences of ten consecutive photographs per trigger, with no delay between triggers. A 1-GB flash memory card stored the photographs with the time and dates of each event.

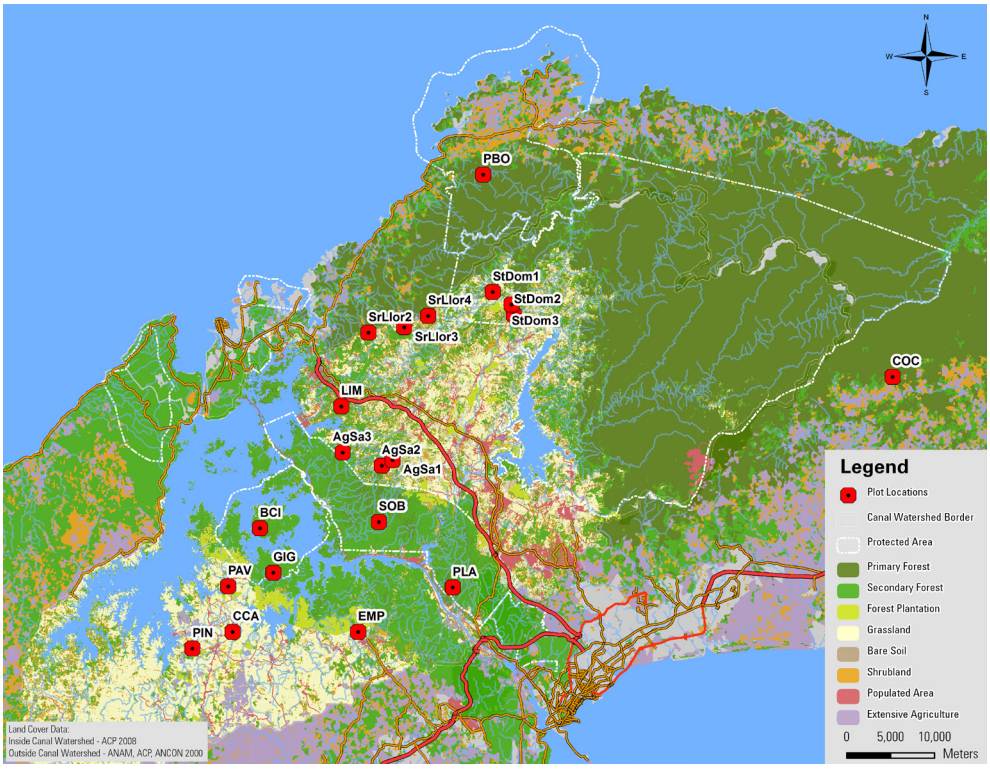


Figure 6.1 Locations of the 21 study sites central Panama.

All photographs were uploaded to and processed in a custom-made database (Kays *et al.* 2011). Photographs were manually grouped into sequences, with each sequence representing the capture of one individual animal or a group of social animals. Animal identifications were based on Reid (2009). All reptiles, bats, and birds were excluded, except for great tinamou (*Tinamus major*), great currasow (*Crax rubra*), and crested guan (*Penelope purpurascensi*). The final dataset included 15,055 animal captures of 39 species that were recorded during 11,647 camera trapping days. We used capture rates as a proxy for relative abundance at each site, as cameras record a species more often where it is more abundant (O'Connell et al. 2010). Capture rates of each species were calculated for each site as the

average number of animal captures per 100 days per meter of maximum detection distance. We used the first-order Jackknife (Jack1) index, an asymptotic estimator of species richness, to compute the number of species that would be expected under exhaustive sampling using EstimateS version 9.1.0 (Colwell 2013). This index is considered more suitable for camera trapping data than the Chao indices (Tobler *et al.* 2008).

Ticks

Questing ticks were collected from the environment using a combination of the dragging and walking techniques (Ginsberg and Ewing 1989). The drag-sampling method involved dragging a white cotton cloth of 1x1 m over leaf litter and through vegetation. In each site, we established ten 50m transects and sampled both sides of each transect, totalling 1000 m^2. The cloth was checked for the presence of ticks every 5 m. Ticks were removed from the cloth with masking tape and stored in 95% ethanol. The walking technique involved removing all ticks found on the clothes of investigators during drag sampling. All adult ticks were morphologically identified to species using an extensive reference collection at the Gorgas Memorial Institute and via taxonomic keys in Fairchild et al. (1966) and Onofrio et al. (2006). *Haemaphysalis juxtakochi* nymphs and larvae were identified using Fairchild *et al.* (1966). *Ixodes affinis* nymphs and larvae were identified using Oliver et al. (1987). Nymphs of *Amblyomma ovale* and *A. longirostre* were identified using Martins *et al.* (2010). All other *Amblyomma* immature ticks could not be morphologically identified to species.

Ticks were also collected from small mammals that were live-trapped in 40x13x13 cm Tomahawk traps. At each site, 100 traps were placed in a grid with 10 m interspacing and baited with ripe banana. Traps were covered with leaf litter for shelter and checked daily during morning hours for five consecutive days. Trapped mammals were weighed, sexed, and searched for ectoparasites across their whole body. All collected ticks were stored in 95% ethanol and identified to either species or genus using the aforementioned taxonomic keys. In addition, Venzal et al. (2008) was used to identify *Ornithodoros puertoricensis* ticks. Ticks that could not be morphologically identified to species were molecularly identified using either the 16S rDNA or the mtDNA COI barcode fragment following Miller et al. (2016).

Microbes

A total of 799 ticks (443 larvae and 356 nymphs) directly collected from small mammals were used to evaluate the presence of tick-borne pathogens and to characterize tick microbiome community composition. All ticks were individually processed under open benchtop conditions. We used surface sterilization techniques during tick sample preparation prior to DNA extractions, as described by Clay et al (2008). Genomic DNA was extracted from whole ticks using the QIAGEN DNeasy kit, following the manufacturer's protocol (Qiagen, Valencia, CA, USA). PCR products were obtained targeting the V1 to V3 hypervariable regions of the 16S rRNA genes using the forward primer 5'-ATTACCGCGGCTGCTG-3' (Muyzer et al. 1993) and reverse primer 5'-GTTTGATCCTGGCTCAG-3' (Kane et al. 1993), covering ~500 bp. These hypervariable regions have previously been shown to be most suitable for distinguishing bacterial species to the genus level (Chakravorty et al. 2007). Individual barcode sequences were added to each tick sample, which allowed us to pool samples into one Illumina MiSeq paired-end sequencing run. Sequencing and demultiplexing was carried out at the Bioinformatics Core facility of the University of California Davis Genome Center.

Raw sequence data were processed using the VSEARCH tool (Rognes et al. 2016). We first merged overlapping read-pairs using PEAR v0.9.8 (Zhang et al. 2014), removed primers and barcodes using CutAdapt v1.13 (Martin 2011), and filtered low quality reads using a maximum expected error threshold of 1 (Edgar and Flyvbjerg 2015). We then dereplicated both at the sample level (sequences within each individual tick) and at the study level (pooled sequences across sites), and removed all singletons and chimeras. Chimeras were detected *de novo* using the UCHIME algorithm (Edgar et al. 2011). A total of 10,768,663 clean reads of 1,177,000 unique sequences were clustered *de novo* at the standard 97% similarity threshold, with each cluster representing an Operational Taxonomic Unit (OTU). A representative sequence of each OTU was taxonomically identified to genus using the Classifier tool (Wang et al. 2007) of the Ribosomal Database Project v11 (Cole et al. 2009).

Statistical analyses

Wildlife – All statistical analyses were performed in R version 3.3.3 (R Core Team 2017). In order to understand how ticks and bacterial microbes respond to increasingly disturbed wildlife communities, we first had to identify the strongest disturbance gradients. We did so by comparing wildlife capture rates of each species between sites with a Principal Component

Analyses (PCA) on the sites x species correlation matrix, using the R package *stats*. The first two axes of a PCA, henceforward PCA1 and PCA2, were uncorrelated and explained most of the variability in a dataset (see Jolliffe 2002). Thus, PCA1 and PCA2 described the two most important, independent gradients in the wildlife community composition. The site scores of PCA1 and PCA2 were entered as predictor variables in further analyses. As host density and species richness are often used to explain patterns of disease risk, we tested if log10-transformed total capture rates and wildlife species richness correlated with PCA1 and PCA2.

Ticks – We used generalized linear mixed models (GLMMs) to test how the number of questing ticks per meter drag sampling were related to biotic (PCA1, PCA2) and abiotic (elevation, rainfall) factors. We included a random intercept term for site to account for repeated measurements in each location (n=39). Year was partially crossed with site, but as there were only 3 levels (2012, 2013, and 2014) the among-year variance could not be reliably estimated and year was therefore treated as a fixed categorical factor (see Bolker et al. 2009). All variables were standardized prior to analyses by extracting the mean and dividing by two standard deviations following Gelman (2008). Counts of adults, nymphs, and larvae were overdispersed, and adult ticks were absent in 23 out of 39 samples. We therefore compared a zero-inflated with a standard negative binomial model for adult ticks using the Akaike's Information Criterion corrected for small sample size, and found that the latter model performed better (ΔAICc = 22.60). Variance inflation factors were <2, indicating that multicollinearity was not an issue. Thus, we included all variables without interactions and fitted negative binomial-distributed error terms with log-link function using the *glmmADMB* package (Fournier et al. 2012).

We used a GLMM with a binomial distribution and logit link function to test how tick prevalence on small mammals was related to intrinsic (individual) and extrinsic (plot) factors. Tick prevalence was defined as the presence (1) or absence (0) of ticks on an individual small mammal. Re-captured individuals were omitted from the analyses. Intrinsic fixed factors included sex and body mass. Extrinsic fixed factors included PCA1, PCA2, and year. Body mass was log10-transformed prior to analysis. Sex was centred and all other variables were standardized by extracting the mean and dividing by two standard deviations (Gelman 2008). Site and species were entered as random factors with varying intercept. Variance inflation factors were all ~1, and there was no interaction between sex and body mass. We therefore included all variables without interactions in the model.

We used a general linear model (GLM) with a Gaussian distribution to test the relationship between tick species richness and biotic (PCA1, PCA2) and abiotic (elevation, rainfall) factors. The number of identified ticks was entered as weighting factor and all variables were standardized prior to analysis as described above. Variance inflation factors for the entered variables were all <4.

Microbes – We used a GLMM with a negative binomial distribution to test how tick microbial diversity (no. of OTUs in each tick) was related to the wildlife gradients (PCA1, PCA2), individual host factors (taxon), and individual tick factors (life stage). Host taxon was either rodent or opossum and tick life stage was either larva or nymph. Year was entered as a fixed factor and site was entered as a random factor. PCA scores were standardized as previously described while host taxon and tick life stage were centred. Variance inflation factors were all ~1.

We used a GLMM with a binomial distribution to test how pathogen prevalence in ticks was related to PCA1, PCA2, host taxon, and tick life stage. Pathogen prevalence was defined as the presence (1) or absence (0) of an OTU that was assigned to a genus of pathogenic tick-borne bacteria. These included *Anaplasma*, *Bartonella*, *Borrelia*, *Coxiella*, *Diplorickettsia*, *Francisella*, *Orientia*, and *Rickettsia* (note: *Orientia* is represented by two species, *O. tsutsugamushi* and *O. chuto*, both of which cause scrub typhus. Although it is transmitted by larvae of trombiculid mites (chiggers), we have included it here as this life-threatening disease has only recently been reported for Latin America (Weitzel et al. 2016) and because the role for ticks as potential vectors has not been explored). Separate GLMMs were run for each of these genera, except *Bartonella*, which was observed in only one site. Year was entered as an additional fixed factor and site was entered as a random factor. PCA scores were standardized as previously described while host taxon and tick life stage were centred. Variance inflation factors were all ~1.

We used a GLM with a Gaussian distribution to test the relationship between potential pathogen richness (number of OTUs assigned to the aforementioned genera) in each site and PCA1, PCA2, tick species richness, and species richness of live-trapped small mammals. The number of sequenced ticks was entered as weighting factor. All variables were standardized and variance inflation factors were <3.

Results

Wildlife

Wildlife community composition varied widely among sites (Fig 6.2). Together, the first two axes of the ordination diagram explained ca. 36% of the total variation in the wildlife capture rates among sites. The first axis (18.4% of the total variation) separated the isolated national parks in central Panama – Barro Colorado Island (BCI), Gigante (GIG), Pipeline Road (PLR), and Plantation Road (PLA) – from all other sites (Fig 6.2). Species that increased in relative abundance along this axis included the Central American agouti (*Dasyprocta punctata*), lowland paca (*Cuniculus paca*), collared peccary (*Pecari tajacu*), Central American red brocket (*Mazama temama*), white-tailed deer (*Odocoileus virginianus*), Baird's tapir (*Tapirus bairdii*), white-nosed coati (*Nasua narica*), and ocelot (*Leopardus pardalis*). Thus, the first PCA axis represents a gradient of increasing relative abundance of medium- to large-sized frugivores and herbivores and one mesopredator. The strong increase of these species along the first axis may be explained by release from predation and/or competition, as jaguar (*Panthera onca*) and puma (*Puma concolor*) are rare or absent in each of the four sites, while game wardens intensively patrol BCI and GIG to prevent illegal hunting (Moreno et al. 2006, Willis 2009, Meyer et al. 2015).

The second axis (17.3% of the total variation) separated sites according to their distance from Chagres National Park, a vast stretch of old-growth forest. Sites adjacent to Chagres NP – Cocobolo Nature Reserve (COC) and Portobelo National Park (PBO) – had species of all trophic levels, including apex predators, whereas sites that were furthest from Chagres NP were dominated by small habitat generalists, such as Tome's spiny rat (*Proechimys semispinosus)*, common opossum (*Didelphis marsupialis*), and nine-banded armadillo (*Dasypus novemcinctus*). Moreover, the three most disturbed sites along this gradient – Piña (PIN), Cerro Cama (CCA), and Las Pavas (PAV) – were frequently visited by cattle. Domestic cats and dogs were also more common in sites that were further from Chagres NP. Thus, the second PCA axis represents a gradient of decreasing trophic complexity of wildlife communities. The results of the PCA were not affected by excluding domestic animals (cattle, dog, cat) and wildlife species that are principally aquatic (i.e. Neotropical river otter *Lontra longicaudis*), arboreal (white-faced capuchin monkey *Cebus capucinus*, crested guan *Penelope purpurascensi*, Rothschild's porcupine *Coendou rothschildi*, and red-tailed squirrel *Sciurus granatensis*) or very small in size (mouse opossums *Marmosa sp.* and mouse sp.) (Figure not shown).

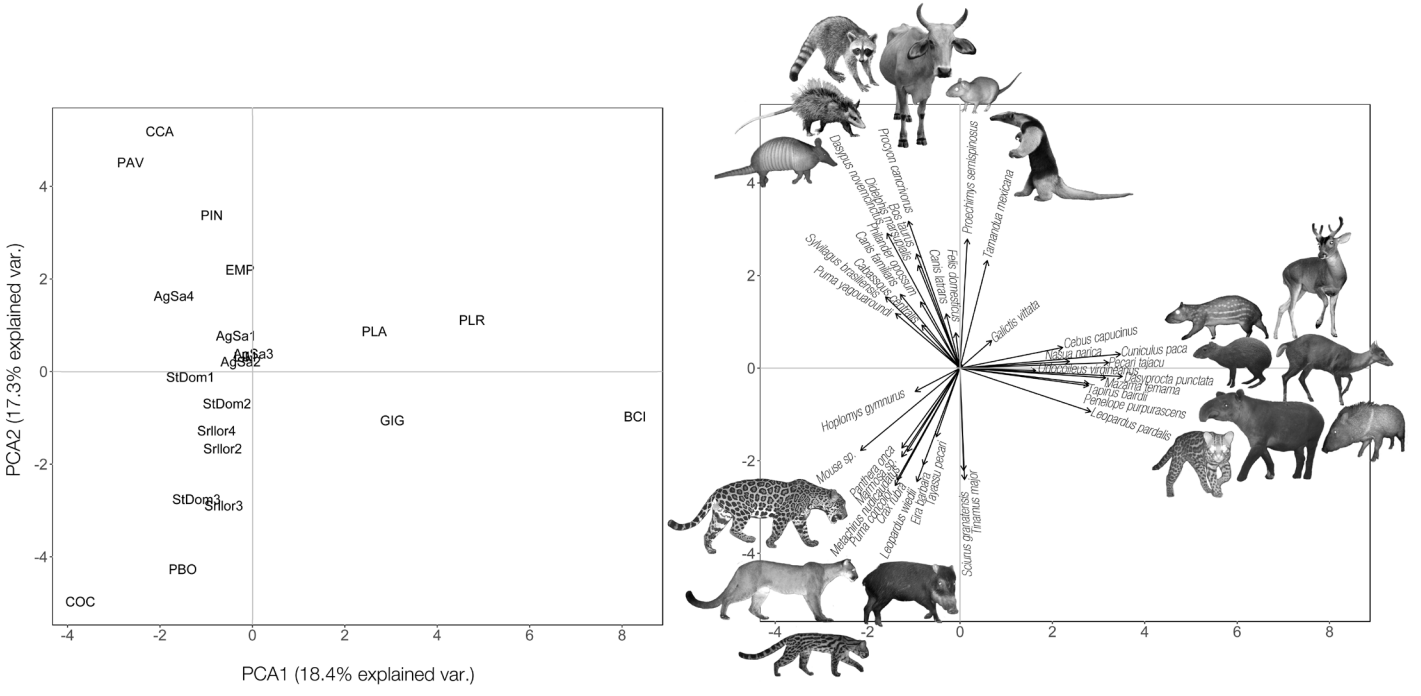


Figure 6.2 Principal Component Analysis showing differences in wildlife community composition between sites. Sites (left) and species (right) are shown separately for clarity. The first axis sets apart the rather isolated national parks in central Panama (BCI, GIG, PLR, PLA) from all other sites, and is strongly related to the relative abundance of medium- to large-sized frugivores and herbivores and ocelot. The second axis reflects a trophic gradient, ranging from communities that have species of all trophic levels including apex predators (bottom), to communities where apex predators are missing (centre), to communities dominated by small habitat generalists and domestic animals (top).

Wildlife species richness ranged from 11.9 in StDom2 to 27.8 in EMP (see Appendix 6: Table A6.1), but was neither correlated with the first (r_{19} = 0.08, P = 0.74) nor with the second PCA axis (r_{19} = -0.10, P = 0.67). Thus, while the identity of species and hence community composition of wildlife differed substantially among sites, species richness was not related to the two strongest gradients (see Appendix 6: Fig A6.1a-b). Total capture rates ranged from 11 animals per m per 100 d in StDom2 to 103 animals per m per 100 days in BCI (see Appendix 6: Table A6.1) and was positively correlated with PCA1 (r_{19} = 0.74, P < 0.001) but not with PCA2 (r_{19} = 0.20, P = 0.38)(see Appendix 6: Fig A6.1c-d). We will henceforward refer to PCA1 as the wildlife abundance gradient and PCA2 as the trophic complexity gradient.

Ticks

A total of 9573 larvae, 913 nymphs, and 135 adult ticks were collected from the free environment via drag sampling, while 508 larvae and 382 nymphs were collected from small mammals (see Appendix 6: Table A6.2). Trapped mammals included five species of rodent: *Proechimys semispinosus* (n=203), *Hoplomys gymnurus* (n=96), *Oryzomys* sp. (n=24), *Heteromys* sp. (n=8), and *Melanomys* sp. (n=1), and four species of opossum: *Didelphis marsupialis* (n=82), *Marmosa* sp. (n=35), *Metachirus nudicaudatus* (n=18), and *Philander opossum* (n=16). We used barcoding for 609 immature ticks that could not be morphologically identified to species and were successful in 599 cases. In total, 2722 ticks (24%) could be assigned to 16 species of 4 genera, including: *Amblyomma auricularium*, *A. dissimile*, *A. geayi*, *A. longirostre*, *A. mixtum*, *A. naponense*, *A. oblongoguttatum*, *A. ovale*, *A. pacae*, *A. sabanerae*, *A. tapirellum*, *A. varium*, *Haemaphysalis juxtakochi*, *Ixodes affinis*, and *Ornithodoros puertoricensis*. In addition, we had 1 unidentified *Amblyomma* larva for which the DNA sequence yielded no match to any reference in GenBank despite the high quality of the sequence.

The GLMMs showed that densities of questing adults, nymphs, and larvae were all positively related to PCA1, but not to PCA2 (Table 6.1), i.e. the relative abundance of medium- to large-sized frugivores and herbivores was a significant predictor for questing tick densities, but the trophic complexity of the wildlife community was not (Fig 6.3a-b). Densities of questing immature ticks were also significantly related to abiotic factors, with nymphal densities declining with elevation and larval densities increasing with rainfall (Table 6.1). In addition, we found an effect of year for each life stage (Table 6.1). Tick prevalence on small mammals was positively related to both PCA1 and PCA2 (Table 6.2). Thus, the proportion of tick-infested small

Table 6.1 Results of negative binomial GLMMs for densities of questing adult, nymphal, and larval ticks. Reported values include standardized regression coefficients (β) and 95% confidence intervals (CI) for fixed factors, and variance and standard deviation (SD) for random factors.

	Density of adults		Density of nymphs		Density of larvae	
Fixed factors	β	95% CI	β	95% CI	β	95% CI
PCA1	**3.08****	(0.99 – 5.18)	**1.07***	(0.01 – 2.14)	**2.72***	(2.20 – 5.03)
PCA2	-0.48	(-3.55 – 2.60)	-0.28	(-1.90 – 1.34)	1.06	(-0.81 – 2.94)
Rainfall	1.57	(-0.94 – 4.07)	-0.12	(-1.48 – 1.26)	**2.98*****	(1.42 – 4.55)
Elevation	-1.39	(-4.10 – 1.32)	**-1.75***	(-3.11 – -0.38)	-0.56	(-2.27 – 1.15)
Year2013[a]	1.49	(-1.03 – 4.00)	-1.06	(-2.39 – 0.27)	**2.16***	(0.33 – 3.98)
Year2014[a]	**2.49***	(0.08 – 4.90)	**-1.26***	(-2.45 – -0.06)	-0.02	(-1.72 – 1.68)
Random factor	Variance	SD	Variance	SD	Variance	SD
Site	1.73	1.32	<0.001	<0.001	<0.001	0.001

[a] standardized regression coefficients as compared to zero for 2012

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

mammals increased with increasing abundance of medium- to large-sized frugivores and herbivores, and with decreasing structural complexity of wildlife communities (Fig 6.3c-d). Tick prevalence also increased with host body mass, and male individuals were more often parasitized than females (Table 6.2). Species richness of ticks increased significantly with PCA1, but was not related to PCA2, rainfall or elevation ($F_{(4,14)} = 4.26$, $R^2_{adj} = 0.42$, Table 6.3, Fig 6.3e-f).

Table 6.2 Results of a binomial GLMM for tick prevalence on small mammals. Reported values include odds ratios and 95% confidence intervals (CI) for fixed factors, and variance and standard deviation (SD) for random factors.

	Tick prevalence	
Fixed factors	Odds	95% CI
PCA1	**2.94****	(1.32 – 6.53)
PCA2	**3.64*****	(1.76 – 7.54)
Sex[a]	**2.27***	(1.19 – 4.35)
Body mass	**2.53***	(1.09 – 5.88)
Year2013[b]	1.67	(0.70 – 3.98)
Year2014[b]	2.57	(0.98 – 6.75)
Random factors	Variance	SD
Site	<0.001	<0.001
Species	0.40	0.64

[a] centered odds ratios for males as compared to females

[b] standardized odds ratios as compared to zero for 2012

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

Table 6.3 Results of a general linear model for tick species richness per site. Reported values include standardized regression coefficients (β), 95% confidence intervals (CI), and *t*-test statistic.

