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The more the merrier : experimental evidence for density-dependent feeding facilitation in the bird-specialised tick *Ixodes arboricola*

Reference:

Van Oosten Raoul, Matthysen Erik, Heylen Dieter.- The more the merrier : experimental evidence for density-dependent feeding facilitation in the bird-specialised tick *Ixodes arboricola*

International journal for parasitology - ISSN 0020-7519 - 46:3(2016), p. 187-193

Full text (Publishers DOI): <http://dx.doi.org/doi:10.1016/j.ijpara.2015.11.002>

To cite this reference: <http://hdl.handle.net/10067/1318760151162165141>

1 The more the merrier – experimental evidence for density-dependent feeding facilitation in the bird-
2 specialised tick, *Ixodes arboricola*

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Abstract

Similar to many other parasites, the distribution of ticks among hosts is strongly skewed, with few hosts harbouring the majority of parasites. Because parasite-induced impairment of host health, parasite population growth and pathogen transmission are density-dependent, understanding why tick distributions are skewed is important for the population and evolutionary dynamics of both parasite and host. However, there is currently no knowledge concerning parasites that strongly depend on individual hosts. Here, we investigated the effects of tick density on feeding performance in the nidicolous tree-hole tick, *Ixodes arboricola*, which feeds on cavity-nesting birds and is the carrier of several tick-borne pathogens. Nidicolous ticks reside in or close to their hosts' nests and therefore depend strongly on individual hosts and their offspring. Increased feeding success at higher densities (facilitation) may therefore be detrimental to the ticks themselves. We investigated the effects of tick density on feeding performance of *I. arboricola* by infesting great tit nestlings with one to five adult ticks, which is within the natural range. There was no effect of tick density on initial attachment success, attachment after 48 h or engorgement weight, but tick recovery rates increased significantly with tick density. We also found a modest increase in nestling body mass with tick density, suggesting that birds over-compensate resource drainage by the ticks and, by doing this, anticipate the costs of a tick-rich environment. Our results indicate that nidicolous ticks perform better when feeding in aggregation. This may have important consequences for population dynamics and consequently pathogen transmission.

Keywords: *Ixodes arboricola*, Density-dependent facilitation, Competition, Population dynamics, Pathogen transmission, Co-feeding

1. Introduction

From microparasites such as viruses and parasitic protozoa, to macroparasites such as helminthes and ticks, aggregation on hosts is common, with relatively few host individuals harbouring the large majority of parasites (Shaw and Dobson, 1995; Woolhouse et al., 1997; Wilson et al., 1998; Poulin, 2007; Calabrese et al., 2011). The proximate explanation is that parasite aggregation is the result of heterogeneity either in exposure to parasites among individual hosts, which results from the uneven distribution of parasites in space and time relative to hosts, or in susceptibility to parasitism, arising from differences among hosts in behaviour or immune resistance (Poulin, 2013). It has also been suggested that aggregation is beneficial for parasites, and that traits promoting aggregation are favoured by selection. Such ultimate explanations of aggregation include facilitation of feeding and mating, and overwhelming host defences (Sonenshine, 1991; Kiszewski et al., 2001; Poulin, 2007). However, aggregating may also be detrimental for parasite individuals by increasing intraspecific competition, pathogen prevalence, host resistance and mortality, and attraction of predators (Jaenike, 1990; Poulin, 2007). For parasites that have low mobility or transmission, or which strongly depend on individual hosts, selection can operate against traits that promote aggregation when it strongly reduces host survival.

It is largely unknown which underlying mechanisms determine whether aggregation leads to density-dependent facilitation, i.e. increased feeding success with increased parasite density on a host, or competition. Such knowledge is important because many parasites are directly detrimental to their hosts and/or are of potential significance as pathogen vectors for both humans and livestock (Clayton and Moore, 1997; Poulin, 2007). There are three aspects of aggregation that have important implications for the population and evolutionary dynamics of the parasite and its host. First, aggregation may lead to increased parasite population growth due to increased reproduction and interspecific competitive potential, but there may also be intraspecific competition for space or nutrients (Wilson et al., 1998; Poulin, 2007). Second, high parasite densities may have negative effects on hosts (even for bird-associated ticks; see e.g. Luttrell et al., 1996; Monks et al., 2006; Heylen and Matthysen, 2008, 2011a;

Pfäffle et al., 2009) or lead to increased host resistance (Randolph, 1994; Jones et al., 2015) and thus aggregation can have strong effects on host and parasite fitness (Wilson et al., 1998). Finally, co-feeding, i.e. parasites feeding on the same host in time or space, can increase pathogen transmission, even in the absence of systemic infection (Randolph et al., 1996).

