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Which forest bird species are the main hosts of the tick, *Ixodes ricinus*, the vector of *Borrelia burgdorferi* sensu lato, during the breeding season?

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ABSTRACT

Wild birds are important hosts for vector–borne pathogens, especially those borne by ticks. However, few studies have been conducted on the role of different bird species within a community as hosts of vector–borne pathogens. This study addressed individual and species factors that could explain the burden of *Ixodes ricinus* on forest birds during the reproductive periods of both vectors and hosts. The goal was to identify which bird species contribute the most to the tick population at the community level. Birds were mist-netted on four plots in 2008 and on seven plots in 2009 in two forests (Sénart and Notre Dame, near Paris, France). The dependence of the tick load per bird upon environmental conditions (questing nymph density, year and plot) and on host species traits (species, age, sex, body size, vertical space use, level of innate and acquired immunity) was analysed. Finally, the relative contribution of each bird species to the local dynamics of ticks was estimated, while accounting for their respective abundance. Tick burden differed markedly between bird species and varied according to questing nymph density. Bird species with a high body mass, those that forage low in the vegetation, and those that had a high innate immune response and a high spleen mass were more likely to have a high tick burden. Four species (the Common Blackbird, *Turdus merula*, the European Robin, *Erithacus rubecula*, the Song Thrush, *Turdus philomelos,* and the Winter Wren, *Troglodytes troglodytes*) hosted more than 90% of the ticks in the local bird community. These species, and particularly *T. merula* which was host to a high proportion of the nymphs, are likely to contribute significantly to the circulation of pathogens for which they are competent, such as the agent of Lyme borreliosis.

1. Introduction

The increasing emergence of zoonotic vector–borne diseases over the last 15 years has drawn attention to their complex ecology (Jones et al., 2008). In particular, it has highlighted the necessity to better evaluate the rate of contact between vectors, reservoir hosts and humans (Jones et al., 2008). Wild birds are important hosts for vector–borne pathogens in North America and Eurasia, especially those borne by ticks (Ogden et al., 2008). However, the role of bird species in the local dynamics of tick-borne pathogens during their reproductive seasons has been poorly studied (but see Battaly et al., 1987; Battaly and Fish, 1993; Hamer et al., 2011; James et al., 2011).

The majority of studies on bird-borne ticks has focused on the role of migrating birds in disseminating tick-transmitted pathogens over long distances, and consequently has examined tick burdens during bird migrations (Olsen et al., 1995; Comstedt et al., 2006; Ogden et al., 2008; Dubska et al., 2009). Avian mobility and migration are epizootiologic factors that potentially increase the dissemination of vector–borne pathogens (Hubalek, 2004; Ogden et al., 2008; Brinkerhoff et al., 2011). However, in addition to disseminating ticks – and tick-borne pathogens – over long distances during migration periods, birds may also play a major role in the dynamics of local tick populations and associated pathogens during the reproductive periods of both birds and ticks. Most larvae and nymphs are found between May and June (Gray, 2002), i.e. the same time that birds are sedentary and settled in their reproductive territories.

Epidemiological research on rodents has shown that the tick burden is influenced by three main variables: the density of questing ticks on vegetation (Dorn et al., 1999; Schmidt et al.,

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1999; Randolph, 2004), and the age and sex of individual hosts (Tälleklint and Jaenson, 1997; Dorn et al., 1999). Tick burden also varies among species (Kurtenbach et al., 1995). This inter-specific variation is explained by: differences in body size (Harrison et al., 2010) – bigger host species have more ticks; differences in immunocompetency (Hughes and Randolph, 2001) – some species defend themselves against ticks better than others; and space use (Boyer et al., 2010) – the larger their home range, the more likely they are to encounter ticks.