	Tick species richness		
Predictors	β	95% CI	*t*
PCA1	**5.98****	(2.06 – 9.89)	2.99
PCA2	7.46	(-2.68 – 17.60)	1.44
Rainfall	3.63	(-6.32 – 13.58)	0.72
Elevation	1.49	(-5.38 – 8.36)	0.43

** $P < 0.01$

Microbes

A total of 26,122 OTUs from 1609 bacterial genera of 404 families and 53 phyla were detected in 799 ticks (larvae n=443, nymphs n=356) collected from small mammals. Tick microbiomes were dominated by *Proteobacteria* (78% of all reads), followed by *Firmicutes* (7.4%), *Actinobacteria* (2.2%), and *Bacteroidetes* (1.9%). Genera with potential tick-borne pathogens included *Anaplasma*, *Bartonella, Borrelia*, *Coxiella*, *Diplorickettsia*, *Francisella*, *Orientia*, and *Rickettsia* (see Appendix 6: Table A6.3), which were present in a wide variety of tick species (see Appendix 6: Table A6.4). In fact, *Francisella* and *Rickettsia* were the two most common bacterial genera, accounting for 45.3% and 11.3% of all reads respectively. PCR results indicated that all *Rickettsia*-positive *A. mixtum* ticks were infected with *R. amblyommii*, which has been implicated as a potential cause of human rickettsiosis (Apperson et al. 2008).

Microbial diversity in ticks was neither related to PCA1 nor to PCA2 (Table 6.4). Rather, microbial diversity depended on tick life stage, with nymphs having higher OTU diversity than larvae, and on host taxon, with ticks collected from rodents having higher OTU diversity than those collected from opossums. There was also a significant effect of year (Table 6.4). In contrast, the proportion of ticks infected with OTUs assigned to pathogenic genera did change along the wildlife disturbance gradients (Table 6.4). Specifically, the prevalence of *Anaplasma* and *Borrelia* increased along PCA1 (Fig. 6.4a,c) whereas the prevalence of *Rickettsia*-infected ticks decreased along the same axis (Fig 6.4e) and with *Diplorickettsia* prevalence decreasing along PCA2 (Fig 6.4h). Thus, although individual ticks harboured equally diverse microbial communities across sites, their odds of being infected with potentially pathogenic bacteria either increased or decreased with changes in wildlife community composition, depending on the type of pathogen. In addition, tick life stage was a significant predictor for *Coxiella*, *Diplorickettsia*, *Francisella*, and *Rickettsia*, with a higher prevalence of these genera in nymphs than in larvae. Host family also explained pathogen prevalence, with a higher prevalence of *Anaplasma*, *Francisella*, and *Orientia* in ticks collected from rodent hosts, and a higher prevalence of *Coxiella* and *Rickettsia* in ticks collected from opossums. Year was a significant predictor for several pathogens (Table 6.4).

Finally, total microbial richness per site increased with tick species richness and the richness of live-trapped small mammals, but not with PCA1 or PCA2 ($F_{(4,14)} = 13.95$, $R^2_{adj} = 0.74$, Table 6.5). Richness of potential pathogens in each site, i.e. the number of OTUs assigned to

pathogenic genera, increased with PCA1, tick species richness, and the richness of live-trapped small mammals, but did not with PCA2 ($F_{(4,14)}$ = 16.68, R^2_{adj} = 0.78, Table 6.5, Fig 6.5).

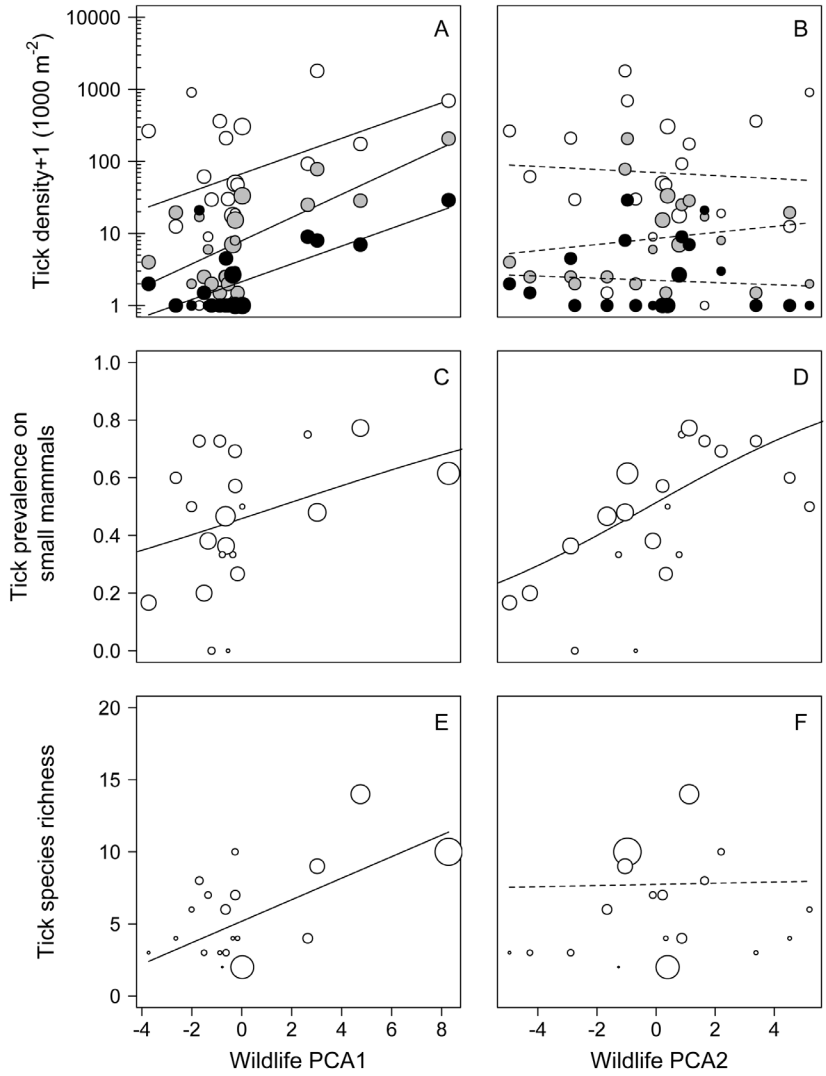


Figure 6.3 Densities of questing larvae (*white*), nymphs (*grey*), and adult ticks (*black*) increased along the first PCA axis of the wildlife community composition **(a)**, but were not related to PCA2 **(b)**. Tick prevalence on small mammals increased along PCA1 **(c)** and PCA2 **(d)**. Tick species richness also increased along PCA1 **(e)**, but was not related to PCA2 **(f)**. Bubble size reflects drag sampling effort **(a-b)**, the number of small mammals **(c-d)**, or the number of identified ticks **(e-f)**.

Table 6.4 Results of a binomial GLMM for pathogen prevalence and a negative binomial GLMM for microbial OTU richness in ticks. Reported values include odds ratios (for pathogen prevalence), standardized regression coefficients (β, for microbial richness), 95% confidence intervals for fixed factors, and variance and standard deviation (SD) for random factors.

	Anaplasma		*Borrelia*		*Coxiella*		*Diplorickettsia*	
Fixed factors	Odds	95% CI	Odds	95% CI	Odds	95% CI	Odds	95% CI
PCA1	**1.85***	(1.03 – 3.34)	**7.46****	(1.69 – 32.88)	1.23	(0.28 – 5.35)	0.81	(0.42 – 1.57)
PCA2	1.24	(0.71 – 2.17)	0.71	(0.17 – 2.89)	0.87	(0.32 – 2.36)	**0.53***	(0.31 – 0.90)
Tick stage[a]	1.19	(0.71 – 1.99)	1.02	(0.47 – 2.21)	**0.54***	(0.33 – 0.88)	**2.08****	(1.19 – 3.61)
Host family[b]	**1.99***	(1.05 – 3.75)	0.67	(0.19 – 2.40)	**0.25*****	(0.13 – 0.50)	1.31	(0.67 – 2.58)
Year2013[c]	1.30	(0.64 – 2.64)	0.66	(0.21 – 2.11)	**0.11*****	(0.06 – 0.22)	**2.53***	(1.22 – 5.23)
Year2014[c]	0.59	(0.29 – 1.20)	**0.22***	(0.06 – 0.84)	**0.11*****	(0.05 – 0.24)	0.84	(0.34 – 2.03)
Random factors	Variance	SD	Variance	SD	Variance	SD	Variance	SD
Site	<0.001	<0.01	0.10	0.32	1.03	1.01	<0.01	0.08

a centered odds ratios for nymphs as compared to larvae

b centered odds ratios for rodents as compared to opossums

c standardized odds ratios as compared to zero for 2012

* P < 0.05

** P < 0.01

*** P < 0.001

Table 6.4 Continued

	Francisella		*Orientia*		*Rickettsia*		Microbial OTU richness	
Fixed factors	Odds	95% CI	Odds	95% CI	Odds	95% CI	β	95% CI
PCA1	0.48	(0.12 – 2.00)	0.60	(0.19 – 1.88)	**0.49***	(0.27 – 0.87)	0.08	(-0.22 – 0.38)
PCA2	1.23	(0.37 – 4.13)	0.93	(0.43 – 2.01)	0.97	(0.64 – 1.47)	0.13	(-0.06 – 0.31)
Tick stage[a]	**5.47*****	(2.33 – 12.89)	2.05	(0.99 – 4.26)	**1.39***	(1.01 – 1.91)	**0.13***	(0.03 – 0.24)
Host family[b]	**6.49*****	(2.17 – 19.44)	**3.73****	(1.54 – 9.02)	**0.64***	(0.42 – 0.99)	**0.25*****	(0.10 – 0.40)
Year2013[c]	**7.06*****	(2.80 – 17.80)	1.67	(0.66 – 4.24)	0.68	(0.43 – 1.08)	**0.32*****	(0.17 – 0.47)
Year2014[c]	**7.72*****	(2.48 – 24.06)	0.36	(0.08 – 1.52)	**1.75***	(1.02 – 2.98)	**-0.33*****	(-0.52 – -0.14)
Random factors	Variance	SD	Variance	SD	Variance	SD	Variance	SD
Site	0.61	0.78	0.23	0.48	0.10	0.32	0.04	0.21

[a] centered odds ratios for nymphs as compared to larvae

[b] centered odds ratios for rodents as compared to opossums

[c] standardized odds ratios as compared to zero for 2012

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

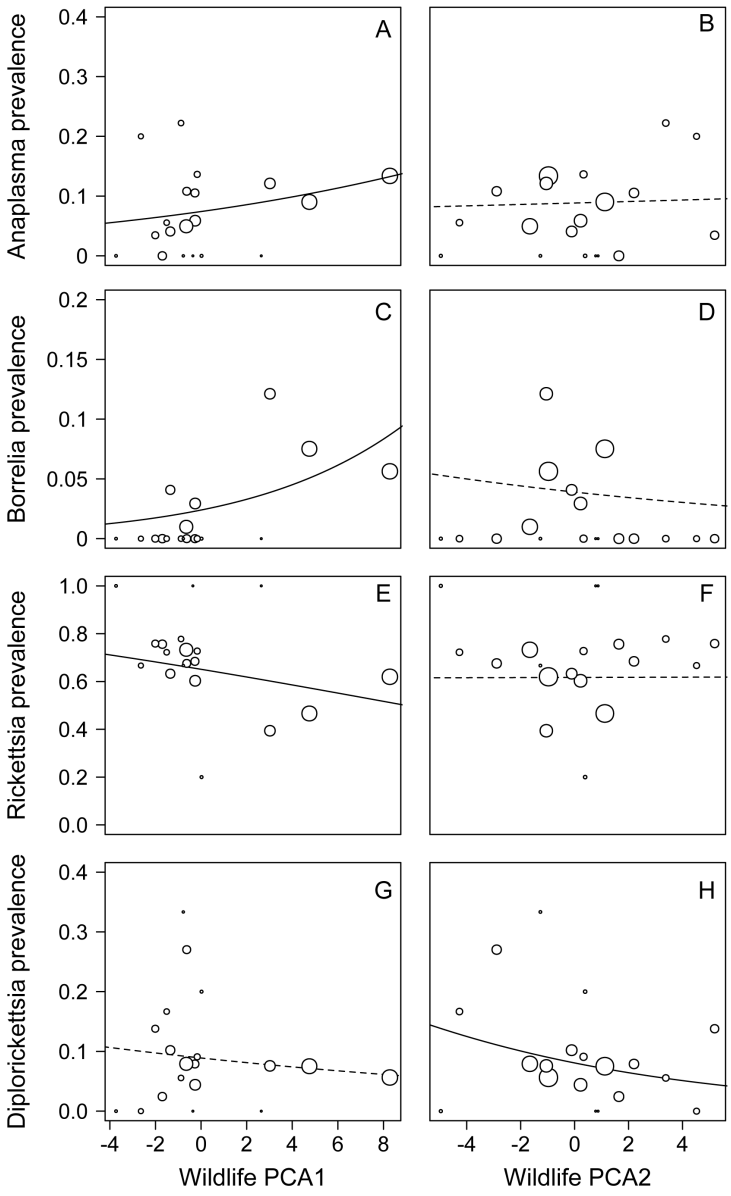


Figure 6.4 The infection prevalence of *Anaplasma* **(a)** and *Borrelia* **(c)** increased along the first wildlife PCA axis, whereas the infection prevalence of *Rickettsia* **(e)** decreased and that of *Diplorickettsia* **(g)** did not change. In contrast, the infection prevalence of *Diplorickettsia* **(h)** decreased along the second wildlife PCA axis, whereas the prevalence of *Anaplasma* **(b)**, *Borrelia* **(d)**, and *Rickettsia* **(f)** did not. Bubble size reflects the number of sequenced ticks.

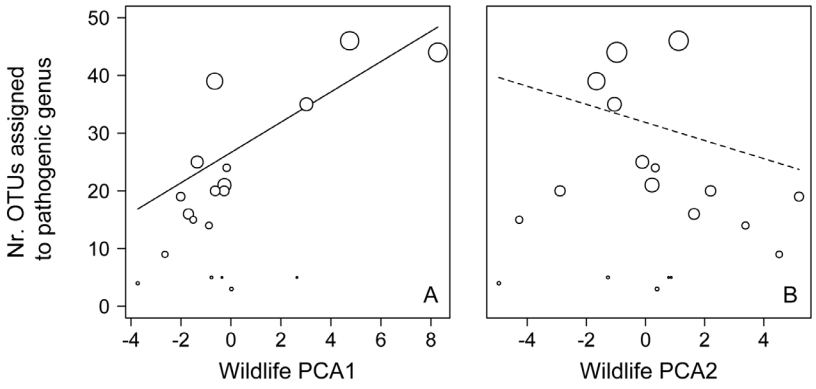


Figure 6.5 The total number of OTUs that were assigned to a pathogenic genus (i.e. *Anaplasma*, *Bartonella*, *Borrelia*, *Coxiella*, *Diplorickettsia*, *Francisella*, *Orientia*, *Rickettsia*) increased along the first wildlife PCA axis **(a)** but did not change significantly along the second axis **(b)**.

Table 6.5 Results of a general linear model for total microbial OTU richness and OTU richness of potential pathogens per site. Reported values include standardized regression coefficients (β), 95% confidence intervals (CI), and *t*-test statistic.

	Microbial richness			Pathogen richness		
	β	95% CI	*t*	β	95% CI	*t*
PCA1	1317.8	(-69.24 – 2704.94)	1.86	**7.41***	(1.08 – 13.73)	2.30
PCA2	-402.0	(-2409.44 – 1605.36)	-0.39	-6.27	(-15.42 – 2.88)	-1.34
Tick species richness	**2567.8***	(740.07 – 4395.60)	2.75	**11.39***	(3.06 – 19.73)	2.68
Host species richness	**1736.4***	(297.90 – 3174.86)	2.37	**7.83***	(1.27 – 14.39)	2.34

* $P < 0.05$

Discussion

Anthropogenic disturbance of tropical forests can have cascading effects on tick communities and tick-borne disease risk through changes in wildlife community composition. In our study, sites where initially only apex predators were lost had higher abundances of medium- to large-sized frugivores and herbivores, which in turn was related to higher tick densities, tick species richness, and tick prevalence on small mammals. Although the diversity of bacterial microbes in individual ticks did not change, the proportion of ticks infected with potential tick-borne pathogens did: infection prevalence of *Anaplasma* and *Borrelia* increased with wildlife

abundance, whereas *Rickettsia* prevalence decreased. When medium- to large-sized frugivores and herbivores also disappeared, wildlife communities were dominated by small- to medium-sized habitat generalists. Rats and opossums were increasingly more likely to be parasitized by ticks in such degraded habitats than in sites with more trophically complex wildlife communities. However, *Diplorickettsia* prevalence decreased with loss of trophic complexity, whereas the prevalence of other pathogenic genera did not change. Thus, anthropogenic changes to wildlife communities either diluted, amplified, or had no effect on infection prevalence, depending on the pathogen and degree of disturbance.

The increase in tick abundance and species richness with the first (wildlife abundance) but not the second (trophic complexity) disturbance gradient can be explained by host-feeding preferences. While the immature stages tend to parasitize a wide range of host species, the adults of most tick species in Panama are quite host-specific (Chapter 2) particularly to larger-bodied host species (Chapter 4). The majority of ticks collected via drag sampling preferentially feed on medium- to large-sized frugivores and herbivores (i.e. *A. naponense*, *A. pacae*, *A. tapirellum*, *H. juxtakochi*, *I. affinis*) and/or carnivores (i.e. *A. ovale*, *I. affinis*) in the adult stage. Deer, peccary, paca, agouti, coati and ocelot all increased in abundance across the first disturbance gradient, providing female ticks with more opportunities to feed, mate, and produce viable eggs. Previous studies have shown that forest fragmentation and loss of apex predators may augment wildlife densities as a result of predatory and competitive release (Michalski and Peres 2007, Ripple et al. 2014), which may explain the hyperabundance of the aforementioned species, and that of their associated ticks, in large forest fragments that lacked apex predators.

Higher densities of questing ticks in the vegetation may explain why a higher proportion of rats and opossums were parasitized by ticks in sites with more wildlife. But how can the increased tick prevalence with loss of trophic complexity be explained? Neither wildlife species richness, nor abundance, or densities of questing ticks changed along this gradient. Keesing et al. (2006) suggested that predators have the ability to reduce encounter rates between vectors and hosts through their impact on host behaviour. It is well established that rodents and other prey species reduce their activity level in response to (perceived) risk of predation (Díaz et al. 2005, Borowski and Owadowska 2010, Haapakoski et al. 2015, Hegab et al. 2015), which in turn may decrease their chances of encountering parasites and/or pathogens (Keesing et al. 2006). Indeed, Hofmeester et al. (Hofmeester et al. 2017) recently showed that tick prevalence on rodents decreased with increasing predator abundance. We

tested this hypothesis and found that the proportion of hyper-carnivores (species whose diet consists of at least 70% meat) was negatively related to tick prevalence on small mammals (deviance difference = 14.62, odds = 0.80, $P < 0.001$). Although alternative hypotheses could explain increased encounter rates between ticks and small mammals in more disturbed fragments (e.g. increased foraging activity), these findings merit further investigation into the role of host movement behaviour in tick-host interactions and tick-borne disease dynamics (Hofmeester et al. 2017).

In line with the 'diversity begets diversity' hypothesis, we found that total microbial richness as well as OTU richness of potential pathogens per site increased with tick species richness. High parasite diversity is thus a source of infectious diseases (Morand et al. 2014). Surprisingly however, microbial diversity in individual ticks did not change across the two disturbance gradients, possibly due to competitive or facilitative interactions among bacteria within ticks that may affect colonization and transmission of other microbial species (Burgdorfer et al. 1980, Macaluso et al. 2002, de la Fuente et al. 2003, Lively et al. 2005). Future studies should evaluate the potential role of such microbial interactions in structuring tick microbial communities (see also Clay and Fuqua 2010). In contrast, infection prevalence of specific pathogens in individual ticks either increased (*Borrelia*, *Anaplasma*) or decreased (*Rickettsia*, *Diplorickettsia*), depending on the disturbance gradient. Thus, while the richness of individual tick microbiomes was unaffected by changes in wildlife community composition, the identity of the bacterial species and hence pathogen prevalence did change.

The availability of competent reservoir hosts may play an important role in the observed changes in pathogen prevalence. For example, rodents, opossums, and dogs have been identified as reservoir hosts for *Rickettsia* (Burgdorfer et al. 1962, Bozeman et al. 1967, Nicholson et al. 2010, Bermúdez et al. 2017), and while small rodents are also reservoir for a range of other pathogens such as *Borrelia* and *Anaplasma* (Meerburg et al. 2009), various strains of relapsing fever group *Borrelia* and *Anaplasma* also circulate in deer and other large ungulates (Massung et al. 2005, Kawahara et al. 2006, Moyer et al. 2006, Yparraguirre et al. 2007, Nieto et al. 2012, Sacchi et al. 2012, Stuen et al. 2013, Lee et al. 2014). Changes in the prevalence of these pathogens reflected changes in the abundance of their respective reservoir hosts: *Rickettsia* and small mammals both decreased, whereas *Borrelia, Anaplasma* and ungulates both increased along the first disturbance gradient. As for *Diplorickettsia*: this genus was only recently discovered (Mediannikov et al. 2010) and a potential vertebrate reservoir host

remains to be elucidated. Its type species however, *D. massiliensis*, has been found to be pathogenic for humans (Subramanian et al. 2012). Our results indicate that pathogen-specific differential host use patterns may cause certain host species (e.g. deer) to simultaneously dilute the prevalence of some pathogens (e.g. *Rickettsia*) while amplifying the prevalence of others (e.g. *Borrelia* and *Anaplasma*) (Randolph and Dobson 2012).

However, the most important metric to quantify human risk to tick-borne diseases is the density of infected ticks (Wood and Lafferty 2013). This requires simultaneous consideration of both pathogen prevalence and vector abundance. By combining these data, it becomes clear that any reduction in pathogen prevalence across the first gradient, such as for *Rickettsia*, is offset by the tremendous increase in densities of questing ticks (see Appendix 6: Fig A6.2). Thus, vector augmentation via high wildlife abundance is likely to negate any dilution effect across the first disturbance gradient, resulting in high densities of infected ticks (Randolph and Dobson 2012). In contrast, densities of infected ticks do not increase across the second disturbance gradient due to a combination of low infection prevalence and constant tick densities (see Appendix Fig A6.2). This suggests that the relationship between biodiversity loss and disease risk may be non-linear: loss of apex predators initially increases tick-borne disease risk through vector augmentation by medium- to large-sized frugivores and herbivores, but subsequent loss of these important reproduction hosts decreases disease risk again by reducing tick abundance. Non-linear relationships between biodiversity and disease risk have previously been hypothesized (Wood and Lafferty 2013, Gottdenker et al. 2014, Wood et al. 2016), but this study is among the first to have found evidence for this hypothesis.

Our analyses of tick microbiota and potential disease risk also have limitations. Blood feeding increases microbial diversity in ticks, including the proportion of OTUs identified as *Rickettsia* (Heise et al. 2010). Subsequent transmission of pathogens to susceptible hosts depends on the tick's ability to maintain infection transstadially, i.e. after molting from larva to nymph and from nymph to adult (Clay and Fuqua 2010). Unfortunately, very few data are available on the vector capacity of most tick species in Central America, and the role of reservoir hosts is only starting to unfold (Bermúdez et al. 2016, Bermúdez et al. 2017). In addition, ticks host a large variety of endosymbiotic, transovarially-transmitted bacteria, including species within *Coxiella*, *Francisella*, and *Rickettsia* (Noda et al. 1997, Scoles 2004, Perlman et al. 2006). It remains unclear which of the OTUs that were assigned to pathogenic genera are non-pathogenic endosymbionts and which are in fact pathogenic to humans or wildlife. Other

pathogenic microbes, such as protozoan parasites (e.g. *Babesia*) or tick-borne Flaviviruses were not detected because of our focus on bacterial 16S rRNA. However, we consider that patterns of response of these pathogens to biodiversity may have been similar to those we describe for bacteria and should not strongly affect our conclusions.

