

Seasonal feeding activity of the tree-hole tick, *Ixodes arboricola*

D. J. A. HEYLEN^{1*}, A. R. VAN OOSTEN¹, N. DEVRIENDT², J. ELST¹, L. DE BRUYN^{1,3}
and E. MATTHYSEN¹

¹Evolutionary Ecology Group, Department of Biology, University of Antwerp, Groenenborgerlaan 171, 2020 Antwerp, Belgium

²Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium

³Research Institute for Nature and Forest (INBO), Kliniekstraat 25, 1070 Brussels, Belgium

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SUMMARY

Bird-specific ticks do not infest humans and livestock, but these ticks often share their avian hosts with generalist ticks that do. Therefore, their feeding activity may have an impact on the transmission of pathogens outside bird–tick transmission cycles. Here we examined the seasonal feeding activity of the tree-hole tick (*Ixodes arboricola*) in relation to the activity of its hole-breeding hosts (*Parus major* and *Cyanistes caeruleus*). We analysed data on ticks derived from birds, on the abundance of engorged ticks inside nest boxes, and on bird nests that were experimentally exposed to ticks. We observed a non-random pattern of feeding associated with the tick instar and host age. The majority of adult ticks fed on nestlings, while nymphs and larvae fed on both free-flying birds and nestlings. Due to their fast development, some ticks were able to feed twice within the same breeding season. The highest infestation rates in free-flying birds were found during the pre-breeding period and during autumn and winter when birds roost inside cavities. Except during winter, feeding of *I. arboricola* overlapped in time with the generalist *Ixodes ricinus*, implying that tick-borne microorganisms that are maintained by *I. arboricola* and birds could be bridged by *I. ricinus* to other hosts.

Key words: *Ixodes arboricola*, *Ixodes ricinus*, phenology, *Borrelia*, *Rickettsia*, Lyme borreliosis.

INTRODUCTION

In parasitology, there is increasing interest in host-specialized ticks that support enzootic cycles of pathogens of medical and veterinary importance (Piesman and Gern, 2004; Bown *et al.* 2006; Norte *et al.* 2013). Although these ticks generally do not infest humans and livestock, their hosts are often shared by generalist ticks that do. Consequently, pathogens of host-specialized ticks may be bridged via generalist ticks to other hosts outside the enzootic cycles (Piesman and Gern, 2004). To gain more insight into the transmission dynamics of tick-borne pathogens, knowledge is required concerning host use and seasonal feeding activity of specialized ticks in comparison with that of the co-occurring generalist ticks. Information about the temporal overlap in feeding activity is all the more important when pathogen transmission is limited in time due to the acquisition of host resistance after an infection, or in the situation when transmission depends on co-occurring ticks that feed in close proximity on the same host (i.e. ‘co-feeding’ transmission) (Gern and Rais, 1996; Randolph *et al.* 1996). More generally,

understanding the life history of ticks is essential to predict which hosts are most likely to be exposed to pathogens. In addition, since ixodid ticks have a very low intrinsic mobility and disperse mainly through the host, the timing of feeding and selection of hosts (e.g. nestlings versus adult birds) may determine the dispersal of pathogens.

Ixodes arboricola Schulze and Schlottko (1929) is an endophilic bird-specialized tick of potential significance as a vector of pathogens for both livestock and humans, as it carries *Rickettsia* sp. and *Borrelia burgdorferi* s.l. bacteria (Thorud, 1999; Spitalska *et al.* 2011; Heylen *et al.* 2013c) and shares several host species with the generalist tick *Ixodes ricinus* L., which is the main European vector of tick-borne diseases (Gray, 1991, 1998; Hillyard, 1996; Heylen *et al.* 2013a). *Ixodes arboricola* is widely distributed in the Palearctic region, from central, western and northern Europe, eastward to Latvia and the European parts of Russia (Hudde and Walter, 1988; Liebisch, 1996). Its entire life cycle is restricted to natural and man-made cavities, where it infests roosting and breeding birds (Arthur, 1963; Hillyard, 1996) and where it detaches after feeding (Heylen and Matthysen, 2010; Heylen *et al.* 2012; White *et al.* 2012). As in all ixodid ticks, every instar (larva, nymph and adult female; adult males do not feed) takes a single blood meal lasting several days before

