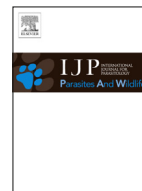


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Parasites and Wildlifejournal homepage: www.elsevier.com/locate/ijppawTesting the robustness of transmission network models to predict ectoparasite loads. One lizard, two ticks and four years [☆]Caroline K. Wohlfiel ^a, Stephan T. Leu ^a, Stephanie S. Godfrey ^{a,b}, C. Michael Bull ^{a,*}^a School of Biological Sciences, Flinders University, GPO Box 2100, Adelaide, SA, Australia^b School of Veterinary and Life Sciences, Murdoch University, 90 South St, Murdoch, WA, Australia

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ABSTRACT

We investigated transmission pathways for two tick species, *Bothriocroton hydrosauri* and *Amblyomma limbatum*, among their sleepy lizard (*Tiliqua rugosa*) hosts in a natural population in South Australia. Our aim was to determine whether a transmission network model continued to predict parasite load patterns effectively under varying ecological conditions. Using GPS loggers we identified the refuge sites used by each lizard on each day. We estimated infectious time windows for ticks that detached from a lizard in a refuge. Time windows were from the time when a detached tick molted and become infective, until the time it died from desiccation while waiting for a new host. Previous research has shown that *A. limbatum* molts earlier and survives longer than *B. hydrosauri*. We developed two transmission network models based on these differences in infective time windows for the two tick species. Directed edges were generated in the network if one lizard used a refuge that had previously been used by another lizard within the infectious time window. We used those models to generate values of network node in-strength for each lizard, a measure of how strongly connected an individual is to other lizards in the transmission network, and a prediction of infection risk for each host. The consistent correlations over time between *B. hydrosauri* infection intensity and network derived infection risk suggest that network models can be robust to environmental variation among years. However, the contrasting lack of consistent correlation in *A. limbatum* suggests that the utility of the same network models may depend on the specific biology of a parasite species.

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1. Introduction

Hosts within a population typically vary in the intensity of parasite infestation. In a previous study, Leu et al. (2010a) reported that the patterns of infestation of one tick species, *Amblyomma limbatum*, on its host lizard, *Tiliqua rugosa*, in 1 year, could be explained by a transmission network model. Our aim in the current study was to determine whether that model was sufficiently robust to predict patterns of infection in two tick species across multiple years.

Variation among hosts in the intensity of infection can result from post-infection differences among hosts in levels of resistance or immunity to the parasite, from interactions with the already established parasite community of the host, or from differences among hosts in their exposure to infestation (Poulin, 2007). Expo-

sure can be determined by host behavior. For instance behavioral differences between males and females (Zuk and McKean, 1996; Grear et al., 2012) or between adults and juveniles (Griffing et al., 2007) can influence their exposure to infection and their subsequent parasite loads. Transmission of parasites from one host to another host depends on behavioral processes and is now often described in terms of social networks. These recognize the role of social structures that influence contacts among hosts. Social networks describe the links between individuals that contact each other, and that are potential pathways for directly transmitted parasites (McCallum et al., 2001; Altizer et al., 2003; Clay et al., 2009). Network properties help in modelling the spread of parasites within a population (Hamede et al., 2012).

For parasites that have indirect transmission, transmission networks can still be constructed that reflect the ecological process of a parasite moving from one host to another. If a parasite leaves one host or sheds propagules that then wait to infest another host, then transmission can occur between hosts that share the same space, and space sharing transmission networks can be derived to predict infection patterns (Godfrey et al., 2010). Sometimes the dynamics of parasite transmission are unclear where it is difficult to make empirical observations of critical stages in the

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transmission process. In those cases, exploring alternative network models that reflect different plausible transmission processes, and comparing the predictions of each model with the empirically observed patterns of parasite infection can help to understand transmission ecology (Fenner et al., 2011). Our central hypothesis was that transmission networks adequately model pathways for the passage of parasites around a population. A prediction of that hypothesis is that individuals that are more connected in the network are at higher risk of infection, and are likely to have higher parasite loads.

Additionally, the dynamics of parasite transmission might vary over time, for instance in different seasons of the year (Hamede et al., 2009) or under different ecological conditions, and an important question is how robust are transmission models to ecological change. Conservation managers, who will often not know the mechanisms or dynamics of transmission of an exotic pathogen, will need to know if models of transmission derived from one time and place will translate to other times and places.

