Parasitol Res (2015) 114:2069–2078
DOI 10.1007/s00436-015-4391-5

ORIGINAL PAPER

Seasonal variation in infestations by ixodids on Siberian chipmunks: effects of host age, sex, and birth season

Christie Le Coeur · Alexandre Robert · Benoît Pisanu · Jean-Louis Chapuis

Received: 5 December 2014 / Accepted: 17 February 2015 / Published online: 1 March 2015

Abstract In mammalian hosts, macroparasite aggregation is highly heterogeneous over space and time and among individuals. While the exact causes of this heterogeneity remain unclear, it has mainly been attributed to individual differences in exposure and susceptibility. Although some extrinsic (e.g., parasite availability) and intrinsic (e.g., sex or age) factors are well known to affect infestation patterns, the joint and possibly interacting effects of these factors are poorly understood. Here, we study the infestation of hard ticks (mainly *Ixodes ricinus*) in a small rodent, the Siberian chipmunk (*Tamias sibiricus*), introduced to an oak-hornbeam forest in France. We investigate the seasonal variation in infestation according to the sex, age, birth season (spring-born or summer-born), and body weight of individual hosts while controlling for interannual variability. During the 10-year study period, 3421 tick count events were recorded involving 1017 chipmunks monitored by the capture-mark-recapture procedure. Our results reveal a male-biased parasitism in the Siberian chipmunk, which is not consistent among individuals born in different seasons. This sex bias is observed among spring-born juveniles from July to the beginning of hibernation. For adults, this difference becomes apparent along the reproduction period (May–September) for summer-born adults only. These complex interactions between sex, age, and birth season suggest overall that the seasonal variation of tick load is critically linked to the reproductive behavior of this small ground sciurid.

Keywords Birth season · Host-intrinsic factors · *Ixodes ricinus* · Rodent · Sex-biased parasitism · *Tamias sibiricus*

Introduction

Understanding the ecological processes of how arthropod vectors are temporally and spatially distributed in the environment and on their host species is fundamental to anticipating and managing vector-borne diseases (Keesing et al. 2010). Many macroparasites are highly aggregated on population hosts, with a few individuals hosting the majority of parasites (Anderson and May 1978; Shaw et al. 1998). According to the 80-20 rule (Woolhouse et al. 1997), only a small proportion of the host population (20 %) is responsible for the majority of parasite transmission (80 %). While the exact causes of aggregation remain unclear, heterogeneity in infestations has been mainly attributed to differences between individuals in exposure and susceptibility to parasites (Wilson et al. 2002). Indeed, these effects are difficult to disentangle, as most obvious demographic sources of heterogeneity in a host population (e.g., sex, age, reproductive status, or population density) are expected to affect both exposure and susceptibility to parasites through behavioral and physiological effects. Moreover, individual heterogeneity in infection can induce heterogeneous individual fitness costs to the host (Burns et al. 2005; Careau et al. 2010) and parasites (Krasnov et al. 2005; Tschirren et al. 2007) themselves and can have profound effects on both host and parasite population dynamics (e.g., Krasnov et al. 2012; Miller et al. 2007; Poulin et al. 2006).

In hard ticks (Ixodidae), aggregation is a complex function of temporal, spatial, and host-intrinsic factors, which may interact with each other (Brunner and Ostfeld 2008). Seasonal variation in host infestation is intimately linked to the

Electronic supplementary material The online version of this article (doi:10.1007/s00436-015-4391-5) contains supplementary material, which is available to authorized users.

C. Le Coeur (✉) · A. Robert · B. Pisanu · J.-L. Chapuis
Centre d'Ecologie et des Sciences de la Conservation (CESCO UMR 7204), Sorbonne Universités, MNHN, CNRS, UPMC, CP51, 57 rue Cuvier, 75005 Paris, France
e-mail: christie.lecoeur@mnhn.fr

availability of questing larvae and nymphs on the vegetation, which depends upon microclimatic and day-length conditions influencing tick behavior and mortality (e.g., Randolph and Storey 1999; Randolph et al. 2002; Randolph 2004). The spatial distribution of questing stages and host density also influence contact rates and hence infestations (Calabrese et al. 2011; Kiffner et al. 2011).

Within most mammalian hosts, males tend to be more heavily infested by hard ticks than females; this may be explained by sex differences in size (Harrison et al. 2010), behavior (Harrison et al. 2010; Boyer et al. 2010), and/or in physiology linked to the immunocompetence handicap hypothesis (Hughes and Randolph 2001). These explanatory processes and factors are often difficult to disentangle, as body mass, ranging patterns, and steroid hormone levels tend to be correlated with each other in mammals, especially in non-monogamous species (Moore and Wilson 2002). Tick burden often tends to increase with host age to reach a threshold or decline gradually in the oldest individuals according to age-dependent exposure and/or immunocompetence (Wilson et al. 2002). Another factor that may contribute to the observed heterogeneities in tick burdens within host populations is body mass (e.g., Dallas et al. 2012; Neuhaus 2003).

Although body mass, sex, and age differences in parasite infections have been commonly reported in mammals (Moore and Wilson 2002), no universal rule has been found with respect to the presence and signs of these effects. Kiffner et al. (2013), for example, studied flea infestation among nine rodent species and found that sex and body mass patterns are not consistent among species even though they are common determinants of parasite aggregation.

In this study, we investigated the spatial, temporal, and host-intrinsic factors related to variation in hard tick infestations at a monthly scale over 10 years in a population of Siberian chipmunks introduced to the suburban French forest of Sénart (Vourc'h et al. 2007; Marmet 2008; Chapuis et al. 2011). We took advantage of several potential factors explaining heterogeneity in tick infestation in our model host population. First, individuals do not breed in their year of birth, becoming sexually mature only in their second year of life (i.e., after first hibernation, Chapuis et al. 2011). This situation involves two distinct periods in an individual's life (before and after its first winter) with distinct physiological states and sexual behaviors among these periods. Second, although no sexual size dimorphism occurs, males move more than females (Marmet et al. 2012), increasing their exposure to questing stages of hard ticks in the environment (Boyer et al. 2010). Third, individuals can reproduce twice a year, involving two birth cohorts—one emerging in spring (May–June) and the second one in summer (August–October)—which in turn involves potential intra-sexual heterogeneity in resource acquisition, development, and seasonal exposure to ticks between these two cohorts.