Our study corroborates the need to move beyond simple measures of biodiversity loss, such as fragment size or species richness, and to directly measure the composition and structure of wildlife communities (LoGiudice et al. 2008, Randolph and Dobson 2012). The use of camera traps allowed us to do this and to subsequently identify the two strongest disturbance gradients. Wildlife species richness did not change across either of these gradients, suggesting substitution of lost species. Thus, sites that varied from small secondary fragments to large old-growth forests had similar number of species, but very different wildlife communities in terms of abundance and trophic complexity. Reanalysing our data showed that only tick species richness increased with wildlife species richness. None of the other measures (i.e. tick density and prevalence, microbial diversity, and pathogen prevalence or richness) could be explained by wildlife species richness. This emphasizes the importance of considering the identity of each suite of species and suggests that densities of specific (reproduction) hosts may be a more important factor than species richness *per se* for tick population and tick-borne disease dynamics.

Appendix 6

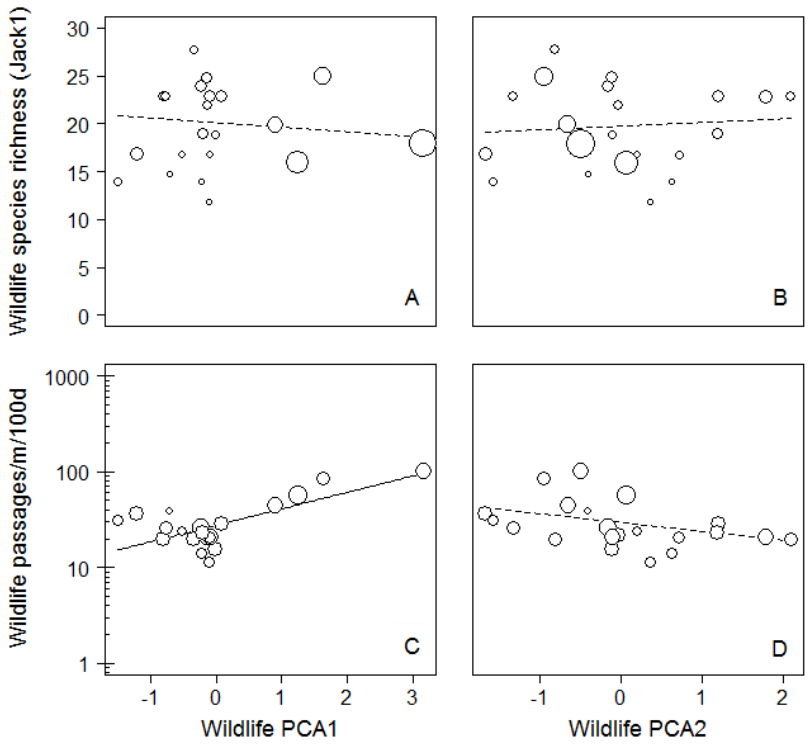


Figure A6.1 Wildlife species richness did not change along the first two principal component axes, PCA1 **(a)** and PCA2 **(b)**. Wildlife abundance increased along PCA1 **(c)** but not along PCA2 **(d)**. Bubble size reflects the number of animal passages recorded per site **(a-b)** or the number of camera deployment days **(c-d)**.

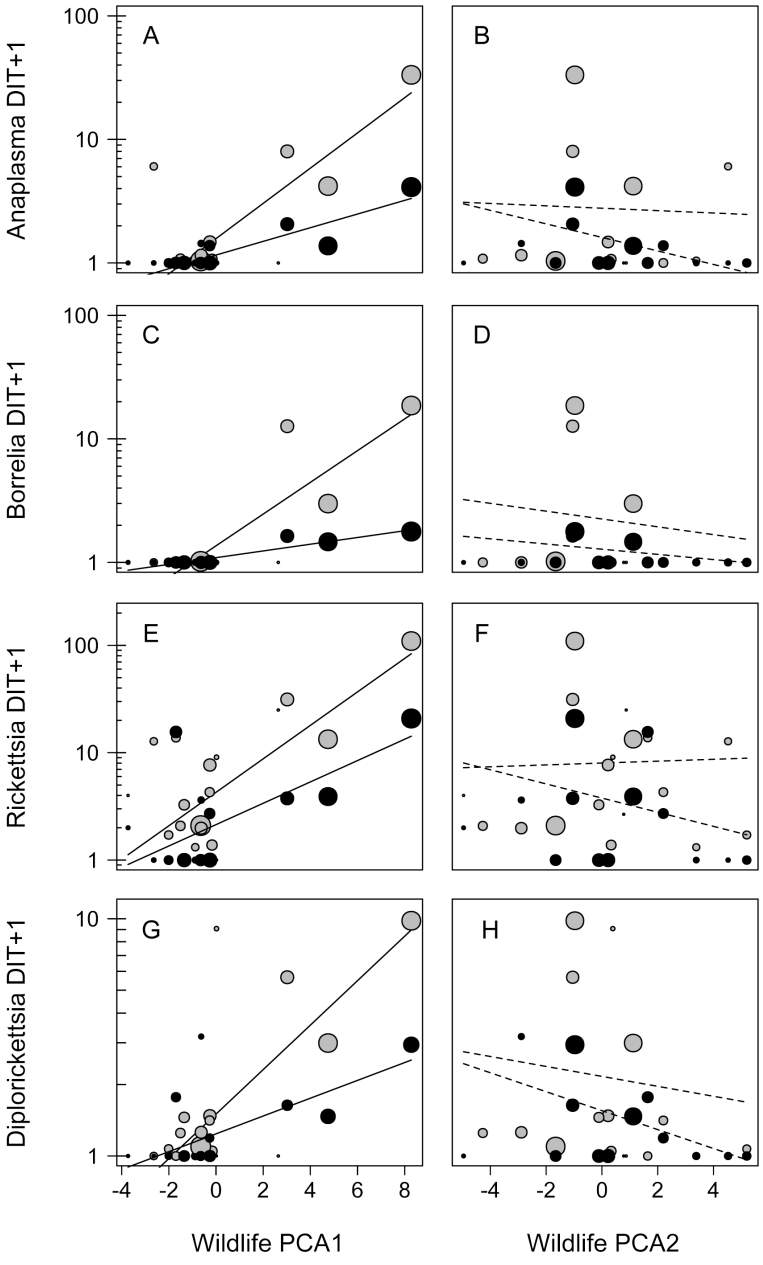


Figure A6.2 The predicted density of infected nymphs (*grey*) and adults (*black*) as a combination of questing tick densities and infection prevalence in respectively larvae and nymphs from small mammals. Bubble size reflects the number of sequenced ticks.

Table A6.1 Summary of camera trapping data: sampling effort (in days), total number of animal captures, observed species richness (S_{obs}), estimated total species richness (Jack1) with lower and upper confidence intervals (CI_{Jack1}), and total capture rate (m^{-1} per 100 days) of wildlife for each site.

Site	Sampling effort	No. captures	S_{obs}	Jack1	CI_{Jack1}	Capture rate
AgSa1	798	681	21	23.9	(23.14 – 24.66)	25.8
AgSa2	571	426	19	21.9	(21.22 – 22.52)	21.6
AgSa3	576	302	16	18.8	(18.07 – 19.55)	15.6
AgSa4	131	181	11	14.9	(13.34 – 16.16)	39.0
BCI	715	3320	16	17.9	(17.44 – 18.42)	103.3
CCA	609	749	15	16.9	(16.45 – 17.41)	36.6
COC	565	415	21	22.9	(22.23 – 23.51)	19.8
EMP	561	354	22	27.8	(27.00 – 28.58)	19.7
GIG	938	2246	15	16.0	(15.63 – 16.31)	56.9
LIM	710	562	20	24.8	(23.69 – 25.97)	21.2
PAV	352	315	13	13.9	(13.48 – 14.40)	30.9
PBO	740	690	20	22.9	(22.31 – 23.49)	21.0
PIN	529	416	17	22.8	(21.96 – 23.58)	25.9
PLA	702	1131	17	19.9	(19.29 – 20.49)	44.6
PLR	509	1317	22	24.9	(24.29 – 25.49)	86.2
Srllor2	395	271	14	16.8	(16.04 – 17.56)	20.5
Srllor3	647	612	18	22.8	(22.08 – 23.56)	28.1
Srllor4	334	179	13	13.9	(13.44 – 14.42)	14.1
StDom1	282	239	14	16.8	(16.01 – 17.57)	23.9
StDom2	356	143	10	11.9	(10.89 – 12.83)	11.3
StDom3	626	506	18	19.0	(18.60 – 19.32)	23.2

Table A6.2 Summary data on ticks: average number of questing ticks (per 1000 m^2), tick prevalence (no. hosts with/without ticks), number of nymphs and larvae collected from small mammals, total number of identified ticks, and species richness of ticks for each site.

Site	Drag sampling			Live trapping			Total	
	No. adults	No. nymphs	No. larvae	Tick prevalence	No. nymphs	No. larvae	No. ticks identified	Species richness
AgSa1	1.67	6	16.67	1/2	1	0	14	4
AgSa2	0	14.33	48.67	6/6	40	34	97	7
AgSa3	0	32.33	303.67	1/1	1	4	589	2
AgSa4	20	16	0	8/3	27	15	66	8
BCI	28	205.5	692.5	24/15	73	75	860	10
CCA	0	1	905	4/4	23	14	29	6
COC	1	3	263.5	3/15	3	3	6	3
EMP	2	7	18	8/3	21	18	45	10
GIG	7	77	1800.5	15/13	41	52	246	9
LIM	0	0.5	46.5	4/10	2	21	22	4
PAV	0	18.5	11.5	6/4	5	11	15	4
PBO	0.5	1.5	60.5	3/15	4	46	20	3
PIN	0	0.5	360.5	6/2	7	11	18	3
PLA	8	24	91.5	3/1	0	1	105	4
PLR	6	27.5	173.5	17/6	74	178	402	14
Srllor2	0	1.5	0.5	13/14	25	235	108	6
Srllor3	3.5	1.5	209	6/14	8	31	38	3
Srllor4	-	-	-	1/2	3	0	3	2
StDom1	0	5	8	8/14	64	34	48	7
StDom2	0	1	29	0/1	0	0	0	-
StDom3	0	1	28.5	0/5	0	0	0	-

Table A6.3 Summary of sequencing data per site: number of ticks sequenced, number of OTUs identified, and number of ticks that tested positive for pathogenic genera. The number of OTUs that were placed within a bacterial genus are indicated between brackets.

	No. ticks	No. OTUs	*Anaplasma* (n=20)	*Bartonella* (n=1)	*Borrelia* (n=6)	*Coxiella* (n=7)	*Diplorickettsia* (n=22)	*Francisella* (n=23)	*Orientia* (n=17)	*Rickettsia* (n=7)
AgSa1	1	22	0	0	0	1	0	1	0	1
AgSa2	68	3,400	4	0	2	5	3	62	0	41
AgSa3	5	469	0	0	0	0	1	5	0	1
AgSa4	41	2,295	0	0	0	7	1	39	2	31
BCI	142	7,293	19	3	8	35	8	127	1	88
CCA	29	2,973	1	0	0	1	4	28	2	22
COC	4	331	0	0	0	1	0	4	0	4
EMP	38	2,384	4	0	0	3	3	37	0	26
GIG	66	5,073	8	0	8	4	5	66	5	26
LIM	22	3,215	3	0	0	6	2	20	1	16
PAV	15	2,493	3	0	0	1	0	15	0	10
PBO	18	1,519	1	0	0	1	3	18	1	13
PIN	18	1,988	4	0	0	2	1	18	1	14
PLA	1	233	0	0	0	0	0	1	0	1
PLR	133	8,678	12	0	10	10	10	131	11	62
SrIlor2	103	6,570	5	0	1	11	8	97	7	74
SrIlor3	37	1,728	4	0	0	1	10	35	1	25
SrIlor4	3	462	0	0	0	2	1	3	0	2
StDom1	57	3,382	2	0	2	24	5	49	5	31
StDom2	0	-	-	-	-	-	-	-	-	-
StDom3	0	-	-	-	-	-	-	-	-	-

Table A6.4 Summary of sequencing data per tick species: number of sequenced individuals, number of OTUs detected, and the number of ticks that tested positive for pathogenic bacterial genera. The number of OTUs that were detected within a specific genus are indicated between brackets.

	No. ticks	No. OTUs	*Anaplasma*	*Bartonella*	*Borrelia*	*Coxiella*	*Diplorickettsia*	*Francisella*	*Orientia*	*Rickettsia*
Amblyomma										
auricularium	75	3,053	7(4)	0	0	3(2)	6(3)	74(5)	5(2)	66(5)
dissimile	28	2,276	2(2)	0	0	1(1)	1(1)	28(5)	1(1)	16(4)
geayi	28	1,865	1(1)	0	1(1)	23(1)	10(3)	21(3)	0	23(6)
longirostre	1	193	0	0	0	0	0	1(1)	0	1(1)
mixtum	44	1,325	2(1)	0	2(2)	39(1)	1(1)	35(5)	2(2)	37(4)
naponense	5	799	0	0	0	3(1)	0	5(1)	1(1)	2(1)
oblongoguttatum	1	26	0	0	0	0	0	1(1)	0	0
ovale	103	3,870	6(3)	0	7(4)	2(1)	7(5)	102(18)	14(2)	60(3)
pacae	45	2,532	1(1)	0	0	2(2)	4(5)	44(5)	4(3)	42(5)
sabanerae	252	8,240	19(9)	2(1)	0	7(3)	29(10)	241(14)	3(3)	135(4)
tapirellum	1	184	0	0	0	1(1)	0	1(1)	0	1(1)
varium	9	1,233	2(1)	0	0	0	0	9(1)	0	5(2)
sp.	1	14	0	0	0	0	0	0	0	0
Haemaphysalis										
juxtakochi	164	6,504	22(10)	0	4(4)	7(5)	6(4)	164(12)	6(5)	81(4)
Ixodes										
affinis	2	108	0	0	0	0	0	1(1)	0	2(1)
Ornithodoros										
puertoricensis	32	1,475	8(4)	1(1)	17(2)	25(1)	1(2)	21(1)	1(1)	13(2)

Chapter 7

General discussion

Introduction

The ongoing loss of biodiversity and increasing emergence of infectious diseases has sparked great interest in the possibility of a causal link between these concurrent patterns. A negative relationship between biodiversity and disease risk could offer a win-win situation for nature conservation and human health (Kilpatrick et al. 2017). However, the generality of this relationship and the underlying mechanisms are still subject of a contentious debate (Randolph and Dobson 2012, Salkeld et al. 2013, Huang 2014, Civitello et al. 2015, Hofmeester 2016). The aim of this thesis was to contribute to a better understanding of the interactions between ticks and their vertebrate hosts in a biodiversity hotspot, and how loss of biodiversity affects these interactions and ultimately, tick-borne disease risk. My study was among the first to simultaneously consider and directly assess broader communities of wildlife, ticks, and tick-borne pathogens across an anthropogenic disturbance gradient in tropical forests. I used a combination of camera trapping to monitor wildlife communities, live trapping of small mammals, drag sampling of questing ticks, and molecular techniques for the identification of ticks and tick-borne pathogens. In this final chapter I synthesize the results of this thesis and discuss how they contribute to the biodiversity-disease discussion.

Host-use patterns of ticks

To understand whether and how biodiversity loss affects tick-borne disease risk in tropical forests, I first needed to quantify the ecological relationships between ticks and their hosts. Ticks are not distributed randomly across vertebrate hosts (Wilson et al. 2002). The number and diversity of ticks carried by a host species depends on tick feeding preferences as well as host biological and ecological traits (Randolph 2004). These factors contribute to pathogen transmission dynamics in a number of ways. For example, the degree to which parasites such as ticks are host-specific is a key determinant of their local abundance (Krasnov et al. 2004b) and the potential routes by which pathogens can be transmitted across vertebrate host taxa (McCoy et al. 2013). Generalist ticks are more likely to transmit pathogens among a wide range of host species (Ostfeld and Keesing 2012), and may feed proportionally more from disease reservoir hosts in forest fragments with impoverished wildlife communities (Allan et al. 2003, Keesing et al. 2010, Gottdenker et al. 2012). Therefore, generalized host-feeding of vectors is a

prerequisite for the dilution effect hypothesis, which states that loss of host diversity should increase disease risk by redistributing vector meals among more competent host species (Ostfeld and Keesing 2000, Keesing et al. 2006). However, theory predicts that specialization should be high in species-rich communities such as those in tropical forests (MacArthur 1972, May 1973, Chesson 2000). Whether tropical ticks indeed tend to be host-specific has received relatively little attention, and studies so far have found mixed patterns (Cumming 1998, Nava and Guglielmone 2013, Wells et al. 2013, Espinaze et al. 2015).

My results indicate that the ticks from Panama, a biodiversity hotspot, are highly host-specific in the adult stage. Using a combination of quantitative network analyses and phylogenetic tools, I have found evidence for 1) structural differences in the distribution of adult ticks across their vertebrate hosts, indicating that some host species are ecologically more important than others (*structural specificity*), and 2) phylogenetic relatedness among exploited host species, which is an important aspect of evolutionary specialization and indicates that adult ticks tend to feed on more closely related host species (*phylogenetic specificity*) (Chapter 2). Moreover, species assemblages of adult ticks became increasingly diverse on larger-bodied host species, indicating that adult ticks in Panama tend to select for large reproduction hosts (Chapter 4). This is in agreement with studies from Europe, where the vector of Lyme disease, *Ixodes ricinus*, principally feeds from roe deer and other ungulates during the adult life stage (Hofmeester et al. 2016), and with studies from Africa, where wild ungulates were the most important hosts for adult ticks (Gallivan and Horak 1997).

Immature ticks showed rather different host-feeding patterns. We collected larvae and nymphs of numerous tick species from a wide variety of birds (Chapter 3), small rodents, and opossums (Chapter 6): host species on which adult ticks were less common (Chapter 2, 4). Many of the larvae and nymphs that were collected from small vertebrates feed on entirely different hosts during the adult stage. But immature ticks also fed from larger vertebrates. In fact, my analyses from chapter 4 showed that assemblages of immature ticks were equally diverse across a large number of host taxa (ranging in body size by 3 orders of magnitude). This suggests that across vertebrate host taxa, larvae and nymphs may feed more opportunistically than their adult counterparts. Other studies also found that immature ticks were less host-specific than adults (Nava and Guglielmone 2013, Espinaze et al. 2015). There are several mutually non-exclusive explanations for this difference. First, adult ticks quest in higher vegetation layers than immature ticks, where they may miss small host species

(Randolph and Storey 1999). Second, adult ticks need to maximize their chances of encountering a mating partner, which they often find on hosts and hence should drive host specificity (Espinaze et al. 2015). Immature ticks in contrast, need to maximize their chances of acquiring a blood-meal, which should drive host generality. Finally, host-immune responses or host grooming could potentially affect adult ticks more strongly than immature ticks feeding on small vertebrates, although I am not aware of any study that tested this hypothesis.

Besides analyses across host taxa, I also examined the distribution of larvae and nymphs across host individuals. Among live-trapped small mammals, tick prevalence increased with host body mass and males were more likely to have ticks than females (Chapter 6). However, because males tend to be larger than females, host body mass may be confounded with sex. Reanalysis of the data showed that host body mass was no longer a significant predictor of tick prevalence when only males (GLMM: $\beta = 0.52$, $P = 0.29$) or females (GLMM: $\beta = 1.27$, $P = 0.07$) were included in the analyses. Thus, sex-biased parasitism was driven by sex as opposed to body mass. Male-biased tick infestation can be explained by males having higher testosterone levels, which reduces both innate and required resistance to tick feeding (Hughes and Randolph 2001), and larger home ranges, which increasing their chances of encountering ticks (Tew and Macdonald 1994). In contrast, no effect of host sex was found for avian hosts: male and female birds were equally likely to be parasitized (Chapter 3). Apparently, behavioural differences between male and female birds are too small or not important enough to result in differences in exposure to ticks (Marsot et al. 2012). Avian ecological traits across species however, did predict tick parasitism: forest habitation, terrestrial foraging, bark insectivory, and lowland residency all increased risk of acquiring ticks (Chapter 3). Further, resident birds were more often parasitized than migratory birds, possibly reflecting differences in host quality and immune response. Migratory birds are exposed to more diverse parasite faunas and, in response, have evolved larger immune defence organs than resident birds (Møller and Erritzøe 1998), which could explain their lower tick prevalence.

Host diversity drives tick and pathogen diversity

High tick-host specificity implies that ticks should be sensitive to loss of host species following anthropogenic disturbances such as habitat fragmentation and hunting. Lack of specific reproduction hosts for adult ticks should strongly limit tick abundance and could make them

susceptible to secondary extinction, even if larvae and nymphs are able to feed from a larger variety of host species (Lafferty 2012). The previously-connected islands and peninsulas of the Barro Colorado Nature Monument (BCNM) offered the ideal opportunity to test this hypothesis (Chapter 5). As the BCNM is well-protected from hunting and largely surrounded by water, the size of these forest fragments was a strong predictor for wildlife abundance and species richness, concordant with island biogeography theory (MacArthur and Wilson 1967). In turn, the abundance and species richness of ticks was tightly linked to that of wildlife. These results contribute to a growing consensus that host diversity begets parasite diversity (Hechinger and Lafferty 2005). In a recent meta-analysis, Kamiya et al. (2014b) demonstrated that species richness of a wide range of endo- and ectoparasites increased with that of their hosts. Chapter 5 extends these findings by providing the first empirical evidence for a positive relationship between host and tick species richness.

The linearity of this relationship is in agreement with theoretical models predicting host-parasite coextinction risk (Lafferty 2012). Several studies have suggested that parasites could make up the unseen majority of species extinctions (Koh et al. 2004, Dobson et al. 2008, Dunn et al. 2009, Lafferty 2012), but empirical evidence of host-parasite coextinction events are relatively scarce. Using data on endoparasitic helminths in fish, Strona et al. (2013) argued that this is because specialist parasites use hosts with low extinction risk. My results demonstrate that the opposite is true for ectoparasitic ticks and their hosts in tropical forests. Adult ticks tended to feed from larger-bodied vertebrates (Chapter 3), which are more likely to suffer primary extinctions (Purvis et al. 2000, Cardillo et al. 2005). Indeed, specialist tick species were only found in larger fragments of the BCNM where their specific reproduction hosts (i.e. peccary and deer) were captured by camera traps. As specialist tick species disappeared, tick communities became increasingly dominated by a generalist species, *Amblyomma oblongoguttatum* (Chapter 5). Susceptibility of specialist ticks but robustness of generalist ticks to local community disassembly is in agreement with theoretical predictions (Lafferty 2012), and suggests that questing ticks can be used as bioindicators of wild fauna (Marcogliese 2005, Ogrzewalska et al. 2011).

A related study by Bush et al. (2013) showed that species richness of lice on birds declined with forest fragment size and that some parasite genera were absent from the smallest fragments. Although neither the lice in that study nor the ticks of the BCNM became actually extinct, the absence of specialist ticks in locations where their specific host species

disappeared provides a clear example of local host-parasite coextirpation. The presence of multiple life stages, high host specificity, and higher extinction risk for hosts than non-hosts should all increase parasite extinction risk (Lafferty 2012). All of these risk factors exist in my study system. First, ticks have complex life cycles with multiple developmental stages. Second, adult ticks showed high host specificity (Chapter 2). Third, principal reproduction hosts for adult ticks were large vertebrates (Chapter 4), which have higher extinction risks than smaller vertebrates (Purvis et al. 2000, Cardillo et al. 2005).