Our question in this study concerned the robustness of transmission network models. We asked whether a transmission network model continued to predict parasite load patterns effectively under alternative ecological conditions. Our study system was a natural population of Australian sleepy lizards (*T. rugosa*), hosting two ectoparasitic ticks, *Bothriocroton hydrosauri* and *A. limbatum*. Both of these are three host ticks. The three life stages, larvae, nymphs and adults, each feed on a single lizard host, then detach to molt to the next stage, or, for adult females, to lay a single clutch of eggs. Both ticks are reptile specific (Belan and Bull, 1995), and both have the sleepy lizard as their main host for all life stages in our study site (Smyth, 1973; Bull et al., 1981; Bull and Burzacott, 2001). Leu et al. (2010a) developed a transmission network model for *A. limbatum* on sleepy lizards based on asynchronous refuge sharing by the host lizards. In this model a lizard could become infected if it used a refuge previously used by another lizard that might have shed ticks there. The model included a time window of infection risk that was set by a pre-molt period and a survival duration for the unattached tick. Leu et al. (2010a) reported a significant correlation between transmission network node in-strength (a measure of the level of network connection) and *A. limbatum* tick load in a network of 18 sleepy lizards, in 1 year. Lizards that more often used refuges previously used by other lizards, were at higher risk of infection and that was reflected in higher tick loads.

This study tests the robustness of that relationship by considering patterns of infestation of two tick species over four ecologically contrasting years. Our aim was to determine whether the transmission network model remained an effective descriptor of infection patterns for different species in different years. Our hypothesis was that transmission networks represent pathways for the passage of parasites, in this case ticks, around a population. Our transmission network models were constructed from our understanding of the host–parasite system, and the test of our hypothesis was whether specific predictions from the network models fitted empirical data of parasite loads. In this case, we predicted that a higher in-strength of individual lizards, that is a higher cross infection risk, should lead to a higher infestation load. Lizards exposed to more infection risk should become more infected, and if the transmission network models are correct we should find a positive correlation between node in-strength of individual lizards and their tick loads. Leu et al. (2010a) reported a positive correlation for a smaller sample of lizards in 1 year, infested only with *A. limbatum*. In this study, we aimed to expand the generality of that previous result by conducting equivalent analyses on a larger sample of lizards, at a different location, over four (new) years, and with two tick species.

2. Materials and methods

2.1. Study site

The study was conducted in a 1.0 × 1.5 km area of semi-arid chenopod shrubland near Bunday Bore Station (33° 54' S, 139° 20' E) in South Australia. At this site, sleepy lizards are active over the spring and early summer (September–December), and almost completely inactive at other times of each year (Kerr and Bull, 2006, 2008). The study took place over four consecutive activity seasons, from 2008 to 2011. The long-term average annual rainfall in the study area is about 250 mm, and the actual rainfall at Bunday Bore Station was lower than average in 2008, but average or above average in the other 3 years (Table 1).

2.2. Data recording

The procedures for measuring lizard activity, storing GPS locations, and deriving social networks have been previously described (Kerr et al., 2004a; Leu et al., 2010b; Bull et al., 2012; Godfrey et al., 2012). Briefly, at the start of each season (September), we located all resident adult lizards within the study site (2008, $n = 48$ lizards; and $n = 61$ lizards in each of 2009, 2010, and 2011), and attached data logger units to the dorsal surface of the tails of each lizard. These recorded synchronous GPS locations for all lizards that were active, every 10 min, over the following 4–5 months (2008, $n = 159$ days; 2009, $n = 127$ days; 2010, $n = 115$ days; 2011, $n = 81$ days). The data logger units included a radio transmitter that allowed each lizard to be located every 12 days to download data and change batteries. Data downloads were conducted at times before or after the diurnal period of lizard activity, to avoid interfering with normal behaviors and to reduce the impact of handling on lizard behavior that was reported by Kerr et al. (2004b). Some units malfunctioned in some of the 12 day periods and some lizards were not fitted with data logger units early in the season. For analyses, we only included lizards with data from more than 30 days (2008, $n = 45$ lizards; 2009, $n = 56$ lizards; 2010, $n = 60$ lizards; 2011, $n = 59$ lizards). For most of those lizards we had over 100 days of data in each of the first 3 years (Table 1). The season was shorter in 2011 when equipment failure prematurely ended monitoring in late November.