With an emphasis on finely characterizing intra-annual seasonal patterns of infestations while controlling for interannual variability, the temporal variation in tick burdens on chipmunks was analyzed as a function of the interactive effect of sex, birth season (host-intrinsic factors), and month, as well as an additive effect of habitat (e.g., Pisanu et al. 2010), separately for three age classes (juveniles, yearlings, and older individuals). After characterizing the dynamics of infestation for this set of observations, we investigated the interactive effects of host body weight, birth season, and month with an additive effect of habitat on the variation of tick infestation for each sex and age class separately.

Material and methods

Species and study site

The Siberian chipmunk (*Tamias sibiricus* [Laxmann, 1769]) is a short-lived (average life expectancy about 1.5–2 years; Chapuis et al. 2011; pers. com.), ground-dwelling sciurid native to northern and southeastern parts of Eurasia (Obolenskaya et al. 2009) that has been introduced to Europe to be sold as a pet since the 1960s (Chapuis et al. 2011). Eleven settled populations of Siberian chipmunks have been recorded in France since the 1970s (Chapuis 2005; Marmet 2008), nearly half of them originating from the southern region of the Korean peninsula (*Tamias sibiricus barberi*; Pisanu et al. 2013). In France, chipmunks hibernate from October to March and can reproduce twice a year (Chapuis et al. 2011). The mating season of this promiscuous species extends from late February until the first half of July with two peaks of births, one in spring (i.e., late March to late April with juveniles emerging from their birth burrow from late April to June) and the second in summer (i.e., late June to late July with emergence from late August to October; Marmet et al. 2012). Regardless of birth season, individuals become sexually mature in their second year (after their first hibernation) with no sexual size dimorphism (Chapuis et al. 2011; Table 1).

The study was conducted in the site of 'La Faisanderie' (14 ha; 48° 39′ N, 02° 29′ E), an area located in the suburban forest of Sénart (3200 ha), 22 km southeast from Paris, France (Marmet et al. 2009; Marmet et al. 2011). Three distinct habitats differing in plant cover were characterized: a 6-ha semi-open oak grove (hereafter, semi-open habitat), a 6.5-ha closed oak-hornbeam grove (closed habitat), and a mix of managed grassland and permanently inhabited buildings representing an additional 1.5 ha (wall-building habitat; Marmet et al. 2009). From 2004 to 2013, the population was monitored using a capture-mark-recapture procedure. Throughout the study site, chipmunks were live-trapped in baited Sherman traps during their activity period from March to October. A grid made of 80 geo-localized traps was set in 2004 and was

Table 1 Mean seasonal host body mass and number of ixodid immature stages feeding on Siberian chipmunks according to chipmunk age ('juvenile,' 'yearlings,' 'older adults,' and 'all adults' datasets), birth season (spring-born or summer-born), and sex, in the forest of Sénart over 10 years (2004–2013). Seasons were chosen according to the intra-annual population dynamics of the questing ticks *Ixodes ricinus* in the study site (Pisanu et al. 2010)

Chipmunk age/birth season	Sex	March–April				May–July				August–October			
		N_{hosts}	N_{counts}	Mean±SE		N_{hosts}	N_{counts}	Mean±SE		N_{hosts}	N_{counts}	Mean±SE	
				Mass (g)	Ticks			Mass (g)	Ticks			Mass (g)	Ticks
Juvenile													
Spring-born	♀	4	4	69±4	1±1	248	466	76±1	25±2	141	258	94±1	23±2
	♂	5	5	67±3	1±1	255	426	75±1	30±2	105	207	93±1	31±2
Summer-born	♀	–	–		–	–	–		–	201	277	71±1	8±1
	♂	–	–		–	–	–		–	227	286	68±1	9±1
Yearling													
Spring-born	♀	59	80	95±1	8±1	71	193	101±1	51±4	35	63	105±1	26±3
	♂	51	72	96±1	10±1	58	149	99±1	60±5	20	30	101±1	23±3
Summer-born	♀	55	89	93±1	6±1	64	157	97±1	38±3	37	61	101±1	20±2
	♂	31	60	93±1	6±1	37	87	96±1	66±7	15	19	97±1	24±7
Older adult													
Spring-born	♀	24	49	105±1	9±1	29	106	105±1	55±5	14	29	107±2	33±6
	♂	13	16	103±2	4±1	14	32	103±1	78±11	1	2	110±1	5±0
Summer-born	♀	24	38	98±2	8±2	25	90	105±1	43±5	13	32	105±1	21±3
	♂	8	11	98±2	18±7	11	22	98±1	115±32	4	5	99±2	48±20
All adults (total)		218	415		8±1	240	836		54±2	120	241		25±2

The number of different hosts (N_{host}) and the number of tick-counted individuals (N_{count}) are detailed as each chipmunk can be tick-counted several times during its life time

surrounded by 24 extra traps during the subsequent years. Two monthly capture sessions were performed, each consisting of three and two consecutive trapping days at 15-day intervals in 2004 and three and five consecutive days between 2005 and 2007. From 2008 on, only a 5-day-long trapping session was carried out each month.

Captured individuals were ear-tagged and implanted with a subcutaneous transponder chip (Marmet et al. 2009). At each trapping event, the date, trap location, and habitat types were recorded, as well as chipmunk identity, birth season (spring-born or summer-born individuals), age (adult or juvenile, determined by the crossed-criteria of capture date and weight [i.e., Marmet et al. 2009] and head morphology), sex, and body mass. After being handled, the chipmunks were released at their point of capture.