The positive relationship between host and parasite diversity that exists for vertebrates and ticks should also apply to ticks and their microbiome. Indeed, in chapter 6 I found that sites with higher species richness of ticks had higher numbers of Operational Taxonomic Units (OTUs), a measure of microbial species richness. Likewise, the number of OTUs that were assigned to potentially pathogenic taxa increased with tick species richness. Other studies have also shown that tick community composition strongly regulates pathogen community composition (Cumming and Guégan 2006). From this I conclude that species richness of wildlife is positively related to species richness of ticks, which in turn is positively related to species richness of microbial endosymbionts and (potential) tick-borne pathogens. This suggests that high biodiversity is a source of infectious diseases (Morand et al. 2014).

Tropical biodiversity loss and tick-borne disease risk

So what are the implications of the tick-host relationships described so far for pathogen transmission dynamics and the impact of biodiversity loss thereupon? On the one hand, the tight link between species richness of host, ticks, and pathogens suggests that tick-borne disease risk should decrease with wildlife community disassembly. Tick abundance was also lower where vertebrate hosts were less abundant. On the other hand, generalist ticks persisted in impoverished wildlife communities where they may feed relatively more from small mammals, which tend to be less susceptible to extinction (Cardillo 2003) and are important disease reservoir hosts (Meerburg et al. 2009). Moreover, defenders of the dilution effect hypothesis argue that host density and hence vector density should not change with wildlife diversity loss, as remaining host species compensate for species loss by increasing in abundance (Keesing et al. 2010, Levi et al. 2016). Although I found that wildlife species richness and abundance both decreased with forest fragment size in the BCNM, these fragments were

largely surrounded by water. More commonly however, they are embedded in agricultural and sub-urban landscapes, where additional nutrient subsidies may elevate the abundance of remaining species, and/or where synanthropic species may be added to the host community (Mendenhall et al. 2014). Therefore, I decided to compare communities of hosts, ticks, and pathogens in forest fragments across a gradient of anthropogenic land-use change in central Panama (Chapter 6).

My study sites ranged from vast forests that had members of all trophic levels, including apex predators such as jaguar and puma, to forest remnants that were dominated by habitat generalists such as rats and opossums. Consistent with previous studies, initial loss of apex predators was associated with increased abundances of medium- to large-sized frugivores and herbivores (Michalski and Peres 2007, Ripple et al. 2014). As the latter species are important reproduction hosts for adult ticks, their increased abundance was accompanied by a dramatic increase in questing tick abundance as well as tick prevalence on small mammals. Wood et al. (2016) argued that reductions in large predators should increase diseases carried by ungulates, but decrease diseases carried by rodents, which are negatively related to ungulate densities (Keesing and Young 2014). My results support this hypothesis. As populations of ungulates increased, so did the prevalence of *Anaplasma* and *Borrelia*, of which several strains circulate in deer (Massung et al. 2005, Kawahara et al. 2006, Moyer et al. 2006, Yparraguirre et al. 2007, Nieto et al. 2012, Sacchi et al. 2012, Stuen et al. 2013, Lee et al. 2014). At the same time, the density of rodents and opossums decreased, as did the prevalence of *Rickettsia*, which circulates in these small mammals (Meerburg et al. 2009, Bermúdez et al. 2017). However, the exponential increase in tick density negated any reduction in pathogen prevalence. Thus, non-competent hosts may increase disease risk by sustaining large vector populations (Wood and Lafferty 2013). This is in agreement with other studies that found that vector augmentation by non-competent hosts is stronger than their diluting effect (Ogden and Tsao 2009, Hofmeester 2016), so that the density of infected ticks can increase even if tick infection prevalence decreases (Wood and Lafferty 2013).

As anthropogenic land-use change became more intense, and medium- to large-sized frugivores and herbivores also disappeared, wildlife communities became dominated by habitat generalists. This is concordant with previous studies from the Neotropics (Chiarello 1999, Michalski and Peres 2007, Canale et al. 2012). Importantly however, neither species richness nor the total abundance of host communities changed significantly across the entire

disturbance gradient. This is because the loss of extinction-prone species was counterbalanced by increased abundances of synanthropic mammals (e.g. opossums, raccoons, armadillos) and the addition of domestic animals to host communities. These results indicate that even though species richness and total abundance of host communities did not change with increasing anthropogenic disturbance, the identity of species in these communities changed dramatically. My study thus corroborates the need to move beyond the use of species richness in biodiversity-disease studies, and to directly assess the composition and structure of wildlife communities (LoGiudice et al. 2008, Randolph and Dobson 2012, Hofmeester 2016). Camera traps allowed me to do this, and to detect changes in the abundance of specific host species that would otherwise have gone unnoticed. Camera trapping therefore provides a promising new tool in the field of disease ecology.

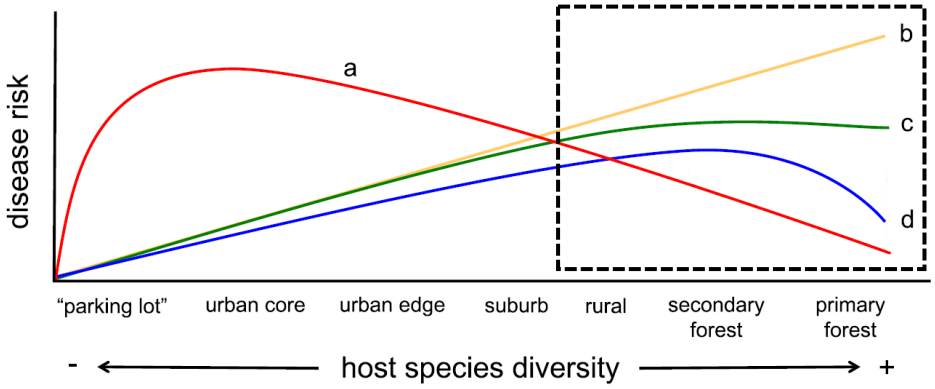


Figure 7.1 Theoretical models for the effect of biodiversity loss/land- use type on disease risk (adapted from Wood et al. 2016). Disease risk should be zero where host species diversity is zero (the "parking lot ecosystem"). Possible relationships include (a) dilution effect, (b) amplification effect, (c) amplification effect that saturates at high levels of biodiversity, and (d) amplification effect that shifts to dilution at high levels of biodiversity. The dashed box represents the gradient considered in Chapter 6. Evaluation of possible relationships indicates that (d) best describes tick-borne disease risk in tropical forests. Note: this model assumes that host species diversity and land-use type are linearly related (see Wood et al. 2016), but the results from Chapter 6 indicate that this assumption is likely to be incorrect for tropical forests.

Densities of infected ticks were much lower in the most disturbed sites compared to sites that had high abundances of ungulates and large caviomorph rodents (Chapter 6). This

suggests a non-linear relationship between biodiversity and disease risk (Fig 7.1): loss of apex predators initially increases tick-borne disease risk through vector augmentation by medium- to large-sized frugivores and herbivores. Subsequent loss of these important reproduction hosts decreases disease risk by reducing tick abundance. Non-linear relationships between biodiversity and disease risk have previously been hypothesized (Wood and Lafferty 2013, Gottdenker et al. 2014, Wood et al. 2016), but this study is among the first to have found evidence for this hypothesis. In conclusion, my results suggest that densities of specific (reproduction) hosts are a more important factor than species richness *per se* for tick population and tick-borne disease dynamics.

Biodiversity conservation needs parasites

Whether control of infectious diseases truly is a general ecosystem service of high biodiversity remains disputable. In contrast, parasites are increasingly recognized for performing several important ecosystem services. For example, parasites help keep population sizes under control (Koplow 2003, Dobson et al. 2008) and are themselves important food sources (Johnson et al. 2010). They dominate food webs (Lafferty et al. 2008) and can influence competitive and predatory interactions, thereby promoting species exclusion or coexistence (Hatcher et al. 2006). Some parasites play important roles in selectively removing pollutants such as heavy metals from the environment (Sures 2004), or allergens from the human gut (Yazdanbakhsh et al. 2002). Parasites can even alter ecosystem structure (Thomas et al. 2005), and are fundamental drivers of co-evolutionary radiation and biodiversity (Marcogliese 2004, Hudson et al. 2006). Yet parasites are often uniquely portrayed as threats to biodiversity conservation (Nichols and Gómez 2011).

We should carefully reconsider the way we look at parasites and their relationship with biodiversity. Parasites are ubiquitous components of healthy ecosystems in terms of species richness, biomass, and significance in food webs (Marcogliese 2005, Hudson et al. 2006, Gómez and Nichols 2013). As parasites need minimum thresholds of host abundance to maintain viable populations, many parasites will go extinct before their hosts disappear (Dobson et al. 2008). In fact, host-parasite coextinctions may account for a significant portion of the current biodiversity crisis (Koh et al. 2004, Dunn et al. 2009). An estimated 63 species of ixodid ticks are considered to be co-endangered with their hosts and at least one species has

already become extinct (Mihalca et al. 2011). While few will mourn their loss, ticks and other parasites have their place in the wider ecosystem. Losing parasites would strongly affect ecosystem functioning and the persistence of their hosts on the long run (Gómez and Nichols 2013). An increasing number of studies therefore highlight the importance of parasite-inclusive conservation (Windsor 1995, Nichols and Gómez 2011, Gómez and Nichols 2013, Dougherty et al. 2016, Spencer and Zuk 2016). However, due to their negative impact on human and animal health, food security, and economics, parasites are usually the target of eradication programs rather than conservation efforts. Yet ignoring parasites in conservation is ignoring the majority of life forms on Earth (Dobson et al. 2008, Gómez and Nichols 2013). Moreover, parasitized individuals sometimes gain fitness advantages over unparasitized conspecifics, so that the benefits may outweigh the costs of being parasitized (Thomas et al. 2000). If not for their intrinsic value, parasites should be recognized in biodiversity conservation for their key roles in ecological and evolutionary processes, but this will require a paradigm shift in our perception and valuation of parasites (Dougherty et al. 2016).

Bibliography

ACP. 2016. The meteorology and hydrology branch, Panama Canal Authority, Republic of Panama.

Agnarsson, I., C. M. Zambrana-Torrelio, N. P. Flores-Saldana, and L. J. May-Collado. 2011. A time-calibrated species-level phylogeny of bats (Chiroptera, Mammalia). PLoS Currents **3**:RRN1212.

Allan, B. F., F. Keesing, and R. S. Ostfeld. 2003. Effect of forest fragmentation on Lyme disease risk. Conservation Biology **17**:267-272.

Altizer, S., C. L. Nunn, P. H. Thrall, J. L. Gittleman, J. Antonovics, A. A. Cunningham, A. A. Cunnningham, A. P. Dobson, V. Ezenwa, K. E. Jones, A. B. Pedersen, M. Poss, and J. R. C. Pulliam. 2003. Social organization and parasite risk in mammals: integrating theory and empirical studies. Annual Review of Ecology, Evolution, and Systematics **34**:517-547.

Anderson, K., V. O. Ezenwa, and A. E. Jolles. 2013. Tick infestation patterns in free ranging African buffalo (Syncercus caffer): Effects of host innate immunity and niche segregation among tick species. International Journal for Parasitology: Parasites and Wildlife **2**:1-9.

Anderson, R. M. and R. M. May. 1978. Regulation and stability of host-parasite population interactions: I. Regulatory processes. The Journal of Animal Ecology:219-247.

Andrén, H. 1994. Effects of habitat fragmentation on birds and mammals in landscapes with different proportions of suitable habitat: A review. Oikos **71**:355-366.

Apanaskevich, D. A. and S. E. Bermúdez. 2013. Description of a new *Dermacentor* (Acari: Ixodidae) species, a parasite of wild mammals in Central America. Journal of Medical Entomology **50**:1190-1201.

Apperson, C. S., B. Engber, W. L. Nicholson, D. G. Mead, J. Engel, M. J. Yabsley, K. Dail, J. Johnson, and D. W. Watson. 2008. Tick-borne diseases in North Carolina: is "*Rickettsia amblyommii*" a possible cause of rickettsiosis reported as Rocky Mountain spotted fever? Vector-Borne and Zoonotic Diseases **8**:597-606.

Araya-Anchetta, A., J. D. Busch, G. A. Scoles, and D. M. Wagner. 2015. Thirty years of tick population genetics: A comprehensive review. Infection, genetics and evolution **29**:164-179.

Argüello, A. P., L. Hun, P. Rivera, and L. Taylor. 2012. A fatal urban case of Rocky Mountain spotted fever presenting an eschar in San Jose, Costa Rica. The American journal of tropical medicine and hygiene **87**:345-348.

Arneberg, P. 2002. Host population density and body mass as determinants of species richness in parasite communities: comparative analyses of directly transmitted nematodes of mammals. Ecography **25**:88-94.

Arner, A. J. 2012. The curious case of the cane toad (*Rhinella marina*): An assessment of exploratory behavior and foraging success of an invasive vertebrate in a novel environment. Master of Science thesis. Texas Tech University.

Asquith, N. M., S. J. Wright, and M. J. Clauss. 1997. Does mammal community composition control recruitment in Neotropical forests? Evidence from Panama. Ecology **78**:941-946.

Bardeleben, C., R. L. Moore, and R. K. Wayne. 2005. A molecular phylogeny of the Canidae based on six nuclear loci. Molecular Phylogenetics and Evolution **37**:815-831.

Begon, M. 2008. Effects of host diversity on disease dynamics. Pages 12-29 *in* R. S. Ostfeld, F. Keesing, and V. T. Eviner, editors. Infectious Disease Ecology: Effects of Ecosystems on Disease and of Disease on Ecosystems. Princeton University Press.

Bermúdez, S., H. J. Esser, R. Miranda, and R. Moreno. 2015a. Wild carnivores (Mammalia) as hosts for ticks (Ixodida) in Panama. Systematic & Applied Acarology **20**:13-19.

Bermúdez, S., R. Miranda, Y. Zaldívar, P. González, G. Berguido, D. Trejos, J. M. Pascale, and M. Labruna. 2012a. Detección de *Rickettsia* sp. en ectoparasitos de animales domésticos y silvestres de la Reserva Natural Privada Cerro Chucantí y communidades aledañas, Panamá (2007-2010). Biomedica **32**:189-195.

Bermúdez, S. E., A. Castro, H. Esser, Y. Liefting, G. García, and R. J. Miranda. 2012b. Ticks (Ixodida) on humans from central Panama, Panama (2010–2011). Experimental and Applied Acarology **58**:81-88.

Bermúdez, S. E., A. M. Castro, D. Trejos, G. G. García, A. Gabster, R. J. Miranda, Y. Zaldívar, and L. E. Paternina. 2016. Distribution of spotted fever group Rickettsiae in hard ticks (Ixodida: Ixodidae) from Panamanian urban and rural environments (2007–2013). EcoHealth **13**:274-284.

Bermúdez, S. E., M. E. Eremeeva, S. E. Karpathy, F. Samudio, M. L. Zambrano, Y. Zaldivar, J. A. Motta, and G. A. Dasch. 2009. Detection and identification of Rickettsial agents in ticks from domestic mammals in eastern Panama. Journal of Medical Entomology **46**:856-861.

Bermúdez, S. E., D. González, and G. García. 2013. Ticks (Acari: Ixodidae, Argasidae) of coyotes in Panama. Systematic & Applied Acarology **18**:112-115.

Bermúdez, S. E., N. Gottdenker, A. Krishnvajhala, A. Fox, H. K. Wilder, K. González, D. Smith, M. López, M. Perea, and C. Rigg. 2017. Synanthropic mammals as potential hosts of tick-borne pathogens in Panama. PLoS ONE **12**:e0169047.
Bermúdez, S. E., R. J. Miranda, and D. Smith. 2010. Tick species (Ixodida) in the Summit Municipal Park and adjacent areas, Panama City, Panama. Experimental and Applied Acarology **52**:439-448.
Bermúdez, S. E., S. Torres, Y. Aguirre, L. Domínguez, and J. Bernal. 2015b. A review of *Ixodes* (Acari: Ixodidae) parasitizing wild birds in Panama, with the first records of *Ixodes auritulus* and *Ixodes bequaerti*. Systematic and Applied Acarology **20**:847-853.
Bermúdez, S. E., Y. Zaldivar, M. G. Spolidorio, J. Moraes-Filho, R. J. Miranda, C. M. Caballero, Y. Mendoza, and M. B. Labruna. 2011. Rickettsial infection in domestic mammals and their ectoparasites in El Valle de Antón; Colcé; Panamá. Veterinary Parasitology **177**:134-138.
Bersier, L.-F., C. Banašek-Richter, and M.-F. Cattin. 2002. Quantitative descriptors of food-web matrices. Ecology **83**.
Blackburn, T. M., V. K. Brown, B. M. Doube, J. J. Greenwood, J. H. Lawton, and N. E. Stork. 1993. The relationship between abundance and body size in natural animal assemblages. Journal of Animal Ecology:519-528.
Blüthgen, N., J. Fründ, D. P. Vázquez, and F. Menzel. 2008. What do interaction network metrics tell us about specialization and biological traits? Ecology **89**:3387-3399.
Blüthgen, N., F. Menzel, and N. Blüthgen. 2006. Measuring specialization in species interaction networks. BMC Ecology **6**:9.
Bolker, B. M., M. E. Brooks, C. J. Clark, S. W. Geange, J. R. Poulsen, M. H. H. Stevens, and J.-S. S. White. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. Trends in Ecology & Evolution **24**:127-135.
Borowski, Z. and E. Owadowska. 2010. Field vole (*Microtus agrestis*) seasonal spacing behavior: the effect of predation risk by mustelids. Naturwissenschaften **97**:487-493.
Bozeman, F. M., A. Shirai, J. W. Humphries, and H. S. Fuller. 1967. Ecology of Rocky Mountain spotted fever. The American journal of tropical medicine and hygiene **16**:48-59.
Bradshaw, C. J., N. S. Sodhi, and B. W. Brook. 2009. Tropical turmoil: a biodiversity tragedy in progress. Frontiers in Ecology and the Environment **7**:79-87.
Brooks, D. R. and E. P. Hoberg. 2007. How will global climate change affect parasite–host assemblages? Trends in Parasitology **23**:571-574.
Brownstein, J., D. Skelly, T. Holford, and D. Fish. 2005. Forest fragmentation predicts local scale heterogeneity of Lyme disease risk. Oecologia **146**:469-475.
Brunner, J. L. and R. S. Ostfeld. 2008. Multiple causes of variable tick burdens on small-mammal hosts. Ecology **89**:2259-2272.
Burgdorfer, W., S. Hayes, and A. Mavros. 1980. Nonpathogenic rickettsiae in *Dermacentor andersoni*: a limiting factor for the distribution of *Rickettsia rickettsii*. Rickettsiae and rickettsial diseases.
Burgdorfer, W., V. F. Newhouse, E. G. Pickens, and D. B. Lackman. 1962. Ecology of Rocky Mountain spotted fever in western Montana: Isolation of *Rickettsia rickettsii* from wild mammals. American journal of hygiene **76**:293-301.
Burger, T. D., R. Shao, and S. C. Barker. 2014. Phylogenetic analysis of mitochondrial genome sequences indicates that the cattle tick, *Rhipicephalus (Boophilus) microplus*, contains a cryptic species. Molecular Phylogenetics and Evolution **76**:241-253.
Bush, S., M. Reed, and S. Maher. 2013. Impact of forest size on parasite biodiversity: implications for conservation of hosts and parasites. Biodiversity and Conservation **22**:1391-1404.
Calero, C. M., J. M. Núñez, and R. S. Goytía. 1952. Rocky Mountain spotted fever in Panama. The American journal of tropical medicine and hygiene **1**:631-636.
Canale, G. R., C. A. Peres, C. E. Guidorizzi, C. A. F. Gatto, and M. C. M. Kierulff. 2012. Pervasive defaunation of forest remnants in a tropical biodiversity hotspot. PLoS ONE **7**:e41671.
Cardillo, M. 2003. Biological determinants of extinction risk: why are smaller species less vulnerable? Animal Conservation **6**:63-69.
Cardillo, M., G. M. Mace, K. E. Jones, J. Bielby, O. R. Bininda-Emonds, W. Sechrest, C. D. L. Orme, and A. Purvis. 2005. Multiple causes of high extinction risk in large mammal species. Science **309**:1239-1241.
Cardinale, B. J., J. E. Duffy, A. Gonzalez, D. U. Hooper, C. Perrings, P. Venail, A. Narwani, G. M. Mace, D. Tilman, D. A. Wardle, A. P. Kinzig, G. C. Daily, M. Loreau, J. B. Grace, A. Larigauderie, D. S. Srivastava, and S. Naeem. 2012. Biodiversity loss and its impact on humanity. Nature **486**:59-67.
Ceballos, G., P. R. Ehrlich, A. D. Barnosky, A. García, R. M. Pringle, and T. M. Palmer. 2015. Accelerated modern human–induced species losses: Entering the sixth mass extinction. Science advances **1**:e1400253.
Chakravorty, S., D. Helb, M. Burday, N. Connell, and D. Alland. 2007. A detailed analysis of 16S ribosomal RNA gene segments for the diagnosis of pathogenic bacteria. Journal of microbiological methods **69**:330-339.
Chao, A. 1984. Nonparametric estimation of the number of classes in a population. Scandinavian Journal of Statistics **11**:265-270.
Chao, A. 1987. Estimating the population size for capture-recapture data with unequal catchability. Biometrics **43**:783-791.
Chapin, F. S., III, E. S. Zavaleta, V. T. Eviner, R. L. Naylor, P. M. Vitousek, H. L. Reynolds, D. U. Hooper, S. Lavorel, O. E. Sala, and S. E. Hobbie. 2000. Consequences of changing biodiversity. Nature **405**:234-242.
Chesson, P. 2000. Mechanisms of maintenance of species diversity. Annual Review of Ecology and Systematics **31**:343-366.