At each data download we also counted the numbers of naturally attached adult, nymphal and larval ticks of each of the two tick species on each lizard. Then we determined the maximum tick count both for all stages of each tick species, and for just larvae plus nymphs, for each lizard over all of the counts in the season. We used those maximum numbers as a measure of the tick load of each lizard in subsequent analyses to test whether lizards that

Table 1

The rainfall in each year, and the mean, standard error and range of the number of days each lizard was surveyed, the number of different refuges used by each lizard, and the number of times each lizard used its most commonly occupied refuge.

	2008	2009	2010	2011
Rainfall (mm)	159	266	332	459
No. of lizards	45	56	60	59
<i>Days surveyed</i>				
Mean (SE)	79.2 (3.2)	81.9 (2.2)	86.7 (2.0)	56.4 (1.8)
Range	33–122	44–112	53–112	30–79
<i>Number of different refuges used</i>				
Mean (SE)	15.6 (0.8)	14.6 (0.7)	14.5 (0.6)	12.2 (0.6)
Range	6–27	6–26	7–27	4–27
<i>Number of times in most commonly used refuge</i>				
Mean (SE)	20.0 (1.4)	19.8 (1.2)	23.9 (1.4)	15.3 (0.9)
Range	7–56	8–50	8–51	5–34

were more strongly connected in a transmission network had higher tick loads. Adult ticks (and particularly male ticks) of these two species can remain attached to a host lizard for over 12 months and in this paper we report on patterns of larval and nymphal attachments, which we considered were more likely to be affected by short-term behaviors of the hosts. The analyses of total tick loads (adults, nymphs and larvae) produced identical trends but are not reported here.

All lizards were treated using procedures formally approved by the Flinders University Animal Welfare Committee in compliance with the Australian Code of Practice for the Use of Animals for Scientific Purposes and conducted under permits from the South Australian Department of Environment and Heritage to Undertake Scientific Research.

2.3. Transmission networks

Our hypothesis was that transmission networks represent pathways for the passage of parasites, in this case ticks, around a population. We constructed models of transmission networks for the ticks following Leu et al. (2010a). Engorged larval and nymphal ticks detach from their lizard host and then take a period of time to moult to the next infectious stage. Unattached ticks will subsequently die from desiccation or predation if the next host does not come past within another period of time. Our network models assumed that tick transmission from one host to the next takes place in the refuges used by lizards, since ticks exposed outside those shelters will quickly desiccate in the hot summer conditions (Chilton and Bull, 1993a). Thus there is a time window after ticks have detached from one lizard when there is a risk of infection for a second lizard that uses the same refuge. The time window begins after ticks have molted, and ends when they have died. Transmission can occur through the asynchronous use of the same refuge site by the second lizard within that time window of infection.

Leu et al. (2010a) estimated that the duration of this infectious time window for *A. limbatum* was 9–39 days. This was derived from reports that, after detachment, engorged larval *A. limbatum* take an average of 8 days to molt to unfed (and infectious) nymphs (Chilton et al., 2000), and that unfed nymphs survive at 30 °C for an average of 31 days after molting (Chilton and Bull, 1993a). Similarly, for this study, we estimated a time window of infection for *B. hydrosauri* of 11–24 days. This was based on previous data reporting that *B. hydrosauri* at 30 °C takes 2 days longer than *A. limbatum* to molt, and survives desiccation for a shorter period than *A. limbatum* (Bull and Smyth, 1973; Chilton and Bull, 1993; Chilton et al., 2000). We also assumed that the time window for infection by larvae was similar, based on previous measures of the period from detachment of engorged females to egg laying (Chilton and Bull, 1994), the development time of eggs to hatching (Chilton and Bull, 1994), and the survival time of larvae (Chilton and Bull, 1993). Again these values are highly variable as temperature and humidity change, but larvae of *A. limbatum* become infectious consistently faster than *B. hydrosauri*, and can become infectious within 14 days and survive desiccation for up to 14 days at warm temperatures (Chilton and Bull, 1993, 1994).