Ixodid ticks are haematophagous ectoparasites and important vectors of pathogens including bacteria (e.g. *Rickettsia* spp. and *Borrelia burgdorferi* sensu lato (s.l.)), viruses (e.g. tick-borne encephalitis virus) and protozoans (e.g. *Babesia* spp.) (Gray, 1991; Jongejan and Uilenberg, 2005). Their low intrinsic mobility, lack of adaptations to facilitate direct transmission from one host to the next and the need for blood during a single long-lasting period of several days per instar (larva, nymph and adult) make ticks highly dependent on individual hosts (Sonenshine, 1991). Therefore fitness loss due to host death would be substantial and, indeed, ixodid ticks generally have limited effects on their hosts (Sonenshine, 1991; but see e.g. Luttrell et al., 1996; Monks et al., 2006; Heylen and Matthysen, 2008, 2011a; Pfäffle et al., 2009). Yet, in nature relatively few individual hosts harbour the majority of ixodid ticks (Davidar et al., 1989; Harrison et al., 2012; Moshaverinia et al., 2012; Heylen et al., 2013a).

There are conflicting results from studies that investigated how tick density affects their feeding success. On the one hand, there may have been selection of traits that promote aggregation on the same host due to selective advantages such as increased feeding performance due to shared activities of salivary compounds that suppress host inflammatory responses and prevent haemostasis (Davidar et al., 1989; Wang et al., 2001; Ogden et al., 2002). Several studies on ticks have found increased tick feeding success at higher feeding densities, i.e. feeding facilitation (Sutherst et al., 1978; Davidar et al., 1989; Hazler and Ostfeld, 1995; Wang et al., 2001; Ogden et al., 2002). On the other hand, high densities of feeding ticks can result in a decrease in feeding success due to elevated awareness of the host or resource competition among ticks (Hazler and Ostfeld, 1995). Other studies found competition such that tick feeding success decreased due to increasing tick densities (Sutherst et al., 1979; Randolph, 1994; Levin and Fish, 1998; Bartosik and Buczek, 2012; Jones et al., 2015). The underlying mechanisms that determine whether tick

aggregation on individual hosts leads to facilitation or competition among feeding ticks are not fully understood, but knowledge concerning the effects of aggregation on ticks and their hosts is essential to understand the population dynamics and the epidemiology of disease transmission by ticks.

To date, the effects of aggregation on feeding performance have been investigated in non-nidicolous ticks, but to the best of our knowledge no such data are available for nidicolous ticks (see references concerning non-nidicolous ticks in the previous paragraph). Non-nidicolous ticks can find a new, unrelated host relatively easily and are often exposed to genetically unrelated hosts during the different developmental stages in the tick's life cycle (Hoogstraal and Aeschlimann, 1982; Sonenshine, 1991; Hillyard, 1996). Therefore, if feeding aggregation leads to decreased host health, the effect on tick fitness is relatively limited. In contrast, nidicolous ticks remain hidden inside or near the hosts' nests or burrows and encounter only a limited variety of hosts (Sonenshine, 1991; Hillyard, 1996; Gray et al., 2014). If fitness of nidicolous ticks increases at the expense of host health, ticks may kill or drive away hosts and ultimately seal their own fate (Gray et al., 2014). Therefore, for nidicolous ticks, feeding aggregation may be largely detrimental.