Chiarello, A. G. 1999. Effects of fragmentation of the Atlantic forest on mammal communities in south-eastern Brazil. Biological Conservation **89**:71-82.
Chivian, E. 2002. Biodiversity: its importance to human health. Center for Health and the Global Environment, Harvard Medical School, Cambridge, MA.
Civitello, D. J., J. Cohen, H. Fatima, N. T. Halstead, J. Liriano, T. A. McMahon, C. N. Ortega, E. L. Sauer, T. Sehgal, and S. Young. 2015. Biodiversity inhibits parasites: broad evidence for the dilution effect. Proceedings of the National Academy of Sciences **112**:8667-8671.
Clark, H. C. 1918. Piroplasmosis of cattle in Panama: Value of the brain film in diagnosis. The Journal of Infectious Diseases:159-168.
Clay, K. and C. Fuqua. 2010. The tick microbiome: diversity, distribution and influence of the internal microbial community for a blood-feeding disease vector.*in* Critical needs and gaps in understanding prevention, amelioration, and resolution of Lyme and other tick-borne diseases: the short-term and long-term outcomes. Workshop report. National Academies Press, Washington, DC.
Clay, K., C. Fuqua, C. Lively, and M. J. Wade. 2006. Microbial community ecology of tick-borne human pathogens. Pages 41-57 *in* S. K. Collinge and C. Ray, editors. Disease ecology: Community structure and pathogen dynamics. Oxford University Press, Oxford.
Clay, K., O. Klyachko, N. Grindle, D. Civitello, D. Oleske, and C. Fuqua. 2008. Microbial communities and interactions in the lone star tick, *Amblyomma americanum*. Molecular Ecology **17**:4371-4381.
Clayton, D. and B. A. Walther. 2001. Influence of host ecology and morphology on the diversity of Neotropical bird lice. Oikos **94**:455-467.
Clayton, D. H. and D. M. Drown. 2001. Critical evaluation of five methods for quantifying chewing lice (Insecta: Phthiraptera). Journal of Parasitology **87**:1291-1300.
Cleaveland, S., M. Laurenson, and L. Taylor. 2001. Diseases of humans and their domestic mammals: pathogen characteristics, host range and the risk of emergence. Philosophical Transactions of the Royal Society of London B: Biological Sciences **356**:991-999.
Cohen, E. B., L. D. Auckland, P. P. Marra, and S. A. Hamer. 2015. Avian migrants facilitate invasions of Neotropical ticks and tick-borne pathogens into the United States. Applied and Environmental Microbiology **81**:8366-8378.
Cohen, J. M., D. J. Civitello, A. J. Brace, E. M. Feichtinger, C. N. Ortega, J. C. Richardson, E. L. Sauer, X. Liu, and J. R. Rohr. 2016. Spatial scale modulates the strength of ecological processes driving disease distributions. Proceedings of the National Academy of Sciences **113**:E3359-E3364.
Cole, J. R., Q. Wang, E. Cardenas, J. Fish, B. Chai, R. J. Farris, A. Kulam-Syed-Mohideen, D. M. McGarrell, T. Marsh, and G. M. Garrity. 2009. The Ribosomal Database Project: improved alignments and new tools for rRNA analysis. Nucleic acids research **37**:D141-D145.
Colwell, R. K. 2013. EstimateS: Statistical estimation of species richness and shared species from samples. Page User's Guide and application published at: http://purl.oclc.org/estimates.
Colwell, R. K. and J. A. Coddington. 1994. Estimating terrestrial biodiversity through extrapolation. Philosophical Transactions of the Royal Society of London B: Biological Sciences **345**:101-118.
Colwell, R. K., R. R. Dunn, and N. C. Harris. 2012. Coextinction and persistence of dependent species in a changing world. Annual Review of Ecology, Evolution, and Systematics **43**:183-203.
Combes, C. 2001. Parasitism: the ecology and evolution of intimate interactions. University of Chicago Press.
Condit, R., W. D. Robinson, R. Ibáñez, S. Aguilar, A. Sanjur, R. Martínez, R. F. Stallard, T. García, G. R. Angehr, and L. Petit. 2001. The Status of the Panama Canal Watershed and Its Biodiversity at the Beginning of the 21st Century Long-term ecological studies reveal a diverse flora and fauna near the Panama Canal, harbored within a corridor of forest stretching from the Caribbean to the Pacific, but deforestation, land degradation, erosion, and overhunting remain threats. BioScience **51**:389-398.
Cumming, G. S. 1998. Host preference in African ticks (Acari: Ixodida): a quantitative data set. Bulletin of Entomological Research **88**:379-406.
Cumming, G. S. 1999. Host distributions do not limit the species ranges of most African ticks (Acari: Ixodida). Bulletin of Entomological Research **89**:303-327.
Cumming, G. S. 2004. On the relevance of abundance and spatial pattern for interpretations of host–parasite association data. Bulletin of Entomological Research **94**:401-409.
Cumming, G. S. and J.-F. Guégan. 2006. Food webs and disease: is pathogen diversity limited by vector diversity? EcoHealth **3**:163-170.
Dantas-Torres, F. 2007. Rocky Mountain spotted fever. The Lancet Infectious Diseases **7**:724-732.
Darling, S. T. 1913. Equine piroplasmosis in Panama. Journal of Infectious Diseases **13**:197-202.
Daszak, P. 2000. Emerging infectious diseases of wildlife - threats to biodiversity and human health. Science **287**:443-449.
Daszak, P., A. A. Cunningham, and A. D. Hyatt. 2001. Anthropogenic environmental change and the emergence of infectious diseases in wildlife. Acta Tropica **78**:103-116.
de la Fuente, J., E. F. Blouin, and K. M. Kocan. 2003. Infection exclusion of the rickettsial pathogen Anaplasma marginale in the tick vector Dermacentor variabilis. Clinical and Diagnostic Laboratory Immunology **10**:182-184.
De Magalhães, J. and J. Costa. 2009. A database of vertebrate longevity records and their relation to other life-history traits. Journal of Evolutionary Biology **22**:1770-1774.

de Rodaniche, E. C. 1953. Natural infection of the tick, *Amblyomma cajennense*, with *Rickettsia rickettsii* in Panama. American Journal of Tropical Medicine and Hygiene **2**:696-699.
de Rodaniche, E. C. and A. Rodaniche. 1950. Spotted fever in Panama: Isolation of the etiological agent from a fatal case. American Journal of Tropical Medicine **30**:511-517.
Devictor, V., J. Clavel, R. Julliard, S. Lavergne, D. Mouillot, and W. Thuiller. 2010. Defining and measuring ecological specialization. Journal of Applied Ecology **47**.
Diaz-Uriarte, R. and T. Garland. 1998. Effects of branch length errors on the performance of phylogenetically independent contrasts. Systematic Biology **47**:654-672.
Díaz, M., I. Torre, A. Peris, and L. Tena. 2005. Foraging behavior of wood mice as related to presence and activity of genets. Journal of Mammalogy **86**:1178-1185.
Díaz, S., J. Fargione, F. S. Chapin, III, and D. Tilman. 2006. Biodiversity loss threatens human well-being. PLoS Biology **4**:e277.
Dietrich, M., E. Lobato, T. Boulinier, and K. McCoy. 2014. An experimental test of host specialisation in a ubiquitous polar ectoparasite: a role for adaptation? Journal of Animal Ecology **83**:576-587.
Dobson, A., K. D. Lafferty, A. M. Kuris, R. F. Hechinger, and W. Jetz. 2008. Homage to Linnaeus: How many parasites? How many hosts? Proceedings of the National Academy of Sciences **105**:11482-11489.
Dormann, C. F. 2011. How to be a specialist? Quantifying specialisation in pollination networks. Network Biology **1**:1-20.
Dormann, C. F., J. Fründ, N. Blüthgen, and B. Gruber. 2009. Indices, graphs and null models: Analyzing bipartite ecological networks. The Open Ecology Journal **2**:7-24.
Dormann, C. F., B. Gruber, and J. Fründ. 2008. Introducing the bipartite package: analysing ecological networks. R News **8**:8-11.
Dormann, C. F. and R. Strauss. 2014. A method for detecting modules in quantitative bipartite networks. Methods in Ecology and Evolution **5**:90-98.
Dougherty, E. R., C. J. Carlson, V. M. Bueno, K. R. Burgio, C. A. Cizauskas, C. F. Clements, D. P. Seidel, and N. C. Harris. 2016. Paradigms for parasite conservation. Conservation Biology **30**:724-733.
Dunn, L. H. 1923. The ticks of Panama, their hosts, and the diseases they transmit. American Journal of Tropical Medicine **3**:91-104.
Dunn, L. H. 1934. Ticks from tapirs of Panama. The Journal of Parasitology **20**:312-312.
Dunn, L. H. and H. C. Clark. 1933. Notes on relapsing fever in Panama with special reference to animal hosts. American Journal of Tropical and Medical Hygiene **s1-13**:201-209.
Dunn, R. R., T. J. Davies, N. C. Harris, and M. C. Gavin. 2010. Global drivers of human pathogen richness and prevalence. Proceedings of the Royal Society of London B: Biological Sciences:rspb20100340.
Dunn, R. R., N. C. Harris, R. K. Colwell, L. P. Koh, and N. S. Sodhi. 2009. The sixth mass coextinction: are most endangered species parasites and mutualists? Proceedings of the Royal Society B: Biological Sciences **276**:3037-3045.
Dunning, J. B. J. 2007. CRC handbook of avian body masses. 2nd edition. CRC Press, Boca Raton, Florida.
Edgar, R. C. and H. Flyvbjerg. 2015. Error filtering, pair assembly and error correction for next-generation sequencing reads. Bioinformatics **31**:3476-3482.
Edgar, R. C., B. J. Haas, J. C. Clemente, C. Quince, and R. Knight. 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics **27**:2194-2200.
Eisenberg, J. F. 1989. Mammals of the Neotropics. vol. 1. The northern neotropics: Panama, Colombia, Venezuela, Guyana, Suriname, French Guiana. The University of Chicago Press, Chicago.
Eremeeva, M. E. and G. A. Dasch. 2015. Challenges posed by tick-borne rickettsiae: eco-epidemiology and public health implications. Frontiers in public health **3**.
Eremeeva, M. E., S. E. Karpathy, M. L. Levin, C. M. Caballero, S. Bermudez, G. A. Dasch, and J. A. Motta. 2009. Spotted fever rickettsiae, *Ehrlichia* and *Anaplasma*, in ticks from peridomestic environments in Panama. Clinical Microbiology and Infection **15**:12-14.
Espinaze, M. P., E. Hellard, I. G. Horak, and G. S. Cumming. 2015. Analysis of large new South African dataset using two host-specificity indices shows generalism in both adult and larval ticks of mammals. Parasitology:1-8.
Esser, H. J., J. E. Foley, F. Bongers, E. A. Herre, M. J. Miller, H. H. Prins, and P. A. Jansen. 2016a. Host body size and the diversity of tick assemblages on Neotropical vertebrates. International Journal for Parasitology: Parasites and Wildlife **5**:295-304.
Esser, H. J., E. A. Herre, N. Blüthgen, J. R. Loaiza, S. E. Bermúdez, and P. A. Jansen. 2016b. Host specificity in a diverse Neotropical tick community: an assessment using quantitative network analysis and host phylogeny. Parasites & Vectors **9**:372.
Estrada-Peña, A., A. A. Guglielmone, and A. J. Mangold. 2004. The distribution and ecological 'preferences' of the tick *Amblyomma cajennense* (Acari: Ixodidae), an ectoparasite of humans and other mammals in the Americas. Annals of Tropical Medicine & Parasitology **98**:283-292.
Estripeaut, D., M. G. Aramburú, X. Sáez-Llorens, H. A. Thompson, G. A. Dasch, C. D. Paddock, S. R. Zaki, and M. E. Eremeeva. 2007. Rocky Mountain spotted fever, Panama. Emerging Infectious Diseases **13**:1763-1765.
Ezenwa, V. O., M. S. Godsey, R. J. King, and S. C. Guptill. 2006a. Avian diversity and West Nile virus: testing associations between biodiversity and infectious disease risk. Proceedings of the Royal Society of London B: Biological Sciences **273**:109-117.

Ezenwa, V. O., S. A. Price, S. Altizer, N. D. Vitone, and K. C. Cook. 2006b. Host traits and parasite species richness in even and odd-toed hoofed mammals, Artiodactyla and Perissodactyla. Oikos **115**:526-536.

Fahrig, L. 2003. Effects of habitat fragmentation on biodiversity. Annual Review of Ecology, Evolution, and Systematics **34**:487-515.

Fairchild, G. B. 1943. An annotated list of the bloodsucking insects, ticks and mites known from Panama. American Journal of Tropical Medicine **s1-23**:569-591.

Fairchild, G. B., G. M. Kohls, and V. J. Tipton. 1966. The ticks of Panama (Acarina: Ixodidea). Pages 167-219 *in* R. L. Wenzel and V. J. Tipton, editors. Ectoparasites of Panama. Field Museum of Natural History, Chicago.

Falco, R. C. and D. Fish. 1992. A comparison of methods for sampling the deer tick, I*xodes dammini*, in a Lyme disease endemic area. Experimental and Applied Acarology **14**:165-173.

Feinsinger, P., E. E. Spears, and R. W. Poole. 1981. A simple measure of niche breadth. Ecology **62**:27-32.

Feldman, A. and S. Meiri. 2013. Length–mass allometry in snakes. Biological Journal of the Linnean Society **108**:161-172.

Feliu, C., F. Renaud, F. Catzeflis, J.-P. Hugot, P. Durand, and S. Morand. 1997. A comparative analysis of parasite species richness of Iberian rodents. Parasitology **115**:453-466.

Felsenstein, J. 1985. Phylogenies and the comparative method. The American Naturalist **125**:1-15.

Felsenstein, J. 2008. Comparative methods with sampling error and within-species variation: contrasts revisited and revised. The American Naturalist **171**:713-725.

Fielden, L., Y. Rechav, and N. Bryson. 1992. Acquired immunity to larvae of *Amblyomma marmoreum* and *Ahebraeum* by tortoises, guinea-pigs and guinea-fowl. Medical and Veterinary Entomology **6**:251-254.

Fournier, D. A., H. J. Skaug, J. Ancheta, J. Ianelli, A. Magnusson, M. N. Maunder, A. Nielsen, and J. Sibert. 2012. AD Model Builder: using automatic differentiation for statistical inference of highly parameterized complex nonlinear models. Optimization Methods and Software **27**:233-249.

Freckleton, R. P., P. H. Harvey, and M. Pagel. 2002. Phylogenetic analysis and comparative data: a test and review of evidence. The American Naturalist **160**:712-726.

Fry, B. G., N. Vidal, J. A. Norman, F. J. Vonk, H. Scheib, S. F. R. Ramjan, S. Kuruppu, K. Fung, S. Blair Hedges, M. K. Richardson, W. C. Hodgson, V. Ignjatovic, R. Summerhayes, and E. Kochva. 2006. Early evolution of the venom system in lizards and snakes. Nature **439**:584-588.

Gallivan, G. and I. Horak. 1997. Body size and habitat as determinants of tick infestations of wild ungulates in South Africa. South African Journal of Wildlife Research **27**:63-70.

García, G. G., A. Castro, I. Rodriguez, and S. E. Bermúdez. 2014. Ixodid ticks of *Hydrochaeris isthmius* Goldman 1912 (Rodentia: Caviidae) in Panama. Systematic & Applied Acarology **19**:404-408.

Garland, T., P. H. Harvey, and A. R. Ives. 1992. Procedures for the analysis of comparative data using phylogenetically independent contrasts. Systematic Biology **41**:18-32.

Gelman, A. 2008. Scaling regression inputs by dividing by two standard deviations. Statistics in medicine **27**:2865-2873.

Ginsberg, H. S., P. Buckley, M. G. Balmforth, E. Zhioua, S. Mitra, and F. G. Buckley. 2005. Reservoir competence of native North American birds for the Lyme disease spirochete, Borrelia burgdorferi. Journal of Medical Entomology **42**:445-449.

Ginsberg, H. S. and C. P. Ewing. 1989. Comparison of flagging, walking, trapping, and collecting from hosts as sampling methods for northern deer ticks, *Ixodes dammini*, and lone-star ticks, *Amblyomma americanum* (Acari: Ixodidae). Experimental and Applied Acarology **7**:313-322.

Gómez, A. and E. Nichols. 2013. Neglected wild life: Parasitic biodiversity as a conservation target. International Journal for Parasitology: Parasites and Wildlife **2**:222-227.

Gottdenker, N. L., L. F. Chaves, J. E. Calzada, A. Saldaña, and C. R. Carroll. 2012. Host life history strategy, species diversity, and habitat influence *Trypanosoma cruzi* vector infection in changing landscapes. PLoS Negl Trop Dis **6**:e1884.

Gottdenker, N. L., D. G. Streicker, C. L. Faust, and C. Carroll. 2014. Anthropogenic land use change and infectious diseases: a review of the evidence. EcoHealth **11**:619-632.

Gratz, N. G. 1999. Emerging and resurging vector-borne diseases. Annual Review of Entomology **44**:51-75.

Greer, K. A., S. C. Canterberry, and K. E. Murphy. 2007. Statistical analysis regarding the effects of height and weight on life span of the domestic dog. Research in Veterinary Science **82**:208-214.

Gregory, R. D., A. E. Keymer, and P. H. Harvey. 1996. Helminth parasite richness among vertebrates. Biodiversity & Conservation **5**:985-997.

Gubler, D. 1998. Resurgent vector-borne diseases as a global health problem. Emerging Infectious Diseases **4**:442-450.

Guernier, V., M. E. Hochberg, and J.-F. Guégan. 2004. Ecology drives the worldwide distribution of human diseases. PLoS Biology **2**:e141.

Guglielmone, A. A., R. G. Robbins, D. A. Apanaskevich, T. N. Petney, A. Estrada-Peña, and I. Horak. 2014. The hard ticks of the world. Springer, Dordrecht.

Guglielmone, A. A., R. G. Robbins, D. A. Apanaskevich, T. N. Petney, A. Estrada-Pena, and I. G. Horak. 2010. The Argasidae, Ixodidae and Nuttalliellidae (Acari: Ixodida) of the world: a list of valid species names. Zootaxa **2528**.

Haapakoski, M., J. Sundell, and H. Ylönen. 2015. Conservation implications of change in antipredator behavior in fragmented habitat: Boreal rodent, the bank vole, as an experimental model. Biological Conservation **184**:11-17.

Hamer, S. A., T. L. Goldberg, U. D. Kitron, J. D. Brawn, T. K. Anderson, S. R. Loss, E. D. Walker, and G. L. Hamer. 2012. Wild birds and urban ecology of ticks and tick-borne pathogens, Chicago, Illinois, USA, 2005–2010. Emerging Infectious Diseases **18**:1589.
Han, B. A., J. P. Schmidt, S. E. Bowden, and J. M. Drake. 2015. Rodent reservoirs of future zoonotic diseases. Proceedings of the National Academy of Sciences **112**:7039-7044.
Harmon, R. 2005. An Introduction to the Panama Canal Watershed. Pages 19-28 *in* R. Harmon, editor. The Río Chagres, Panama. Springer, Dordrecht.
Harrison, A., M. Scantlebury, and W. I. Montgomery. 2010. Body mass and sex-biased parasitism in wood mice *Apodemus sylvaticus*. Oikos **119**:1099-1104.
Harvey, P. H. and M. D. Pagel. 1991. The comparative method in evolutionary biology. Oxford university press Oxford.
Hatcher, M. J., J. T. Dick, and A. M. Dunn. 2006. How parasites affect interactions between competitors and predators. Ecology Letters **9**:1253-1271.
Hebert, P. D., E. H. Penton, J. M. Burns, D. H. Janzen, and W. Hallwachs. 2004. Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. Proceedings of the National Academy of Sciences of the United States of America **101**:14812-14817.
Hechinger, R. F. and K. D. Lafferty. 2005. Host diversity begets parasite diversity: bird final hosts and trematodes in snail intermediate hosts. Proceedings of the Royal Society of London B: Biological Sciences **272**:1059-1066.
Hegab, I. M., S. Kong, S. Yang, W. I. Mohamaden, and W. Wei. 2015. The ethological relevance of predator odors to induce changes in prey species. acta ethologica **18**:1-9.
Heise, S. R., M. Elshahed, and S. Little. 2010. Bacterial diversity in *Amblyomma americanum* (Acari: Ixodidae) with a focus on members of the genus *Rickettsia*. Journal of Medical Entomology **47**:258-268.
Heylen, D., F. Adriaensen, S. Van Dongen, H. Sprong, and E. Matthysen. 2013. Ecological factors that determine *Ixodes ricinus* tick burdens in the great tit (*Parus major*), an avian reservoir of *Borrelia burgdorferi* s.l. International Journal for Parasitology **43**:603-611.
Hidalgo, M., J. Miranda, D. Heredia, P. Zambrano, J. F. Vesga, D. Lizarazo, S. Mattar, and G. Valbuena. 2011. Outbreak of Rocky Mountain spotted fever in Cordoba, Colombia. Memórias do Instituto Oswaldo Cruz **106**:117-118.
Hofmeester, T. R. 2016. The wild life of tick-borne pathogens. Wageningen University, Wageningen, the Netherlands.
Hofmeester, T. R., E. C. Coipan, S. E. van Wieren, H. H. T. Prins, W. Takken, and H. Sprong. 2016. Few vertebrate species dominate the Borrelia burgdorferi s.l. life cycle. Environmental Research Letters **11**:043001.
Hofmeester, T. R., P. A. Jansen, H. J. Wijnen, E. C. Coipan, M. Fonville, H. H. Prins, H. Sprong, and S. E. van Wieren. 2017. Cascading effects of predator activity on tick-borne disease risk. Page 20170453 *in* Proc. R. Soc. B. The Royal Society.
Holt, R. D., A. P. Dobson, M. Begon, R. G. Bowers, and E. M. Schauber. 2003. Parasite establishment in host communities. Ecology Letters **6**:837-842.
Hoogstraal, H. and A. Aeschlimann. 1982. Tick-host specificity. Bulletin de la Société Entomologicue Suisse **55**:5-32.
Huang, S., O. R. Bininda-Emonds, P. R. Stephens, J. L. Gittleman, and S. Altizer. 2014. Phylogenetically related and ecologically similar carnivores harbour similar parasite assemblages. Journal of Animal Ecology **83**:671-680.
Huang, Z. 2014. Effect of species richness on disease risk: dilution effect and underlying mechanisms. Wageningen University.
Huang, Z., F. Van Langevelde, A. Estrada-Peña, G. Suzán, and W. De Boer. 2016. The diversity–disease relationship: evidence for and criticisms of the dilution effect. Parasitology **143**:1075-1086.
Huang, Z. Y., W. F. de Boer, F. van Langevelde, V. Olson, T. M. Blackburn, and H. H. Prins. 2013. Species' life-history traits explain interspecific variation in reservoir competence: a possible mechanism underlying the dilution effect. PLoS ONE **8**:e54341.
Huang, Z. Y., Y. YU, F. van Langevelde, and W. F. de Boer. 2017. Does the dilution effect generally occur in animal diseases? Parasitology:1-4.
Huchon, D. and E. J. P. Douzery. 2001. From the Old World to the New World: A molecular chronicle of the phylogeny and biogeography of hystricognath rodents. Molecular Phylogenetics and Evolution **20**:238-251.
Hudson, P. J., A. P. Dobson, and K. D. Lafferty. 2006. Is a healthy ecosystem one that is rich in parasites? Trends in Ecology & Evolution **21**:381-385.
Hughes, J. and R. D. Page. 2007. Comparative tests of ectoparasite species richness in seabirds. BMC evolutionary biology **7**:1.
Hughes, V. L. and S. E. Randolph. 2001. Testosterone depresses innate and acquired resistance to ticks in natural rodent hosts: a force for aggregated distributions of parasites. Journal of Parasitology **87**:49-54.
Jarvis, E. D., S. Mirarab, A. J. Aberer, B. Li, P. Houde, C. Li, S. Y. W. Ho, B. C. Faircloth, B. Nabholz, J. T. Howard, and e. al. 2014. Whole-genome analyses resolve early branches in the tree of life of modern birds. Science **346**:1320-1331.
Johnson, P. T., J. C. De Roode, and A. Fenton. 2015a. Why infectious disease research needs community ecology. Science **349**:1259504.
Johnson, P. T., A. Dobson, K. D. Lafferty, D. J. Marcogliese, J. Memmott, S. A. Orlofske, R. Poulin, and D. W. Thieltges. 2010. When parasites become prey: ecological and epidemiological significance of eating parasites. Trends in Ecology & Evolution **25**:362-371.