In this study, we analysed individual and species variables which could explain the tick burden on territorial, reproductive birds and we estimated which species contribute most to the tick population. Extending the findings on rodents to birds, the following set of predictions was formulated. First, bird tick burdens would be determined largely by questing nymph density (Dorn et al., 1999; Schmidt et al., 1999). Second, tick burdens would be higher in adults than in juveniles, given that nestlings are free of ticks (Gregoire et al., 2002) and that the colonization of young hosts takes time (although this time lag is not known). Third, during the reproductive period, female passerines spend more time incubating the eggs and less time patrolling the territory than males. Therefore, active males were predicted to bear more ticks than females during the reproductive period. At the community level, species are not likely to be equally infested. A set of variables most likely modulates the probability that a species bears ticks. Our fourth prediction therefore was that larger species would be more likely to host a greater number of ticks than small species because they expose larger areas of skin suitable for tick attachment and cope better with parasitism than smaller individuals. Fifth, since questing ticks do not climb higher than 1.5 m (Meljon and Jaenson, 1997), species spending much of their active period close to the ground would be more heavily infested by ticks than others. Breeding birds are expected to be particularly exposed to ticks during two main activities: when foraging and/or when accessing their nest to care for their chicks. Therefore the effects of average foraging height and of average nest height on tick burden were tested separately: the use of lower strata was predicted to be associated with a higher tick burden. Moreover, hosts defend themselves against tick infestation by launching immune responses when a tick tries to feed. The innate immunity, and particularly the local inflammatory response, probably helps to deter ticks (Prosdocimi et al., 2010). Acquired immunity is also known to contribute to protection against tick infestation (Trager, 1939; Akhtar et al., 2011). The sixth prediction therefore was that, since allocation of resources to immune defences varies among species (Møller and Erritzoe, 2002), the more a species allocates to innate and/or acquired immunity, the lower would be its tick burden. The size of the skin swelling elicited by the injection of mitogen phytohemagglutinin (referred to hereafter as the PHA response) was interpreted as an indicator of the strength of the inflammatory response (Martin et al., 2006), the first major component of the innate immune response. Furthermore, the mass of the spleen was used as an index of the investment in the acquired immune response at the species level (Møller et al., 2000; Møller and Erritzoe, 2002). The tick burden was predicted to be negatively related to PHA values and to spleen mass.

Lyme borreliosis is the most prevalent vector–borne disease affecting humans in temperate zones of the northern hemisphere (Smith et al., 2006; Bacon et al., 2008; Rizzoli et al., 2011). In Europe, it is transmitted by *Ixodes ricinus*. This tick species is a generalist parasite which goes through three active developmental stages (larval, nymph and adult). During each stage, with the exception of adult males, the tick takes one blood meal on a vertebrate host either to moult (larvae, nymphs) or to reproduce (Gern, 2008). Lyme borreliosis is caused by pathogenic bacteria belonging to the genospecies complex of *Borrelia burgdorferi* sensu lato (sl), mostly *Borrelia burgdorferi* sensu stricto (ss), *Borrelia afzelii*, *Borrelia bavariensis*, *Borrelia garinii*, *Borrelia spielmanii* and *Borrelia valaisiana* (van Dam et al., 1993; Stanek and Reiter, 2011). Reservoir hosts which maintain *B. burgdorferi* sl are normally infected by nymphs and transmit it to uninfected larvae (Gern and Humair, 2002). The role of rodents as reservoir hosts is crucial for some *Borrelia* spp. such as *B. afzelii*, *B. bavariensis* (in Europe) and *B. burgdorferi* ss (in the USA and to a lesser extent in Europe) (Mather et al., 1989; Humair et al., 1995; Peavey et al., 1997; Humair and Gern, 1998; Hanincovà et al., 2003a), but birds are also important in maintaining certain genospecies such as *B. garinii* and *B. valaisiana* in Europe (Humair et al., 1998; Kurtenbach et al., 1998; Hanincovà et al., 2003b) and *B. burgdorferi* ss in the USA (Brinkerhoff et al., 2011; Hamer et al., 2011).

Understanding the contribution of alternative reservoirs to rodents such as birds in an enzootic area for *B. burgdorferi* sl is a prerequisite for advancing prevention strategies for Lyme borreliosis (Tsao et al., 2004; Hamer et al., 2011). This is particularly true in areas where host habitats overlap with those of humans. Because infected nymphs are the source of infection to reservoir hosts, we also tested whether the proportion of nymphs differed between species. Species bearing more nymphs than average would contribute the most to the dynamics of *B. burgdorferi* sl. Then, the relative contribution of each bird species to the local dynamics of tick populations was evaluated using an "index of importance" for each bird species, corresponding to the product of the estimated mean tick burden and the relative abundance of the bird species (from James et al., 2011, but we corrected for between-species variation in capturability). For a given bird species in a community, a high tick burden or a high abundance of the host were assumed to be of equal importance in determining the contribution of the species to the local tick dynamics. This index of importance was also stratified to reflect the stage distribution of ticks (nymphs versus larvae) on each host species. These parameters characterizing the host-parasite association were assessed, in 2008–2009, on a community of breeding forest birds in northern France, where *I. ricinus* is the main vector of Lyme borreliosis.

2. Materials and methods

2.1. Study sites

The study was conducted 22 km southeast of Paris (France) at two sites, Sénart forest (3200 hectares (ha), 02°29′E, 48°40′N, 80 m above sea level) and Notre Dame forest (2200 ha, 02°35′E, 48°45′N, 100 m), both oak-hornbeam forests. The study plots were essentially composed of broad-leaved trees dominated by oaks (*Quercus robur, Quercus pedunculata*) and European hornbeams (*Carpinus betulus*), with two other forest tree species, the sweet chestnut (*Castanea sativa*) and the lime tree (*Tilia vulgaris*) in certain areas (Marmet, 2008).