In our study site, the Siberian chipmunk feed four species of hard ticks, of which *Ixodes ricinus* represents the majority of immature stages (>90 % of larvae and nymphs; Vourc'h et al. 2007). Adult ticks are mostly hosted by roe deer (*Capreolus capreolus*) for which varying annual densities were recorded (ranging from 0.3 to 1.0 ind/ha; pers. com) on our wire-fenced study site. Because very few adult stages of ticks were found on chipmunks (all identified as *Ixodes acuminatus*; 2005–2008: Pisanu et al. 2010; 2009–2013, pers.com), only immature stages were considered in this study. At the first capture of a chipmunk within a trapping session between 2005 and 2013, we counted out ticks by direct observation of attached larvae and nymphs to the head of the host, using eye lenses (×3 magnification; Pisanu et al. 2010). The differentiation between nymph and larvae when performing counts was primarily based on the difference in size between these two stages, allowing to determine a proxy of their burden. Ticks were not counted when chipmunks were recaptured several times within a trapping session. Larvae and nymph counts were summed up in 2004 at the initial phase of the monitoring of the chipmunk population. The mean number of tick count events per marked chipmunk was 3.4 (range 1–34, n=1017 chipmunks). Throughout the 10-year study period (2004–2013), tick counting was conducted by the same observer (J.-L.C.), at each capture session, helped by an additional one (J. Marmet) from 2005 to 2007. Comparisons in the number of ticks counted per chipmunk obtained by the two observers were regularly assessed on the field and were of the same magnitude. Two consecutive tick counts during two consecutive trapping sessions were therefore

separated by at least 15 days and were considered independent of each other (see Boyer et al. 2010).

The infestation ratio of larvae and nymphs was about c.10:1 regardless of chipmunk age (Pisanu et al. 2010), and we only considered the total number of ticks (larvae and nymphs) per individual in the main results (see also Online Resource 1 for a complementary analysis performed on larvae and nymphs separately). We analyzed tick burdens (i.e., sum of larvae and nymphs) of juvenile and adult chipmunks separately and distinguished yearlings as individuals in the year following the first hibernation period (hereafter, referred to as "yearlings") from older adults (referred to as "older adults").

Statistical analysis

During the 10-year study period, 4578 tick counts events were recorded involving 1298 marked animals. In our analysis, we only considered individuals with known age, sex, birth season, and weight, and the remaining dataset consisted of 3421 tick count events for 1017 marked chipmunks (see details in Table 1).

Tick burdens (log-transformed) were analyzed using a linear mixed-effects model (Pinheiro and Bates 2000) for the datasets of (i) juveniles, (ii) yearlings, (iii) older adults, and (iv) all adults (yearlings and older adults). Because tick load is expected to vary consistently within each year according to the tick's phenology (generating intra-annual temporal autocorrelation), we modeled intra-annual dynamics by considering month as a quantitative variable (and tested its quadratic component). An alternative modeling considering month as a factorial variable was performed, which yielded similar qualitative results (Online Resource 2). Because tick availability may vary across years (Randolph et al. 2002), especially with varying roe deer densities on our study site (e.g., Rosà et al. 2007), we considered the year in all subsequent analyses. Additionally, individual tick burdens were found to be repeatable over capture sessions (Boyer et al. 2010). Consequently, the random effects of chipmunk identity (ID), year, and the random slope of month (nested in year) were fitted to account for multiple records per individual and per month within and between years, allowing a control for potential bias in parameter estimates due to autocorrelation in residual errors (e.g., Zuur et al. 2010). The random effect of year also allowed controlling for yearly variation due to different observers (the same two observers from 2005 to 2007 and only one of them for 2004 and 2008–2013).

Two distinct analyses of tick burdens were conducted. First, we modeled tick burdens as a function of birth season, sex, and month (quadratic) and their interaction with an additional effect of habitat (hereafter referred to as the "main analysis"). This design was run separately for each age class.

As a second step, due to sex-specific differences in the seasonal variation of body weight (due to pregnancy for females), we separately tested for the two sexes the effect of body weight, birth season, and month (quadratic) in interaction, again including an additive effect of the habitat (hereafter referred to as "weight analysis"). This design was run separately for the adult class only (i.e., 'yearlings,' 'older adults,' and 'all adults' datasets separately) because strong cohort effects occurred at the juvenile stage (Table 1). To check potential biases of between-cohort tick load differences, a complementary analysis of juvenile tick loads was completed from August to October during which both cohorts overlapped (Online Resource 3).

To generate the 'best' predictive model, Akaike's information criterion (AIC)-based model selection was performed ('dredge' function, MuMIn package, Bartoń 2013), and the model with the lowest AIC value was retained for inferences (Burnham and Anderson 2002). At the starting and final points of the selection, model-checking plots were conducted for normality distribution of residuals and homogeneity. For the main analysis, we departed from a general model including the three-way interaction between birth season, sex, and month (quadratic) with an additional effect of habitat. For the weight analysis, the starting model included a three-way interaction between body weight, birth season, and month (quadratic) with an additive effect of habitat.

All models were performed using R version 3.0.3 (R Core Team 2014) with the packages lme4 v.1.1–5 ('lmer' function; Bates et al. 2014), and graphics were generated using the ggplot2 package v.1.0.0 (Wickham 2009).

Results

A total of 996 juveniles, 282 yearlings, and 93 older adults, involving 1929, 1060, and 432 tick count events, respectively, were taken into account in the 10-year analysis (see detailed Table 1). The mean number of ticks on chipmunks varied with season, host age, and sex (Table 1). At the adult age ('all adults' dataset), the seasonal fluctuation of chipmunk tick burdens ranged from 8±1 in spring (March–April) and 25±2 in late summer (August–October) to 54±2 in early summer (May–July; see details in Table 1). For juveniles, tick burdens were much lower than adults but also varied across seasons with high infestation levels during early summer (May–July): the mean number varied from less than 1 (i.e., 0.7±0.4) in spring to 28±1 and 16±1 in early summer and late summer, respectively.