Johnson, P. T., R. S. Ostfeld, and F. Keesing. 2015b. Frontiers in research on biodiversity and disease. Ecology Letters **18**:1119-1133.
Johnson, P. T., D. L. Preston, J. T. Hoverman, and B. E. LaFonte. 2013a. Host and parasite diversity jointly control disease risk in complex communities. Proceedings of the National Academy of Sciences **110**:16916-16921.
Johnson, P. T., D. L. Preston, J. T. Hoverman, and K. L. Richgels. 2013b. Biodiversity decreases disease through predictable changes in host community competence. Nature **494**:230-233.
Johnson, P. T., J. R. Rohr, J. T. Hoverman, E. Kellermanns, J. Bowerman, and K. B. Lunde. 2012. Living fast and dying of infection: host life history drives interspecific variation in infection and disease risk. Ecology Letters **15**:235-242.
Johnson, W. E., E. Eizirik, J. Pecon-Slattery, W. J. Murphy, A. Antunes, E. Teeling, and S. J. O'Brien. 2006. The Late Miocene radiation of modern Felidae: a genetic assessment. Science **311**.
Jolliffe, I. 2002. Principal component analysis and factor analyses. Principal component analysis. Springer, New York.
Jones, K. E., N. G. Patel, M. A. Levy, A. Storeygard, D. Balk, J. L. Gittleman, and P. Daszak. 2008. Global trends in emerging infectious diseases. Nature **451**:990-993.
Jongejan, F. and G. Uilenberg. 2004. The global importance of ticks. Parasitology **129**:S3-S14.
Jorge, L. R., P. I. Prado, M. Almeida-Neto, and T. M. Lewinsohn. 2014. An integrated framework to improve the concept of resource specialisation. Ecology Letters **17**:1341-1350.
Joseph, M. B., J. R. Mihaljevic, S. A. Orlofske, and S. H. Paull. 2013. Does life history mediate changing disease risk when communities disassemble? Ecology Letters **16**:1405-1412.
Jost, L. 2006. Entropy and diversity. Oikos **113**:363-375.
Kamiya, T., K. O'Dwyer, S. Nakagawa, and R. Poulin. 2014a. What determines species richness of parasitic organisms? A meta-analysis across animal, plant and fungal hosts. Biological reviews **89**:123-134.
Kamiya, T., K. O'dwyer, S. Nakagawa, and R. Poulin. 2014b. Host diversity drives parasite diversity: meta-analytical insights into patterns and causal mechanisms. Ecography **37**:689-697.
Kane, M. D., L. K. Poulsen, and D. A. Stahl. 1993. Monitoring the enrichment and isolation of sulfate-reducing bacteria by using oligonucleotide hybridization probes designed from environmentally derived 16S rRNA sequences. Applied and Environmental Microbiology **59**:682-686.
Kawahara, M., Y. Rikihisa, Q. Lin, E. Isogai, K. Tahara, A. Itagaki, Y. Hiramitsu, and T. Tajima. 2006. Novel genetic variants of *Anaplasma phagocytophilum*, *Anaplasma bovis*, *Anaplasma centrale*, and a novel *Ehrlichia* sp. in wild deer and ticks on two major islands in Japan. Applied and Environmental Microbiology **72**:1102-1109.
Kays, R. W., S. Tilak, B. Kranstauber, P. A. Jansen, C. Carbone, J. M. Rowcliffe, T. Fountain, J. Eggerst, and Z. He. 2011. Camera traps as sensor networks for monitoring animal communities. International Journal of Research and Reviews in Wireless Sensor Networks **1**:19-29.
Keesing, F., L. K. Belden, P. Daszak, A. Dobson, C. D. Harvell, R. D. Holt, P. Hudson, A. Jolles, K. E. Jones, C. E. Mitchell, S. S. Myers, T. Bogich, and R. S. Ostfeld. 2010. Impacts of biodiversity on the emergence and transmission of infectious diseases. Nature **468**:647-652.
Keesing, F., J. Brunner, S. Duerr, M. Killilea, K. LoGiudice, K. Schmidt, H. Vuong, and R. S. Ostfeld. 2009. Hosts as ecological traps for the vector of Lyme disease. Proceedings of the Royal Society B: Biological Sciences **276**:3911-3919.
Keesing, F., R. D. Holt, and R. S. Ostfeld. 2006. Effects of species diversity on disease risk. Ecology Letters **9**:485-498.
Keesing, F. and T. P. Young. 2014. Cascading consequences of the loss of large mammals in an African savanna. BioScience **64**:487-495.
Kembel, S. W., P. D. Cowan, M. R. Helmus, W. K. Cornwell, H. Morlon, and D. D. Ackerly. 2010. Picante: R tools for integrating phylogenies and ecology. Bioinformatics **26**.
Kiffner, C., C. LÖDige, M. Alings, T. Vor, and F. RÜHe. 2011a. Body-mass or sex-biased tick parasitism in roe deer (*Capreolus capreolus*): A GAMLSS approach. Medical and Veterinary Entomology **25**:39-45.
Kiffner, C., M. Stanko, S. Morand, I. S. Khokhlova, G. I. Shenbrot, A. Laudisoit, H. Leirs, H. Hawlena, and B. R. Krasnov. 2014. Variable effects of host characteristics on species richness of flea infracommunities in rodents from three continents. Parasitology Research **113**:2777-2788.
Kiffner, C., T. Vor, P. Hagedorn, M. Niedrig, and F. Rühe. 2011b. Factors affecting patterns of tick parasitism on forest rodents in tick-borne encephalitis risk areas, Germany. Parasitology Research **108**:323-335.
Kilpatrick, A. and S. Altizer. 2010. Disease ecology. Nature Education Knowledge **1**:13.
Kilpatrick, A. M., A. A. Chmura, D. W. Gibbons, R. C. Fleischer, P. P. Marra, and P. Daszak. 2006. Predicting the global spread of H5N1 avian influenza. Proceedings of the National Academy of Sciences **103**:19368-19373.
Kilpatrick, A. M., D. J. Salkeld, G. Titcomb, and M. B. Hahn. 2017. Conservation of biodiversity as a strategy for improving human health and well-being. Philosophical Transactions of the Royal Society B: Biological Sciences **372**.
Kilpatrick, H. J., A. M. Labonte, and K. C. Stafford III. 2014. The relationship between deer density, tick abundance, and human cases of Lyme disease in a residential community. Journal of Medical Entomology **51**:777-784.
Kinnaird, M. F., E. W. Sanderson, T. G. O'Brian, H. T. Wibisono, and G. Woolmer. 2003. Deforestation trends in a tropical landscape and implications for endangered large mammals. Conservation Biology **17**:245-257.
Klompen, J. S. H., W. C. Black, J. E. Keirans, and J. H. Oliver. 1996. Evolution of ticks. Annual Review of Entomology **41**:141-161.

Koepfli, K.-P., K. A. Deere, G. J. Slater, C. Begg, K. Begg, L. Grassman, M. Lucherini, G. Veron, and R. K. Wayne. 2008. Multigene phylogeny of the Mustelidae: Resolving relationships, tempo and biogeographic history of a mammalian adaptive radiation. BMC Biology **6**:1-22.

Koepfli, K. P., M. E. Gompper, E. Eizirik, C. C. Ho, L. Linden, J. E. Maldonado, and R. K. Wayne. 2007. Phylogeny of the Procyonidae (Mammalia: Carnivora): molecules, morphology and the Great American Interchange. Mol Phylogenet Evol **43**.

Koh, L. P., R. R. Dunn, N. S. Sodhi, R. K. Colwell, H. C. Proctor, and V. S. Smith. 2004. Species coextinctions and the biodiversity crisis. Science **305**:1632-1634.

Koplow, D. A. 2003. Smallpox: the fight to eradicate a global scourge. University of California Press.

Krasnov, B. R., F. Bordes, I. S. Khokhlova, and S. Morand. 2012a. Gender-biased parasitism in small mammals: patterns, mechanisms, consequences. Mammalia **76**:1-13.

Krasnov, B. R., M. A. Fortuna, D. Mouillot, I. S. Khokhlova, G. I. Shenbrot, and R. Poluin. 2012b. Phylogenetic signal in module composition and species connectivity in compartmentalized host-parasite networks. The American Naturalist **179**:501-511.

Krasnov, B. R., D. Mouillot, I. S. Khokhlova, G. I. Shenbrot, and R. Poulin. 2008a. Scale-invariance of niche breadth in fleas parasitic on small mammals. Ecography **31**.

Krasnov, B. R., D. Mouillot, G. I. Shenbrot, I. S. Khokhlova, and R. Poulin. 2004a. Geographical variation in host specificity of fleas (Siphonaptera) parasitic on small mammals: the influence of phylogeny and local environmental conditions. Ecography **27**.

Krasnov, Boris R., Robert Poulin, Georgy I. Shenbrot, David Mouillot, and Irina S. Khokhlova. 2004b. Ectoparasitic "Jacks-of-All-Trades": Relationship between abundance and host specificity in fleas (Siphonaptera) parasitic on small mammals. The American Naturalist **164**:506-516.

Krasnov, B. R., G. I. Shenbrot, I. S. Khokhlova, and A. A. Degen. 2004c. Flea species richness and parameters of host body, host geography and host 'milieu'. Journal of Animal Ecology **73**:1121-1128.

Krasnov, B. R., G. I. Shenbrot, I. S. Khokhlova, D. Mouillot, and R. Poulin. 2008b. Latitudinal gradients in niche breadth: empirical evidence from haematophagous ectoparasites. Journal of Biogeography **35**:592-601.

Krasnov, B. R., G. I. Shenbrot, I. S. Khokhlova, and R. Poulin. 2005. Nested pattern in flea assemblages across the host's geographic range. Ecography **28**.

Kumar, N. P., A. Rajavel, R. Natarajan, and P. Jambulingam. 2007. DNA barcodes can distinguish species of Indian mosquitoes (Diptera: Culicidae). Journal of Medical Entomology **44**:1-7.

Kuris, A. M., A. R. Blaustein, and J. J. Alio. 1980. Hosts as islands. American Naturalist **116**:570-586.

Labruna, M., L. Sanfilippo, C. Demetrio, A. Menezes, A. Pinter, A. Guglielmone, and L. Silveira. 2007. Ticks collected on birds in the state of São Paulo, Brazil. Experimental and Applied Acarology **43**:147-160.

Labruna, M. B. 2009. Ecology of *Rickettsia* in South America. Annals of the New York Academy of Sciences **1166**:156-166.

Labruna, M. B., R. R. Cabrera, and A. Pinter. 2009. Life cycle of *Ixodes luciae* (Acari: Ixodidae) in the laboratory. Parasitology Research **105**:1749-1753.

Labruna, M. B., R. C. Leite, J. L. H. Faccini, and F. Ferreira. 2000. Life cycle of the tick *Haemaphysalis leporispalustris* (Acari: Ixodidae) under laboratory conditions. Experimental & applied acarology **24**:683-694.

Labruna, M. B., S. Mattar, S. Nava, S. Bermudez, J. M. Venzal, G. Dolz, A. Katia, L. Romero, R. De Sousa, and J. Oteo. 2011. Rickettsioses in Latin America, Caribbean, Spain and Portugal. Revista MVZ Córdoba **16**:2435-2457.

Labruna, M. B., J. W. McBride, D. H. Bouyer, L. M. A. Camargo, E. P. Camargo, and D. H. Walker. 2004. Molecular evidence for a spotted fever group *Rickettsia* species in the tick *Amblyomma longirostre* in Brazil. Journal of Medical Entomology **41**:533-537.

Labruna, M. B., S. L. Souza, A. C. Menezes, M. C. Horta, A. Pinter, and S. M. Gennari. 2002. Life-cycle and host specificity of *Amblyomma tigrinum* (Acari: Ixodidae) under laboratory conditions. Experimental & applied acarology **26**:115-125.

Lado, P., S. Nava, M. B. Labruna, M. P. J. Szabo, L. A. Durden, S. Bermudez, M. Montagna, A. C. Sánchez Quirós, and L. Beati. 2016. *Amblyomma parvum* Aragão, 1908 (Acari: Ixodidae): Phylogeography and systematic considerations. Ticks and Tick-borne Diseases ***in press***.

Lafferty, K. D. 2012. Biodiversity loss decreases parasite diversity: theory and patterns. Philosophical Transactions of the Royal Society B: Biological Sciences **367**:2814-2827.

Lafferty, K. D., S. Allesina, M. Arim, C. J. Briggs, G. De Leo, A. P. Dobson, J. A. Dunne, P. T. J. Johnson, A. M. Kuris, D. J. Marcogliese, N. D. Martinez, J. Memmott, P. A. Marquet, J. P. McLaughlin, E. A. Mordecai, M. Pascual, R. Poulin, and D. W. Thieltges. 2008. Parasites in food webs: the ultimate missing links. Ecology Letters **11**:533-546.

Lafferty, K. D. and C. L. Wood. 2013. It's a myth that protection against disease is a strong and general service of biodiversity conservation: Response to Ostfeld and Keesing. Conservation Biology **14**:722-728.

Lareschi, M. and B. R. Krasnov. 2010. Determinants of ectoparasite assemblage structure on rodent hosts from South American marshlands: the effect of host species, locality and season. Medical and Veterinary Entomology **24**:284-292.

Laurance, W. F. 2007. Have we overstated the tropical biodiversity crisis? Trends in Ecology & Evolution **22**:65-70.

Lee, K., A. Takano, K. Taylor, M. Sashika, M. Shimozuru, S. Konnai, H. Kawabata, and T. Tsubota. 2014. A relapsing fever group *Borrelia* sp. similar to *Borrelia lonestari* found among wild sika deer (*Cervus nippon yesoensis*) and *Haemaphysalis* spp. ticks in Hokkaido, Japan. Ticks and Tick-borne Diseases **5**:841-847.

Lee, K. A. 2006. Linking immune defenses and life history at the levels of the individual and the species. Integrative and Comparative Biology **46**:1000-1015.
Lee, M. S. Y. 2013. Turtle origins: insights from phylogenetic retrofitting and molecular scaffolds. Journal of Evolutionary Biology **26**:2729-2738.
Léger, E., G. Vourc'h, L. Vial, C. Chevillon, and K. D. McCoy. 2013. Changing distributions of ticks: causes and consequences. Experimental and Applied Acarology:1-26.
Leigh, J., E.G. 1999. Tropical forest ecology: A view from Barro Colorado Island. Oxford University Press, New York.
Levi, T., A. L. Massey, R. D. Holt, F. Keesing, R. S. Ostfeld, and C. A. Peres. 2016. Does biodiversity protect humans against infectious disease? Comment. Ecology **97**:536-542.
Lindeborg, M., C. Barboutis, C. Ehrenborg, T. Fransson, T. G. Jaenson, P.-E. Lindgren, Å. Lundkvist, F. Nyström, E. Salaneck, and J. Waldenström. 2012. Migratory birds, ticks, and Crimean-Congo hemorrhagic fever virus. Emerging Infectious Diseases **18**:2095.
Lively, C. M., K. Clay, M. J. Wade, and C. Fuqua. 2005. Competitive co-existence of vertically and horizontally transmitted parasites. Evolutionary Ecology Research **7**:1183-1190.
LoGiudice, K., S. T. K. Duerr, M. J. Newhouse, K. A. Schmidt, M. E. Killilea, and R. S. Ostfeld. 2008. Impact of host community composition on Lyme disease risk. Ecology **89**:2841-2849.
LoGiudice, K., R. S. Ostfeld, K. A. Schmidt, and F. Keesing. 2003. The ecology of infectious disease: effects of host diversity and community composition on Lyme disease risk. Proceedings of the National Academy of Sciences of the United States of America **100**:567-571.
Macaluso, K. R., D. E. Sonenshine, S. M. Ceraul, and A. F. Azad. 2002. Rickettsial infection in *Dermacentor variabilis* (Acari: Ixodidae) inhibits transovarial transmission of a second *Rickettsia*. Journal of Medical Entomology **39**:809-813.
MacArthur, R. H. 1965. Patterns of species diversity. Biological reviews **40**:510-533.
MacArthur, R. H. 1972. Geographical ecology: patterns in the distribution of species. Princeton University Press, Princeton.
MacArthur, R. H. and E. O. Wilson. 1967. The theory of island biogeography. Princteon University press, Princeton.
Mace, G. M., K. Norris, and A. H. Fitter. 2012. Biodiversity and ecosystem services: a multilayered relationship. Trends in Ecology & Evolution **27**:19-26.
Madinah, A., F. Abang, A. Mariana, M. Abdullah, and J. Mohd-Azlan. 2014. Interaction of ectoparasites-small mammals in tropical rainforest of Malaysia. Community Ecology **15**:113-120.
Magalhães, S., M. Forbes, A. Skoracka, M. Osakabe, C. Chevillon, and K. McCoy. 2007. Host race formation in the Acari. Experimental and Applied Acarology **42**:225-238.
Marcogliese, D. 2004. Parasites: small players with crucial roles in the ecological theater. EcoHealth **1**:151-164.
Marcogliese, D. J. 2005. Parasites of the superorganism: Are they indicators of ecosystem health? International Journal for Parasitology **35**:705-716.
Marini, M. Â., B. L. Reinert, M. R. Bornschein, J. C. Pinto, and M. A. Pichorim. 1996. Ecological correlates of ectoparasitism on Atlantic Forest birds, Brazil. Ararajuba **4**:93-102.
Marsot, M., P.-Y. Henry, G. Vourc'h, P. Gasqui, E. Ferquel, J. Laignel, M. Grysan, and J.-L. Chapuis. 2012. Which forest bird species are the main hosts of the tick, *Ixodes ricinus*, the vector of *Borrelia burgdorferi* sensu lato, during the breeding season? International Journal for Parasitology **42**:781-788.
Martin, M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet. journal **17**:pp. 10-12.
Martins, T. F., V. C. Onofrio, D. M. Barros-Battesti, and M. B. Labruna. 2010. Nymphs of the genus *Amblyomma* (Acari: Ixodidae) of Brazil: descriptions, redescriptions, and identification key. Ticks and Tick-borne Diseases **1**:75-99.
Massung, R. F., J. W. Courtney, S. L. Hiratzka, V. E. Pitzer, G. Smith, and R. L. Dryden. 2005. *Anaplasma phagocytophilum* in white-tailed deer. Emerging Infectious Diseases **11**:1604.
May, R. M. 1973. Stability and complexity in model ecosystems. Princeton UniversityPress, Princeton.
May, R. M. and R. M. Anderson. 1979. Population biology of infectious diseases: Part I. Nature **280**:455-461.
McCoy, K. D., T. Boulinier, S. Schjørring, and Y. Michalakis. 2002. Local adaptation of the ectoparasite *Ixodes uriae* to its seabird host. Evolutionary Ecology Research **4**:441-456.
McCoy, K. D., E. Léger, and M. Dietrich. 2013. Host specialization in ticks and transmission of tick-borne diseases: a review. Frontiers in Cellular and Infection Microbiology **3**:57.
McFarlane, R., A. Sleigh, and T. McMichael. 2012. Synanthropy of wild mammals as a determinant of emerging infectious diseases in the Asian–Australasian region. EcoHealth **9**:24-35.
Mediannikov, O., Z. Sekeyová, M.-L. Birg, and D. Raoult. 2010. A novel obligate intracellular gamma-proteobacterium associated with ixodid ticks, *Diplorickettsia massiliensis*, gen. nov., sp. nov. PLoS ONE **5**:e11478.
Meerburg, B. G., G. R. Singleton, and A. Kijlstra. 2009. Rodent-borne diseases and their risks for public health. Critical reviews in microbiology **35**:221-270.
Meiri, S. 2010. Length–weight allometries in lizards. Journal of Zoology **281**:218-226.
Mendenhall, C. D., D. S. Karp, C. F. Meyer, E. A. Hadly, and G. C. Daily. 2014. Predicting biodiversity change and averting collapse in agricultural landscapes. Nature **509**:213-217.
Meyer, N., H. J. Esser, R. Moreno, F. van Langevelde, Y. Liefting, D. R. Oller, C. B. F. Vogels, A. D. Carver, C. K. Nielsen, and P. A. Jansen. 2015. An assessment of the terrestrial mammal communities in forests of Central Panama, using camera-trap surveys. Journal for Nature Conservation **26**:28-35.

Michalski, F. and C. A. Peres. 2007. Disturbance-mediated mammal persistence and abundance-area relationships in Amazonian forest fragments. Conservation Biology **21**:1626-1640.
Mihalca, A., C. Gherman, and V. Cozma. 2011. Coendangered hard-ticks: threatened or threatening? Parasites & Vectors **4**:1-7.
Miller, M. J., H. J. Esser, J. R. Loaiza, E. A. Herre, C. Aguilar, D. Quintero, E. Alvarez, and E. Bermingham. 2016. Molecular ecological insights into Neotropical bird–tick interactions. PLoS ONE **11**:e0155989.
Mills, J. N. 2006. Biodiversity loss and emerging infectious disease: an example from the rodent-borne hemorrhagic fevers. Biodiversity **7**:9-17.
Miranda, J., A. Portillo, J. A. Oteo, and S. Mattar. 2012. *Rickettsia* sp. strain colombianensi (Rickettsiales: Rickettsiaceae): a new proposed *Rickettsia* detected in *Amblyomma dissimile* (Acari: Ixodidae) from iguanas and free-living larvae ticks from vegetation. Journal of Medical Entomology **49**:960-965.
Møller, A. P. and J. Erritzøe. 1998. Host immune defence and migration in birds. Evolutionary Ecology **12**:945-953.
Moraes-Filho, J., A. Marcili, F. A. Nieri-Bastos, L. J. Richtzenhain, and M. B. Labruna. 2011. Genetic analysis of ticks belonging to the *Rhipicephalus sanguineus* group in Latin America. Acta Tropica **117**:51-55.
Morand, S., S. Jittapalapong, Y. Suputtamongkol, M. T. Abdullah, and T. B. Huan. 2014. Infectious diseases and their outbreaks in Asia-Pacific: biodiversity and its regulation loss matter. PLoS ONE **9**:e90032.
Morand, S. and R. Poulin. 1998. Density, body mass and parasite species richness of terrestrial mammals. Evolutionary Ecology **12**:717-727.
Moreno, R. S., R. W. Kays, and R. Samudio Jr. 2006. Competitive release in diets of ocelot (*Leopardus pardalis*) and puma (*Puma concolor*) after jaguar (*Panthera onca*) decline. Journal of Mammalogy **87**:808-816.
Moreno, S. H. 1993. Impact of development on the Panama Canal environment. Journal of Interamerican Studies and World Affairs **35**:129-149.
Morens, D. M., G. K. Folkers, and A. S. Fauci. 2004. The challenge of emerging and re-emerging infectious diseases. Nature **430**:242-249.
Moyer, P., A. Varela, M. Luttrell, V. Moore, D. Stallknecht, and S. Little. 2006. White-tailed deer (*Odocoileus virginianus*) develop spirochetemia following experimental infection with *Borrelia lonestari*. Veterinary microbiology **115**:229-236.
Münkemüller, T., S. Lavergne, B. Bzeznik, S. Dray, T. Jombart, K. Schiffers, and W. Thuiller. 2012. How to measure and test phylogenetic signal. Methods in Ecology and Evolution **3**:743-756.
Murgas, l. L., A. M. Castro, and S. E. Bermúdez. 2013. Current status of *Amblyomma ovale* (Acari: Ixodidae) in Panama. Ticks and Tick-borne Diseases **4**:164-166.
Muyzer, G., E. C. De Waal, and A. G. Uitterlinden. 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Applied and Environmental Microbiology **59**:695-700.
Myers, N., R. A. Mittermeier, C. G. Mittermeier, G. A. B. da Fonseca, and J. Kent. 2000. Biodiversity hotspots for conservation priorities. Nature **403**:853-858.
Nava, S., L. Beati, M. B. Labruna, A. G. Cáceres, A. J. Mangold, and A. A. Guglielmone. 2014. Reassessment of the taxonomic status of *Amblyomma cajennense* (Fabricius, 1787) with the description of three new species, *Amblyomma tonelliae* n. sp., *Amblyomma interandinum* n. sp. and *Amblyomma patinoi* n. sp., and reinstatement of *Amblyomma mixtum* Koch, 1844, and *Amblyomma sculptum* Berlese, 1888 (Ixodida: Ixodidae). Ticks and Tick-borne Diseases **5**:252-276.
Nava, S. and A. A. Guglielmone. 2013. A meta-analysis of host specificity in Neotropical hard ticks (Acari: Ixodidae). Bulletin of Entomological Research **103**:216-224.
Nichols, E. and A. Gómez. 2011. Conservation education needs more parasites. Biological Conservation **144**:937-941.
Nicholson, W. L., K. E. Allen, J. H. McQuiston, E. B. Breitschwerdt, and S. E. Little. 2010. The increasing recognition of rickettsial pathogens in dogs and people. Trends in Parasitology **26**:205-212.
Nieto, N. C., M. B. Teglas, K. M. Stewart, T. Wasley, and P. L. Wolff. 2012. Detection of relapsing fever spirochetes (*Borrelia hermsii* and *Borrelia coriaceae*) in free-ranging mule deer (*Odocoileus hemionus*) from Nevada, United States. Vector-Borne and Zoonotic Diseases **12**:99-105.
Noda, H., U. Munderloh, and T. Kurtti. 1997. Endosymbionts of ticks and their relationship to *Wolbachia* spp. and tick-borne pathogens of humans and animals. Applied and Environmental Microbiology **63**:3926-3932.
Nunn, Charles L., S. Altizer, K. E. Jones, and W. Sechrest. 2003. Comparative tests of parasite species richness in primates. The American Naturalist **162**:597-614.
O'Connell, A. F., J. D. Nichols, and K. U. Karanth. 2010. Camera traps in animal ecology: methods and analyses. Springer Science & Business Media.
Ogden, N. and J. Tsao. 2009. Biodiversity and Lyme disease: dilution or amplification? Epidemics **1**:196-206.
Ogden, N. H., L. R. Lindsay, K. Hanincova, I. K. Barker, M. Bigras-Poulin, D. F. Charron, A. Heagy, C. M. Francis, C. J. O'Callaghan, and I. Schwartz. 2008. Role of migratory birds in introduction and range expansion of *Ixodes scapularis* ticks and of *Borrelia burgdorferi* and *Anaplasma phagocytophilum* in Canada. Applied and Environmental Microbiology **74**:1780-1790.