Although we imposed two specific time windows into our transmission network models, we recognize that molting and survival times for each tick species will be different under different climatic conditions over an activity season, and for different life stages, and with different levels of predation pressure, largely from ants (Dawes-Gromadzki and Bull, 1997a,b). Thus, although we developed two transmission network models, one with short (11–24 days) and the other with long (9–39 days) windows of infection, these are only two of a range of possible models that we could have used to determine relationships between network connections and tick loads on lizards.

2.4. Refuge locations

To integrate infectious time windows into a transmission network, we needed to know when lizards were using individual refuge sites. We deduced that the last active GPS location for each day was close to the site of the refuge that the lizard used for that night (Kerr et al., 2004a). In most cases in this study, and in that of Leu et al. (2010b), lizards occupied a refuge for one night, and then moved into another refuge after activity on the next day. If a lizard remained inactive in the same refuge over consecutive days, for instance when climatic conditions inhibited normal diurnal activity, the refuge location was only recorded once. In those cases, our models assumed that all transmission events, such as detachment of engorged ticks and attachment of waiting, unfed ticks, happened during the first night of refuge use. We have previously reported that these ticks do not move to seek new hosts, but stay close to where they detached (Petney et al., 1983), and our models assumed that unfed ticks remained in the lizard refuge where they had detached. In our models, most transmission occurred when a lizard moved into a refuge that contained waiting, unfed ticks.

Then, in each model, we compared the refuge location of each lizard on each night with the refuge locations of each other lizard on all of the subsequent nights within the time window of infection. Following Leu et al. (2010a), and taking into account the dimensions of refuge bushes and the 6 m precision of the GPS locations (Leu et al., 2010b), two lizards in refuges recorded as up to 14 m apart, were considered to have occupied the same refuge. This conservative approach probably overestimated both the level of refuge sharing and the opportunities for parasite transmission. We also estimated the number of different refuges used by each lizard by inspecting the distances between all possible pairs of recorded refuge locations over the activity season, and by assuming that records within 14 m of each other were the same refuge. Again this probably underestimated the actual number of different refuges used.

2.5. Analysis of network models

A prediction of our hypothesis was that lizards that were more connected in the network would have higher parasite loads. The following analyses were designed to test that prediction. For each model (short and long time windows of infection) we constructed a weighted directed transmission network, based on the asynchronous overnight refuge sharing events (described above), and on the infection risk of each of these events. The network consisted of directed edges from lizard A to lizard B, if lizard A used a refuge and then lizard B used the same refuge on at least one night within the time window of infection. Edge weight was defined as the probability of transmission of ticks from lizard A (node of origin) to lizard B (node of destination). At each refuge use (or re-use) ticks could detach from lizard A and, after they had molted, be waiting to attach to lizard B. For lizard B, the probability of infection with ticks from lizard A, from a single night in a refuge, increased with the number of nights that lizard A had previously used this refuge, up to the duration of the time window of infection. The total probability that lizard B would become infected with ticks from lizard A (the edge weight) was the sum of all the infection risks of all of the refuge sharing events over the activity season, when lizard B used a refuge after lizard A. The asynchronous timing of refuge use required for transmission meant that edge weights in opposite directions between two individual lizards were different.

We then calculated node strength, a parameter that defines the connectedness of each individual in the network, and incorporates both the number of edges from the node (i.e. how many other lizards that lizard has interacted with) and the weight of those edges (as described above). Node strength is defined as the total weight

of all edges connected to a node (Croft et al. 2008). In our analyses we used node in-strength, the sum of the weights of all edges directed towards the node, as a measure of the total risk of an individual lizard to become infected from sharing refuges with any other lizard in the population.

Measurements of both node strength and node in-strength can increase with the number of days of observation. Therefore, because individual lizards were observed for different numbers of days, we standardized the in-strength value by dividing by the number of overnight refuge records, and called this standardized in-strength the “cross-infection risk”, as in Leu et al. (2010a). We used PopTools (Hood, 2006) to analyze our transmission networks.