From the main analysis, the model that included a significant interaction between birth season, sex, and month (quadratic) with an additive effect of habitat best explained the variation in juvenile tick loads (ΔAIC=9.39; Table 2). Tick loads of juvenile chipmunks varied greatly among birth cohort, and summer-born juveniles carried lower tick loads. Among new spring-born individuals, ixodid infestations

Table 2 Model selection results of tick burden variation according to age ('juvenile,' 'yearlings,' 'older adults,' and 'all adults' datasets), sex, birth season (birth seas), habitat (hab), month as a quadratic component (month^2), and weight from the "main" and the "weight" analyses

Sources of variations	Model	np	AICc	ΔAICc
Main analysis				
Juveniles	***birth seas × month^2 × sex + hab***	***19***	***5300***	
	birth seas : month^2+sex: month^2+hab	16	5309.4	9.39
Yearlings	***birth seas × month^2 × sex+hab***	***19***	***2885.2***	
	birth seas : month^2+sex : month^2+hab	16	2892.4	7.15
Older adults	**birth seas × month^2 × sex**	**17**	**1139.5**	
	birth seas × month^2 × sex + hab	*19*	*1142.8*	*3.37*
All adults	***birth seas × month^2 × sex + hab***	***19***	***3970.2***	
	birth seas: month^2+birth seas : sex+month^2 : sex	17	3976.3	6.16
Female weight				
Yearlings	**birth seas : month^2+weight : month^2+hab**	**16**	**1778.8**	
	weight : month^2+hab+birth seas	14	1783.9	5.12
	birth seas × month^2 × weight + hab	19	1790.3	11.46
Older adults	**birth seas × month^2**	**11**	**904.8**	
	month^2	**8**	**905.9**	**1.04**
	birth seas+month^2	**9**	**906.8**	**1.99**
	birth seas × month^2 × weight+ hab	19	931.0	26.22
All adults	**birth seas : month^2+weight : month^2+hab**	**16**	**2634.8**	
	weight × month^2+hab+birth seas	14	2639.5	4.69
	birth seas × month^2 × weight+ hab	19	2646.1	11.30
Male weight				
Yearlings	**birth seas × month^2+hab**	**13**	**1126.2**	
	month^2+hab	**10**	**1127.7**	**1.54**
	birth seas × month^2 × weight+ hab	19	1150.9	24.75
Older adults	**birth seas × month^2+hab**	**11**	**250.4**	
	month^2+hab	**12**	**251.8**	**1.34**
	birth seas × month^2 × weight+ hab	19	270.8	20.37
All adults	**month^2+hab**	**10**	**1360.3**	
	Birth seas × month^2+hab	**13**	**1361.1**	**0.078**
	birth seas × month^2 × weight+ hab	19	1383.2	22.93

All models referred to linear mixed-effects models with chipmunk identity (ID), year, and the random slope of month (nested in year) as random effects. Model selection is based on second-order Akaike's information criterion (AICc): number of parameters (np), AICc values, and AICc differences between the best model and the next candidate model or starting model (ΔAICc) were reported. Best models occur in bold (ΔAICc<2), and starting models are in italic. Only the models close to the best models were detailed in this table

increased from March to June, remained relatively stable for males or still increased for females during summer and slightly decreased in October, for both sexes (Fig. 1). In contrast, throughout the active period of summer-born individuals (i.e., from August to October), male and female shapes displayed similar temporal patterns and were not quantitatively different (Fig. 1; Table 1). Additionally, juvenile chipmunks trapped in the wall-building habitat had significantly lower average tick loads (N_{hosts}=162, N_{counts}=233, mean=7±1) than juveniles trapped in open or closed forested habitats (N_{hosts}= *399, N_{counts}= 752, mean= 23±1; N_{hosts}= 944, N_{counts}= 528, mean= 24±1; respectively).*

Adults displayed heavier tick burdens than juveniles although adult burden leveled off between yearlings and older adults (Fig. 2). The best models assumed a quadratic effect of month on the tick load of adults ('yearlings,' 'older adults,' and 'all adults' analyses respectively; Table 2). A significant interaction between birth season and sex was revealed for all adults, including both yearlings and older individuals (Fig. 3); there was no significant difference between sexes for adults born in spring, whereas for summer-born adults, males were more heavily infested than females and peaked during May to September. The same pattern was found separately in yearlings (N_{host}=282, N_{counts}=1060) and older adults (N_{host}=93, N_{counts}=432); however, sex differences in older summer-born

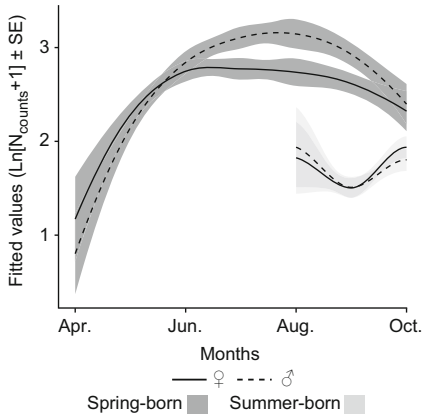

Fig. 1 Monthly infestations by ixodid ticks (fitted values of logged tick burden+1 derived from the best model, see Table 2) according to the sex and birth season of juvenile Siberian chipmunks. Month is a quadratic component

adults were marked over 5 months of each year, from April to September (supplementary figures in Online Resource 4). As observed for juveniles, we found habitat to affect the variation of tick burdens with overall adult chipmunks trapped in the building habitat harboring on average 15±3 ticks (N_{host}=54, N_{counts}=118), those in the closed habitat 40±2 ticks (N_{host}=177, N_{counts}=862), and those in the open habitat 35±2 ticks (N_{host}=129, N_{counts}=512). Most of the effects on the tick load of Siberian chipmunks were confirmed by the alternative analysis considering month as a factorial variable except the three-way interaction between birth season, month, and sex (see details in Online Resource 2).

