

# The relationships between *Ixodes ricinus* and small mammal species at the woodland–pasture interface

Chloé Boyard · Gwenaél Vourc'h · Jacques Barnouin

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**Abstract** *Ixodes ricinus*, as vector, and small mammals, as reservoirs, are implicated in pathogen transmission between wild fauna, domestic animals and humans at the woodland–pasture interface. The ecological relationship between ticks and small mammals was monitored in 2005 on four bocage (enclosed pastureland) sites in central France, where questing ticks were collected by dragging and small mammals were trapped. Questing *I. ricinus* tick and small mammal locations in the environment were assessed through correspondence analysis. *I. ricinus* larval burden on small mammals was modeled using a negative binomial law. The correspondence analyses underlined three landscape features: grassland, hedgerow, and woodland. Seven small mammal species were trapped, while questing ticks were all *I. ricinus*, with the highest abundance in woodland and the lowest in pasture. The small mammals were overall more abundant in hedgerow, less present in woodland and sparse in grassland. They carried mainly *I. ricinus*, and secondarily *I. acuminatus* and *I. trianguliceps*. The most likely profile for a tick-infested small mammal corresponded to a male wood mouse (*Apodemus sylvaticus*) in woodland or hedgerow during a dry day. *A. sylvaticus*, which was the only species captured in grassland, but was also present in hedgerow and woodland, may be a primary means of transfer of *I. ricinus* larvae from woodland to pasture.

**Keywords** *Ixodes ricinus* · Small mammal · Woodland · Hedgerow · Pasture · Ecotone

## Introduction

The emergence of tick-borne disease in temperate regions of the Northern hemisphere appears to be mainly related to forest management involving reforestation and conditions favouring an increase in populations of deer (Barbour and Fish 1993; Morse 1995; George

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and Chastel 2002; Steere et al. 2004). Because many tick-borne pathogens are shared between domestic animals, wildlife, and humans (e.g. *Borrelia burgdorferi* s.l., *Anaplasma phagocytophilum*, tick-borne encephalitis virus), understanding how ticks circulate between wildlife and domestic animals is important to evaluate the risk of disease outbreak (Chomel 1998).

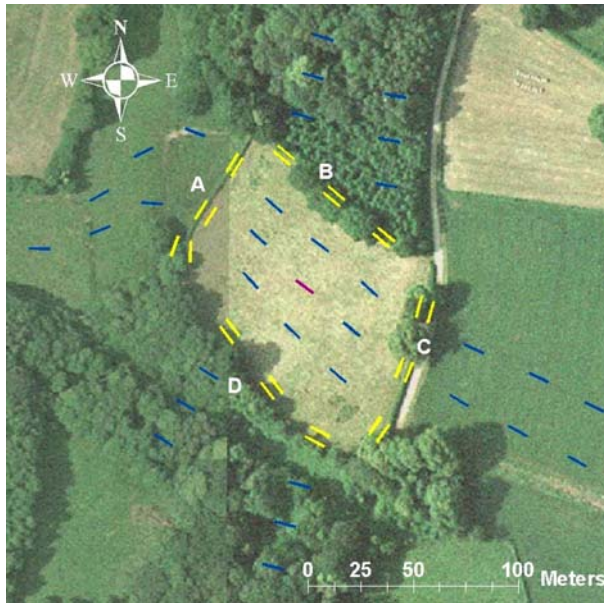
The abundance of the pathogen-transmitting *Ixodes ricinus* tick is affected by abiotic (habitat structure, climate) and biotic (host community composition) conditions (Gray 2002). Woodlands offer the most suitable habitat for *I. ricinus* (Gilot et al. 1975; Aeschlimann 1981; Gray 1998), providing both a humid shelter protecting the tick from desiccation and a host community ensuring tick feeding success. Grassland is regarded as a less suitable habitat for *I. ricinus* than woodland, most likely because of the dryer atmosphere (Daniel et al. 1977; L'Hostis et al. 1995b; L'Hostis and Seegers 2002). Nevertheless, *I. ricinus* is present on grazed pastures located in a bocage landscape (a countryside with woodland and pasture enclosed by hedgerows, earth banks or rows of trees) (L'Hostis et al. 1995b; Mémeteau et al. 1998; Boyard et al. 2007). We hypothesized that immigration from neighbouring woodland is critical for the maintenance of tick population on pastures (Boyard et al. 2007). Small mammals such as the bank vole (*Myodes [Clethrionomys] glareolus*) and the wood mouse (*Apodemus sylvaticus*), are potential tick- and pathogen-carriers since they are permanent inhabitants of bocage (Le Louarn and Quéré 2003), important hosts for *I. ricinus* larvae (Gray 2002) and reservoirs for *Borrelia burgdorferi* s.l., the agent of Lyme disease (Humair et al. 1999).

The aim of the paper was to identify, through a field study, small mammal species that could convey *I. ricinus* at the woodland–pasture interface. These small mammals should be a priori characterized by high infestation levels and by using woodland, hedgerow and grassland habitats. The study was conducted in a bocage region in France that was endemic for human Lyme disease (Arzouni 1990) and bovine babesiosis (L'Hostis et al. 1995a). The following questions were addressed: (1) where was *I. ricinus* located in the bocage landscape? (2) What was the distribution of small mammal species within this landscape? (3) Which small mammals could be responsible for *I. ricinus* transport at the woodland–pasture interface?