In all correlation analyses, we used Spearman rank correlations because the data were not usually normally distributed. Because network derived measurements, such as node in-strength, are relational, non-independent data (Croft et al., 2008), we used randomization tests to estimate the probability that the observed correlation coefficient was obtained by chance. Since our network was based on dyadic interactions (asynchronous refuge sharing by pairs of lizards), we randomized node labels (tick load or number of different refuges) among nodes (lizards) and re-calculated the correlation coefficient (James et al., 2009). We repeated this procedure 10,000 times to achieve a consistent frequency distribution for values of the randomly generated correlations (Bejder et al., 1998). Following Croft et al. (2008), we calculated Monte Carlo *P*-values as the quotient of the number of times the randomly generated values exceeded or were below the observed value, depending on our hypothesis. *P*-values were corrected for multiple testing as described by Holm (1979). We considered that each year produced independent datasets, and corrected for multiple testing separately for the set of four analyses within each year. Corrected *P*-values are presented in the results.

3. Results

The surveyed lizards were recorded using an average of 12–16 different refuges in the activity season of each year of the study (Table 1). Values were not directly comparable because there were different numbers of days of observation among lizards and years. Individual refuges were used by a lizard on up to 56 nights within the observation period (Table 1). Each year, between 79% and 95% of surveyed hosts were infected with ticks of each species (Table 2). The mean intensity of infection (measured as the mean of the maximum recorded tick load per lizard) for *B. hydrosauri* (larvae and nymphs) peaked in 2010, and was higher than for *A. limbatum* in the last 2 years (Table 2). In each year some lizards were recorded with loads of each tick species very much higher than the mean,

and in the last 3 years, there were significant positive correlations between the infection intensity of *B. hydrosauri* and of *A. limbatum* on each host (Table 2).

To test our prediction, that stronger connections in the transmission network led to higher infection levels, we derived eight transmission networks, one for each model (short or long time window of infection), in each of the 4 years. Two of those, for the long and short time window of infection in 1 year, are shown in Fig 1. In each season, the models differed in the distribution of nodal in-strengths with higher values for the long time window of infection models (Table 3). The nodal in-strengths generated by the two models were highly correlated within years ($r = 0.99$; $P = 0.009$). There were significant positive correlations between the standardized in-strength of each lizard node in the network (the cross infection risk) and the *B. hydrosauri* tick load, for each transmission network model, in the activity season of each year (Table 4), as predicted by our hypothesis. There was no consistent trend across the four study years for the correlations to be stronger when we applied one or the other model.

For *A. limbatum* there were significant positive correlations between the standardized in-strength of each lizard node in the network and tick load for the lizards in 2011, but not in the other 3 years (Table 4).

4. Discussion

Our hypothesis was that transmission networks represent pathways for the passage of parasites, in this case ticks, around a host population, in a range of ecological conditions. Leu et al. (2010a) reported a transmission network based on asynchronous sharing of refuge sites that predicted the patterns of tick infestation in a population of sleepy lizards. That previous study reported that the tick load on lizards was positively correlated with the node in-strength of the network, called the cross infection risk. The conclusions of Leu et al. (2010a) were derived from one tick species, *A. limbatum*, in a social network of 18 lizards, in 1 year.

Here, we considered a larger network of lizards, over four (different) years and with two tick species. Although the study site was about 5 km north of the previous study, average climatic conditions, vegetation and lizard behavior were similar across the two sites. The only major difference was that there were two tick species at the new site, while there was only one at the site of the previous study. In the current study, we also developed two model transmission networks based on a short and a long duration of the time window of infection. These alternative time windows were derived from empirical data on the survival times of one life stage of each of the two tick species, under desiccation at one tem-

Table 2

The prevalence (percentage of lizards that are infected) and intensity of infection (mean number of larvae and nymphs per lizard (SE)) for each tick species in each season of the study. Intensity was measured as the maximum tick load recorded on each lizard over a season. Also shown is the range of maximum tick loads recorded on all lizards for each tick species in each season, and the Spearman rank correlation values for total tick infestation intensity of the two tick species.