Ogrzewalska, M., I. Literák, M. Capek, O. Sychra, V. Á. Calderón, B. C. Rodríguez, C. Prudencio, T. F. Martins, and M. B. Labruna. 2015. Bacteria of the genus *Rickettsia* in ticks (Acari: Ixodidae) collected from birds in Costa Rica. Ticks and Tick-borne Diseases **6**:478-482.

Ogrzewalska, M., I. Literak, J. M. Cardenas-Callirgos, M. Capek, and M. B. Labruna. 2012. *Rickettsia bellii* in ticks *Amblyomma varium* Koch, 1844, from birds in Peru. Ticks and Tick-borne Diseases **3**:254-256.

Ogrzewalska, M., T. Martins, M. Capek, I. Literak, and M. B. Labruna. 2013. A *Rickettsia parkeri*-like agent infecting *Amblyomma calcaratum* nymphs from wild birds in Mato Grosso do Sul, Brazil. Ticks and Tick-borne Diseases **4**:145-147.

Ogrzewalska, M., R. C. Pacheco, A. Uezu, L. J. Richtzenhain, F. Ferreira, and M. B. Labruna. 2009. Ticks (Acari: Ixodidae) infesting birds in an Atlantic rain forest region of Brazil. Journal of Medical Entomology **46**:1225-1229.

Ogrzewalska, M., A. Uezu, C. N. Jenkins, and M. B. Labruna. 2011. Effect of forest fragmentation on tick infestations of birds and tick infection rates by *Rickettsia* in the Atlantic forest of Brazil. EcoHealth **8**:320-331.

Ogrzewalska, M., A. Uezu, and M. B. Labruna. 2010. Ticks (Acari: Ixodidae) infesting wild birds in the eastern Amazon, northern Brazil, with notes on rickettsial infection in ticks. Parasitology Research **106**:809-816.

Olegário, M., M. Gerardi, S. Tsuruta, and M. Szabó. 2011. Life cycle of the tick *Amblyomma parvum* Aragão, 1908 (Acari: Ixodidae) and suitability of domestic hosts under laboratory conditions. Veterinary Parasitology **179**:203-208.

Oliver Jr, J. H., J. E. Keirans, D. R. Lavender, and H. J. Hutcheson. 1987. *Ixodes affinis* Neumann (Acari: Ixodidae): new host and distribution records, description of immatures, seasonal activities in Georgia, and laboratory rearing. The Journal of Parasitology:646-652.

Onofrio, V., M. B. Labruna, A. Pinter, F. Giacomin, and D. M. Barros-Battesti. 2006. Comentários e chaves as espécies do gênero *Amblyomma*. Pages 53-82 *in* D. Barros-Battesti, M. Arzua, and G. Bechara, editors. Carrapatos de importância médico-veterinária da regiao Neotropical. Instituto Butantan, Brasil.

Ostfeld, R. S., C. D. Canham, K. Oggenfuss, R. J. Winchcombe, and F. Keesing. 2006. Climate, deer, rodents, and acorns as determinants of variation in Lyme-disease risk. PLoS Biology **4**:e145.

Ostfeld, R. S. and F. Keesing. 2000. Biodiversity and disease risk: the case of Lyme disease. Conservation Biology **14**:722-728.

Ostfeld, R. S. and F. Keesing. 2012. Effects of host diversity on infectious disease. Annual Review of Ecology, Evolution, and Systematics **43**:157-182.

Ostfeld, R. S. and F. Keesing. 2013. Straw men don't get Lyme disease: response to Wood and Lafferty. Trends Ecol Evol **28**:502-503.

Pacheco, R. C., M. Arzua, F. A. Nieri-Bastos, J. Moraes-Filho, A. Marcili, L. J. Richtzenhain, D. M. Barros-Battesti, and M. B. Labruna. 2012. Rickettsial infection in ticks (Acari: Ixodidae) collected on birds in southern Brazil. Journal of Medical Entomology **49**:710-716.

Pagel, M. 1999. Inferring the historical patterns of biological evolution. Nature **401**:877-884.

Pagel, M. D. 1992. A method for the analysis of comparative data. Journal of theoretical biology **156**:431-442.

Paradis, E., J. Claude, and K. Strimmer. 2004. APE: analyses of phylogenetics and evolution in R language. Bioinformatics **20**:289-290.

Parola, P., C. D. Paddock, C. Socolovschi, M. B. Labruna, O. Mediannikov, T. Kernif, M. Y. Abdad, J. Stenos, I. Bitam, and P.-E. Fournier. 2013. Update on tick-borne rickettsioses around the world: a geographic approach. Clinical Microbiology Reviews **26**:657-702.

Parola, P., C. Socolovschi, L. Jeanjean, I. Bitam, P.-E. Fournier, A. Sotto, P. Labauge, and D. Raoult. 2008. Warmer weather linked to tick attack and emergence of severe rickettsioses. PLoS neglected tropical diseases **2**:e338.

Patefield, W. 1981. Algorithm AS 159: an efficient method of generating random R× C tables with given row and column totals. Applied Statistics:91-97.

Patterson, B. D. and L. P. Costa. 2012. Introduction to the history and geography of Neotropical mammals. Page 1 *in* B. D. Patterson and L. P. Costa, editors. Bones, Clones, and Biomes: The History and Geography of Recent Neotropical Mammals. The University of Chicago Press, Chicago & London.

Perkins, S. E., I. M. Cattadori, V. Tagliapietra, A. P. Rizzoli, and P. J. Hudson. 2003. Empirical evidence for key hosts in persistence of a tick-borne disease. International Journal for Parasitology **33**:909-917.

Perlman, S. J., M. S. Hunter, and E. Zchori-Fein. 2006. The emerging diversity of *Rickettsia*. Proceedings of the Royal Society of London B: Biological Sciences **273**:2097-2106.

Pinheiro, J., D. Bates, S. DebRoy, D. Sarkar, and R. C. Team. 2017. nlme: Linear and Nonlinear Mixed Effects Models. .

Pollock, N. B., L. K. Vredevoe, and E. N. Taylor. 2012. How do host sex and reproductive state affect host preference and feeding duration of ticks? Parasitology Research **111**:897-907.

Pongsiri, M. J., J. Roman, V. O. Ezenwa, T. L. Goldberg, H. S. Koren, S. C. Newbold, R. S. Ostfeld, S. K. Pattanayak, and D. J. Salkeld. 2009. Biodiversity loss affects global disease ecology. BioScience **59**:945-954.

Poulin, R. 1995. Phylogeny, ecology, and the richness of parasite communities in vertebrates. Ecological Monographs **65**:283-302.

Poulin, R. 1997. Parasite faunas of freshwater fish: the relationship between richness and the specificity of parasites. International Journal for Parasitology **27**:1091-1098.

Poulin, R. 2004. Macroecological patterns of species richness in parasite assemblages. Basic and Applied Ecology **5**:423-434.

Poulin, R. 2010. Network analysis shining light on parasite ecology and diversity. Trends in Parasitology **26**.
Poulin, R. 2011. Evolutionary ecology of parasites. 2nd edition. Princeton University Press, Princeton.
Poulin, R. and D. B. Keeney. 2008. Host specificity under molecular and experimental scrutiny. Trends in Parasitology **24**:24-28.
Poulin, R., B. R. Krasnov, and S. Morand. 2006. Patterns of host specificity in parasites exploiting small mammals. Pages 233-256 *in* S. Morand, B. R. Krasnov, and R. Poulin, editors. Micromammals and Macroparasites. Springer, Tokyo.
Poulin, R., B. R. Krasnov, and D. Mouillot. 2011. Host specificity in phylogenetic and geographic space. Trends in Parasitology **27**:355-361.
Purvis, A. and T. Garland. 1993. Polytomies in comparative analyses of continuous characters. Systematic Biology **42**:569-575.
Purvis, A., J. L. Gittleman, G. Cowlishaw, and G. M. Mace. 2000. Predicting extinction risk in declining species. Proceedings of the Royal Society of London B: Biological Sciences **267**:1947-1952.
R Core Team. 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
Ramirez, D. G., G. A. Landulfo, V. C. Onofrio, S. M. Simons, J. Reck, J. R. Martins, M. B. Labruna, and D. M. Barros-Battesti. 2016. Laboratory life cycle of *Ornithodoros brasiliensis* (Acari: Argasidae): An endemic tick from southern Brazil. Ticks and Tick-borne Diseases.
Rand, P. W., C. Lubelczyk, G. R. Lavigne, S. Elias, M. S. Holman, E. H. Lacombe, and R. P. Smith Jr. 2003. Deer density and the abundance of *Ixodes scapularis* (Acari: Ixodidae). Journal of Medical Entomology **40**:179-184.
Randolph, S. 2004. Tick ecology: processes and patterns behind the epidemiological risk posed by ixodid ticks as vectors. Parasitology **129**:S37-S65.
Randolph, S. E. 1979. Population regulation in ticks: the role of acquired resistance in natural and unnatural hosts. Parasitology **79**:141-156.
Randolph, S. E. 1998. Ticks are not insects: consequences of contrasting vector biology for transmission potential. Parasitology Today **14**:186-192.
Randolph, S. E. 2010. To what extent has climate change contributed to the recent epidemiology of tick-borne diseases? Veterinary Parasitology **167**:92-94.
Randolph, S. E. and A. Dobson. 2012. Pangloss revisited: a critique of the dilution effect and the biodiversity-buffers-disease paradigm. Parasitology **139**:847-863.
Randolph, S. E. and K. Storey. 1999. Impact of microclimate on immature tick-rodent host interactions (Acari: Ixodidae): implications for parasite transmission. Journal of Medical Entomology **36**:741-748.
Rappole, J. and Z. Hubalek. 2003. Migratory birds and West Nile virus. Journal of applied microbiology **94**:47-58.
Ratnasingham, S. and P. D. Hebert. 2007. BOLD: The Barcode of Life Data System (http://www. barcodinglife. org). Molecular Ecology Resources **7**:355-364.
Ratnasingham, S. and P. D. Hebert. 2013. A DNA-based registry for all animal species: the Barcode Index Number (BIN) system. PLoS ONE **8**:e66213.
Reid, F. 2009. A field guide to the mammals of Central America and Southeast Mexico. Oxford University Press, New York.
Revell, L. J. 2012. phytools: an R package for phylogenetic comparative biology (and other things). Methods in Ecology and Evolution **3**:217-223.
Ridgely, R. S. and J. A. Gwynne. 1989. A guide to the birds of Panama: with Costa Rica, Nicaragua, and Honduras. 2nd edition. Princeton University Press, Princeton.
Ripple, W. J., J. A. Estes, R. L. Beschta, C. C. Wilmers, E. G. Ritchie, M. Hebblewhite, J. Berger, B. Elmhagen, M. Letnic, and M. P. Nelson. 2014. Status and ecological effects of the world's largest carnivores. Science **343**:1241484.
Rivas, J. J., A. Moreira-Soto, G. Alvarado, L. Taylor, O. Calderón-Arguedas, L. Hun, E. Corrales-Aguilar, J. A. Morales, and A. Troyo. 2015. Pathogenic potential of a Costa Rican strain of 'Candidatus Rickettsia amblyommii' in guinea pigs (*Cavia porcellus*) and protective immunity against *Rickettsia rickettsii*. Ticks and Tick-borne Diseases **6**:805-811.
Robinson, W. D., J. D. Brawn, and S. K. Robinson. 2000. Forest bird community structure in central Panama: influence of spatial scale and biogeography. Ecological Monographs **70**:209-235.
Rocha, L. A., A. Aleixo, G. Allen, F. Almeda, C. Baldwin, M. V. Barclay, J. M. Bates, A. Bauer, F. Benzoni, and C. Berns. 2014. Specimen collection: An essential tool. Science **344**:814-815.
Rognes, T., T. Flouri, B. Nichols, C. Quince, and F. Mahé. 2016. VSEARCH: a versatile open source tool for metagenomics. PeerJ **4**:e2584.
Romer, Y., A. C. Seijo, F. Crudo, W. L. Nicholson, A. Varela-Stokes, R. R. Lash, and C. D. Paddock. 2011. *Rickettsia parkeri* rickettsiosis, Argentina. Emerging Infectious Diseases **17**:1169.
Rottstock, T., J. Joshi, V. Kummer, and M. Fischer. 2014. Higher plant diversity promotes higher diversity of fungal pathogens, while it decreases pathogen infection per plant. Ecology **95**:1907-1917.
Rowcliffe, J. M., C. Carbone, P. A. Jansen, R. Kays, and B. Kranstauber. 2011. Quantifying the sensitivity of camera traps: an adapted distance sampling approach. Methods in Ecology and Evolution **2**:464-476.
Sacchi, A., J. Duarte, M. André, and R. Machado. 2012. Prevalence and molecular characterization of Anaplasmataceae agents in free-ranging Brazilian marsh deer (*Blastocerus dichotomus*). Comparative immunology, microbiology and infectious diseases **35**:325-334.

Salkeld, D. J., K. A. Padgett, and J. H. Jones. 2013. A meta-analysis suggesting that the relationship between biodiversity and risk of zoonotic pathogen transmission is idiosyncratic. Ecology Letters **16**:679-686.

Santamaria, A., J. E. Calzada, A. Saldana, M. J. Yabsley, and N. L. Gottdenker. 2014. Molecular diagnosis and species identification of *Ehrlichia* and *Anaplasma* infections in dogs from Panama, Central America. Vector-Borne and Zoonotic Diseases **14**:368-370.

Schmidt, K. A. and R. S. Ostfeld. 2001. Biodiversity and the dilution effect in disease ecology. Ecology **82**:609-619.

Schoener, T. W. 1968. The Anolis lizards of Bimini: resource partitioning in a complex fauna. Ecology **49**:704-726.

Scoles, G. A. 2004. Phylogenetic analysis of the *Francisella*-like endosymbionts of *Dermacentor* ticks. Journal of Medical Entomology **41**:277-286.

Scott, J. D. and L. A. Durden. 2015. *Amblyomma dissimile* Koch (Acari: Ixodidae) parasitizes bird captured in Canada. Systematic and Applied Acarology **20**:854-860.

Shannon, C. E. 1948. A mathematical theory of communication. The Bell System Technical Journal **27**:379-423.

Smith, F. A., S. K. Lyons, S. M. Ernest, K. E. Jones, D. M. Kaufman, T. Dayan, P. A. Marquet, J. H. Brown, and J. P. Haskell. 2003. Body mass of late quaternary mammals: Ecological archives E084-094. Ecology **84**:3403-3403.

Smith, K. F., M. Goldberg, S. Rosenthal, L. Carlson, J. Chen, C. Chen, and S. Ramachandran. 2014. Global rise in human infectious disease outbreaks. Journal of the Royal Society Interface **11**.

Sonenshine, D. E. 1991. Biology of ticks. Oxford University Press, New York.

Spencer, H. G. and M. Zuk. 2016. For host's sake: the pluses of parasite preservation. Trends in Ecology & Evolution **31**:341-343.

Stanko, M., D. Miklisová, J. Goüy de Bellocq, and S. Morand. 2002. Mammal density and patterns of ectoparasite species richness and abundance. Oecologia **131**:289-295.

Strona, G., P. Galli, and S. Fattorini. 2013. Fish parasites resolve the paradox of missing coextinctions. Nature communications **4**:1718.

Stuen, S., E. G. Granquist, and C. Silaghi. 2013. *Anaplasma phagocytophilum* — a widespread multi-host pathogen with highly adaptive strategies. Frontiers in Cellular and Infection Microbiology **3**.

Subramanian, G., O. Mediannikov, E. Angelakis, C. Socolovschi, G. Kaplanski, L. Martzolff, and D. Raoult. 2012. *Diplorickettsia massiliensis* as a human pathogen. European journal of clinical microbiology & infectious diseases **31**:365-369.

Sures, B. 2004. Environmental parasitology: relevancy of parasites in monitoring environmental pollution. Trends in Parasitology **20**:170-177.

Szabó, M. P. J., M. B. Labruna, M. V. Garcia, A. Pinter, K. C. Castagnolli, R. C. Pacheco, M. B. Castro, V. A. Veronez, G. M. Magalhães, A. Vogliotti, and J. M. B. Duarte. 2009. Ecological aspects of the free-living ticks (Acari: Ixodidae) on animal trails within Atlantic rainforest in south-eastern Brazil. Annals of Tropical Medicine and Parasitology **103**:57-72.

Tagliapietra, V., R. Rosà, D. Arnoldi, F. Cagnacci, G. Capelli, F. Montarsi, H. Hauffe, and A. Rizzoli. 2011. Saturation deficit and deer density affect questing activity and local abundance of *Ixodes ricinus* (Acari, Ixodidae) in Italy. Veterinary Parasitology **183**:114-124.

Tälleklint, L. and T. G. T. Jaenson. 1997. Infestation of mammals by *Ixodes ricinus* ticks (Acari: Ixodidae) in south-central Sweden. Experimental and Applied Acarology **21**:755-771.

Tamura, K., D. Peterson, N. Peterson, G. Stecher, M. Nei, and S. Kumar. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular biology and evolution **28**:2731-2739.

Tew, T. and D. Macdonald. 1994. Dynamics of space use and male vigour amongst wood mice, *Apodemus sylvaticus*, in the cereal ecosystem. Behavioral Ecology and Sociobiology **34**:337-345.

Thomas, F., R. Poulin, J.-F. Guégan, Y. Michalakis, and F. Renaud. 2000. Are there pros as well as cons to being parasitized? Parasitology Today **16**:533-536.

Thomas, F., F. Renaud, and J.-F. Guégan. 2005. Parasitism and ecosystems. Oxford University Press on Demand.

Thompson, J. N. 2005. The geographic mosaic of coevolution. University of Chicago Press, Chicago.

Tobler, M. W., S. E. Carrillo-Percastegui, R. Leite Pitman, R. Mares, and G. Powell. 2008. An evaluation of camera traps for inventorying large- and medium-sized terrestrial rainforest mammals. Animal Conservation **11**:169-178.

Tribaldos, M., Y. Zaldivar, S. Bermudez, F. Samudio, Y. Mendoza, A. A. Martinez, R. Villalobos, M. E. Eremeeva, C. D. Paddock, K. Page, R. E. Smith, and J. M. Pascale. 2011. Rocky Mountain spotted fever in Panama: a cluster description.

Troughton, D. R. and M. L. Levin. 2007. Life cycles of seven ixodid tick species (Acari: Ixodidae) under standardized laboratory conditions. Journal of Medical Entomology **44**:732-740.

Tucker Lima, J. M., A. Vittor, S. Rifai, and D. Valle. 2017. Does deforestation promote or inhibit malaria transmission in the Amazon? A systematic literature review and critical appraisal of current evidence. Philosophical Transactions of the Royal Society B: Biological Sciences **372**.

Turner, I. M. 1996. Species loss in fragments of tropical rain forest: A review of the evidence. Journal of Applied Ecology **33**:200-209.

Tylianakis, J. M., T. Tscharntke, and O. T. Lewis. 2007. Habitat modification alters the structure of tropical host-parasitoid food webs. Nature **445**:202-205.