The avian community is composed of common forest birds, and is dominated by passerines (Laury, 2007). Birds were mist-netted on four plots in the Sénart forest in 2008, and on five plots (including those of 2008) in the Sénart forest and two plots in the Notre Dame forest in 2009. The plots were chosen so that they were representative of the average forest cover. A plot was a 4 ha square of 16 vertical mist-nets (12 m long and 2.5 m high) located 50 m from each other. Mist-nets intercepted birds between 0.4 and 2.9 m from the ground. Bird sampling was conducted during the peak of activity of questing ticks, namely between June and July 2008 and between May and June 2009, with a frequency of one morning capture session per week per plot over 4 weeks.

Table 1

The number of bird individuals sampled, the number of *Ixodes ricinus* collected on those birds, the mean tick burden per bird individual, and life-history traits per bird species within a community of forest bird hosts from France, 2008–2009. Species-level life-history traits were the average guesstimates for heights at which birds forage (mean foraging height) or nest (mean nest height), the average response to an injection of phytohemagglutinin (PHA) to trigger an innate immune response (from Soler et al. 2007), and the average spleen size (from Møller et al., 1998).

Bird species	No of birds examined	Estimated abundance [CI] (group)	Mean body mass (g) (S.D.)	No of ticks collected	Mean no of ticks per individual (CI)	Mean prop. of nymphs per individual (CI)	Mean foraging height (m)	Mean nest height (m)	Mean PHA response (mm)	Mean spleen mass (mg)
Erithacus rubecula	99	98 [67;150] (A)	16 (1.4)	532	6.2 [4.3;9.0]	0.11 [0.08;0.17]	0.8	0.8	0.29	–
Parus major	98	61 [45;88] (B)	17 (1.0)	173	1.4 [1.0;2.0]	0.21 [0.11;0.30]	6.3	2.8	0.18	28
Turdus merula	55	52 [37;84] (C)	89 (5.3)	1080	14.7 [10.1;21.5]	0.63 [0.56;0.67]	2.5	1.6	0.20	221
Sylvia atricapilla	41	37 [27;60] (D)	17 (1.4)	39	0.7 [0.4;1.2]	0.49 [0.33;0.64]	2.6	1.5	0.13	3
Troglodytes troglodytes	32	55 [40;77] (E)	9 (0.9)	161	3.7 [2.2;6.1]	0.11 [0.05;0.20]	1.0	1.1	0.13	17
Phylloscopus collybita	28	42 [31;59] (E)	7 (0.7)	5	0.1 [0.04;0.4]	0.25 [0.01;0.90]	5.6	0.2	–	7
Fringilla coelebs	14	–	21 (1.1)	9	0.7 [0.3;1.9]	0.30 [0.04;0.74]	6.9	4.4	0.20	37
Poecile palustris	14	10 [7;14] (B)	10 (0.6)	0	0	–	6.4	3.8	–	–
Cyanistes caeruleus	13	12 [9;18] (B)	11 (0.8)	5	0.3 [0.1;0.9]	1 [–]	5.8	2.9	0.13	13
Turdus philomelos	13	35 [25;49] (E)	65 (2.6)	193	9.5 [4.5;20.2]	0.29 [0.19;0.39]	4.0	2.6	–	124
Dendrocopos major	11	22 [16;32] (B)	77 (4.6)	0	0	–	7.3	5.0	–	48.5
Prunella modularis	8	13 [10;18] (E)	18 (1.2)	46	5.8 [2.2;15.1]	0.71 [0.50;0.89]	0.7	1.0	0.21	26.5
Certhia brachydactyla	7	10 [7;14] (B)	9 (0.6)	0	0	–	5.3	3.1	–	12
Sitta europaea	7	10 [7;14] (B)	22 (1.0)	1	0.1 [0.01;1.0]	1 [–]	7.6	4.2	–	24
Sturnus vulgaris	4	–	74 (2.7)	61	–	0.87 [0.64;0.94]	4.3	4.9	–	89
Turdus viscivorus	4	–	104 (7.9)	77	–	0.35 [0.20;0.49]	5.5	5.6	–	245
Phoenicurus phoenicurus	3	–	13 (0.6)	45	–	0.17 [0.05;0.52]	4.7	4.7	0.13	29
Sylvia borin	3	–	17 (0.9)	0	0	–	2.5	1.5	0.26	–
Garrulus glandarius	2	–	167 (2.1)	243	–	0.30 [0.17;0.37]	6.6	5.1	0.61	18.5
Picus viridis	2	–	189 (6.4)	1	–	1 [–]	3.6	4.1	–	88
Total	458			2671						

No, number; CI, 95% confidence interval.
Dashes indicate missing values.

2.2. Data collection

For each bird captured, the species, body mass, sex (male versus female versus unknown) and age (juvenile versus adult, Svensson, 1992) were recorded. Birds were then banded, and inspected carefully for ticks. In 2008, the bird's entire body was systematically inspected. Basically all (99.7%) of the ticks found were located on the head, as found in other birds (Humair et al., 1998; Gregoire et al., 2002) and in mammals (Nilsson and Lundqvist, 1978), with only one tick located on another part of the body (tibio-tarsal articulation). Consequently in 2009, ticks were counted only on the head. All ticks were removed during inspection and placed in 70% ethanol for further identification.