The complementary analysis investigating the monthly variation in larvae and nymph burdens separately (2005–2013 dataset) according to birth season and sex of juvenile and adult chipmunks (yearlings, older adults, and total adults datasets) yielded the same result than the main analysis (total tick burden; see Online Resource 1). The important part of larvae numbers on the total tick burden pattern (nymphs and larvae added up) was emphasized.

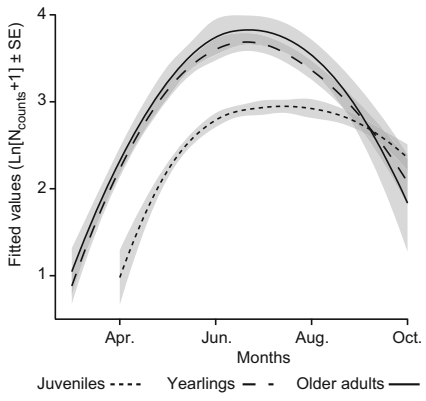

Fig. 2 Monthly variation of tick infestations according to the age of the chipmunk hosts. The fitted values (logged tick burden+1) representing the spring-born individuals only, derived from the best model of each age class

According to the second analysis on adult weights, a significant interaction between month and body weight was found to explain tick burdens of yearling and adult (all) females (Table 2). During the summer period (May to June), heavier females appeared to be less infested by ixodids than lighter females (Online Resource 5). In contrast, male tick burdens were not related to weight, and similar patterns of tick burdens between groups were found for the three adult datasets (ΔAIC >>2 between best model and models including weight effect for 'yearlings,' 'older adults,' and 'all adults' datasets; Table 2).

Discussion

Male-biased parasitism is commonly observed among mammal species and has been linked to a range of explanations, including sex differences in morphology (e.g., body mass), behavior (e.g., spatial behavior), and physiology (involving immunosuppressive hormones, such as testosterone; Folstad and Karter 1992) or has been associated with fundamental differences in male and female life histories (Rolff 2002; Zuk and Stoehr 2002). In the present study, we uncovered a male-biased tick load in the Siberian chipmunk. However, this sex difference becomes apparent only between May and September of each year in summer-born adult individuals and from July to the beginning of hibernation at the juvenile stage. These complex interactions between sex, age, season, and birth season suggest overall that tick load variation is critically linked to the reproductive behavior of this small ground sciurid.

Reproductive activity and variation in tick load

In our study population, we underlined differences in tick infestations between juveniles and adults. At the juvenile stage, seasonality in tick load is much less marked than for adults. A male-biased infestation only occurs in spring-born juveniles starting from July to the beginning of hibernation, and it disappears at the adult stage. The dispersal distances of juveniles (i.e., non-reproductive individuals) were found to be farther in males than in females (Marmet et al. 2011), which could involve higher exposure to parasites due to behavioral differences. Summer-born juveniles were less infested because of lower availability in the questing stages of ticks after emerging from their birth-nest (Pisanu et al. 2010).

At the adult stage (i.e., reproductive individuals), male Siberian chipmunks were found to be more heavily infested than females during the summer period, when tick burdens peak on adult hosts. These findings are in agreement with previous findings on Siberian chipmunks and other mammal species suggesting that sex differences in parasite loads are related to different uses of space by males and females in relation

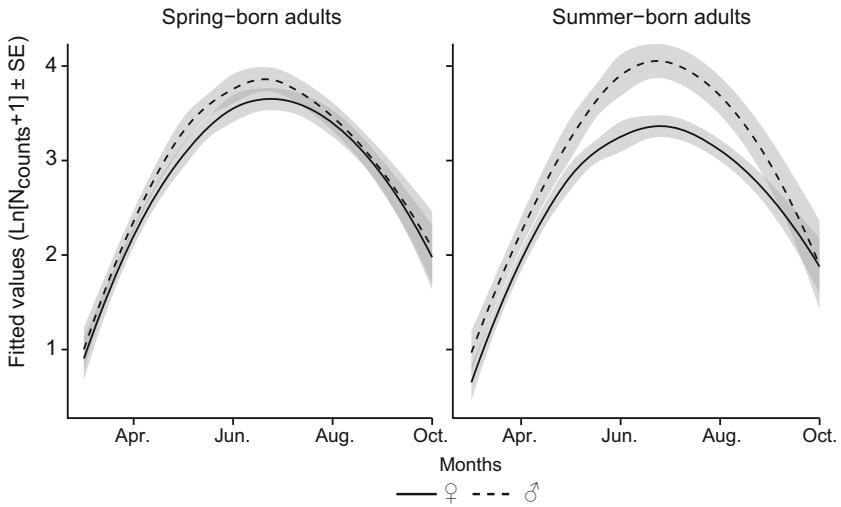

Fig. 3 Monthly variation of tick load according to the birth season and sex of adult chipmunks. Best model fitted values (logged tick burden+1) were shown for both sexes of spring-born (*shown at left*) and summer-born (*shown at right*) individuals as adults, including yearlings and older adults

with reproduction. In particular, in promiscuous or polygynous mammals, males are more mobile and have larger home ranges than females (Greenwood 1980; Frank and Heske 1992; Shier and Randall 2004). The higher mobility and larger home ranges of males allow them to increase their mating chances but with the side effect of increasing their exposure to parasites through higher parasite encounter probabilities, close proximity, and higher contact rates among individuals (Altizer et al. 2003; Bordes et al. 2009). In the promiscuous Siberian chipmunk, the tick load was found to increase with space use (Boyer et al. 2010), which is known to be higher for males than for females (Marmet et al. 2009). The home-range size of males was found to be positively associated with their reproductive success (Marmet et al. 2012), confirming that behavior and reproduction are closely linked.