Van Oosten, A. R., J. Dieter, A. Heylen, J. Elst, S. Philtjens, and E. Matthysen. 2016. An experimental test to compare potential and realised specificity in ticks with different ecologies. Evolutionary Ecology **30**:487.
Vázquez, D. P., C. J. Melián, N. M. Williams, N. Blüthgen, B. R. Krasnov, and R. Poulin. 2007. Species abundance and asymmetric interaction strength in ecological networks. Oikos **116**:1120-1127.
Vellend, M., W. K. Cornwell, K. Magnuson-Ford, and A. Ø. Mooers. 2011. Measuring phylogenetic biodiversity. Pages 194-207 Biological diversity: frontiers in measurement and assessment. Oxford University Press, Oxford.
Venzal, J. M., A. Estrada-Peña, A. J. Mangold, D. González-Acuña, and A. A. Guglielmone. 2008. The *Ornithodoros* (*Alectorobius*) *talaje* species group (Acari: Ixodida: Argasidae): description of *Ornithodoros* (*Alectorobius*) *rioplatensis* n. sp. from southern South America. Journal of Medical Entomology **45**:832-840.
Vor, T., C. Kiffner, P. Hagedorn, M. Niedrig, and F. Rühe. 2010. Tick burden on European roe deer (*Capreolus capreolus*). Experimental and Applied Acarology **51**:405-417.
Voss, R. S. and S. A. Jansa. 2009. Phylogenetic relationships and classification of didelphid marsupials, an extant radiation of New World metatherian mammals. Bulletin of the American Museum of Natural History:1-177.
Wang, Q., G. M. Garrity, J. M. Tiedje, and J. R. Cole. 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Applied and Environmental Microbiology **73**:5261-5267.
Webb, C. O., D. D. Ackerly, M. A. McPeek, and M. J. Donoghue. 2002. Phylogenies and community ecology. Annual Review of Ecology and Systematics **33**.
Webb, S. D. 2006. The great American biotic interchange: patterns and processes. Annals of the Missouri Botanical Garden **93**:245-257.
Weitzel, T., S. Dittrich, J. López, W. Phuklia, C. Martinez-Valdebenito, K. Velásquez, S. D. Blacksell, D. H. Paris, and K. Abarca. 2016. Endemic scrub typhus in South America. New England Journal of Medicine **375**:954-961.
Weksler, M. 2006. Phylogenetic relationships of oryzomine rodents (Muroidea: Sigmodontinae): Separate and combined analyses of morphological and molecular data. Bulletin of the American Museum of Natural History:1-149.
Wells, K., R. O'Hara, M. Pfeiffer, M. Lakim, T. Petney, and L. Durden. 2013. Inferring host specificity and network formation through agent-based models: tick–mammal interactions in Borneo. Oecologia **172**:307-316.
West, G. B., J. H. Brown, and B. J. Enquist. 1999. The fourth dimension of life: fractal geometry and allometric scaling of organisms. Science **284**:1677-1679.
Wickner, P. 2014. The first 4 Central American cases of delayed meat allergy with Galactose-Alpha-1, 3-Galactose positivity clustered among field biologists in Panama. Journal of Allergy and Clinical Immunology **133.2**:AB212.
Willis, J. 2009. Jaguars at the crossroads of the continents. Jaguar News **28**.
Wilson, D. E. and D. M. Reeder. 2005. Mammal species of the world: A taxonomic and geographic reference. 3 edition. John Hopkins University Press, Baltimore.
Wilson, K., O. Bjørnstad, A. Dobson, S. Merler, G. Poglayen, S. Randolph, A. Read, and A. Skorping. 2002. Heterogeneities in macroparasite infections: patterns and processes. Pages 6-44 The ecology of wildlife diseases.
Windsor, D. A. 1995. Equal rights for parasites. Conservation Biology **9**:1-2.
Wood, C. L. and K. D. Lafferty. 2013. Biodiversity and disease: a synthesis of ecological perspectives on Lyme disease transmission. Trends in Ecology & Evolution **28**:239-247.
Wood, C. L., K. D. Lafferty, G. DeLeo, H. S. Young, P. J. Hudson, and A. M. Kuris. 2014. Does biodiversity protect humans against infectious disease? Ecology **95**:817-832.
Wood, C. L., K. D. Lafferty, G. DeLeo, H. S. Young, P. J. Hudson, and A. M. Kuris. 2016. Does biodiversity protect humans against infectious disease? Reply. Ecology **97**:543-546.
Woolhouse, M. E. J. 2002. Population biology of emerging and re-emerging pathogens. Trends in Microbiology **10**:s3-s7.
Woolhouse, M. E. J., C. Dye, J.-F. Etard, T. Smith, J. D. Charlwood, G. P. Garnett, P. Hagan, J. L. K. Hii, P. D. Ndhlovu, R. J. Quinnell, C. H. Watts, S. K. Chandiwana, and R. M. Anderson. 1997. Heterogeneities in the transmission of infectious agents: Implications for the design of control programs. Proceedings of the National Academy of Sciences **94**:338-342.
Woolhouse, M. E. J., L. H. Taylor, and D. T. Haydon. 2001. Population biology of multihost pathogens. Science **292**:1109-1112.
Yazdanbakhsh, M., P. G. Kremsner, and R. Van Ree. 2002. Allergy, parasites, and the hygiene hypothesis. Science **296**:490-494.
Young, H., R. H. Griffin, C. L. Wood, and C. L. Nunn. 2013. Does habitat disturbance increase infectious disease risk for primates? Ecology Letters **16**:656-663.
Young, H. S., D. J. McCauley, R. Dirzo, C. L. Nunn, M. G. Campana, B. Agwanda, E. R. Otarola-Castillo, E. R. Castillo, R. M. Pringle, K. E. Veblen, D. J. Salkeld, K. Stewardson, R. Fleischer, E. F. Lambin, T. M. Palmer, and K. M. Helgen. 2017. Interacting effects of land use and climate on rodent-borne pathogens in central Kenya. Philosophical Transactions of the Royal Society B: Biological Sciences **372**.
Yparraguirre, L. A., E. Machado-Ferreira, A. J. Ullmann, J. Piesman, N. S. Zeidner, and C. A. Soares. 2007. A hard tick relapsing fever group spirochete in a Brazilian *Rhipicephalus* (*Boophilus*) *microplus*. Vector-Borne and Zoonotic Diseases **7**:717-722.
Zhang, J., K. Kobert, T. Flouri, and A. Stamatakis. 2014. PEAR: a fast and accurate Illumina Paired-End reAd mergeR. Bioinformatics **30**:614-620.

Summary

The ongoing loss of global biodiversity is unprecedented in both magnitude and pace, raising urgent questions as to how this loss will affect ecosystem functioning and human well-being. Control of infectious diseases has been proposed as an important ecosystem service that is likely to be affected by biodiversity loss. A negative relationship between biodiversity and disease risk could offer a win-win situation for nature conservation and human health. However, the generality of this relationship remains the subject of contentious debate. The aim of this thesis was to contribute to a better understanding of the interactions between ticks and their vertebrate hosts in a biodiversity hotspot, and how loss of biodiversity affects these interactions and ultimately, tick-borne disease risk. My study was unique in that I simultaneously considered and directly assessed broader communities of Neotropical wildlife, ticks, and tick-borne pathogens across an anthropogenic disturbance gradient.

Determining whether and how biodiversity loss affects tick-borne disease risk in tropical forests requires a thorough understanding of tick-host associations, which are a function of tick-host specificity as well as host biological and ecological traits. In chapter 2, I therefore quantified the degree to which adult ticks are host-specific in my study region: Panama. Using quantitative network analyses and phylogenetic tools with null model comparisons, I found that the adult life stages of most tick species were specific to a limited number of host species that were phylogenetically closely related. In Chapter 4 I showed that species assemblages of adult ticks became increasingly diverse on larger-bodied host species, indicating that adult ticks in Panama tend to select for large reproduction hosts.

In contrast to adult ticks, understanding the ecological interactions between immature ticks and their hosts in the tropics has long been hampered by a lack of morphological identification keys. Therefore, in Chapter 3, I describe the development of a DNA barcode reference library for the molecular identification of larvae and nymphs. This reference library was highly effective in species-level identification of immature ticks collected from birds (Chapter 3) and small mammals (Chapter 4 and 6). Several avian ecological traits were

positively associated with tick parasitism, but the potential role of wild birds in tick-borne disease transmission seems to be limited in Panama. Immature ticks did not show any specificity to particular bird species or avian ecological traits (Chapter 3), and species assemblages of immatures ticks were equally diverse across a large number of host taxa (Chapter 4). This suggests that larvae and nymphs may feed more opportunistically than their adult counterparts.

High host specificity in adult ticks implies high susceptibility to tick-host coextinction, even if immature ticks feed opportunistically. In chapter 5, I tested this hypothesis by surveying tick and vertebrate host communities across a forest fragmentation gradient. Forest fragments consisted of previously connected islands and peninsulas in the Panama Canal and ranged 1000-fold in size. Abundance and species richness of ticks was positively related to that of wildlife, which in turn was related to the size of the forest fragment. Specialist tick species were only present in fragments where their specific reproduction hosts were captured by camera traps. Further, less diverse tick communities were dominated by a generalist tick species. These results indicate that loss of wildlife had cascading effects on tick communities through local host-parasite coextinction.

In Chapter 6, I studied how communities of wildlife, ticks, and tick-borne microbes changed along a more 'typical' disturbance gradient, in which forest fragments were embedded in an agricultural and sub-urban landscape, rather than surrounded by water. I found that wildlife community disassembly either diluted, amplified, or had no effect on infection prevalence in ticks, depending on the pathogen and degree of disturbance. However, hyperabundance of medium- to large-sized frugivores and herbivores (important reproduction hosts for adult ticks) in sites that lacked apex predators was related to exponential increases in tick density, negating any effect of reduced pathogen prevalence. Moreover, high tick species richness in these sites was related to high microbial and pathogen richness. High parasite diversity is thus a source of infectious diseases. When medium- to large-sized frugivores and herbivores also disappeared, densities of infected ticks declined, suggesting a non-linear relationship between biodiversity loss and tick-borne disease risk, in which initial loss of apex predators increases disease risk, but further loss of species decreases disease risk again.

In this thesis, I have quantified host-feeding relationships of adult and immature Neotropical ticks, many of which (in the case of larvae and nymphs) were largely unknown. I have shown that adult ticks tend to be highly host-specific, particularly to larger-bodied

vertebrates, whereas immature ticks appear to have broader host-use patterns. I found that ticks are susceptible to local host-tick coextirpation, and that the relationship between biodiversity loss and tick-borne disease risk is non-linear. My results emphasize the importance of directly assessing host community composition and suggest that the presence of specific (reproduction) hosts are a more important factor than species richness *per se* for tick population and tick-borne disease dynamics.

Samenvatting

De snelheid en mate waarmee biodiversiteit wereldwijd verloren gaat is ongekend. Hoe zal dit verlies het functioneren van ecosystemen beïnvloeden, en uiteindelijk het welzijn van de mens? Een mogelijk gevolg van biodiversiteitsverlies is een toename in infectieziekten. Een negatieve relatie tussen biodiversiteit en het risico op infectieziekten zou een win-winsituatie betekenen voor zowel natuurbescherming als de menselijke gezondheid. De hypothese dat een hoge biodiversiteit in zijn algemeenheid beschermt tegen infectieziekten is echter omstreden. Dit proefschrift beoogt een bijdrage te leveren aan deze discussie en richt zich hierbij op de ecologische interacties tussen teken en hun gastheren in Panama, een biodiversiteitshotspot. Mijn onderzoek was uniek doordat ik tegelijkertijd bredere gemeenschappen van Neotropische teken, hun gastheren, en pathogenen direct bestudeerde langs een gradiënt van biodiversiteitsverlies.

Om vast te stellen of en hoe biodiversiteitsverlies in de tropen het risico op tekenziekten beïnvloedt, is een gedegen kennis nodig van de relatie tussen teken en hun gastheren. Deze relatie is afhankelijk van de mate waarin teken een voorkeur hebben voor een specifieke gastheer (gastheer-specificiteit) en de biologische en ecologische kenmerken van gastheren. In hoofdstuk 2 heb ik daarom de gastheer-specificiteit van de teken in Panama gekwantificeerd. Door gebruik te maken van netwerkanalyse, de fylogenie van gastheren, en uitkomsten te vergelijken met nul-modellen, vond ik dat de adulte levensstadia van de meeste tekensoorten een sterke voorkeur hadden voor specifieke gastheren die evolutionair nauw verwant zijn. In hoofdstuk 4 liet ik daarnaast zien dat populaties adulte teken op een gastheer steeds soortenrijker werden naarmate de gastheer groter was. De adulte teken in Panama zijn dus vooral specifiek voor grotere soorten gastheren.

Waar adulte teken gemakkelijk te identificeren zijn door middel van morfologische kenmerken, is dit in grote delen van de tropen niet of nauwelijks mogelijk voor larven en nimfen: de onvolwassen levensstadia van de teek. Dit heeft onze kennis van de ecologische relaties tussen de onvolwassen tekenstadia en hun gastheren sterk belemmerd. In hoofdstuk 3

beschrijf ik daarom de ontwikkeling van een zogenaamde referentie-bibliotheek voor DNA barcodes waarmee de teken uit Panama moleculair geïdentificeerd kunnen worden. Deze referentie-bibliotheek bleek zeer effectief in het tot op soort identificeren van larven en nimfen die verzameld waren van vogels (hoofdstuk 3) en kleine zoogdieren (hoofdstuk 4 en 6). Een aantal ecologische eigenschappen van vogels was positief gecorreleerd met de aanwezigheid van teken, maar de potentiële rol van vogels in het overbrengen van tekenziekten lijkt gering in Panama. De onvolwassen tekenstadia vertoonden geen specificiteit voor specifieke vogelsoorten of ecologische kenmerken (hoofdstuk 3), en hun soortenrijkdom veranderde niet met de grootte van de gastheer (hoofdstuk 4). Dit suggereert dat larven en nimfen wellicht meer opportunistisch voeden dan de adulte levensstadia.

De sterke mate waarin adulte teken een voorkeur hebben voor specifieke gastheren maakt dat ze erg gevoelig zouden moeten zijn voor lokale uitroeiing van hun gastheren, zelfs wanneer de larven en nimfen van andere soorten gastheren kunnen voeden. In hoofdstuk 5 heb ik deze hypothese getest door gemeenschappen van teken en gastheren te vergelijken tussen eilanden van verschillende grootte in het Panama Kanaal. De dichtheid en soortenrijkdom van teken was positief gecorreleerd aan die van de fauna, en gespecialiseerde tekensoorten waren alleen aanwezig op eilanden waar hun specifieke gastheer voor het adulte stadium ook aanwezig was. Bovendien werden minder diverse tekengemeenschappen gedomineerd door een generalistische tekensoort. Deze resultaten tonen aan dat het verlies van fauna een kettingreactie kan veroorzaken waardoor ook tekensoorten verdwijnen.

In hoofdstuk 6 bestudeerde ik hoe gemeenschappen van teken, hun gastheren, en pathogenen veranderden langs een gradiënt van menselijke verstoring, waarbij steeds kleiner wordende bosfragmenten omringd waren door landbouwgrond, dorpen, en andere menselijke landschappen. Mijn resultaten lieten zien dat met de bijbehorende veranderingen in fauna gemeenschappen de infectieprevalentie in teken ofwel toenam, afnam, of gelijk bleef. Echter, de enorme toename aan middelgrote tot grote frugivoren en herbivoren (welke belangrijke gastheren zijn voor adulte teken) in locaties waar toppredatoren zoals jaguars en poema's afwezig zijn, was gerelateerd aan een exponentiële toename in tekendichtheden, waardoor enige afname in infectieprevalentie volledig teniet werd gedaan. Bovendien was de soortenrijkdom van teken positief gecorreleerd aan de soortenrijkdom van microben en pathogenen. Een hoge diversiteit aan teken vormt dus een bron voor infectieziekten. Wanneer middelgrote tot grote frugivoren en herbivoren ook verdwenen omdat bosfragmenten te klein

werden, namen tekendichtheden ook af. Dit suggereert een niet-lineaire relatie tussen biodiversiteit en het risico op infectieziekten, waarbij het initiële verlies van toppredatoren leidt tot een toename aan het risico op infectieziekten, maar verder verlies van biodiversiteit het risico op infectieziekten juist weer verlaagt.

In dit proefschrift heb ik de relaties tussen Neotropische teken en hun gastheren gekwantificeerd, waarvan die voor larven en nimfen tot nu toe veelal onbekend waren. Mijn resultaten lieten zien dat adulte teken een sterke voorkeur vertonen voor specifieke gastheren, vooral grotere soorten frugivoren en herbivoren, terwijl de larven en nimfen minder selectief bleken. Teken waren gevoelig voor co-extinctie met specifieke gastheren, en de relatie tussen biodiversiteitsverlies en het risico op infectieziekten was niet lineair. Bovendien bleekt dat voor het risico op tekenziekten de aanwezigheid van specifieke gastheren (voor de adulte teken) een belangrijkere factor was dan puur de soortenrijkdom aan gastheren. Deze resultaten benadrukken het belang van het direct bepalen van de relatieve dichtheid en soortensamenstelling van lokale fauna.

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Short biography

Helen Joan Esser was born on the 26th of June 1986 on Aruba. She spent her early childhood in pursuit of iguanas, geckos, and hermit crabs, but switched to rearing butterflies after moving to the less exotic town of Kerkrade, the Netherlands, in 1993. When asked what she wanted to be after growing up she'd reply "nature researcher" until she learned that this profession was better known as "biologist". After completing vwo at the Scholengemeenschap Lelystad in 2004, it came to no one's surprise that she chose to study Biology at Wageningen University. During her BSc she specialized in Ecology and obtained a minor in Basics of Infectious Diseases. Her first MSc thesis project was about the impact of hunting on the regeneration of tropical palm trees. For her second MSc thesis project she studied the impact of tropical forest fragmentation on communities of wildlife and ticks. After she finished her MSc (*cum laude*), Helen immigrated to Panama to work on the Smithsonian Institution Grand Challenges Awards project '*Tropical vertebrate diversity loss and the emergence of tick-borne diseases*' as a junior researcher. After obtaining a personal grant from the Wageningen Graduate School for Production Ecology & Resource Conservation, she was able to turn this project into her doctoral study in 2012. From April 2016 – 2017, she became involved in the Eco-alert project, for which she wrote a systematic review on the ecological risk factors associated with the circulation of six zoonotic arboviruses. Based on this review, she helped perform a spatial risk analyses for the Netherlands and aided in the selection of surveillance sites as part of an early warning system. Helen currently works as a postdoctoral researcher at the Laboratory of Entomology, Wageningen University, where she studies the recent emergence and ecology of tick-borne encephalitis virus in the Netherlands.

Publications

Published articles

Esser, H.J., J.E. Foley, F.J.J.M. Bongers, E.A. Herre, M.J. Miller, H.H.T. Prins, and P.A. Jansen (2016) Host body size and the diversity of tick assemblages in Neotropical vertebrates, International Journal for Parasitology: Parasites and Wildlife, 5: 295-304

Esser, H.J., E.A. Herre, N. Blüthgen, J.R. Loaiza, S.E. Bermúdez, and P.A. Jansen (2016) Host-specificity in a diverse Neotropical tick community: an assessment using quantitative network analysis and host phylogeny, Parasites and Vectors, 9(372): DOI 10.1186/s13071-016-1655-6

Miller, M.J., **H.J. Esser**, J.R. Loaiza, E.A. Herre, C. Aguilar, D. Quintero, E. Alvarez, and E. Birmingham (2016) Molecular ecological insights into Neotropical bird-tick interactions, PLoS ONE, 11(5): e0155989

Meyer, N., **H.J. Esser**, R. Moreno, F. van Langevelde, Y. Liefting, D. Ros Oller, C.B.F. Vogels, A.D. Carver, C.K. Nielsen, and P.A. Jansen (2015) An assessment of the terrestrial mammal communities in forests of Central Panama, using camera-trap surveys, Journal of Nature Conservation, 26: 28-35

Bermúdez, S.E., **H.J. Esser**, R.J. Miranda, and R. Moreno, (2015) Wild carnivores (Mammalia) as hosts for ticks (Ixodida) in Panama, Systematic and Applied Acarology, 20: 13-19

Beaty, L., **H.J. Esser**, R.J. Miranda, and R.A. Norton (2013) First report of phoresy by an oribatid mite (Trhypochthoniidae: *Archegozetes magnus*) on a frog (Leptodactylidae: *Engystomops pustulosus*), International Journal of Acarology, 39: 325-326

Esser, H.J., Y. Liefting, R. Kays, and P.A. Jansen (2012) A record of striped hog-nosed skunk *Conepatus semistriatus* in Central Panama, between two known sub-ranges, Small Carnivore Conservation, 47: 62-64

Bermúdez, S.E., A. Castro, H. Esser, Y. Liefting, G. García, and R.J. Miranda (2012) Ticks (Ixodidae) on humans from central Panama, Panama (2010-2011), Experimental and Applied Acarology, 58: 81–88

Esser, H., D. Brown, and Y. Liefting (2010) Swimming in the Northern tamandua (Tamandua mexicana) in Panama, Edentata, 11: 70–72

In preparation

Esser, H.J., E.A. Herre, R. Kays, Y. Liefting, and P.A. Jansen. Host-parasite coextinction: evidence from tick-host communities and implications for disease transmission

Esser, H.J., J.E. Foley, Y. Liefting, N. Stephenson, M.R. Miller, E.A. Herre, F.J.J.M. Bongers, H.H.T. Prins, and P.A. Jansen. Host-tick-pathogen interactions across a Neotropical disturbance gradient

Esser, H.J., R. Mögling, N.B. Cleton, H. van der Jeugd, C. van Maanen, H. Sprong, A. Stroo, M.P.G. Koopmans, C.B.E.M. Reusken, and W.F. de Boer. Ecological risk factors associated with sustained circulation of six arboviral diseases: a systematic review for selection of surveillance sites

Esser, H.J., Y. Liefting, A. Ibáñez-Justicia, R. Mögling, H. van der Jeugd, H. Sprong, A. Stroo, M.P.G. Koopmans, C.B.E.M. Reusken, and W.F. de Boer. Development of risk maps for six arboviruses for the Netherlands

Esser, H.J., J. Hody, S. Bermúdez, N. Meyer, R. Moreno, P.A. Jansen, and R. Kays. Current distribution of the crab-eating fox *Cerdocyon thous* (Mammalia: Canidae) in Panama

Liddell, C., H.J. Esser, F.J.J.M Bongers, and P.A. Jansen (2017) Wildlife community composition along a gradient of forest structural complexity

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PE&RC Training and Education Statement

With the training and education activities listed below the PhD candidate has complied with the requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review of literature (4.5 ECTS)

- Community ecology of ticks, hosts and their pathogens under anthropogenic environmental change (2012)

Writing of project proposal (4.5 ECTS)

- Wildlife diversity loss and the emergence of tick-borne diseases in central Panama (2012)

Post-graduate courses (5.5 ECTS)

- Consumer-resource interactions; PE&RC/SENSE/RSEE (2014)
- Principals of ecological genomics; PE&RC/SENSE/RSEE (2015)
- Structural equation modelling; PE&RC (2015)

Laboratory training and working visits (2.1 ECTS)

- Next generation sequencing of the microbial community in ticks from Panama; oral presentation; University of California Davis, USA (2014)

Invited review of (unpublished) journal manuscript (4 ECTS)

- International Journal of Acarology: assembly behaviour by the lone star tick, *Amblyomma americanum* (*Acari: Ixodidae*), influenced by relative humidity (2013)
- International Journal of Acarology: the emergence of Lyme disease in China (2014)
- Parasites & Vectors: nested coevolutionary networks shape the ecological relationships of ticks, hosts and the Lyme disease bacteria *Borrelia burgdorferi* (2016)
- Parasitology: tick host specificity: an analysis based on host phylogeny and tick ecological features using *Amblyomma* triste and *Amblyomma tigrinum* immatures as target (2017)

Deficiency, refresh, brush-up courses (3 ECTS)

- Laboratory animal science course; EZO, WUR (2012)

Competence strengthening / skills courses (4.9 ECTS)

- Competence assessment; WGS (2012)
- Reviewing a scientific paper; WGS (2013)
- How to write a world-class paper; Wageningen UR Library & Elsevier Science (2013)
- How to write a convincing research proposal; Wageningen in'to Languages (2013)
- Entrepreneurship in and outside Science; WGS (2015)
- Writing grant proposals; WGS (2015)
- Career perspectives; WGS (2015)

PE&RC Annual meetings, seminars and the PE&RC weekend (3 ECTS)

- PE&RC First year PhD weekend (2012)
- PE&RC Day (2012, 2013)

- PE&RC Midterm PhD weekend (2014)
- PED&RC Last year PhD weekend (2015)
- WGS PhD Workshop carousel (2015)

Discussion groups / local seminars / other scientific meetings (6.5 ECTS)

- Tupper/Bambi/Gamboa/BCI guides seminars; oral presentations; Smithsonian Tropical Research Institute, Panama (2012-2014)
- Ecological Theory and Application PhD discussion group; oral presentation; WUR (2012-2016)
- Lecture: the Bonobo and the Atheist; WUR (2013)
- Food Web Ecology PhD discussion group; WUR (2013-2016)
- Current Themes in Ecology; NERN (2014)
- Symposium infectious diseases in natural populations; NIOO (2014)
- Wageningen Evolution and Ecology Seminars PhD discussion group; WUR (2014-2016)
- Studium Generale; WUR (2014-2016)
- Netherlands Annual Ecology Meeting; poster presentation; NERN (2015)
- Symposium "Wildlife Conservation"; oral presentation; Archeopteryx, Utrecht University (2015)

International symposia, workshops and conferences (7 ECTS)

- Fellow's symposium; poster presentation; Smithsonian Tropical Research Institute, Panama City, Panama (2014)
- 6th European Wildlife Disease Association (EWDA) Student Workshop on "human drivers of emerging diseases"; oral presentation; Veyrier du Lac, France (2015)
- 13th Ecology and Evolution of Infectious Diseases annual conference; poster presentation; Athens, USA (2015)
- STRI Science Symposium: tropical microbial ecology and evolution; oral presentation; Panama City, Panama (2016)

Lecturing / supervision of practicals / tutorials (7.5 ECTS)

- Princeton parasitology course, Panama (2012-2013)
- Ecological methods (2012- 2015)
- Animal ecology (2014-2015)

Supervision of a MSc student (3 ECTS)

- The composition of wildlife communities along a gradient of forest structural complexity in Central Panama (2014)

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