* Corresponding author: Evolutionary Ecology Group, Department of Biology, University of Antwerp, Groenenborgerlaan 171, 2020 Antwerp, Belgium. E-mail: Dieter.Heylen@uantwerpen.be

detaching and moulting to the next development stage, and thus spends at least 90% of its life off-host (Hillyard, 1996). After feeding and detachment, fertilized females deposit a clutch of several hundreds of eggs (160–800 eggs per female; Liebis, 1996; Heylen, 2011), from which larvae will emerge. *Ixodes arboricola* has been found on birds throughout the year, even during the coldest winter months (Walter *et al.* 1979; Literak *et al.* 2007), but details on the seasonal feeding activity of the different life stages are lacking.

The great tit (*Parus major* L.) and blue tit (*Cyanistes caeruleus* L.) are amongst the principal hosts of *I. arboricola* in NW Europe (Walter *et al.* 1979; Hudde and Walter, 1988), and are also frequently infested by the generalist *I. ricinus* (Heylen, 2011; Heylen *et al.* 2013a). These small songbirds inhabit woodlands and gardens, and nest and roost in natural cavities and nest boxes (Perrins, 1979). During the pre-breeding period (early March–early April) birds prospect potential nest sites and establish breeding territories. Nest building starts in the beginning of April, and females lay their eggs once the nest is finished. Incubation, lasting approximately 2 weeks, is performed by the female exclusively. The female continues to warm the chicks until they are homoiothermic (i.e. 8–10 days old and able to maintain a relatively constant body temperature of about 30–40 °C; Mertens, 1969; Dunn, 1975). In total, during the breeding season, parents and their young occupy a single cavity for approximately 6 weeks (Gosler, 1993). Nestlings fledge when they are approximately 3 weeks old, after which the cavity is not used any more for the coming months, except in the case of second broods, which have become increasingly rare in western European populations in the past decades (Visser *et al.* 2003; Matthysen *et al.* 2011). In summer (July–September), birds roost outside and seldom make use of cavities (Kluijver, 1951; Hinde, 1952; Perrins, 1979; Cramp and Perrins, 1993; Gosler, 1993). From early autumn until the end of winter (October–February) birds roost individually in cavities (Perrins, 1979; Gosler, 1993). During this period, birds tend to return to the same cavity every day (Tyller *et al.* 2012), although competition for roost sites or external disturbance may cause shifts between cavities (Perrins, 1979; Gosler, 1993).

In the current study we describe the feeding activity of *I. arboricola* in relation to the seasonal activity pattern of great and blue tits. We make use of observational data on ticks found on captured birds, as well as the year-round abundances of engorged and unfed *I. arboricola* stages inside tit nest boxes. In addition, we analysed the tick's feeding activity and developmental rates in nest boxes that were experimentally exposed to ticks of all developmental stages at the start of the birds' breeding cycle and that were monitored with high temporal resolution.

MATERIALS AND METHODS

Observational data

Fieldwork was carried out from 2006 to 2013 in four deciduous woodland areas, all within 50 km from the centre of the city of Antwerp: Boshhoek (BH; 51°08'42"N, 4°30'38"E), Peerdsbos (PB; 51°16'33"N, 4°29'17"E), Park de Warande (WA; 51°17'52"N, 4°43'40"E) and Wortel Kolonie (WK; 51°23'59"N, 4°50'07"E). Two areas (BH and PB) have been subject to long-term population studies on great and blue tits for many years (Matthysen *et al.* 2001, 2011). All study areas are provided with wooden nest boxes, which are used mainly by great and blue tits, and in which *I. arboricola* ticks have been observed. The removable nest box lids allow easy inspection of the nest cup and the nestlings, as well as detection of *I. arboricola* ticks that, after engorgement, typically migrate to the upper zones of the nest box (i.e. 'negative geotropism') (D. Heylen, unpublished observations).

In total, 1153 tit nest boxes were repeatedly screened for engorged and unfed *I. arboricola* ticks in one or more years (BH: Feb. 2008–Feb. 2011; PB: Nov. 2007–Dec. 2010; WA: Jun. 2011–Feb. 2013; WK: Nov. 2011–Mar. 2013). Inspections were done at distinct times with respect to the birds' seasonal activity pattern and cavity use: in March, before the breeding season commences and when birds inspect potential breeding sites ('Pre-breeding'); in April–June, during the breeding season ('Breeding'); in July–September, when birds seldom occupy nest boxes ('Summer'); and in October–February, when nest boxes are frequently used for roosting ('Autumn–Winter'). Even though nest boxes were specifically inspected to find ticks, unfed larval and nymphal stages can still be easily overlooked because of their small size compared with engorged ticks or unfed adults.