The tick *Ixodes arboricola* Schulze and Schlottke 1930 is nidicolous and its entire life cycle is restricted to tree holes and human-made nest boxes (Walter et al., 1979; Heylen et al., 2014). Thus, it chiefly infests cavity-nesting birds, with great and blue tits (*Parus major*, *Cyanistes caeruleus*) as the principal hosts (Walter et al., 1979; Hudde and Walter, 1988; Petney et al., 2011). Because intrinsic mobility is low and hosts show high site fidelity to single cavities (Gosler, 1993), the ticks' fitness depends on the survival and re-use of cavities by the same hosts and their offspring (Heylen and Matthysen, 2011a). This has been argued to be the reason *I. arboricola* has a lower engorgement weight, longer feeding duration and no effect on host health compared with the congeneric non-nidicolous tick *Ixodes ricinus* (Heylen and Matthysen, 2011a). In nature, ticks aggregate and the majority of *I. arboricola* ticks are found on a limited number of hosts and in a limited number of nests (Heylen and Matthysen, 2011b; Heylen et al., 2012, 2014; Van Oosten et al., 2014a). However, the effects of feeding aggregation

on tick fitness remain unexplored. This knowledge is important to gain a better understanding of the population dynamics of this tick, which is the carrier of *Rickettsia* spp. and *B. burgdorferi* s.l. (Špitalská et al., 2011; Heylen et al., 2013b). Knowledge concerning the mechanisms underlying aggregation is also essential for predicting and possibly controlling parasites in general.

In the current study, we infested nestlings of the great tit with increasing densities, within the natural range, of adult female *I. arboricola*. The infestations were carried out with adult female ticks because this is the largest instar and most important in terms of tick reproduction. We investigated tick attachment and feeding success as well as nestling body mass in order to test whether increased tick feeding densities lead to facilitation or competition among feeding ticks.

2. Materials and methods

2.1. Study species

Ixodes arboricola is widely distributed across Europe (Liebisch, 1996; Petney et al., 2011). The tick goes through three active life stages (larva, nymph and adult) and during each stage, with the exception of adult males, takes a single blood meal either to moult (larvae and nymphs) or to reproduce (Sonenshine, 1991). Adult males do not take a blood meal and remain in the nest box where they copulate with unfed and engorged female ticks (Van Oosten et al., unpublished data). Adult female ticks feed primarily on nestling birds, and are most apparent during the breeding season (Heylen et al., 2014). To obtain a blood meal, ticks attach to a host inside the nest and remain attached for several days (Sonenshine, 1991). Detachment is often in the same nest box because its hosts show high nest fidelity (Gosler, 1993). As such, dispersal capabilities between cavities are limited and relatedness of ticks within cavities can be high (Van Oosten et al., 2014a). Because its entire life cycle is restricted to natural and human-made cavities, *I. arboricola* only infests hosts that make use of such cavities, primarily cavity-nesting birds (Walter et al., 1979; Petney et al., 2011; Heylen et al., 2014). While ticks are generally

uncommon in Belgium (Van Oosten et al., 2014a), high local densities can be reached with up to 167 nymphs, 15 adult females and hundreds of larvae found in individual nest boxes, and up to 46 larvae, 22 nymphs and five adult female ticks on individual great tits (Heylen et al., 2014, unpublished data). Although there is seasonality in tick occurrences on hosts and in nest boxes, all three instars can be found throughout the year (Heylen et al., 2014). The natural prevalence of *I. arboricola* ticks infesting adult great tits has been estimated at 7.5% during the pre-breeding season (early March to early April) but much lower throughout the rest of the year (Heylen et al., 2014).

The great tit (family Paridae) is the most common secondary cavity-nesting bird in western Europe and uses available natural or artificial cavities for breeding and roosting (Gosler, 1993). Great tits use cavities during the breeding season (April to June) to raise young and during winter (late September to early March) for roosting (Gosler, 1993). They lay four to 12 eggs which require 15 to 20 days of incubation, and nestlings fledge at a weight of 14 to 22 g after 18 to 21 days (Gosler, 1993). Apart from infestations with *I. arboricola*, great tits are frequently infested with the conspecific ticks *I. ricinus* and *Ixodes frontalis*. Infestations with multiple tick species may occur simultaneously, especially during the breeding season (Heylen et al., 2014).