The density of questing nymphs is used as a proxy for the density of questing larvae and nymphs. This is a reasonable assumption given that, at our study sites, questing larvae and nymphs were synchronous (Pisanu et al., 2010), and given that density of larvae is very difficult to estimate due to their very high aggregation. Questing nymphs were sampled on each plot by dragging a 1 m^2 cotton blanket over the vegetation (McLeod, 1932), once per month from March to November 2008 and 2009. Within each plot, 16 units of 10 m^2 were randomly dragged. The density of questing nymphs per 100 m^2 was calculated from the number of nymphs collected. For each bird capture session, the density of questing nymphs sampled on the closest date was used in the analysis. The maximum time lag was 2 weeks between the date of capture and the date of sampling of questing nymphs.

2.3. Statistical analyses

Only the tick count from the first capture per bird was included in the analyses because ticks were removed while counting them. The number of ticks counted on the head of each sampled bird was analysed with Generalized Linear Models (GLM) (McCullagh and Nelder, 1989) using a negative binomial distribution with a log link (package MASS, function glm.nb, R Development Core Team, 2008). The analysis was done in two steps.

First, the effects of sex, age, questing nymph density (defined as a categorical variable divided into three classes), year, study plot and bird species identity were assessed. Due to collinearity, either study plot or nymph density were used in the models, but the two variables were not included in the same model. The selection of significant effects started by fitting a model with all of the explanatory variables of interest (additive effects only). Then a backward model selection approach was used to delete non-significant variables from the model. The statistical significance of effects was assessed with Likelihood Ratio (LR) tests between nested models, i.e. between models with and models without the variable of interest. LR follows a X^2 distribution and the threshold for significance was set at $P < 0.05$. We successively re-fitted reduced models, applying the same rule, until all remaining variables were statistically significant. Infestations were reported as means, standard errors and 95% confidence intervals.

Second, the effects of species-level traits on tick burden were examined. The species traits were body size, vertical space use and proxies of allocation to immune defences. The log-transformed body mass at capture for each bird was used as an index for body size. Since breeding birds spend much time both in their foraging habitat and in their nesting habitat, we tested separately for the effects of both components in the vertical space use per species. The mean height of foraging and the mean height of nest location per species were computed from guesstimates provided by 14 experienced field ornithologists (essentially licensed bird ringers), who were blind to our predictions (Table 1). These guesstimates were robust since the nest height values were highly correlated to estimates available in the literature ($R^2 = 0.812$ for Géroudet, 1961; $R^2 = 0.783$ for Hoeher, 1972). The dependency of the infestation rate on species-level innate and/or acquired immune competences was tested with two literature datasets on between-species variations in immune functions. The log-transformed PHA response (Table 1; Soler et al., 2007) was used as a proxy for the strength of the innate immune response. The relative mass of the spleen (Table 1) was used as a proxy for the tissue allocation to the acquired immune response. Since the spleen mass depends on the size (and therefore on the mass) of the species (Møller et al., 2000), the dependent variable used in the analyses was the relative size of the spleen per species, measured as residuals from a regression of log-transformed spleen mass on log-transformed body mass (Møller and Erritzoe, 2002).

As species level life-history traits are correlated (Supplementary Table S1), the effect of each trait was tested separately (univariate LR test) by comparisons of nested models which were adjusted for the significant effects determined in the first step of the analysis but excluding the species effect.

2.4. Relative importance of bird species in local tick dynamics

The proportion of ticks that were carried per species within the local community of bird hosts were computed and used to estimate the relative contribution of each bird species to the local dynamics of ticks. This proportion was defined as the average tick burden multiplied by the absolute abundance of adult birds (averaged across years) per species, divided by the sum over all species of the average tick burden times the absolute abundance of adult birds. To compute this index, estimates of bird abundances per species were needed. This was done with closed population capture-mark-recapture models designed for estimating population size and fitted with software CAPTURE (Otis et al., 1978), applied to yearly datasets of capture–recapture per species. For some species (Table 1), the number of recaptures was too small to allow the estimation of the number of individuals only with the data of the species. When this was the case, the capture data were pooled to form groups of species with similar capture probabilities (on the basis of their habitat use and phylogenetic relationships, Table 1). After producing the estimate of the total number of individuals per group of species (N_{group}), the number of individuals per species was computed by multiplying N_{group} by the proportion of individuals per species in the raw capture dataset. For a majority of the tests (six out of 10), the major source of variation of capture probability was the among-individual heterogeneity of capturability. All abundance estimates were therefore produced with this model structure (model M_h, see Otis et al. (1978) for details).