## Materials and methods

### Study sites

The study was conducted in four sites (located at Dontreix [site 1]: 46.0° N, 2.5° E; Bussières [site 2]: 46.1° N, 2.6° E; Marcillat [site 3]: 46.1° N, 3.0° E; Le Quartier [site 4]: 46.1° N, 2.7° E), belonging to commercial bovine farms of the Combrailles, a region of central France. Each study site included a central permanent grazed pasture for cattle of approximately 1 ha, and the neighbouring pastures, hedgerows and woodlands. The central grazed pastures of sites 1, 2, and 4, hosted ten, 14, and nine heifers respectively. The grazing heifers were observed on the whole pasture area (Boyard 2007). The central pastures were selected from a set of previously sampled pastures to study environmental factors affecting *I. ricinus* nymph abundance (Boyard et al. 2007). The pasture selection criteria were: (1) at least a third of the pasture surrounded by woodland, (2) no building or road in the vicinity of the pasture, (3) no marshland in the pasture, (4) no significant variation of questing nymph abundance observed within pasture between 2003 (Boyard et al. 2007) and 2004 (unpublished data), (5) a marked difference in questing nymph abundance between pastures (ranging from <0.1 for site 1–10 nymphs/m<sup>2</sup> for site 4).



**Fig. 1** Layout of subtransects corresponding to site 4 (Le Quartier): 2 transects along inner and outer perimeters of the pasture (yellow rectangles), 2 traverse transects running from **A** to **C** pasture side, and 2 traverse transects running from **B** to **D** pasture side (blue rectangles). Each transect included 10 m<sup>2</sup> subtransects (STs) separated by a gap of 20 m. The traverse transects presented 3 STs at each side of the pasture. STs of the 2 first traverse transects were oriented parallel to the woodland edge or the main hedgerow. STs of the 2 s traverse transects were oriented parallel to STs of the first traverse transects. A distance of 20 m was maintained between STs, traverse transects, and ecotone. If needed, additional STs were sampled to examine the pasture center (purple rectangle). INRA<sup>®</sup> live-traps for small rodents and shrews were set at 1 m before each end of all STs

### Tick and rodent sampling design

Tick collection and small mammal trapping were carried out on six transects, one along the inner pasture perimeter, one along the outer pasture perimeter, and four traverse transects (Fig. 1). The study transects were divided into 10 m<sup>2</sup> (10 m × 1 m) subtransects (STs) separated by a gap of 20 m. Each ST was dragged with a 1 × 1 m white towel to collect ticks (MacLeod 1932; Vassallo et al. 2000). Additionally, INRA<sup>®</sup> live-traps for mice and shrews were set at 1 m before each end of all STs. The traps on the pasture perimeter were set in the undergrowth when present.

### Tick sampling

Questing ticks were collected monthly from May to September 2005. The following variables were assessed at each ST, over the study period: (1) prevalence of *I. ricinus* larvae, defined as the percentage of infested STs, (2) prevalence and total number of *I. ricinus* nymphs, and (3) prevalence and total number of *I. ricinus* adults.

### Trapping of small mammals

Small mammals were captured during three trapping sessions (24th–29th April, 5th–10th June, 18th–3rd September 2005) with INRA<sup>®</sup> traps (a French model live-trap, described in

Guédon et al. (1990), efficient in catching both shrews and rodents) which were baited with a mixture of oat flakes, peanut butter, and sardine oil. Traps were checked each morning at dawn for five consecutive days. Living trapped animals were sacrificed in the field by cervical dislocation and stored in a plastic bag before examination in the laboratory. Attached ticks and those found in the plastic bag, were counted, put in a vial with 70% ethanol, and identified under the microscope (Cotty 1985). The species of small mammals were identified by their morphological traits (Le Louarn and Quéré 2003). For shrews, species identification was confirmed through genetic analysis performed by the ‘Muséum National d’Histoire Naturelle’ (Paris). Beside species, potential factors influencing tick burden considered were: (i) whether the animal had died in the trap, (ii) animal weight, (iii) animal sex and sexual features linked to hormone production (length of the seminal vesicle and testicle diameter for males, and pregnancy and lactating status for females), (iv) indirect assessment of immunity (presence of mites, parasites in liver and intestine, spleen weight, data not shown), and (v) indirect estimation of age (crystalline lens weight). Crystalline lenses were extracted from eye globes after three months soaking in 10% formaldehyde solution. Except for shrews, whose crystalline lens is too small, the lenses were then dried for 24 h at 95°C and weighed (Martinet 1966; Le Louarn 1971; Quéré and Vincent 1989). As juveniles are expected to be absent during the April session, the threshold between adults and juveniles was set, within each species, as the minimum lens weight in April (Le Pesteur et al. 1992).

Small mammal presence in the environment considered trapping effort, i.e. the number of trap-nights corrected by a factor of 0.5 if the trap was closed without capture (Nelson and Clark 1973). An index of abundance was obtained, which was expressed as the number of individuals per 100 trapping efforts. Small mammal presence variations between sites and trapping sessions were tested with chi-square tests.