Moreover, in adults, we found that the male-biased tick burden becomes apparent only in the reproductive season. Variation of gender-biased parasitism across time periods has been already reported among mammals. For example, Krasnov et al. (2005) found that gender differences in the pattern of flea parasitism of desert rodent hosts were manifested mainly during the reproductive period.

Overall, our results suggest that adult male-bias parasitism is related to the reproductive activity of individuals and, in particular, their movement rate during the reproductive period. However, several complementary hypotheses can be developed, such as immunocompetence ('immuno-handicap hypothesis') and/or life histories hypotheses. Because morphometric dimorphism was not observed between male and female chipmunks on our study site, the sexual size dimorphism hypothesis is excluded.

It should be noticed that these results held true when analyzing variation nymph and larvae burdens separately (Online Resource 1). This is not surprising because chipmunks pick up the different questing stages equivalently in the environment, despite the different spatial distribution between questing larvae and nymphs on the vegetation (Pisanu et al. 2010).

Birth season and tick load

Surprisingly, we found that the sex difference in tick burden is not consistent among adult individuals born in different seasons. Tick burdens of spring-born chipmunks did not differ between males and females, whereas significant male-biased burden occurred for summer-born adults.

Assuming that the observed male-biased tick load is related to differences in reproductive behavior and, in particular, to differences in space use, the fact that this effect is only apparent in summer-born individuals may be explained by two main processes. First, the observed pattern may be caused by a heritable birth season. If individuals born in summer tend to systematically reproduce more in summer (i.e., when questing tick availability and the number of ticks feeding on chipmunks are the highest), this category of individuals will exhibit more sex difference in tick load mediated by the reproductive behavior.

Second, if summer-born individuals tend to reproduce with a higher probability than spring-born individuals, then any sex differences mediated by the reproductive behavior should be more marked in summer-born individuals. As previously mentioned, female chipmunks had a much lower space use than males during their activity period (Marmet et al. 2009), and the sex difference is expected to increase during the reproduction periods because female burrowing activity is intensive (Kawamichi and Kawamichi 1993). Thus, if reproductive effort differs, the tick exposure of summer-born females is supposed to be lower than spring-born females as a consequence of more intensive nest activity.

Otherwise, based on the life history theory (Roff 1992; Stearns 1992), the opposite pattern can be expected. The

theory predicts a trade-off between reproductive effort and several traits such as immunity due to the high-energy costs of female reproduction in mammals (i.e., for pregnancy and lactation; Degen 2006; Dlugosz et al. 2014). By investing more in immune defenses, lower tick burden and lower reproductive effort of summer-born females should be observed. Ectoparasites such as ticks can negatively affect the reproductive success of their mammal hosts (Moller 1993; Fitze et al. 2004) including several examples in sciurid hosts (Neuhaus 2003; Patterson et al. 2013 but see Gooderham and Schulte-Hostedde 2011). Indeed, it is well supported that ticks induce immune responses in their mammal hosts which might acquire immunologically based resistance (Wikel and Whelen 1986; Hughes and Randolph 2001; Kovár 2004). Such resistance to tick infestations manifests in reduced numbers and blood meal weights or the prolonged engorgement duration of engorging ticks, decreased viability or production of ova in adults, and tick death on the host (Brossard and Wikel 2004). Thus, by investing more in immunity, a decreasing number of ticks feeding on summer-born females is expected even at the cost of lower reproductive effort.

Additionally, results from the weight analyses revealed that lighter females are more infested by ixodids than heavier females during the reproductive period. Assuming that lighter females are nonreproductive individuals (pregnancy is associated with weight gain in mammals), these results could support the reproductive activity and behavior hypothesis.

Conclusion

The current study highlights the complex interacting effects of sex, age, and birth season on seasonal tick burden dynamics in an introduced Siberian chipmunk population. We hypothesized that gender-biased infestations by ticks are critically related to behavioral processes associated with reproduction. The hypotheses developed should be further investigated by performing complementary analyses on reproduction and mortality patterns of this Siberian chipmunk population. In addition to fundamental knowledge gathered to better understand the determinants of rodent-hard tick relationships, such work will also contribute to a better understanding of the transmission dynamics of Lyme borreliosis on the forest of Sénart, where introduced Siberian chipmunks can play an important role (Pisanu et al. 2010; Marsot et al. 2011; Marsot et al. 2013).

Acknowledgments We thank the National Forests Office (Office National des Forêts, France) for the financial support and for allowing fieldwork in the site 'La Faisanderie' (Forest of Sénart, France). This work was also funded by the Conseil Régional d'Ile-de-France, the Conseil Général des Hauts-de-Seine and the Ministère de l'Ecologie, du Développement durable et de l'Energie (MEDDE). We are thankful to J. Marmet for her contribution in the mark-recapture monitoring and tick counts from 2005 to 2007.

Compliance with ethical standards All procedures performed in studies involving animals were in accordance with the ethical standards of the institutional ethics committee at the MNHN (#6810 Comité Cuvier) at which the studies were conducted.

References

Altizer S, Nunn CL, Thrall PH et al (2003) Social organization and parasite risk in mammals: integrating theory and empirical studies. Annu Rev Ecol Evol Syst 34:517–547. doi:10.1146/annurev.ecolsys.34.030102.151725

Anderson RM, May RM (1978) Regulation and stability of host-parasite population interactions: I. Regulatory processes. J Anim Ecol 47: 219–247

Bartoń KA (2013) MuMIn—R package for model selection and multimodel inference

Bates D, Maechler M, Bolker B et al (2014) Package "lme4."