2.2. Study location

This study took place in two deciduous woodland areas in northern Belgium during the breeding seasons (April to June) of 2014 and 2015: Peerdsbos, Brasschaat (PB; 51°16'29"N, 4°29'03"W) and the Boshoeck area, Lier (BH; 51°07'43"N, 4°31'52"W). Nest boxes have been present in these areas for many years as a part of long-term population studies on great and blue tits (Matthysen et al., 2001, 2011). The following actions were taken to avoid natural infestations with ticks as much as possible. First, prior to the two breeding seasons during which the experiment took place, nest contents from previous years were removed from the nest boxes. Second, prior to the breeding seasons all nest boxes were checked for

naturally occurring ticks and all ticks were removed. Since *I. arboricola* typically climbs to the upper surfaces of a nest box (negative geotropism, Heylen and Matthysen, 2010), it can be easily detected (in particular the adult and engorged stages). Finally, nest boxes showing any indication of non-experimentally added ticks (larvae and nymphs) during the experiment were excluded from all analyses. It is unlikely that parental birds transferred ticks to the nest at a later stage, which would have influenced our results, for three reasons. First, during the breeding season great tits are unlikely to obtain ticks elsewhere because they show high nest fidelity (Gosler, 1993). Second, such introduced ticks would have been unlikely to be adult female ticks because adult ticks prefer to feed on nestlings (Heylen et al., 2014). Finally, in the current study we never observed non-adult ticks (larvae or nymphs) on inspected nestlings (unpublished data).

In total, we used 137 nest boxes. Infestations in PB were, on average, carried out 6 days later because great tits tend to start breeding later in this area than in BH. Ticks came from a laboratory stock that was established in 2007 with ticks from nest boxes in PB and BH (Heylen and Matthysen, 2010), and maintained by allowing ticks to primarily infest great tits in several studies (e.g. Heylen and Matthysen, 2011a; Van Oosten et al., 2014b).

2.3. Experimental infestations and tick recovery

In each nest, a randomly chosen great tit nestling of 8 days old was removed from its nest and one to five adult female *I. arboricola* ticks were placed under the feathers on the occipital side of the head with a small brush. The infested nestling was placed in a cotton bag, which was placed in the nest. After 1 h, the nestling was removed from the bag and replaced in the nest, and the cotton bag was inspected for unattached ticks. All nestlings in the nest were inspected for attached ticks 48 h after infestation by holding them firmly and brushing the feathers apart with tweezers. Ticks were counted but not removed. Nest boxes were inspected every 2 days. Adult *I. arboricola* ticks typically take at least 5 days to

complete a blood meal (Heylen and Matthysen, 2010, 2011a). Engorged ticks usually crawl to the top of the nest box, which is where they were recovered. Once the nestlings had fledged, the nest material was inspected for ticks that had not crawled to the top of the nest box. Engorged ticks were weighed to the nearest 0.1 mg directly after recovery. To correct for initial size differences between ticks, scutums were measured on a 1 x 1 mm grid with a Leica MZ125 stereomicroscope (Leica Microsystems, Wetzlar, Germany), after engorged females finished egg laying, by dissecting the scutum with tweezers, and standardised to the grid with ImageJ v 1.48. Nestling body mass was recorded on the day of infestation and again when they were inspected for attached ticks (48 h after infestation).

2.4. Statistical analyses

All data analyses were done in R v 3.2.2 (R Foundation for Statistical Computing, Vienna, Austria). We used generalised linear models to evaluate the following response variables: (i) attachment success (i.e. the proportion of ticks feeding on a nestling; logit-link, binomial distributed residuals) after 1 h; (ii) attachment success after 48 h; (iii) tick recovery (i.e. the proportion of ticks collected from nest boxes; logit-link, binomial distributed residuals); and (iv) tick engorgement weight (corrected for scutum-size; identity-link, normally distributed residuals). Covariates in all models included: tick density and nestling body mass, and their two-way interaction (tick density : nestling body mass). Depending on the response variable, different measures of tick density were used as covariates: initial tick density (i.e. the number of ticks placed on a nestling) for attachment success after 1 h; tick density after 1 h for attachment success after 48 h; and tick density after 48 h for the proportion of ticks recovered and engorgement weight. Similarly, different measures of nestling body mass were used: nestling body mass at the moment of infestation for attachment success after 1 h; the relative nestling body mass increase over the experimental period (i.e. the difference in body mass between infestation and inspection relative to nestling body mass at infestation) for all other models. Different measures of nestling body mass were

used because body mass increase was not available for attachment success after 1 h, but may provide a more reliable estimate of the effect of tick density on nestlings in the other models. All models were also fitted with nestling body condition (body mass corrected for body size) instead of body mass. Similar results were obtained and therefore these data are not shown.