	2008	2009	2010	2011
No of lizards	45	56	60	59
<i>B. hydrosauri</i>				
Prevalence	82.2%	78.6%	95.1%	82.3%
Intensity (L + N)	3.09 (0.89)	4.50 (1.21)	23.08 (6.56)	7.10 (1.49)
Range of loads	0–32	0–37	0–237	0–58
<i>A. limbatum</i>				
Prevalence	91.1%	94.6%	88.5%	79.0%
Intensity (L + N)	2.04 (0.58)	4.38 (2.22)	5.33 (1.19)	3.63 (1.38)
Range of loads	0–21	0–130	0–55	0–77
Correlation of intensities	$r = 0.05$ $P = 0.73$	$r = 0.45$ $P < 0.001$	$r = 0.53$ $P < 0.001$	$r = 0.67$ $P < 0.001$

P-values in bold were significant.

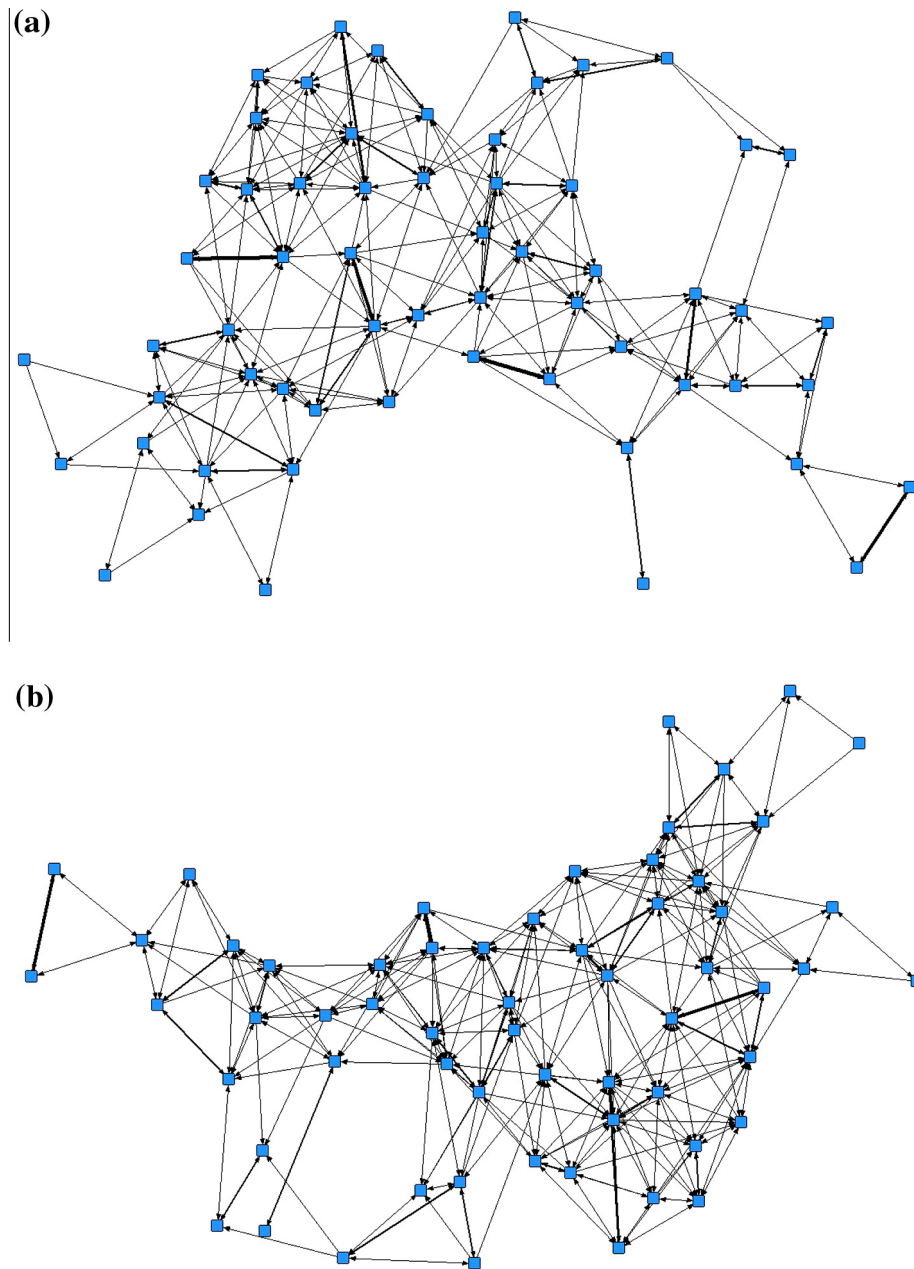


Fig. 1. Transmission networks generated with (a) a short time window of infection; and (b) a long time window of infection, from the GPS location data of the lizards in the study population in 2010. Nodes represent individual lizards and edges between nodes are directed towards the lizard that is at risk of infection. The edges are weighted as described in the main text and the thicker the line the more weight is associated with that edge.