Bordes F, Morand S, Kelt DA, Van Vuren DH (2009) Home range and parasite diversity in mammals. Am Nat 173:467–474. doi:10.1086/597227

Boyer N, Réale D, Marmet J et al (2010) Personality, space use and tick load in an introduced population of Siberian chipmunks *Tamias sibiricus*. J Anim Ecol 79:538–547. doi:10.1111/j.1365-2656.2010.01659.x

Brossard M, Wikel SK (2004) Tick immunobiology. Parasitology 129: S161–S176. doi:10.1017/S0031182004004834

Brunner JL, Ostfeld RS (2008) Multiple causes of variable tick burdens on small-mammal hosts. Ecology 89:2259–2272

Burnham K, Anderson D (2002) Model selection and multi-model inference: a practical information-theoretic approach, 2nd edition. 1–515

Burns C, Goodwin B, Ostfeld R (2005) A prescription for longer life? Bot fly parasitism of the white-footed mouse. Ecology 86:753–761

Calabrese JM, Brunner JL, Ostfeld RS (2011) Partitioning the aggregation of parasites on hosts into intrinsic and extrinsic components via an extended Poisson-gamma mixture model. PLoS One 6:e29215. doi:10.1371/journal.pone.0029215

Careau V, Thomas D, Humphries M (2010) Energetic cost of bot fly parasitism in free-ranging eastern chipmunks. Oecologia 162:303–312. doi:10.1007/s00442-009-1466-y

Chapuis J-L (2005) Répartition en France d'un animal de compagnie naturalisé, le Tamia de Sibérie (*Tamias sibiricus*). Rev Ecol (Terre Vie) 60:239–254

Chapuis J-L, Obolenskaya EV, Pisanu B, Lissovsky AA (2011) Datasheet on *Tamias sibiricus*. CABI, Wellingford, UK (http://www.cabi.org/isc/)

Dallas TA, Foré SA, Kim H-J (2012) Modeling the influence of *Peromyscus leucopus* body mass, sex, and habitat on immature Dermacentor variabilis burden. J Vector Ecol 37:338–341. doi:10.1111/j.1948-7134.2012.00236.x

Degen AA (2006) Effect of macroparasites on the energy budget of small mammals. In: Springer-Japan (ed) Micromammals and macroparasites. pp 371–399

Dlugosz EM, Downs CJ, Khokhlova IS et al (2014) Ectoparasite performance when feeding on reproducing mammalian females: an unexpected decrease when on pregnant hosts. J Exp Biol 217:1058–1064. doi:10.1242/jeb.098376

Fitze PS, Tschirren B, Richner H (2004) Life history and fitness consequences of ectoparasites. J Anim Ecol 73:216–226

Folstad I, Karter AJ (1992) Parasites, bright males, and the immunocompetence handicap. Am Nat 139:603–622

Frank DH, Heske EJ (1992) Seasonal changes in space use patterns in the southern grasshopper mouse, *Onychomys torridus torridus*. J Mammal 73:292–298

Gooderham K, Schulte-Hostedde A (2011) Macroparasitism influences reproductive success in red squirrels (*Tamiasciurus hudsonicus*). Behav Ecol 22:1195–1200. doi:10.1093/beheco/arr112

Greenwood PJ (1980) Mating systems, philopatry and dispersal in birds and mammals. Anim Behav 28:1140–1162

Harrison A, Scantlebury M, Montgomery IW (2010) Body mass and sex-biased parasitism in wood mice *Apodemus sylvaticus*. Oikos 119: 1099–1104. doi:10.1111/j.1600-0706.2009.18072.x

Hughes VL, Randolph SE (2001) Testosterone depresses innate and acquired resistance to ticks in natural rodent hosts: a force for aggregated distributions of parasites. J Parasitol 87:49–54

Kawamichi T, Kawamichi M (1993) Gestation period and litter size of Siberian chipmunk *Eutamias sibiricus lineatus* in Hokkaido, northern Japan. J Mammal Soc Jpn 18:105–109

Keesing F, Belden LK, Daszak P et al (2010) Impacts of biodiversity on the emergence and transmission of infectious diseases. Nature 468: 647–652. doi:10.1038/nature09575

Kiffner C, Vor T, Hagedorn P et al (2011) Factors affecting patterns of tick parasitism on forest rodents in tick-borne encephalitis risk areas, Germany. Parasitol Res 108:323–335. doi:10.1007/s00436-010-2065-x

Kiffner C, Stanko M, Morand S et al (2013) Sex-biased parasitism is not universal: evidence from rodent-flea associations from three biomes. Oecologia 173:1009–1022. doi:10.1007/s00442-013-2664-1

Kovár L (2004) Tick saliva in anti-tick immunity and pathogen transmission. Folia Microbiol (Praha) 49:327–336

Krasnov BR, Morand S, Hawlena H et al (2005) Sex-biased parasitism, seasonality and sexual size dimorphism in desert rodents. Oecologia 146:209–217. doi:10.1007/s00442-005-0189-y

Krasnov BR, Bordes F, Khokhlova IS, Morand S (2012) Gender-biased parasitism in small mammals: patterns, mechanisms, consequences. Mammalia 76:1–13. doi:10.1515/mammalia-2011-0108

Marmet J (2008) Traits d'histoire de vie du Tamia de Sibérie *Tamias sibiricus*, espèce exotique naturalisée dans la forêt de Sénart (Essonne): démographie, biologie de la reproduction, occupation de l'espace et dispersion. Dissertation, Muséum National d'Histoire Naturelle

Marmet J, Pisanu B, Chapuis J-L (2009) Home range, range overlap, and site fidelity of introduced Siberian chipmunks in a suburban French forest. Eur J Wildl Res 55:497–504. doi:10.1007/s10344-009-0266-3

Marmet J, Pisanu B, Chapuis J-L (2011) Natal dispersal of introduced Siberian chipmunks, *Tamias sibiricus*, in a suburban forest. J Ethol 29:23–29. doi:10.1007/s10164-010-0215-3

Marmet J, Pisanu B, Chapuis J-L et al (2012) Factors affecting male and female reproductive success in a chipmunk (*Tamias sibiricus*) with a scramble competition mating system. Behav Ecol Sociobiol 66: 1449–1457. doi:10.1007/s00265-012-1399-z