#### ST characteristics and meteorological data

Each ST was characterized by: (1) herbaceous layer height (nil; <10 cm; 10–30 cm; >30 cm), (2) vegetation type (grass; hedgerow between two pastures; hedgerow between a pasture and woodland; undergrowth), (3) connection status with woodland (no connection; direct connection if the ST was in woodland; indirect connection if the ST bordered a hedgerow running to woodland), and (4) location (middle of the pasture; inner/outer pasture perimeter; or woodland). In addition, the presence of the most represented tree types in the direct ST vicinity was recorded: beech (*Fagus sylvatica*), ash (*Fraxinus excelsior*), oaks (*Quercus* spp.), conifers (*Abies* spp., *Picea* spp. and *Cedrus atlantica*), hazel (*Corylus avellana*), chestnut (*Castanea sativa*) and sweet chestnut (*Aesculus hippocastanum*). The presence of apple and plum trees (*Malus* spp. and *Prunus domestica*) was also recorded, as fruit trees were previously found to correlate with tick abundance (Boyard et al. 2007).

Meteorological data, provided by ‘Météo-France’ from the meteorological station of Saint-Gervais d’Auvergne, were mean values of daily minimum temperature for the two days overlapping the trapping nights, mean values of daily maximum temperature, mean values of daily minimum relative humidity, and mean values of daily maximum relative humidity (2 d max humidity).

#### Database

The data were stored in an Access 2000 (Microsoft France, Courtaboeuf, France) relational database organized in 47 tables. Checking procedures for data validity were conducted,

which entailed inspecting the record forms completed in the field and implementing automatic procedures to detect erroneous entries.

### Statistical analyses

The distributions of questing ticks and small mammals in the local environment were first analyzed by correspondence analysis (CA) models (Hill 1974) using the *ade4* R package (Chessel et al. 2006) of the statistical software ‘R’ (R Development Core Team, 2005).

For questing tick distribution in the environment, CA was performed including the potential explanatory variables related to ST position in the environment (site, vegetation type, connection status with woodland, location, tree types). Then, variables of interest (presence/absence of *I. ricinus* larvae on each ST, total number of nymphs per ST, and total number of adults per ST) were projected on the resulting factorial space. The factorial space was divided into four quadrants according to the two first principal components. Differences in the distribution between the quadrants were assessed for the presence/absence of *I. ricinus* larvae using the chi-square test, and for the number of collected *I. ricinus* nymphs and adults using the Kruskal–Wallis test (Benzécri 1992).

In the CA model for small mammal distribution in the environment, potential explanatory variables included, in addition to those used in the questing tick model, the trapping session and the herbaceous layer height. To correct for variation in sampling effort, each level of explanatory variables was weighted with the corresponding trapping effort. Then, the presence of small mammals and the species were projected on the resulting factorial space. Differences in the small mammal distribution between the quadrants were assessed using a chi-square test (Benzécri 1992).

The association between *I. ricinus* larvae burden on small mammals and explanatory variables was assessed using a negative binomial model with backward elimination at  $P$ -value  $< 0.05$  (*glm.nb* function of R, R Development Core Team 2005). Such models are frequently used to model burden of ticks and other parasites (Shaw et al. 1998; Randolph et al. 1999). Two questions were addressed: (1) what factors explain tick burden variation among all individuals, taking into account ‘species’ as the structuring variable? (2) For each species, does tick burden vary according to the animal location in the landscape? For the first question, beside species, potential explanatory variables included site, presence of questing *I. ricinus* larvae, abundance of questing *I. ricinus* nymphs and adults, trapping session, small mammal characteristics, meteorological data, and small mammal coordinates on first and second principal components of the small mammal CA summarizing ST characteristics. Prior to this negative binomial modeling, variables were selected using univariate tests. Quantitative variables were divided into two or three categories of equal size depending on variable distribution. Kruskal–Wallis tests and rank sum tests were performed for categorical and binary data, respectively, between potential explanatory variables and larvae burden (selection threshold:  $P$ -value  $< 0.1$ ). To avoid redundancy in explanatory variables, correlations among the candidate variables were analysed using chi-square tests. In the present study, the small mammal species was the key structuring explanatory variable, therefore all the variables correlated ( $P$ -value  $< 0.05$ ) with the species were removed. Concerning the other correlated variables, the less significant through the univariate tests were removed. In the final negative binomial model, rate parameter estimates of each significant variable were used to assess the relative change in larval abundance for one level compared to a reference. The adjustment of the predicted negative binomial distribution to the observed distribution was assessed using a chi-square test. For the second question, only species for which tick burden was significantly correlated to small mammal coordinates on the

**Table 1** Prevalence and mean abundance of questing *Ixodes ricinus* ticks per subtransect (10 m<sup>2</sup> surface unit) on four sites of the Combrailles region, France

	Larvae			Nymphs			Adult females			Adult males		
	<i>p</i>	<i>p</i>	<i>x</i>	<i>p</i>	<i>x</i>	<i>c.i.</i>	<i>p</i>	<i>x</i>	<i>c.i.</i>	<i>p</i>	<i>x</i>	<i>c.i.</i>
Site (# of STs)												
Site 1 (52)	7.7	46.2	2.94	1.67–5.17	17.3	0.25	0.12–0.50	21.2	0.23	0.13–0.41		
Site 2 (58)	6.9	46.6	2.21	1.36–3.58	13.8	0.19	0.09–0.40	8.6	0.09	0.04–0.21		
Site 3 (56)	14.3	48.2	8.02	4.20–15.30	19.6	0.38	0.19–0.75	14.3	0.23	0.10–0.51		
Site 4 (57)	33.3	75.4	23.89	15.04–37.96	43.9	1.21	0.77–1.91	43.9	1.33	0.82–2.16		
Quadrant (# of STs)												
Lower left (69)	29.0	95.7	24.14	18.23–31.97	52.2	0.94	0.69–1.28	42.0	0.67	0.46–0.96		
Upper left (60)	20.0	61.7	6.53	3.96–10.78	25.0	0.75	0.38–1.48	26.7	0.93	0.47–1.85		
Upper right (9)	.	33.3	0.44	0.16–1.23	11.1	0.22	0.02–2.44	.	.	.		
Lower right (85)	3.5	17.6	0.35	0.19–0.65	1.2	0.02	0.00–0.31	4.7	0.05	0.02–0.13		
Total (223)	15.7	54.3	9.38	6.95–12.66	23.8	0.51	0.37–0.71	22.0	0.48	0.33–0.67		