The effect of infestation density on relative nestling body mass increase was evaluated with a generalised linear model including tick density after 48 h as a covariate.

In all models, we corrected for potential area and year differences by adding the factors area and year, and their two-way interaction (area : year), to all models. In the model for tick engorgement weight, nest box was fitted as a random effect with package LME-4 v 1.1-7 (Bates et al., 2013) to correct for the non-independence in the response variable. In all models a stepwise selection procedure was used in which the model was iteratively refitted after exclusion of the least significant covariate. Terms were not removed if they were part of a higher order significant interaction. Engorgement weight was corrected for scutum size and a square transformation was carried out to fulfil normality assumptions. Estimates are reported as mean $\pm$ S.E. unless otherwise mentioned. $P < 0.05$ was considered significant.

2.5. Data archiving

Experimental data have been submitted to Mendeley: doi:10.17632/ddy82wg2fw.1.

3. Results

Attachment success 1 h after infestation was 85% ($n = 356$ ticks across 137 birds). There was a significant interaction between tick density and nestling weight; the heavier the nestlings, the stronger the effects of tick density on tick attachment success ($\chi^2_{1,130} = 5.61$, $P = 0.018$; Fig. 1, Table 1).

Of the ticks attached to the birds after 1 h, 81% were attached after 48 h ($n = 306$ ticks across 137 birds). There was no effect of tick density ($\chi^2_{1,122} = 0.95$, $P = 0.329$) or nestling body mass ($\chi^2_{1,124} = 9.51$, $P = 0.056$), nor a significant interaction ($\chi^2_{1,119} = 0.01$, $P = 0.906$; Fig. 2, Table 1).

Of the ticks attached after 48 h, 69% were recovered ($n = 254$ ticks across 126 nests). The proportion of ticks recovered increased significantly with tick density ($\chi^2_{1,106} = 6.67$, $P = 0.009$; Fig. 3, Table 1). There was no effect of nestling body mass ($\chi^2_{1,105} = 1.17$, $P = 0.280$), nor a significant interaction ($\chi^2_{1,103} = 0.20$, $P = 0.657$).

The average engorgement weight of ticks was 38.33 ± 0.73 mg ($n = 182$ ticks across 85 nests). There was no effect of tick density ($\chi^2_{1,177} = 0.09$, $P = 0.770$) or nestling body mass ($\chi^2_{1,177} = 0.78$, $P = 0.378$), nor a significant interaction ($\chi^2_{1,176} = 2.59$, $P = 0.108$; Fig. 4, Table 1). Engorgement weight was significantly correlated with scutum size ($r_{189} = 0.457$, $P < 0.001$).

The relative increase of nestling body mass was significantly correlated with tick density ($\chi^2_{1,122} = 0.21$, $P = 0.035$; Fig. 5).

4. Discussion

The current study is, to our knowledge, the first to have investigated the effects of tick density on attachment and feeding success in a nidicolous tick. Nidicolous ticks are more dependent on individual hosts than non-nidicolous ticks and can therefore be expected to feed more slowly, reach a lower maximum engorgement weight and have a smaller effect on host health (Lehmann, 1993; Clayton and Tompkins, 1994; Heylen and Matthysen, 2011a). In the current study, more ticks were recovered at higher feeding densities. This suggests that feeding nidicolous ticks facilitate each-other's feeding success. We found no effect of feeding density on engorgement weight. This suggests that feeding aggregation is

unlikely to affect tick fecundity, at least at the tested densities, because engorgement weight is correlated with clutch size and hatching success (Gladney and Drummond, 1970; Chen et al., 2009; Ma et al., 2013).

Previous studies have shown *I. arboricola* have a lower engorgement weight, longer feeding duration and no health-impairing effects on their hosts compared with the congeneric non-nidicolous *I. ricinus* (Heylen and Matthysen, 2011a). It has been suggested this is adaptive because nidicolous ticks are strongly dependent on individual hosts and their offspring, and harming them affects the ticks themselves (Heylen and Matthysen, 2011a; Gray et al., 2014). In the current study, there was no increase in engorgement weight due to aggregation. In addition, nestling growth was actually higher for nestlings with higher tick densities. This corroborates a previous study by Heylen and Matthysen (2011a) who found that nestlings or their parents overcompensate the expected negative tick effects, e.g. through increased begging and resultant food provisioning (Heylen and Matthysen, 2011a and references therein). Thus, our results suggest that feeding aggregation of *I. arboricola* ticks has no short-term effects on the host. However, host body mass was only recorded over 48 h and we did not investigate other health-related parameters (e.g. haematocrit levels and albumin/globulin ratios). Therefore further studies are required to investigate whether feeding aggregation of nidicolous ticks leads to long-term health-impairment for their hosts.