Free-flying birds were captured throughout the year (2007–2012) in BH and PB (9172 captures; average time interval between capture session \pm s.d.: 4.8 ± 10.3 days; range: 1–127 days) and inspected for ticks to define the prevalence of ixodid ticks. Birds were captured under license of the Flemish Ministry (Agentschap Natuur en Bos). Breeding birds were captured inside nest boxes with nest traps when feeding 8-day-old nestlings (4243 captures), and mist nets were used through the remainder of the year to capture free-flying birds (2601 captures). During autumn and winter, additional birds were screened at night when roosting in nest boxes (2328 captures). The head region of captured birds was systematically screened for ticks while holding the beak of the bird between thumb and forefinger and blowing and brushing the birds' feathers apart (Heylen *et al.* 2009). Any ticks found on a random subset of 1297 birds were removed using tweezers and stored in 70% ethanol, in order to define the proportion of the tick

species and their developmental stages. Species, developmental stage, sex (only for adult ticks) and engorgement status were determined using a stereomicroscope and identification keys (Arthur, 1963; Hillyard, 1996). After inspection for and removal of ticks, the birds were released.

Experimental data

Field experiments were carried out to investigate feeding activity and developmental rates of each developmental stage of *I. arboricola*. From the start of the birds' breeding season onwards, nest boxes occupied by great tits were monitored for engorged, moulted and unfed ticks after newly constructed nest cups had been exposed to unfed ticks. Experiments were carried out in two woodlots within BH (April 2010–February 2012). To minimize the interference of naturally occurring ticks, nest boxes were checked for ticks and any ticks found were removed just before the start of the breeding season of 2010. In addition, nest material from previous breeding seasons – which could be infested with *I. arboricola* – was removed. We released high numbers of ticks of different stages inside the newly constructed nest cups at the start of the birds' breeding season and counted the number of engorged ticks in the nest box at regular time intervals. Nestlings were not inspected for tick infestations. Although ticks that attached to parental birds were not removed for identification at capture on the 8th day in the nestlings' development, a reliable visual estimate of the conspicuous and morphologically distinctive adult female ticks could still be obtained. We can be sure that these adult females are not *I. ricinus* adults, as latter stages have never been observed on great tits under natural circumstances (Heylen *et al.* 2013a) and only feed successfully on larger mammals (Gray, 1991). During both breeding seasons, nest boxes were screened nine times in succession, with an average interval of 6.4 ± 1.9 days between each check. We considered this time resolution as sufficiently high, because the feeding period (days) for larvae (mean \pm s.e.: 3.64 ± 0.21), nymphs (3.84 ± 0.02) and adult females (5.29 ± 0.16) under field conditions (Heylen and Matthysen, 2011) is only a few days shorter. Outside the breeding season, when the occupancy of the nest boxes is lower, we checked nest boxes 18 times (2010) and 17 times (2011) with an average interval of 15.7 ± 4.7 days.

For the engorged larvae and nymphs, we estimated the number of days until moult to the next developmental stage based on the observations of exuviae and unfed stages. For the engorged adult females, we estimated the duration until egg deposition, and the duration until the emergence of the larvae. For the estimation of the developmental rates under field conditions, the duration of moulting (larvae and nymphs) is based on the moment when 50% of the engorged ticks had moulted.

The ticks used for this experiment originated from a laboratory colony originally collected during the winter of 2007–2008 from nest boxes (BH and PB) (for details, see Heylen *et al.* 2012). At the start of the breeding season of 2010, 25 nymphs were released in each of 11 nest cups. At the start of the breeding season of 2011, in each of 17 nest cups we added a mix of three adult females, two adult males and a batch of larvae (mean \pm s.d.: 101.8 ± 59.3 larvae per nest cup). Males were added to the nest cups because in some ixodid tick species, adult females tend not to attach and/or complete the blood meal unless males of the same species are present (Rechav *et al.* 1997; Sonenshine, 2004; Weiss and Kaufman, 2004; Donohue *et al.* 2009). Before release, adult females and males were put together in vials (15 females with 10 males in each vial) for 2 weeks, to give them the opportunity to mate without the possible interference of the host.