Ticks were collected once a month from May to September 2005. For *I. ricinus* larvae, only presence was recorded. Quadrants refer to the axis-bounded quadrants resulting from the correspondence analysis for tick data (see Fig. 2). *p* = % of infested subtransects, *x* = Mean # of ticks per subtransect, *c.i.* = Confidence interval at 95% computed on the basis of a negative binomial law. ‘.’ Substitutes for zero

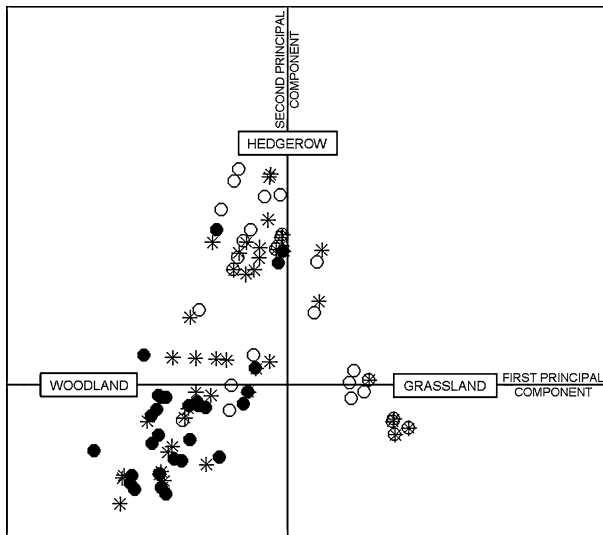
first and/or the second principal component of small mammal CA (*P*-value <0.1) were considered. A ‘one-species’ negative binomial model was fitted, that included the remaining variables of the ‘multi-species’ model and the significant principal component(s) divided into three classes.

## Results

### Questing ticks

The presence of *I. ricinus* larvae was recorded in 35 STs out of 223 (15.4%). 2092 *I. ricinus* nymphs and 220 *I. ricinus* adults were sampled (see prevalences and mean abundances per transect in Table 1). Two nymphs and four adults (three males, one female) of *Dermacentor reticulatus* were also collected. The highest tick density was observed in site 4, whilst the lowest occurred in site 2 (Table 1).

The questing tick CA showed, for every life stage, that tick distribution depended on ST characteristics (*P*-values for differences between quadrants for larvae, nymph and adult <0.001). The first and the second principal components represented, respectively, 19.6% and 14.7% of the projected inertia. Vegetation type, connection status with woodland, and location defined the factorial space (correlation coefficients with components 1 and 2 >0.60). The modalities of these variables characterized three dominant landscape features: woodland, grassland, and hedgerow. The lower left axis-bounded quadrant (Fig. 2) corresponded to woodland (STs lying in undergrowth and direct connection with woodland), the upper left quadrant to hedgerows adjacent to woodland (STs lying along hedgerows with connection with woodland), the upper right quadrant to hedgerows between two pastures, and the lower right quadrant to grassland (STs lying in grass, no connection with woodland). Larvae, nymphs, and adult females were more prevalent/abundant in the lower left quadrant (e.g. see nymphs Fig. 2). Adult males were also more prevalent in the lower left quadrant, but their abundance was higher in the upper left quadrant (Table 1).



**Fig. 2** Spatial distribution in the multivariate factorial space (principal components 1 and 2) of the *Ixodes ricinus* nymph abundance, on 4 sites of the Combrailles region of France, according to subtransect characteristics (see text). The graph shows the relative distance between subtransects in the factorial space according to landscape features. Symbols: ○ = 0 nymphs, \* = 1–10 nymphs, ● = 11–228 nymphs

### Small mammals

A total of 194 animals were captured, belonging to seven different species. Bank vole (*Myodes glareolus*) was the most abundant species, representing 40.7% of all captures. Wood mouse (*Apodemus sylvaticus*) represented 21.6% of all captures, common vole (*Microtus arvalis*) 13.4%, yellow-necked mouse (*Apodemus flavicollis*) 9.3%, common shrew (*Sorex araneus*) and French shrew (*Sorex coronatus*) 8.8%, and greater white-toothed shrew (*Crocidura russula*) 6.2% of captured small mammals.

Small mammal presence varied between sites and session ( $P$ -values = 0.041 and <0.001, respectively). Site 3 showed the greatest number of captured animals relative to trapping effort and 63% of the captures occurred in September (Table 2). In April, only four species were represented: *A. sylvaticus*, *M. glareolus* and *Sorex* spp.