Table 3

Mean (SE) and range of nodal in-strength values for all lizards in the transmission networks derived in each season with a short (11–24 days after tick detachment) or a long (9–39 days after tick detachment) infective time window.

	2008	2009	2010	2011
<i>Short infection window</i>				
Mean (SE)	1.19 (0.18)	0.86 (0.07)	0.94 (0.08)	0.80 (0.07)
Range	0–5.6	0–2.2	0–2.8	0–1.9
<i>Long infection window</i>				
Mean (SE)	2.33 (0.34)	1.63 (0.13)	1.90 (0.17)	1.51 (0.12)
Range	0–10.2	0–4.6	0.1–5.9	0–3.5

perature. They generated models with different frequency distributions of in-strength values. Specifically, there was a consistent trend for the short time window of infection model, where ticks

had a lower time of survival while waiting for hosts, to have fewer edge connections and lower in-strength values. In theory, a less connected network should be less efficient in transmission of the tick parasites from host to host. It is unlikely that either model accurately represented the time that infective stages of either tick species can persist in every refuge under every climatic condition experienced during the four lizard activity seasons of the study. But the results of [Leu et al. \(2010a\)](#) suggested that in 1 year and for one tick species, the long time window for transmission (that we also used in the current study) was sufficiently close to reality to allow the derived model to predict a pattern of tick infestation on lizards close to that observed. An aim of the current study was to test the robustness of that model.

For one tick species, the current results provided strong support for the conclusions of the previous study. For the tick *B. hydrosauri*,

Table 4

Analyses of correlation between tick load and cross-infection risk, for each model (Short = short time window of infection; Long = long time window of infection), and for each tick species, in each season. r_{sp} = Spearman's rank correlation value; 95% CI = the 95% confidence intervals around correlation derived from 10,000 network randomizations; P = probability that the observed r was outside those confidence limits. For analyses, P -values were corrected for multiple testing within each year using the Holm method (Holm 1979). P -values in bold were significant.

	<i>B. hydrosauri</i>			<i>A. limbatum</i>		
	r_{sp}	95% CI	P	r_{sp}	95% CI	P
2008						
Short	0.467	−0.295: 0.293	0.0048	−0.014	−0.297: 0.297	0.99
Long	0.401	−0.289: 0.294	0.0093	−0.019	−0.289: 0.290	0.99
2009						
Short	0.289	−0.265: 0.259	0.0369	0.144	−0.264: 0.262	0.15
Long	0.370	−0.262: 0.258	0.0100	0.218	−0.267: 0.260	0.10
2010						
Short	0.257	−0.258: 0.253	0.0705	0.204	−0.251: 0.255	0.12
Long	0.293	−0.249: 0.259	0.0444	0.192	−0.263: 0.252	0.12
2011						
Short	0.359	−0.260: 0.256	0.0076	0.268	−0.255: 0.248	0.0366
Long	0.348	−0.259: 0.258	0.0135	0.246	−0.256: 0.256	0.0366

there were strong and significant positive correlations between the cross-infection risk derived from the model and the empirically observed tick load. The relationship was consistent across years and with both versions of the transmission network model. Those 4 years differed substantially in rainfall. Previous studies have suggested that low annual rainfall has resulted in a poorer germination and persistence of the annual plants that make up a major part of the diet of these herbivorous lizards, and that in turn has led to lower levels of lizard activity in drier years (Kerr and Bull, 2006). Tick transmission might be adversely affected in low rainfall years both from this reduced lizard activity and from a faster desiccation rate of unfed ticks waiting for hosts in drier conditions. Combining these two effects means lizards are less likely to move into a new refuge, and ticks waiting there will have a shorter time window of infectivity before they die, in a drier year.