Statistical analysis

For the observational data from bird captures, when testing hypotheses on tick prevalences in relation to season and bird species, generalized linear mixed models with logit-link and binomial distributed residuals were fitted (procedure PROC GLIMMIX in SAS v 9.2, SAS Institute, Cary, NC) while controlling for study area and year as a random factor. When residuals showed high over-dispersion (i.e. Pearson Chi square/residual $df \geq 1.5$), standard errors of the main effects were adjusted by the scale parameter (Molenberghs and Verbeke, 2005; Zuur *et al.* 2009). As the ticks of only a random subset of 1297 birds were isolated for identification (see above), we derived the total number of captured birds that matches this subset of infested birds basing ourselves on the prevalence of *Ixodes* sp. infestations that was obtained from the complete set of 9172 bird captures. $\alpha = 0.05$ was chosen as the lowest acceptable level of significance. Odds ratio estimates are reported with their 95% confidence limits. All other estimates are reported as mean \pm s.e., unless otherwise mentioned.

RESULTS

Observational data from bird captures

The marginal prevalences (based on the pooled set of captures over the two study areas) of *I. arboricola* and *I. ricinus* infestations in free-flying birds in relation to the birds' seasonal activity pattern are presented in Fig. 1. There were no differences between great tits (P.m.) and blue tits (C.c.) in the prevalence of *I. arboricola* infestations in any of the seasons (Odds ratio (OR)_(P.m.–C.c.): 1.18 (95% confidence interval limits: 0.75–1.87); $t = 0.73$; $df = 46$; $P = 0.47$). The prevalence of *I. arboricola* infestations differed among seasons ($F_{3,46} = 3.33$, $P = 0.03$). The highest

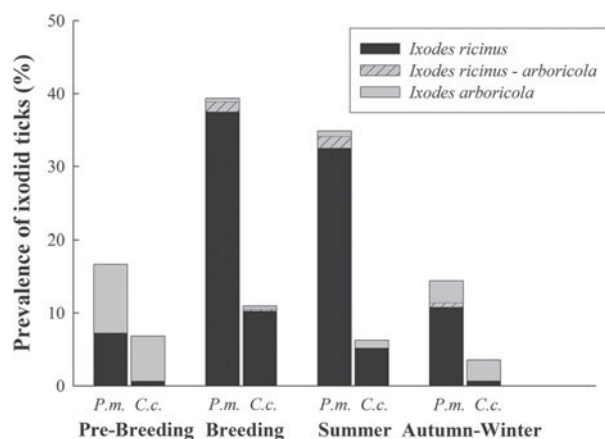


Fig. 1. Infestation prevalence of *Ixodes arboricola* and *I. ricinus* in free-flying great tits (*P.m.*) and blue tits (*C.c.*) in relation to the birds' seasonal activity pattern. *Ixodes* sp. overall prevalence was obtained from 9172 bird captures, while the relative proportion of each tick species is based on tick collections of a random subset of 1297 infested birds.

prevalence was observed during the pre-breeding season (average prevalence over two study areas in great tits: $7.5 \pm 0.4\%$, $N = 80$; blue tits: $3.3 \pm 3.3\%$, $N = 136$), while the ticks on the free-flying birds were least prevalent during the breeding season (great tits: $1.9 \pm 0.4\%$, $N = 1037$; blue tits: $0.8 \pm 0.6\%$, $N = 898$). In both bird species, the prevalence of *I. arboricola* adult females was much lower than that of the immature developmental stages (Fig. 2), whereas the prevalence of larvae tended to be higher than that of the nymphs for any season ($OR_{(larva-nymph)}$: 1.46 ($1.01-2.13$); $t = 2.02$; $df = 86$; $P = 0.05$). Feeding *I. arboricola* adult females were not observed in summer. On one great tit individual (autumn-winter) the three developmental stages were co-feeding. In the infested birds, the mean infestation intensity varied both for great tits (larvae: 4.4 ± 1.1 (range: 1-46); nymphs: 2.5 ± 0.6 (1-22); and adult females: 1.8 ± 0.8 (1-5)) and for blue tits (larvae: 6.4 ± 2.1 (1-47); nymphs: 1.6 ± 0.4 (1-8); and adult females: 1.0 ± 0.0).