Aggregation of ticks (Davidar et al., 1989; Harrison et al., 2012; Moshaverinia et al., 2012; Heylen et al., 2013a) and many other parasites (Shaw and Dobson, 1995; Woolhouse et al., 1997; Wilson et al., 1998; Poulin, 2007; Calabrese et al., 2011) in nature primarily occurs due to heterogeneity in host exposure and susceptibility to parasitism (Wilson et al., 1998; Poulin, 2013). However, there may be adaptations that lead to aggregation and on which selection can operate. In *I. arboricola*, these preferences are most apparent during the breeding season. During the breeding season, *I. arboricola* has the ability to infest both parental birds as well as any of the nestlings, yet adult female ticks feed almost exclusively on nestlings and usually avoid adult birds (Heylen et al., 2014). In addition, *I. arboricola* prefers to feed on the most developed nestlings (Heylen and Matthysen, 2011b; Heylen et al., 2012). Both

observations lead to overdispersion within nests, with the majority of ticks on the most developed nestlings. The current data do not allow testing of whether aggregated feeding occurs because ticks prefer to feed in aggregation or because certain hosts are intrinsically more profitable. It would be worthwhile to investigate this further because it is largely unclear which underlying mechanisms determine whether ticks that feed in aggregation facilitate each other's feeding success (Wilson et al., 1998; Poulin, 2007).

Nestlings with more ticks had higher growth rates but the proportion of ticks attached (as well as recovery rate and engorgement weight) was unaffected. We therefore suggest that hosts compensate for tick burdens (as found in earlier work, see Heylen et al., 2015 for adult birds; Heylen and Matthysen, 2011a for nestlings), rather than that nestlings with higher growth rates are more profitable for parasites because they are more nutritious or invest less in parasite countermeasures. On the other hand, previous research has shown that ticks, when they can choose between nestlings of varying developmental states, prefer more developed nestlings (Heylen and Matthysen, 2011b; Heylen et al., 2012). Because ticks in our experiment were not given a choice and because a tick's host choice is final (Heylen and Matthysen, 2011b), the ticks may have attached regardless of host quality and remained attached even when other hosts became available. Only for the heaviest nestlings did we find that attachment success after 1 h was higher at lower tick densities. Since tick density enhances recovery rates, it may be more critical for ticks to select a profitable, i.e. heavier and more developed, host when feeding in solitude than when feeding in aggregation.

We did not investigate whether feeding duration was affected by tick density because ticks were recovered from the upper surfaces of the nest boxes only once every 2 days. Therefore the time resolution is low and in addition we cannot be sure exactly when the ticks detached from their hosts. We believe the influence of the recovery procedure on tick engorgement weight (due to pre-weighing digestion) should be minimal because egg production takes considerable time under field conditions (Heylen et al., 2014). However feeding duration may have been influenced by tick density. Because shorter feeding times reduce host-induced tick mortality (Clayton and Moore, 1997), at higher densities one would expect a

shorter feeding time if aggregated feeding is facilitative, and a longer feeding time if it is competitive. In *Rhipicephalus appendiculatus* aggregation leads to facilitation and reduces feeding time (Wang et al., 2001), whereas in *Dermacentor reticulatus* it leads to competition and increases feeding time (Bartosik and Buczek, 2012). For *I. arboricola*, the fact that we found no competition due to aggregation makes it unlikely that feeding time would be increased at higher tick densities but further research is required to investigate whether aggregation reduces feeding time.