Marsot M, Sigaud M, Chapuis J-L et al (2011) Introduced Siberian chipmunks (*Tamias sibiricus barberi*) harbor more-diverse *Borrelia burgdorferi* sensu lato genospecies than native bank voles (*Myodes glareolus*). Appl Environ Microbiol 77:5716–5721. doi: 10.1128/AEM. 01846-10

Marsot M, Chapuis J-L, Gasqui P et al (2013) Introduced Siberian chipmunks (*Tamias sibiricus barberi*) contribute more to Lyme borreliosis risk than native reservoir rodents. PLoS One 8:e55377. doi:10.1371/journal.pone.0055377

Miller MR, White A, Wilson K, Boots M (2007) The population dynamical implications of male-biased parasitism in different mating systems. PLoS One 2:e624. doi:10.1371/journal.pone.0000624

Moller AP (1993) Ectoparasites increase the cost of reproduction in their hosts. J Anim Ecol 62:309–322. doi:10.2307/5362

Moore SL, Wilson K (2002) Parasites as a viability cost of sexual selection in natural populations of mammals. Science 297:2015–2018. doi:10.1126/science.1074196

Neuhaus P (2003) Parasite removal and its impact on litter size and body condition in Columbian ground squirrels (*Spermophilus columbianus*). Proc Biol Sci 270:S213–S215. doi:10.1098/rsbl.2003.0073

Obolenskaya EV, Lee M-Y, Dokuchaev NE et al (2009) Diversity of Palaearctic chipmunks (Tamias, Sciuridae). Mammalia 73:281–298. doi:10.1515/MAMM.2009.047

Patterson JEH, Neuhaus P, Kutz SJ, Ruckstuhl KE (2013) Parasite removal improves reproductive success of female North American red squirrels (*Tamiasciurus hudsonicus*). PLoS One 8:e55779. doi:10.1371/journal.pone.0055779

Pinheiro JC, Bates DM (2000) Mixed-effects models in S and S-PLUS. Springer

Pisanu B, Marsot M, Marmet J et al (2010) Introduced Siberian chipmunks are more heavily infested by ixodid ticks than are native bank voles in a suburban forest in France. Int J Parasitol 40:1277–1283. doi:10.1016/j.ijpara.2010.03.012

Pisanu B, Obolenskaya EV, Baudry E et al (2013) Narrow phylogeographic origin of five introduced populations of the Siberian chipmunk *Tamias* (*Eutamias*) *sibiricus* (Laxmann, 1769) (Rodentia: Sciuridae) established in France. Biol Invasions 15:1201–1207. doi:10.1007/s10530-012-0375-x

Poulin R, Krasnov BR, Morand S (2006) Patterns of host specificity in parasites exploiting small mammals. In: Springer-Japan (ed) Micromammals and macroparasites. pp 233–256

R Core Team (2014) R: a language and environment for statistical computing

Randolph SE (2004) Tick ecology: processes and patterns behind the epidemiological risk posed by ixodid ticks as vectors. Parasitology 129:S37–S65. doi:10.1017/S0031182004004925

Randolph SE, Storey K (1999) Impact of microclimate on immature tick-rodent host interactions (Acari: Ixodidae): implications for parasite transmission. J Med Entomol 36:741–748

Randolph SE, Green RM, Hoodless AN, Peacey M (2002) An empirical quantitative framework for the seasonal population dynamics of the tick *Ixodes ricinus*. Int J Parasitol 32:979–989. doi:10.1016/S0020-7519(02)00030-9

Roff DA (1992) Evolution of life histories: theory and analysis, Springer

Rolff J (2002) Bateman's principle and immunity. Proc Biol Sci 269:867–872. doi:10.1098/rspb.2002.1959

Rosà R, Pugliese A, Ghosh M et al (2007) Temporal variation of *Ixodes ricinus* intensity on the rodent host *Apodemus flavicollis* in relation to local climate and host dynamics. Vector Borne Zoonotic Dis 7: 285–295. doi:10.1089/vbz.2006.0607

Shaw DJ, Grenfell BT, Dobson AP (1998) Patterns of macroparasite aggregation in wildlife host populations. Parasitology 117:597–610

Shier DM, Randall JA (2004) Spacing as a predictor of social organization in kangaroo rats (*Dipodomys heermanni arenae*). J Mammal 85:1002–1008

Stearns SC (1992) The evolution of life histories. Oxford University Press, Oxford

Tschirren B, Bischoff LL, Saladin V, Richner H (2007) Host condition and host immunity affect parasite fitness in a bird-ectoparasite system. Funct Ecol 21:372–378. doi:10.1111/j.1365-2435.2007.01235.x

Vourc'h G, Marmet J, Chassagne M et al (2007) *Borrelia burgdorferi* Sensu Lato in Siberian chipmunks (*Tamias sibiricus*) introduced in suburban forests in France. Vector Borne Zoonotic Dis 7:637–641. doi:10.1089/vbz.2007.0111

Wickham H (2009) ggplot2: elegant graphics for data analysis, Springer

Wikel SK, Whelen AC (1986) Ixodid-host immune interaction. Identification and characterization of relevant antigens and tick-induced host immunosuppression. Vet Parasitol 20:149–174

Wilson K, Bjørnstad ON, Dobson AP et al (2002) Heterogeneities in macroparasite infections: patterns and processes. In: Oxford: Oxford University Press (ed) Ecol. Wildl. Dis. pp 6–44

Woolhouse MEJ, Dye C, Etard J-F et al (1997) Heterogeneities in the transmission of infectious agents: implications for the design of control programs. Proc Natl Acad Sci U S A 94:338–342

Zuk M, Stoehr AM (2002) Immune defense and host life history. Am Nat 160:S9–S22. doi:10.1086/342131

Zuur AF, Ieno EN, Elphick CS (2010) A protocol for data exploration to avoid common statistical problems. Methods Ecol Evol 1:3–14. doi: 10.1111/j.2041-210X.2009.00001.x