In contrast to *I. arboricola*, the prevalence of *I. ricinus* infestations (Fig. 1) was consistently higher in great tits ($OR_{(P.m.-C.c.)}$: 5.25 ($3.32-8.29$); $t = 7.33$; $df = 39.89$; $P < 0.001$). *Ixodes ricinus* was most prevalent during the breeding season (great tits: $39 \pm 1\%$, $N = 1037$; blue tits: $11 \pm 1\%$, $N = 898$) and summer (great tits: $39 \pm 28\%$, $N = 400$; blue tits: $6 \pm 3\%$, $N = 74$), and lowest during autumn-winter (great tits: $7 \pm 1\%$, $N = 1812$; blue tits: $0.9 \pm 0.1\%$, $N = 904$). The mean infestation intensity of immature developmental stages varied both for great tits (larvae: 4.4 ± 0.3 (range: 1-48); nymphs: 2.1 ± 0.1 (1-17)) and for blue tits (larvae: 1.5 ± 0.2 (1-4); nymphs: 1.3 ± 0.1 (1-5)). In this tick species, we did not observe any adult female feeding on a bird.

Mixed infestations, i.e. single hosts carrying both *I. arboricola* and *I. ricinus* ticks, were observed for

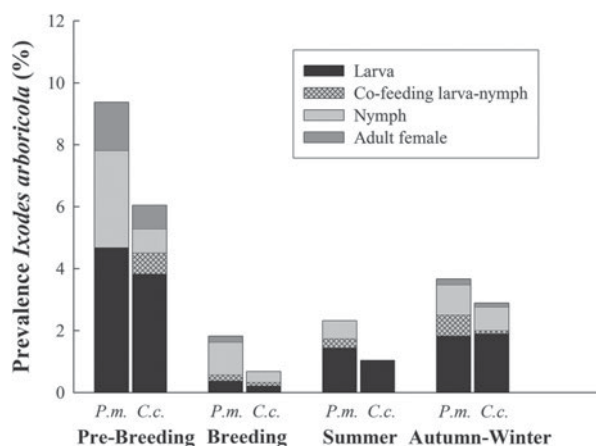


Fig. 2. Infestation prevalence of the different developmental stages of *Ixodes arboricola* in free-flying great tits (*P.m.*) and blue tits (*C.c.*) in relation to the birds' seasonal activity pattern. Larvae or nymphs co-feeding with adult females are not indicated separately.

great tits in all seasons except the pre-breeding season (range prevalence: $0.3-2.7\%$), and for blue tits in the breeding season only (0.5%).

In addition to *I. ricinus* and *I. arboricola*, we collected *Ixodes frontalis* Panzer (1795) from both bird species (prevalence in great tits: 1.6% ; blue tits: 0.5%). On the *I. frontalis* infested birds, we collected individuals belonging to the three developmental stages: larvae (mean infestation intensity great tits: 1.9 ± 0.3 (1-8); blue tits: 1.1 ± 0.1 (1-2)); nymphs (great tits: 1.1 ± 0.1 (1-2); blue tits: 1.0 ± 0.0); and to a lesser extent adult females (great tits: 1.0 ± 0.0 ; blue tits: 1.0 (only one individual)).

For all ixodid tick species, adult males have never been observed on any of the free-flying birds.

Observational data from nest boxes

The average counts per nest box over the four study areas for engorged and unfed developmental stages in relation to the birds' seasonal activity pattern are presented in Fig. 3. The area's proportion of nest boxes in which one or more stages of *I. arboricola* were observed over the total observation period was $30 \pm 8\%$. Of all the observed engorged females ($N = 142$ individuals) and nymphs ($N = 430$), the majority (average proportion: $82 \pm 6\%$ and $64 \pm 13\%$ for females and nymphs, respectively) was found during the birds' breeding season. Of the engorged larvae ($N = 2006$) the highest proportions were found during the pre-breeding period ($47 \pm 11\%$) and autumn-winter period ($32 \pm 11\%$), while very low numbers were found during the summer months ($0.5 \pm 0.5\%$). The four study areas showed similar seasonal distributions of engorged tick stages, as was shown by the high pairwise correlation coefficients of the tick proportions over time (Pearson's rho: $0.52-0.84$; P -values: $0.08-0.003$).