How is it possible that feeding aggregation leads to facilitation in some cases (Davidar et al., 1989; Hazler and Ostfeld, 1995; Wang et al., 2001; Ogden et al., 2002) and to competition in others (Sutherst et al., 1979; Randolph, 1994; Levin and Fish, 1998; Bartosik and Buczek, 2012; Jones et al., 2015)? This may be due to the interaction between the ability of ticks to suppress host immune responses, and the ability of hosts to repel ticks. Ticks introduce salivary compounds that suppress host inflammatory responses and prevent haemostasis. In susceptible hosts, i.e. hosts lacking tick antibodies, although converse effects may be observed due to an innate, tick-unrelated immune response, such immunosuppressants may be too dilute at low tick densities but facilitate feeding at higher densities (Davidar et al., 1989; Wang et al., 2001). At high parasite densities there may be resource competition among parasites and the host may be unable to compensate (Lehmann, 1993). Indeed, some studies have found parasite competition at higher densities in susceptible hosts (Randolph, 1994; Bartosik and Buczek, 2012). In resistant hosts, salivary compounds will be ineffective but activate host grooming and immune responses (Wikel, 1996; Brossard and Wikel, 2004), such that high densities of ticks are likely to result in an elevated immune response by the host and lowered feeding success of ticks (Hazler and Ostfeld, 1995). Indeed, there are examples where tick feeding success decreases because hosts acquired resistance from previous infestations (e.g. Davidar et al., 1989; Hazler and Ostfeld, 1995). However, ticks may also be able to avoid or suppress host resistance responses, for instance due to a long coevolutionary history with specific host species, even adult hosts that acquired resistance (Heylen et al., 2010).

Clearly it is difficult, if not impossible, to predict the outcome of parasite density on feeding success and host health. Because parasite population growth, the effects of parasites on their hosts and pathogen transmission are density-dependent, there is a need for empirical studies that investigate the mechanisms underlying patterns of aggregation. The knowledge such studies may provide is important because parasites may be directly detrimental to their hosts and/or of potential significance as pathogen vectors for both humans and livestock (Clayton and Moore, 1997; Poulin, 2007).

Acknowledgements

We would like to thank Frank Adriaensen, Joris Elst, Sophie Philtjens and Naomi van der Wijk for field assistance and Natalie Van Houtte for laboratory assistance. This research was supported by Fonds Wetenschappelijk Onderzoek (FWO, Belgium) grant G.0049.10 to EM, and an FWO postdoctoral fellowship to DJAH. The experimental protocol was approved by the Ethical Committee of the University of Antwerp, Belgium and experiments on birds were licensed by the Agentschap voor Natuur en Bos, Belgium.

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477

Legends to Figures

Fig. 1. Proportion of attached *Ixodes arboricola* ticks 1 h after infestation of great tit nestlings in relation to tick density and nestling weight (g) at infestation.

Fig. 2. Proportion of attached *Ixodes arboricola* ticks 48 h after infestation of great tit nestlings in relation to tick density 1 h after infestation.

Fig. 3. Proportion of *Ixodes arboricola* ticks recovered from great tit nests in relation to tick density 48 h after infestation of great tit nestlings.

Fig. 4. Engorgement weight of recovered *Ixodes arboricola* ticks corrected for scutum length in relation to tick density 48 h after infestation of great tit nestlings. Whiskers: 1.5 * interquartile range; black circles: outlier values.

Fig. 5. Relative increase of great tit nestling body mass over 48 h in relation to *Ixodes arboricola* tick density 48 h after infestation. Whiskers: 1.5 * interquartile range; black circles: outlier values.

Table 1

Table 1. Summary of the fixed effects in the generalised linear (mixed) models of attachment success of *Ixodes arboricola* proportion of *I. arboricola* ticks recovered and engorgement weight in relation to infestation density on great tits and gre. Significant effects are in bold.

Fixed effect	Attachment 1 h		Attachment 48 h		Recovery rate	
	χ^2	<i>P</i>	χ^2	<i>P</i>	χ^2	<i>P</i>
Tick density			$\chi^2_{1,122} = 0.95$	0.3287	$\chi^2_{1,106} = 6.67$	0.009
Nestling body mass			$\chi^2_{1,124} = 9.51$	0.0562	$\chi^2_{1,105} = 1.17$	0.280
Density : body mass	$\chi^2_{1,130} = 5.61$	0.018	$\chi^2_{1,119} = 0.01$	0.906	$\chi^2_{1,103} = 0.20$	0.657

Figures

Relative increase of nestling body mass

0.2
0.1
0.0
-0.1

0

1

2

3

4

5

Tick density on nestlings 48 h after infestation