Weight, sex ratio, reproductive traits, percentage of juveniles and percentage of individuals found dead in the traps are displayed per species in Table 3. Mean weight per species varied from 6.4 g to 26.1 g. Sex ratio varied from 0.86 for *M. arvalis* to 2.00 for *A. flavicollis*. In this species, no females were found pregnant, while 64% of *M. arvalis* females were pregnant. Moreover, most *Sorex* spp. were found dead in the traps.

The small mammal CA revealed the same landscape features as the questing tick CA, and was defined by the same variables (correlation coefficients with principal components 1 and 2 >0.65), and the descriptions of quadrants were similar. The first and the second principal components represented respectively 17.2% and 11.8% of the projected inertia. Small mammal presence differed between landscape features ( $P$ -values for differences between quadrants: <0.001). Small mammals were more often captured near hedgerows (6.18 individuals per 100 trapping efforts in hedgerows between two pastures, vs. 3.03 in woodland, 3.53 in hedgerows connected with woodland, and 1.50 in grassland). The species also differed

**Table 2** Number of small mammals captured in 2005 on four sites of the Combrailles region, France, per site and trapping session

	<i>As</i>	<i>Af</i>	<i>Mg</i>	<i>Ma</i>	<i>Cr</i>	<i>S</i>	Total # of small mammals	Trapping effort	# of small mammals per 100 trapping efforts
Site									
Site 1	5	6	20	3	1	8	43	1515.5	2.84
Site 2	2	4	32	4	0	4	46	1790.5	2.57
Site 3	21	6	14	12	11	1	65	1600.0	4.06
Site 4	14	2	13	7	0	4	40	1536.0	2.60
Trapping session									
April	11	0	10	0	0	2	23	2201.5	1.04
June	7	4	11	16	5	6	49	2113.0	2.32
September	24	14	58	10	7	9	122	2127.5	5.73
Total	42	18	79	26	12	17	194	6442.0	3.01

Species acronyms: *As* = *Apodemus sylvaticus*, *Af* = *Apodemus flavicollis*, *Mg* = *Myodes glareolus*, *Ma* = *Microtus arvalis*, *Cr* = *Crociodura russula*, *S* = *Sorex* spp. Trapping effort was the number of trap-nights realized, corrected by a factor of 0.5 if the trap was tripped without capture (Nelson and Clark 1973)

**Table 3** Characteristics of the small mammals captured in 2005 on 4 sites of the Combrailles region, France

Small mammal species (# of captured individuals)	Mean weight (s.d.) (g)	Sex ratio <sup>a</sup>	% of pregnant females	% of lactating females	% of juveniles	% found dead in the trap
<i>As</i> (42)	17.7 (5.9)	1.21	10.5	10.5	50.0	7.1
<i>Af</i> (18)	26.1 (4.8)	2.00	0.0	0.0	38.8	0.0
<i>Mg</i> (79)	17.2 (4.4)	0.98	22.5	27.5	27.8	35.4
<i>Ma</i> (26)	20.6 (6.1)	0.86	64.3	23.1	53.8	46.2
<i>Cr</i> (12)	7.1 (1.7)	1.40	20.0	20.0	ND	41.7
<i>S</i> (17)	6.4 (2.5)	1.13	25.0	0.0	ND	88.2

Acronyms: *As* = *Apodemus sylvaticus*, *Af* = *Apodemus flavicollis*, *Mg* = *Myodes glareolus*, *Ma* = *Microtus arvalis*, *Cr* = *Crociodura russula*, *S* = *Sorex* spp

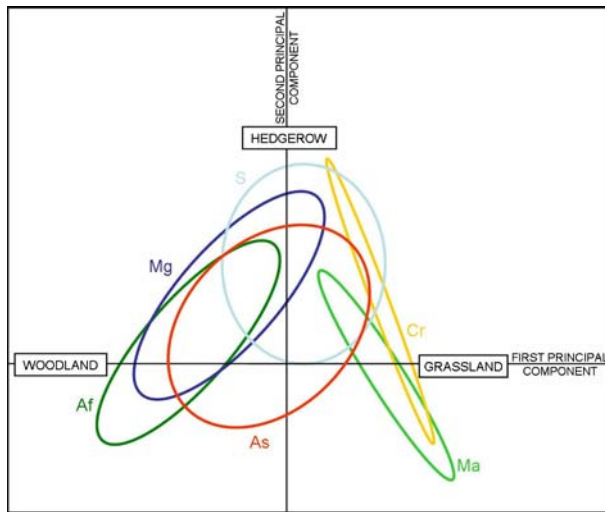
<sup>a</sup> Sex ratio = # of males/# of females; ND: Not Determined

significantly according to landscape features (Fig. 3). *A. sylvaticus* was found overlapping all the landscape features with the highest prevalence in hedgerows. *A. flavicollis* was always captured in STs connected with woodland and *M. glareolus* in hedgerows as well as in woodland. Only 1 *M. glareolus* was trapped in grassland. Moreover, *M. arvalis* was more prevalent on grassland, *C. russula* was captured on grassland and in hedgerows between two pastures, and 70% of *Sorex* spp. were found in hedgerows between two pastures.

#### Tick burden on small mammals

Captured small mammals carried a total of 408 *I. ricinus* ticks (393 larvae and 15 nymphs), 110 *I. acuminatus* (106 larvae, one nymph and three adult females), and 13 *I. trianguliceps* (two larvae and 11 nymphs) (Table 4). Species identification could not be confirmed for six *Ixodes* sp. and one *Dermacentor* sp. (not shown). *I. ricinus* and *I. trianguliceps* were found on the four sites, while *I. acuminatus* was only found on 17 individuals of site 1 and two individuals of site 3. Moreover, *I. acuminatus* larva burden in site 1 reached the level of site 3 and site 4 for *I. ricinus* larva burden.