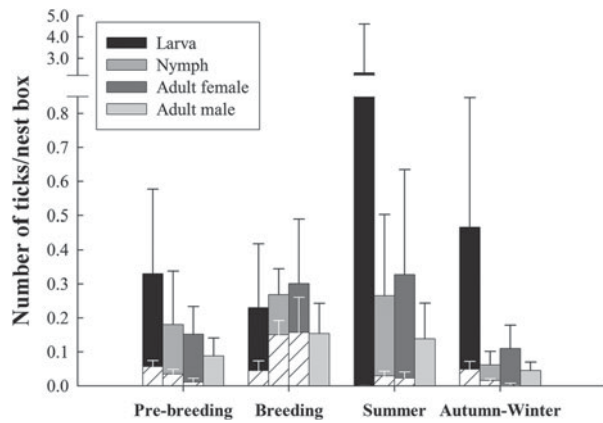


Fig. 3. Average counts of *Ixodes arboricola* stages per nest box in relation to the birds' seasonal activity pattern. Data were obtained from regular checks of 1153 nest boxes used by great and blue tits in four study areas. Shaded white bars represent the average number of fed stages. Counts for larvae have been divided by 10. Note that counts of unfed larvae and nymphs were probably unreliable (See 'Materials and Methods' section for details).

In adult females, the proportion of engorged individuals was higher during the breeding season than in other seasons (range $OR_{(\text{breeding} - \text{other season})}$: 16.1–29.1; all $P < 0.001$; Fig. 3). In the larvae and nymphs, proportions of engorged ticks were higher in both the pre-breeding and breeding season than in summer (range $OR_{(\text{pre-breeding} - \text{summer})}$: 9.1–120.1; $P < 0.069$; range $OR_{(\text{breeding} - \text{summer})}$: 7.8–311.9; $P < 0.02$). Furthermore, the proportion of engorged larvae during the pre-breeding season was higher than that from the autumn–winter period ($OR_{(\text{pre-breeding} - \text{winter})}$: 5.6 (1.8–17.1); $t = 3.22$; $df = 17.32$; $P = 0.005$). None of the other pairwise comparisons between seasons were significant in any of the developmental stages.

Experimental data

The proportion of released ticks that were found engorged was $36 \pm 15\%$ for adult females ($N = 17$ nests), $15 \pm 3\%$ for nymphs ($N = 11$ nests) and $0.9 \pm 0.4\%$ for larvae ($N = 17$ nests). All engorged adult females were found more than one week after hatching of bird eggs. In contrast, most of the engorged immature stages were found during the pre-hatching phase of the breeding cycle (larvae: $80 \pm 2\%$, nymphs: $86 \pm 7\%$). In three nest boxes in which unfed nymphs were introduced, two engorged adult females per nest were observed before the end of the nestling phase, indicating individual ticks that successfully completed two feeding stages. The single observation of a feeding adult female tick that was found on a parental bird when nestlings were 8 days old (in 2010) is likely to have originated from an unfed nymph as well. Similarly, in six of the 17 nests

Table 1. Development durations of *Ixodes arboricola* ticks fed on *Parus major* nestlings

	Field conditions	Lab conditions
Development durations (days)		
Larva–Nymph	34.6 ± 4.3 ($N = 3$)	12.8 ± 0.3 ($N = 24$)
Nymph–Adult	48.0 ± 4.7 ($N = 20$)	13.3 ± 0.1 ($N = 90$)
Female–Eggs	63.0 ± 4.0 ($N = 7$)	11.3 ± 0.7 ($N = 26$)
Eggs–Larvae	91.5 ± 23.5 ($N = 2$)	29.9 ± 0.5 ($N = 23$)

Data are presented as mean \pm s.e. For the development under field conditions, the duration of moulting (for larvae and nymphs) is based on the moment when 50% of the engorged ticks have moulted. N equals the number of nests and the number of ticks for the moulting (larvae and nymphs) and the development of larvae from the engorged female, respectively. For development under lab conditions (data from Heylen and Matthysen, 2011), N equals the number of ticks. Lab conditions: 25 °C, 83% RH, light/dark: 0 h/24 h.

where unfed larvae were introduced, 1–10 engorged nymphs per nest emerged before the end of the nestling phase.