As nymphs are the key stage for infecting reservoir hosts (Gern and Humair, 2002), we tested whether the proportion of nymphs carried per individual bird varied significantly between species, after accounting for between year and between plot variations. The number of nymphs per individual was analyzed with GLM, using a quasi-Poisson distribution to account for moderate overdispersion (with a log-link function, and the log-transformed total number of ticks per individual as offset), and with bird species, year and study plot as explanatory variables. The indices of contribution to tick carriage within the bird community were then multiplied by the estimated proportions of nymphs per species to account for the greater role of birds carrying a high proportion of nymphs in the dynamics of *B. burgdorferi* sl.

3. Results

3.1. Tick burden of birds

A total of 458 birds comprising 20 species was examined for ticks during the study (Table 1). The most frequently captured species were the European Robin (*Erithacus rubecula*), the Great Tit (*Parus major*), the Common Blackbird (*Turdus merula*) and the Eurasian Blackcap (*Sylvia atricapilla*), which accounted, respectively, for 22%, 21%, 12% and 9% of all birds examined (Table 1). Ticks were found on 238 individuals and a total of 2671 ticks were submitted for identification (Table 1). All of the specimens collected were identified as *I. ricinus* larvae and nymphs. Tick counts showed that birds hosted on average 5.8 ticks, of which 78% were larvae and 22% were nymphs. When considering parasitized birds only, the mean load was 11.2 ticks.

3.2. Correlates of tick burden

The most influential driver of tick burden was questing nymph density (LR = 14.91, degrees of freedom (d.f.) = 4, $P < 0.005$). As predicted, the prevalence of ticks on birds was associated with a high questing nymph density. For questing nymph densities per 100 m^2 of 0–63 nymphs, 64–188 nymphs, and 189–331 nymphs, the estimated mean number of ticks per bird were, respectively, 1.4 (95% confidence interval [0.9;2.1]), 2.4 [1.6;3.8] and 2.7 [1.7;4.2]. Contrary to our predictions, age and sex had no influence on tick burden (LR = 0.37, d.f. = 1, $P = 0.54$ and LR = 0.03, d.f. = 1, $P = 0.86$, respectively). The tick burden did not vary significantly between years (LR = 1.02, d.f. = 2, $P = 0.60$), nor between study plots (LR = 13.25, d.f. = 8, $P = 0.10$). Tick burden differed strongly (LR = 276.77, d.f. = 27, $P < 0.001$) between species of birds in this forest community. The five species with the highest average tick burden were three members of the Turdidae, *T. merula*, the Song Thrush, *Turdus philomelos*, and *E. rubecula*, followed by the Dunnock, *Prunella modularis,* and the Winter Wren, *Troglodytes troglodytes* (Fig. 1). The mean tick burden could not be estimated for six species that were represented by fewer than five individuals (Table 1).

3.3. Life-history correlates of among-species variation in tick burden

Larger bird species hosted more ticks than smaller species (LR = 85.7, d.f. = 2, $P < 0.001$, Fig. 2). Tick infestation depended on the vertical use of the habitat during foraging (LR = 7.6, d.f. = 1, $P < 0.001$, Fig. 3), whereas the average height of the nest did not explain among-species variation in tick burden (LR = 2.5, d.f. = 1, $P = 0.11$). A high PHA response was associated with high tick burden (LR = 13.2, d.f. = 1, $P < 0.001$). This positive relationship was robust to the omission of the two upper value outliers (corresponding to two Eurasian Jays, *Garrulus glandarius*; LR = 5.1, d.f. = 1, $P = 0.02$). The relative mass of the spleen was also positively associated with high tick burden (LR = 65.0, d.f. = 1, $P < 0.001$).

3.4. Proportion of nymphs per species

The proportion of nymphs per individual varied significantly between species ($P < 0.001$, Table 1), and to a lesser extent between sites ($P = 0.04$), but not between years ($P = 0.12$). The overall mean proportion of nymphs was 0.29 (95% confidence interval [0.22–0.35]). The Common Starling *Sturnus vulgaris* and *T. merula* hosted a significantly higher proportion of nymphs than the average (0.87, $P < 0.01$, 0.63, $P = 0.02$, respectively), whereas *T. troglodytes*, *E. rubecula* and *P. major* had significantly lower proportions of nymphs than average (0.11, 0.11, and 0.21, respectively; $Ps < 0.001$).