**Fig. 3** Spatial distribution in the multivariate factorial space (principal components 1 and 2) of the small mammals captured on 4 sites of the Combrailles region, France, according to environmental variables (see text). The graph shows the inertia ellipses giving the relative distance between species according to landscape features. Species labels: *As* = *Apodemus sylvaticus*, *Af* = *Apodemus flavicollis*, *Mg* = *Myodes glareolus*, *Ma* = *Microtus arvalis*, *Cr* = *Crocidura russula*, *S* = *Sorex* spp

After univariate variable selection and correlation tests, all small mammal variables but species, sex and 2 d max humidity were dropped. It is worth noticing that trapping session, which was correlated to species, was also correlated to maximum humidity since 28 out of 49 animals captured in June were trapped by ‘dry’ days (2 d max humidity: 82–89%), vs. nine out of 23 in April, and 0 out of 122 in September. The regression model was thus performed with the three explanatory variables which were all significant (Table 5, dispersion parameter  $\emptyset = 1.78$ ). The chi-square statistic indicated that the data fitted the model ( $P$ -value = 0.806, Fig. 4). Mice (*A. sylvaticus* and *A. flavicollis*) had the highest larvae burdens. *M. arvalis* presented an intermediate larvae burden, while *Sorex* spp., *M. glareolus*, and *C. russula* were poorly infested. Males were more infested than females. Higher larvae burdens were observed during the periods of lower relative humidity.

In the one-species approach, *I. ricinus* larvae burden of only *A. sylvaticus* and *C. russula* were significantly correlated to small mammal coordinates on the first and the second principal components of small mammal CA respectively. The negative binomial analysis for *A. sylvaticus* showed that all explanatory variables were significant and the data fitted the model ( $P$ -value = 0.807,  $\emptyset = 1.34$ ). *A. sylvaticus* carried 4.8-fold more larvae in woodland (confidence interval (c.i.) at 95%: 1.72–13.40) and 2.6-fold more larvae in hedgerow (c.i.: 0.90–7.46) than in grassland not connected to woodland. Males carried 3.7-fold more larvae than females (c.i.: 1.44–9.65), and when the weather was drier, *A. sylvaticus* carried 9.4-fold more *I. ricinus* larvae than during high humidity periods (c.i.: 3.53–25.37). For *C. russula*, the only significant explanatory variable was the coordinates on the second principal component. *C. russula* carried 18.0-fold more *I. ricinus* larvae when captured in hedgerows than when in grassland (c.i.: 2.28–142.09,  $P$ -value for goodness of fit = 0.865, dispersion parameter  $\emptyset < 0.001$ ).

**Table 4** Tick burden of small mammal species

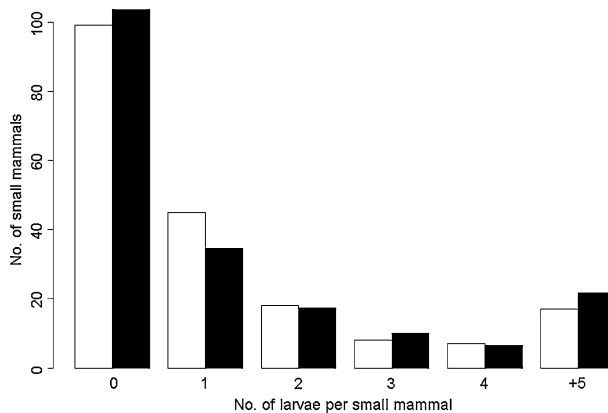
Tick species									
Small mammal species (# of individuals)	Larvae			Nymphs			Adult females		
	<i>p</i>	<i>x</i>	c.i.	<i>p</i>	<i>x</i>	c.i.	<i>p</i>	<i>x</i>	c.i.
<i>Ixodes ricinus</i>									
<i>As</i> (42)	66.7	5.38	3.16–9.17	4.8	0.26	0.02–2.75	.	.	.
<i>Af</i> (18)	55.6	2.72	1.22–6.06	.	.	.	.	.	.
<i>Mg</i> (79)	41.8	0.73	0.51–1.05	1.3	0.01	0.00–0.09	.	.	.
<i>Ma</i> (26)	46.2	1.50	0.73–3.10	7.7	0.12	0.02–0.56	.	.	.
<i>Cr</i> (12)	41.7	0.83	0.34–2.03	.	.	.	.	.	.
<i>S</i> (17)	41.2	0.65	0.33–1.29	.	.	.	.	.	.
<i>Ixodes acuminatus</i>									
<i>As</i> (42)	7.1	0.14	0.03–0.60	.	.	.	.	.	.
<i>Af</i> (18)	.	.	.	.	.	.	.	.	.
<i>Mg</i> (79)	15.2	0.70	0.29–1.66	1.3	0.01	0.00–0.09	1.3	0.01	0.00–0.09
<i>Ma</i> (26)	3.8	1.19	0.84–1.70	.	.	.	.	.	.
<i>Cr</i> (12)	.	.	.	.	.	.	.	.	.
<i>S</i> (17)	5.9	0.82	0.49–1.39	.	.	.	5.9	0.12	0.01–10.42
<i>Ixodes trianguliceps</i>									
<i>As</i> (42)	.	.	.	2.4	0.02	0.00–0.17	.	.	.
<i>Af</i> (18)	.	.	.	.	.	.	.	.	.
<i>Mg</i> (79)	2.5	0.03	0.01–0.10	5.1	0.13	0.03–0.50	.	.	.
<i>Ma</i> (26)	.	.	.	.	.	.	.	.	.
<i>Cr</i> (12)	.	.	.	.	.	.	.	.	.
<i>S</i> (17)	.	.	.	.	.	.	.	.	.