The estimated duration to moult in larvae and nymphs, as well as the duration to egg deposition and hatching of larvae, are presented in Table 1, together with the developmental durations obtained under lab conditions from previous work.

DISCUSSION

The purpose of our study was to investigate the feeding activity in different stages of the tick *I. arboricola* in relation to the seasonal activity pattern of their songbird hosts. We observed a non-random pattern of feeding associated with the tick's developmental stage and the host's age. The most obvious finding was that newly engorged adult females were mainly observed during the breeding season after nestlings hatched, while engorged immature stages (nymphs and larvae) were detected whenever the birds used cavities for either breeding or roosting. At an ultimate level, temporal variation in infestations may reflect adaptive timing adjustments with respect to the presence and biology of the hosts. On the other hand, at a proximate level, these non-random infestation patterns may have resulted from temperature-dependent development rates and host-finding activity. Similar to most physiological processes in poikilothermic animals, mobility and inter-stadial development rates of ticks increase with increasing ambient temperature (Randolph, 2004). Both points of view will be discussed below.

The majority of adult female ticks must have fed on nestlings. Although not directly observed, this is indicated both by the observational data and by the field experiment, where newly engorged females were found mainly during the nestling phase and rarely

attached to parental birds. Also outside the breeding season, the adult female ticks are seldom found on free-flying birds (Walter *et al.* 1979; Hudde and Walter, 1988; Literak *et al.* 2007; Spitalska *et al.* 2011) even though unfed females can be found inside the nest boxes when birds are roosting (Fig. 3). In a previous experiment, it was shown that unfed adult females delay their attachment until the nestlings grow older (Heylen *et al.* 2012). These nestlings are likely to be more profitable hosts than younger ones, because they provide good resources, while showing low immunological resistance. In addition, the older nestlings have better developed feathers under which ticks are sheltered (Heylen and Matthysen, 2011; Heylen *et al.* 2012). On the other hand, the low prevalence of adult ticks in free-flying birds could be the result of the better developed grooming and preening skills, leading to a higher host-induced tick mortality (Heylen and Matthysen, 2010).

In contrast to adult female ticks, feeding and engorged immature developmental stages were found throughout the year. In the field experiment, they showed a high readiness to attach to the parental birds when nestlings were not yet present. However, previous experimental studies have shown that nestlings can become successfully infested as well, even at a young age (Heylen and Matthysen, 2011; Heylen *et al.* 2012). Due to their body size, the smaller immature tick stages may find refuges from early in the bird's development onwards, which may explain their high readiness to attach to recently hatched nestlings as well as older birds like the parents and full-grown birds outside the breeding seasons (Hudde and Walter, 1988; Literak *et al.* 2007; Heylen and Matthysen, 2010).

At a proximate level, the temporal infestation patterns may have resulted from temperature-dependent host-finding activity and development rates. The occupation of nest boxes and tree holes by roosting and brooding birds increases the inside temperature, which in turn may trigger and facilitate larval and nymphal infestations, even during the coldest winter months (Figs 2 and 3). The improvement of thermoregulation with nestling development – which in great tits is believed to start from day 8 after hatching (Perrins, 1979) – further elevates the nest box temperature, stimulating the adult female to search for nestlings. The high temperatures during the breeding season probably also facilitate the inter-stadial developments, and hence make it possible for ticks to feed again before the nestlings fledge (cf. life cycle of the endophilic *I. lividus* L. infesting bank swallows (*Riparia riparia* L.); Balashov, 1972; Ulmanen *et al.* 1977). In our study, several nymphs successfully fed as an adult female within the same breeding cycle, due to their short developmental durations (Table 1; Liebis, 1996). Similarly, larvae show short developmental durations, and hence, can re-infest the birds in the same nest box. We mention that the

obtained information on the feeding of larvae was limited as a result of the unusually low larval infestation success compared with previous experimental field studies (Heylen and Matthysen, 2011; Heylen *et al.* 2012). Unfavourable abiotic conditions or exhaustion of energy reserves could be the reasons for the low infestation success observed here. Unfed larvae have remained for at least 10 months in the climate rooms before release. As larvae age, and energy stores are depleted, so too does their host-finding ability (Gray, 1991; Randolph *et al.* 2002).