3.5. Estimation of bird species abundances

The abundance was estimated for the 13 bird species for which a mean tick burden could be estimated (the Chaffinch, *Fringilla coelebs,* was excluded because we had no recapture; Table 1). Abundances were estimated for each species, which provided us with the relative species abundance within the bird community. The

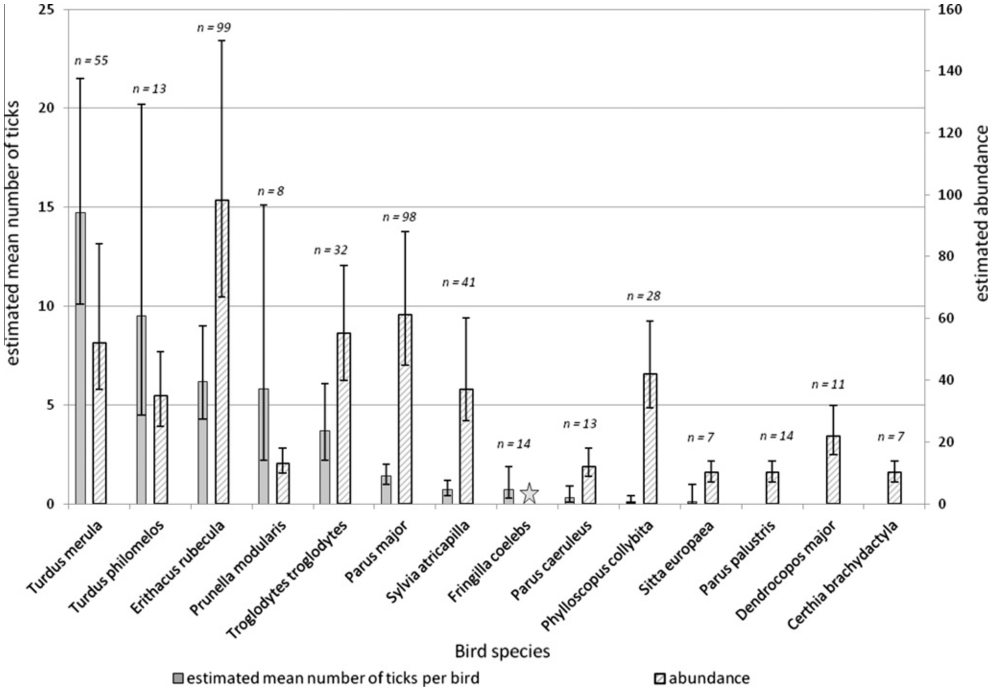

Fig. 1. Estimated mean number of ticks per individual and abundance of adult birds per species in a community of forest bird hosts from France during the 2008–2009 reproductive seasons of birds and ticks. The star indicates that the value is missing; *n* corresponds to the number of birds examined and error bars to 95% confidence intervals.

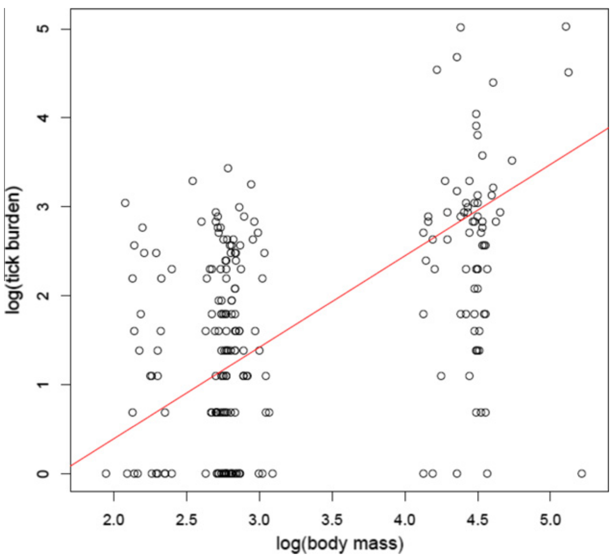

Fig. 2. Relationship between the logarithm of the number of ticks (tick burden) and the logarithm of the body mass (in g) per bird individual in a community of forest birds from France, 2008–2009. The line is the regression line estimated with the model.

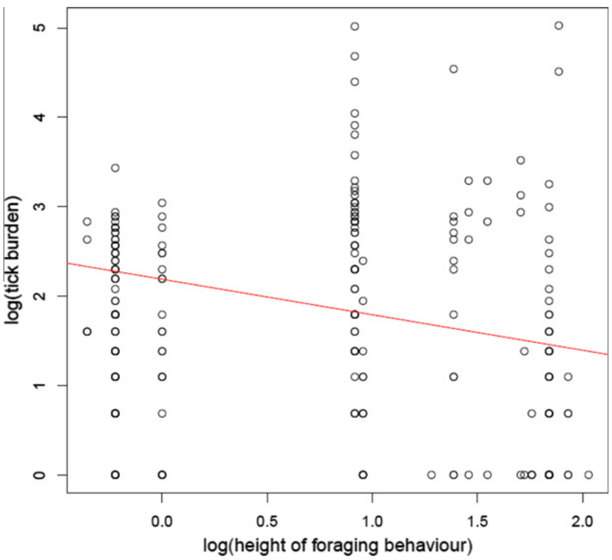

Fig. 3. Relationship between the logarithm of the number of ticks per bird individual and the logarithm of the mean height of foraging behavior per bird species (in m) in a community of forest birds from France, 2008–2009. The line is the regression line estimated with the model.

most abundant species were *E. rubecula* followed by *P. major*, *T. troglodytes* and *T. merula* (Fig. 1).