However, across those climatically different years there was a consistent pattern. Lizards that more often used refuges that other lizards had used had higher loads of the tick species *B. hydrosauri*. The pattern of infection among host lizards closely matched the predictions of the transmission network models and provided support for the assumed mechanisms of parasite transmission among hosts. The pattern of infection suggested that the transmission network models reliably describe the dynamics of the transmission of this tick among its lizard hosts.

But for the other tick species, *A. limbatum*, the pattern was different, and we only found significant correlations between tick load and cross-infection risk in one of the four study years. In that year there was a significant correlation between tick infection and both network models. Two questions arise. One is why the pattern differed between the two tick species. An explanation may be that, while waiting off the host for the next lizard to come by, nymphs and adults of *A. limbatum* tolerate desiccation for longer (Bull and Smyth, 1973; Chilton and Bull, 1993), and have lower mortality from predation (Dawes-Gromadzki and Bull, 1997a,b) than *B. hydrosauri*. This is the reason we allocated a longer time window of infection for that species originally. But another consequence may be that detached *A. limbatum* ticks can persist for longer in a wider range of microhabitats while waiting for their next host. Host refuges provide shelter for detached ticks from predation and desiccating high temperatures (Kerr et al., 2003), but *B. hydrosauri* may be more reliant than *A. limbatum* on host refuges as survival and transmission locations, over a wider range of conditions. As a result, the transmission network models that incorporate asynchronous refuge sharing may be more robust for *B. hydrosauri* than for *A. limbatum*. One conclusion from the current study is that network based models of the transmission dynamics of parasites

among host individuals will not necessarily be consistent for different parasites, even for those with very similar life cycles and transmission mechanisms.

A second question is why the results from three of the 4 years in this study differed from those of the previous study that reported a significant positive correlation between *A. limbatum* tick load and node in-strength (cross-infection risk). Possible explanations include that the environmental conditions differed between the first 3 years of the current study and 2007, when the previous study was conducted. Another factor is that microhabitats available as sites for ticks to wait for hosts may differ between the two sites, such that host refuges are not as important in the site used for the current study. Another possible explanation is that only one tick species infected lizards at the site of the first study, while both tick species were present at the current study site. These two species have a broadly parapatric distribution (Bull and Burzacott, 2001) and the current study site was located in their narrow overlap zone. Although the ecological processes that generate this sharp distributional boundary are not known, some form of negative interspecific interaction between the two tick species must occur (Bull, 1991; Bull and Possingham, 1995). Possible mechanisms include reproductive interference (Andrews et al., 1982; Chilton et al., 1992), and density dependent competition during feeding (Tyre et al., 2003). Whatever the mechanism, it is possible that, for *A. limbatum*, the effects of interspecific interactions on host infection patterns might override those generated by the transmission networks. Our data showing significant positive associations between infection intensities of each species might contradict this interpretation, but a second conclusion we can derive from the current study is that models of the transmission dynamics of parasites among host individuals will not necessarily be consistent for an individual parasite species in different circumstances.

The study tested two network models. Like other investigations of the transmission of parasites and pathogens around wildlife populations, our interpretation relies on correlational patterns, higher parasite loads for hosts with stronger network connections. In most fields of ecology, correlations and associations are the first evidence required to support a model of the ecological process. Significant correlations provide an indication that a particular model process is one possible explanation of the pattern observed. Further studies are then required to explore alternative explanations. A lack of correlation does not necessarily eliminate the model, if other processes mask the effects of the ecological mechanism that is proposed. For instance, in this case, tick loads might be higher in one part of the study area than others, perhaps because, in a heterogeneous habitat, tick survival while waiting for a host is higher in

some microhabitats than others. In that case, a positive relationship between tick load and node in-strength might be masked by high in-strength values for some nodes in local areas where tick densities are low.

Our understanding of the ecological processes that generate the dynamics of host-parasite interactions lags behind our understanding of many other ecological processes. Perhaps this is because of the difficulty of directly observing the process of parasite transmission, and our reliance on patterns of host infection to infer transmission. Nevertheless, the theoretical models of parasite-host dynamics, including those involving social networks, now demand empirical data on actual transmissions to test their rigor.

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