No adult male was observed. *p* = % of infested hosts, *x* = mean # of ticks per host, c.i. = confidence interval at 95% computed on the basis of negative binomial law. Species acronyms: *As* = *Apodemus sylvaticus*, *Af* = *Apodemus flavicollis*, *Mg* = *Myodes glareolus*, *Ma* = *Microtus arvalis*, *Cr* = *Crocidura russula*, *S* = *Sorex* spp. ‘.’ substitutes for zero

**Table 5** Final negative binomial models for *Ixodes ricinus* larvae abundance on small mammals captured on 4 sites of the Combrailles region, France

Variable	Rate parameter	Confidence interval at 95%	
Species			
<i>Myodes glareolus</i>	1	/	
<i>Apodemus sylvaticus</i>	4.39	2.42–7.93	***
<i>Apodemus flavicollis</i>	2.74	1.23–6.10	*
<i>Microtus arvalis</i>	1.55	0.73–3.28	
<i>Crocidura russula</i>	0.79	0.26–2.43	
<i>Sorex</i> spp.	0.86	0.33–2.21	
Sex			
Female	1.00	/	
Male	2.13	1.32–3.43	**
Maximum humidity			
High: 91–98%	1.00	/	
Low: 82–89%	3.10	1.80–5.35	***

The estimation of linear predictors was considered as asymptotically normally distributed. ‘\*’, ‘\*\*’ and ‘\*\*\*’ indicate significant factors at *P*-values of <0.05, <0.01 and <0.001 respectively



**Fig. 4** Observed (white bars) and predicted (black bars) distributions of larval abundance on small mammals captured on 4 sites of the Combrailles region of France. The number of *Ixodes ricinus* larvae per small mammal was predicted by a negative binomial model adjusted with explanatory variables (Table 5)

## Discussion

In the bocage region of the Combrailles, where pasture enclosed by hedgerows alternated with woodland, we found that questing *I. ricinus* abundance decreased from woodland to hedgerow and from hedgerow to the middle of the pasture. This finding is consistent with previous studies showing that in open pasture, ticks suffer from desiccation, resulting in lower survivorship than in woodland or at woodland edges (Daniel et al. 1976, 1977), and that *I. ricinus* were found in higher abundance along hedgerows in pastures tightly linked to woodland vicinity (L’Hostis et al. 1995b; Boyard et al. 2007). Questing tick distribution in the environment is the result of their location when they detached from hosts, of their survival capability, questing activity and lateral movements, although *I. ricinus* ticks are considered to move no more than a few meters (Gray 1985; Carroll and Schmidtman 1996).

The simultaneous observation of small mammals on the study sites led to an index of abundance rather small, but in accordance with the normal fluctuation found in France (Le Louarn and Quéré 2003; Pascal et al. 2005). Only one small mammal species, *A. sylvaticus*, used all three habitat types, woodland, hedgerow and grassland. Furthermore, this species had the highest *I. ricinus* larval burden, which also varied consistently according to habitat types. Thus *A. sylvaticus* appears to be the best candidate of the species we studied to transport tick larvae between habitats and act as an ‘epidemiological bridge’ between woodlands and pastures. The large home range of *A. sylvaticus* (1,000–2,000 m<sup>2</sup>, Le Louarn and Quéré 2003) would allow the species to pick up larvae in woodlands and carry them into pastures, directly or through hedgerow channels. The other small mammal species would be less efficient at crossing the ecotones (Despommier et al. 2007) and spreading the ticks because their home range did not include grassland (*A. flavicollis*, *M. glareolus*), or because these species had low levels of tick infestation (*M. glareolus*, *M. arvalis*, *C. russula* and *Sorex* spp.).

Tick burden on small mammals varied following the same pattern as questing ticks in the landscape. However, it is not easy to distinguish between a species and a habitat effect since mammal species are generally linked to habitat types. Indeed, *I. ricinus* larvae burden was higher in two out of three species living in woodland (*A. sylvaticus* and *A. flavicollis*), whilst the larvae burden was minimal for the shrews (*C. russula* and *Sorex* spp.) which

were rarely trapped in woodland. The lower burden of ticks in shrews has been found in several studies (L'Hostis et al. 1996; Liz et al. 2000) though not all (Tälleklint and Jaenson 1994). The effect of mammal exposure to ticks is apparent in the one-species models, where 80% of tick-infested *C. russula* were trapped in hedgerows, and where *A. sylvaticus* was more heavily infested in woodland and hedgerow than on grassland. Conversely, the effect of species is clearly apparent in the case of *M. glareolus*, which was found mainly in woodland but had a significant lower burden than other woodland species (*Apodemus* spp.) and slightly less than *M. arvalis*, which was pasture-specific.