3.6. Relative importance of bird species to the local dynamics of ticks

Combining species-specific tick loads and species abundances revealed that three bird species bore 81% of all *I. ricinus* ticks within the local forest bird community: *T. merula* (36%), *E. rubecula* (29%) and *T. philomelos* (16%; Fig. 4). Three species contributed to another 18% of the tick carriage in the bird community, which covers the majority of the remaining ticks borne by birds: *T. troglodytes* (10%), *P. major* (4%) and *P. modularis* (4%; Fig. 4). Among them, *T. merula* was the only abundant species that also hosted a high proportion of nymphs (Fig. 4).

4. Discussion

In examining a community of forest birds, our goal was to characterize the relative role of each bird species on the dynamics of *I. ricinus* populations, the vector of Lyme borreliosis in Western

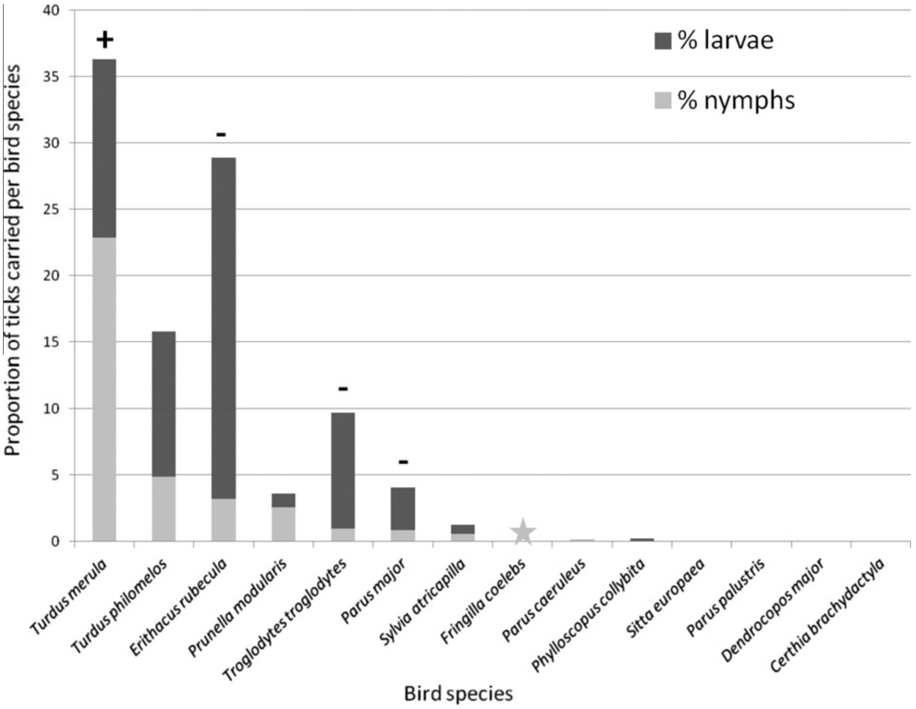

Fig. 4. Estimated relative importance of bird species in terms of tick carriage (proportion of ticks carried per bird species) within a community of forest bird hosts from France during the 2008–2009 reproductive seasons of birds and ticks. Respectively, in grey and black, are the proportions of nymphs and of larvae within the total number of ticks per bird species. The signs indicate that the proportion of nymphs for the species is significantly higher (+) or lower (−) than the average proportion of nymphs across all species. The star indicates that the value is missing.

Europe. The variation in questing nymph density between years and plots was the major determinant of the tick burden on birds. The most logical explanation is that when there are more questing ticks in an environment, the probability of a tick coming into contact with a bird is higher. The same conclusion was reached in comparative studies on rodents (Randolph, 2004), but prior to our research, the relative importance of this source of variation had not been studied in birds. Contrary to our predictions, age and sex classes did not influence tick burden on birds. It suggested that, once fledglings leave their nest, they are rapidly colonized by ticks. This is congruent with the findings of Gregoire et al. (2002) on *T. merula*, showing that the tick load of juveniles was similar to that of adults. The similar tick loads in males and females suggest that, despite the sexes having different levels of activity during the incubation period (i.e. about 15–20 days), this difference is too short (time) or not important enough to generate different tick loads (see also Gregoire et al., 2002). The mean questing tick density (155 nymphs/100 m^2, unpublished data) in our study area places it among the sites with the highest tick density in Europe (Ferquel et al., 2006; Wielinga et al., 2006; Boyard et al., 2011). This high tick availability in the environment probably fosters a rapid colonization of juvenile birds and an equivalent infestation of males and females. Certain bird species were more heavily infested by ticks than others. *Turdus merula* and *T. philomelos* had the highest tick loads, as found by Dubska et al. (2009) and James et al. (2011) (but see Poupon et al., 2006). The third most heavily infested species was *E. rubecula*, whereas in other studies *P. modularis* had a higher tick load than *E. rubecula* (Dubska et al., 2009; James et al., 2011).