The highest *I. arboricola* prevalence in free-flying birds was observed in the pre-breeding season (beginning of March – start of April), which could have resulted from the increasing ambient air temperatures that stimulate the ticks to search. Although the summer temperatures should be sufficiently high for the host-finding activity as well, we observed low numbers of recently engorged ticks in nest boxes (Fig. 3) and low infestation prevalences in the free-flying birds (Figs 1 and 2; see also Hudde and Walter, 1988). An explanation for the latter observation could be the fact that after the nestlings have fledged, birds generally roost in the open (Hinde, 1952), and therefore get less frequently exposed to tree-hole inhabiting *I. arboricola*.

When interpreting the observed feeding patterns in combination with the birds' activity, we hypothesize that most of *I. arboricola*'s dispersal events occur in the immature developmental stages, from autumn (when birds start to roost inside cavities) until the pre-breeding season (when birds prospect potential breeding sites). During the breeding season, free-flying birds are territorial, and although parents and nestlings are frequently infested, they do not leave their small territories. Although the bird's post-fledging dispersal during the summer ranges over a large scale (Van Overveld *et al.* 2011), due to the low *I. arboricola* exposure, birds contribute little to the tick's dispersal. From autumn onwards, birds start to roost inside cavities, resulting in frequent infestations. Birds switch between roosting sites due to competition with other birds (Perrins, 1979; Gosler, 1993) or external disturbances (Tyller *et al.* 2012), which facilitates the tick colonization of new cavities. Since *I. arboricola* has a low intrinsic mobility, the behaviour of the host strongly determines its dispersal. By delayed detachment in response to unsuitable environmental conditions, immature developmental stages have the capability to bridge long periods when birds move between successive home ranges and spend several nights outside cavities (Heylen and Matthysen, 2010; White *et al.* 2012).

The prevalence of *I. arboricola* in free-flying birds was similar for great and blue tits, which can be explained by the fact that both bird species use cavities for roosting and breeding to the same extent (Perrins, 1979). In both bird species, the infestation

pattern of *I. arboricola* was different from the generalist and non-nidicolous *I. ricinus*. The prevalence of *I. ricinus* was consistently higher in free-flying great tits, probably because great tits are more inclined than blue tits to forage at low heights inside the habitat of this generalist tick (Comstedt *et al.* 2006; Heylen *et al.* 2010). *Ixodes ricinus* infestations in nestlings are commonly very low (Gallizzi *et al.* 2008), since this tick species cannot access the nestlings directly as it is restricted to the surface of the litter layer in the woodland (Mejlon and Jaenson, 1997; Gray, 1998) away from the tree holes where the nestlings grow up (Nilsson, 1984). Feeding activities of the two tick species overlapped in time (Fig. 1), except for the winter period, when there is little questing activity of *I. ricinus* (Gray, 1991; Randolph *et al.* 2002). As climatic conditions affect the development and seasonal activity of *I. ricinus* (Gray, 1991; Randolph *et al.* 2002) and possibly also that of *I. arboricola*, the overlap in time may vary among different habitat types and climatic regions.

Previous studies have shown that the great tit is a reservoir for *B. burgdorferi* s.l. spirochaetes (Heylen *et al.* 2013b) and that bird-fed *I. arboricola* ticks carry these bacteria and may possibly transmit them to uninfected hosts (Thorud, 1999; Heylen *et al.* 2013c). *Borrelia* spirochaetes and other tick-borne pathogens (e.g. *Rickettsia* spp.; Spitalska *et al.* 2011) may therefore be maintained partly by *I. arboricola*, and bridged to other hosts outside the bird–*I. arboricola* cycle by generalist ticks such as *I. ricinus*. To assess the importance of *I. arboricola* as a vector of pathogens, experimental studies (cf. Heylen *et al.* in press) are required to evaluate its vector-competence, i.e. its capacity to carry and transmit the pathogens to new hosts.

In conclusion, our findings show significant differences in host use and seasonal activity pattern between the specialized *I. arboricola* and generalist *I. ricinus* ticks that are clearly related to the nidicolous lifestyle of the former. Nevertheless, there is also considerable seasonal overlap leading to cases of co-feeding and potential for transmission of tick-borne pathogens between the two tick species. Further experimental studies are required to get a better insight into the temperature-dependent development rates and host-finding activity of the ticks, and to establish whether the non-random infestation patterns are the result of adaptive timing adjustments that optimize the tick's fitness, or are only due to the development rates and availability of unfed stages.

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