Significant lower larval burden of *M. glareolus* compared to *Apodemus* spp. has been previously reported (L'Hostis et al. 1996; Tälleklint and Jaenson 1997; Hanincova et al. 2003; Michalik et al. 2003; Stanko et al. 2007) and could be due to a greater home range and a better roaming within the home range for *Apodemus* spp. (Tälleklint and Jaenson 1997; Le Louarn and Quéré 2003), and/or a better immunity against *I. ricinus* for *M. glareolus* (Dizij and Kurtenbach 1995; Hughes and Randolph 2001). The tendency (not significant) to have a higher tick burden for *M. arvalis* than *M. glareolus* is surprising compared to other studies (L'Hostis et al. 1996; Sinski et al. 2006; Stanko et al. 2007) and to the fact that *M. arvalis* was not found in woody vegetation and has a smaller home range (from 25 in winter to 500 m<sup>2</sup> in summer, Le Louarn and Quéré 2003).

The other factors influencing larvae burden were sex and 2 d max humidity. The higher infestation of males compared to females is a recurrent finding in parasitology. It has been linked to high testosterone levels which can impair the immune response (Klein 2004) and to different sizes of home range according to sex (Le Louarn and Quéré 2003). The probability for a small mammal of encountering *I. ricinus* larvae appeared to be greater when the weather was relatively dry (2 d max humidity: 82–89%). This could be interpreted as an effect of humidity on questing height or mobility, which in turns affects which species the ticks can feed on (Randolph and Storey 1999). The higher humidity, the higher up the tick would quest on the vegetation and the most likely they are to quest above small mammals height. Moreover, ticks, especially larvae, are far less mobile in wet condition (Perret et al. 2003). Alternatively, the difference in humidity could reflect a trapping session effect, as more small mammals were trapped in dry days in April and June than in September, when tick burden was the lowest in small mammals (trapping session was significantly correlated to tick burden in univariate test,  $P$ -value = 0.036) and when questing larvae are less frequent (data not shown).

The two other main tick species found on small mammals, *I. acuminatus* and *I. trianguliceps*, which inhabit burrows (Hillyard 1996), are never found on the vegetation (Gilot et al. 1992), but could be involved in pathogen transmission (Bown et al. 2003; Bown et al. 2006). In the Combraillies area, *I. trianguliceps* appeared to be of low importance as the species was not abundant, while *I. acuminatus* was located in a single site (site 1). However, the epidemiological role of *I. acuminatus* must not be overlooked in this site where it outnumbered 2-fold *I. ricinus* found on the trapped animals.

In the present study, questing tick and small mammal abundances overall varied the same way in the environment. Although *I. ricinus* were more abundant in woodland and small mammals appeared more concentrated in hedgerow, they were both relatively rare on pasture. However, the site with the highest small mammal abundance (site 3) did not display the highest density of questing ticks (site 4). Such discrepancy has previously been obtained for *Peromyscus leucopus* and *Ixodes scapularis* in the United States (Lindsay et al. 1999). The host role for other species than small mammals should be investigated to have a complete overview of the relative contribution of *I. ricinus* hosts. Using the detection of host DNA in questing nymphs, rodents (Pichon et al. 2006) and artiodactyls (Morán

Cadenas et al. 2007) were shown to be the main larvae hosts in continental European forests. The presence of wild animal species, possibly involved in the *I. ricinus* cycle at the woodland–pasture interface, has been investigated in the study sites (Vourc'h et al. 2008). Roe deer (*Capreolus capreolus*), which was present in the 4 sites and the 3 habitats (pasture, woodland and hedgerow), as did 3 other mammals (*Lepus europaeus*, *Martes martes*, *Vulpes vulpes*) and 6 ground foraging bird species (*Buteo buteo*, *Strix aluco*, *Corvus corone*, *Garrulus glandarius*, *Columba palumbus*, *Fringilla coelebs*, *Picus viridis*), could be hosts for *I. ricinus* in our study conditions. Moreover, grazing cattle, which are important hosts for adult *I. ricinus*, are likely to contribute to the maintenance of tick populations on pastures.

In multi-host pathogen system, assessing the relative contribution of host species to the circulation of the pathogen is one of the major challenges facing epidemiologists (Haydon et al. 2002). The woodland–pasture ecotone could be considered as a model to study the role of the wildlife-human-domestic animal interface (Cleaveland et al. 2001) in the emergence of tick-borne diseases and other epidemiological pathways including a complexity of hosts and pathogens (Despommier et al. 2007). In this current study, *A. sylvaticus* was highlighted as the most important of the small mammal species we studied as tick larvae carrier contributing to the maintenance of tick populations on pastures at the woodland–pasture interface. This species is also known to be a reservoir for *Borrelia burgdorferi* s.l. (Kurtenbach et al. 1998; Humair et al. 1999), for tick-borne encephalitis virus (Randolph et al. 1999) as well as for *Mycobacterium microti* which has been implicated in severe forms of human tuberculosis (Cavanagh et al. 2002). Moreover, this rodent could play a significant role in the ecology of leptospires (Treml et al. 2002). Further effort should focus on the study of the pathogen distribution in the hosts and landscape, the pathogen genetic diversity and its possible link to hosts and landscape, and the potential role of other vertebrate species at different scales. Such studies undoubtedly require a high level of multidisciplinary cooperation (Stephens et al. 1998) and material means, and require solving numerous methodological biases regarding scale, biological sampling, analytical markers and modeling procedures.

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