Whereas many studies have looked at the variables influencing tick burden on rodents (Tälleklint and Jaenson, 1997; Dorn et al., 1999; Schmidt et al., 1999), very few have investigated birds at the community level. An original feature of our work was to consider species life-history traits alongside conventionally investigated factors in our investigation of tick burden on birds. In accordance with our prediction, bird species with high body mass and those that forage low in the vegetation bear more ticks. Heavier birds are also larger (Thompson, 1942) and thus expose larger areas of skin suitable for tick attachment. Their probability of meeting and hosting ticks are therefore higher than for other birds. Indeed, the actual determinant of the body size effect may be the skin surface around the beak and the eyes where ticks aggregate (present study; Humair et al., 1998; Gregoire et al., 2002). Comparative studies on rodents reached the same conclusion (Pisanu et al., 2010), but our study is the first to statistically assess this effect of body size for birds. The tick burden decreased logarithmically with the height of foraging behavior. This has been suggested by other studies, which categorized bird species into two classes of vertical use of habitats: ground-nesting versus arboreal species (Comstedt et al., 2006; James et al., 2011). Our study provides the first quantitative test and statistical characterization of this dependency of tick burden on the vertical use of the habitat per species. Although foraging height and nest location were highly correlated, the height of foraging behavior had a greater explanatory power. This indicates that birds were more likely to become infested by ticks while foraging than when accessing their nests. The closer to the ground the birds forage, the higher the probability that they will encounter ticks. It should be noted that the mist-nets used in our study only permitted the capture of birds to a height of 3 m. The sampling of the forest bird community consequently was biased towards species that mainly dwell close to the ground and in understory shrubs. Nonetheless, since ticks also mainly dwell within 1.5 m of the ground, it is unlikely that strictly arboreal bird species would harbor *I. ricinus*. Eventually, contrary to our predictions, bird species that had a high inflammatory immune response and a high relative spleen mass also had more ticks than others.

Hence, our results do not support the idea that species with the strongest immune defenses better defend themselves (on average) against ticks. On the contrary, it would be better interpreted as an indication that bird species allocating resources to innate and acquired immunity have evolved under a higher exposure to parasites, including ticks.

Although birds are often considered to be an undifferentiated reservoir group for vector–borne pathogens, their competency for hosting vectors and maintaining and transmitting pathogens varies between bird species (Kurtenbach et al., 1998; Taragel'ovà et al., 2008). The three major bird species that contributed to tick dynamics in our study (accounting for 80% of ticks collected), *T. merula*, *E. rubecula* and *T. philomelos* are known to be reservoir hosts of *B. burgdorferi* sl (Humair et al., 1993, 1998), as is *T. troglodytes*, the fourth important contributor species of our study (accounting for 10% of ticks collected) (Olsen et al., 1995). Among these species, *T. merula* was the only species to contribute with a higher proportion of nymphs than the average. This species thus has a higher probability to become infected than the other species. Conversely, *E. rubecula* and *T. troglodytes* may contribute less to the transmission of *B. burgdorferi* sl than expected from their tick load and abundance alone, because they host a low proportion of nymphs. *Turdus merula*, and to a lesser extent *T. philomelos* and *E. rubecula*, thus likely drive the dynamics of the agent of Lyme borreliosis within the bird compartment of the community of vertebrate hosts. In the only other detailed study that addressed the respective importance of species carrying ticks within a community of breeding bird hosts (James et al., 2011), 90% of the ticks were also carried by very few species (six), including *T. merula* (i.e. bearing 21.1% of the ticks). There, *E. rubecula* contributed with only 2.5% of the tick carriage. The respective roles of each species cannot easily be compared between our study and that of James et al. (2011) for two reasons. First, James et al. (2011) did not adjust their indices of relative abundance (the number of individuals caught) for inter-specific variations in capturability. Second, the community composition differed between the two studies. For instance, in contrast to our community, in the community of James et al. (2011), *T. philomelos*, *T. troglodytes* and *P. modularis* were captured in low numbers and therefore made low contributions to the tick population (respectively, 1.9%, 0.6% and 5%). Conversely, species occurring in very low numbers in our study dominated their community (e.g., 26.5% of tick carriage for *F. coelebs*, 18.6% for *P. major*, 16.1% for the Siskin, *Carduelis spinus*). To strengthen future characterizations of tick carriage at the host community level, we recommend that authors estimate host abundances and provide statistics that allow the characterization of the distribution of ticks (distinguishing larvae and nymphs) between host species at the community level.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijpara.2012.05.010.

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